Drosophila *P* Element Transposase Induces Male Recombination Additively and Without a Requirement for *P* Element Excision or Insertion

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ABSTRACT

P element dysgenesis-associated male recombination in Drosophila was examined with a selective system focused upon a section of the third chromosome divided into eight recombination segments. Tests compared crossing over in the presence of none, one and two doses of *P(Δ 2-3)(99B)*, a non-mobile transposase source, in the absence of a mobilizable *P* element target in the genome. In the presence of the *P* transposase source, and without a *P* element target, significant male recombination occurred in genomic regions physically separated from the *P(Δ 2-3)* site. Using two doses of *P(Δ 2-3)* without a *P* element target, the male recombination rate doubled, and 90% of the crossovers occurred in the pericentric region. The distribution of recombination events, in the absence of *P* element targets approximates that seen in studies of radiation induced mitotic crossing over and the metaphase chromosome map. Another experiment examined the effects of one dose of *P(Δ 2-3)* on a genome with a single *P* element target, *P(lArB)(87C9)*, in the third recombination segment. Crossovers increased 58-fold in the immediate region of the *P* element target.

THE first recognized feature of *P* element hybrid dysgenesis was the discovery of significant crossing over among the progeny of hybrid dysgenic males resulting from crosses of natural population samples to a laboratory tester strain (HIRAIZUMI 1971). From the outset, the clustered appearance of the exceptional crossover individuals served to identify the event as occurring largely in gonial mitosis (HIRAIZUMI *et al.* 1973). Subsequent studies demonstrated that these events occurred in dysgenic individuals of both sexes (KIDWELL 1977; SLATKO 1978) and that their chromosomal distribution differed markedly from that of normal female meiotic exchange. Thus, KIDWELL and KIDWELL (1976) and DUTTAROY *et al.* (1990) found that their male recombination maps approximated the polytene chromosomes. SLATKO (1978) suggested that, while the data resembled both the salivary gland and metaphase maps, it also resembled the mitotic recombination maps, allowing for some discrepancy in the centromeric region. SINCLAIR and GRIGLIATTI (1985) reported a non-random distribution which approximated neither the standard genetic map nor the presumably random exchanges of the radiation induced male recombination map.

Following the association of hybrid dysgenesis with the *P* family of transposable elements (BINGHAM *et al.* 1982), efforts to understand their various biological effects focused upon the *P* element transposase, its several truncated forms and their effects upon the Drosophila genome and the *P* elements inserted therein. This topic has been the subject of extensive, recent review (ENGELS 1989; RIO 1990, 1991). With respect to dysgenesis-associated crossing over, models for understanding this phenomenon have placed varying emphasis upon features of the biochemical activities of the transposase and the transpositional behavior of the *P* element itself. Thus, SVED *et al.* (1990, 1991) view the crossover event as a direct result of transposition of a *P* element, which is initiated by transposase action effecting a double-strand break excision of the *P* element. Emphasis here is placed upon the high binding affinity of transposase for the *P* element DNA (KAUFMAN *et al.* 1989). A different model emerged from preliminary studies in this laboratory (MCCARRON *et al.* 1989; DUTTAROY *et al.* 1990), that were unable to associate induced crossovers with specific *P*-element transposition events, and recognized the ability of transposase to bind and cut Drosophila DNA, even in the complete absence of *P* element targets. This report represents a continuation of this work.

MATERIALS AND METHODS

Strains: All mutations and rearrangements not described herein are described in LINDSLEY and ZIMM (1992). Abbreviations have been used for the following mutations, chromosomes and transposons:

M(3)76A = slight *Minute* in *76A3/B2* used here as a selective lethal. Recessive lethal effect in late embryogenesis. *Minute* mutants show delayed development: M^+/M^+ cells produced by mitotic exchange in an M/M^+ fly are reported to proliferate more rapidly than surrounding cells (MORATA and RIPOLL 1975), which may serve to increase cluster size.

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Dfd^{1} = *Antennapedia Complex* mutant in *84A4/5* showing a spectrum of head defects and used here as a selective lethal. Recessive lethal effect at end of embryogenesis.

kar^{2} = eye color mutant in *87C8* used as a visible marker for exchange.

mesB^{7} = spontaneous allele of *messyB* at *87D7/10*. Semilethal. Recessive visible phenotype outspread wings and dark trident-like crown on dorsal thorax. Used as genetic marker.

rosy^{106} = eye color mutant at *87D11/12*, distinct from *kar*^{2}, used as a visible genetic marker.

pic^{23} = recessive lethal at *piccolo* in *87D11/14*. Used as genetic marker.

Ace^{1} = acetylcholinesterase mutant at *87E3*, formerly called *l(3)26*. Late embryonic recessive lethal used as a genetic marker.

Sb^{1} = *Stubble* locus bristle mutant in *89B9/10*, used here as a selective lethal. Recessive lethal effect during early larval development.

Ubx^{1} = *Bithorax Complex* mutant *Ultrabithorax* at *89E1/2*. Shows enlarged halteres. Used here as a selective lethal. Recessive lethal effect in early third instar larvae.

MRS = *Tp(3;3)MRS, M(3)76A ry*^{2} *Sb*: balances proximal regions of both arms of chromosome *3*.

MKRS = *Tp(3;3)MKRS, M(3)76A kar ry*^{2} *Sb*: balances proximal regions of both arms of chromosome *3*.

P18 = *In(3L)P* + *In(3R)P18, kar ry*^{41} *Ubx e*^{4}.

TM3,Ser = *In(3LR)TM3, p*^{p} *bx*^{34e} *e Bd*^{S}: balances all but the distal 3L region of chromosome *3*.

TM6B,Tb = *In(3LR)TM6B, e Tb ca*: balances all of chromosome *3*.

P(Δ2-3) = *P(Δ2-3ry*$^{+}$*)(99B)*, an unusually stable transposase source (Robertson *et al.* 1988) carrying two *P* elements head to head in *99B*. In the mating schemes of Tables 1, 4 and 5 (with the exception of cross 3), *P(Δ2–3)* bearing progeny are excluded. Thus, the crossover exceptions are recovered in a stable genome.

P(lArB) = *P(lacZ Adh*$^{+}$ *rosy*$^{+}$ Bluescript *M13*$^{+}$ *KS) A53.1M3* at *87C9*, a homozygous viable stock with no phenotypic alteration due to the insertion at *87C9*. This transposon stock was obtained from the Bloomington, Indiana, Stock Center as *A53.1M3*, which carries the insert in a *rosy*^{506} background. The *P(lArB)* location in *87C9* was confirmed by *in situ* hybridization and Southern blot, and was mapped by deletion tests to the region between *l(3)87Cd*^{16} and *l(3)87Da*^{2} (Figure 2). The 19 kb *P(lArB)* transposon carries a *P-lacZ-hsp70* fusion gene, a 3.2-kb fragment with the *Adh*$^{+}$ gene, a 7.2-kb fragment containing the *rosy*$^{+}$ gene, and a 3.5-kb fragment from the Bluescript M13^{+} KS vector (Stratagene) which carries the bacterial gene for ampicillin resistance (Wilson *et al.* 1989). The original *P(lArB) rosy*^{506} chromosome is homozygous viable. This chromosome was used in crosses 4 and 5 (Table 4). For crosses 6 and 7 (Table 5), appropriate markers were crossed onto the original chromosome to provide the lethal selective system shown in Figure 1, chromosome B.

Chromosomes used in these experiments, with the exception of *P(lArB) ry*^{506}, were constructed from stocks already in use in this laboratory as a recombination selective system for the *rosy* region and first described in Chovnick *et al.* (1962). The mutants *M(3)76A, Dfd, Ace, Sb* and *Ubx* are the original lethal alleles at these loci: *M(3)76A* and *Ace* are recessive lethals; *Dfd, Sb* and *Ubx* are dominant visibles with recessive lethal effect. The construction of the male parent chromosomes A and B, without the optional *P(lArB)* and *P(Δ2-3)*, and the tester female (Figure 1) was described in McCarron *et al.* (1989). Each stock originated from a single third chromosome in the male, balanced, and checked by *in situ* hybridization and Southern blot for the presence of *P* elements. None were found. Presence of the required markers was checked at each step in construction. *P(Δ2-3)* was placed on the chromosomes by standard recombination techniques. At each step, *in situ* hybridization and Southern blots were used to identify a single third chromosome in the male carrying *P(Δ2-3)99B* to be carried into the next construction step. Each final stock was bred from a single, balanced, fully tested male.

In all recombination experiments flies were reared on standard Drosophila cornmeal-sucrose-yeast medium at 25°, the optimum temperature for *P* element-induced male recombination (Kidwell and Novy 1979). Individual chromosome A and B stocks for the hybrid male of Figure 1 were maintained over the whole chromosome balancers *TM3,Ser* or *TM6B,Tb*. Individual 2–4-day-old hybrid males were mated to six tester females in culture vials. These were transferred onto fresh medium to permit a total of 20 days of egg laying. Crossover exceptions were mated individually to *P18/MKRS* and the exceptional third chromosomes maintained over *MKRS* to balance the region of interest. Exceptions carrying *Sb* were balanced over *TM6B,Tb* or *TM3,Ser*. Estimates of total progeny for the flanking lethal selection experiments (Tables 1 and 5) were determined by crossing the appropriate hybrid males to *rosy*^{506} nonselective females. All progeny survived, were counted, and provided a multiplier to estimate the number of progeny in a given experiment. In cross 4 (Table 4, row 1) the progeny of 15 of the experimental vials were counted during screening to provide a progeny estimate. In cross 5 (Table 4, row 2), all progeny of the experiment were counted.

Molecular techniques: DNA extractions and Southern blots were performed according to standard methodology substantially as described by Rushlow *et al.* (1984) and Sambrook *et al.* (1989). Genomic DNA was prepared from approximately 125 adult male flies. Gels were blotted to nytran membrane (Schleicher & Schuell). Filters were stripped for serial reprobing by the method of Bucheton *et al.* (1984). Probes were prepared both from plasmid DNA and from isolated DNA fragments extracted from low melting point agarose gel. For radiolabeling, both [^{32}P]dCTP and [^{32}P]dGTP were used and nick translation was performed with the DNA labeling system obtained from BRL.

The following probes were used in the study:

pπ25.7 BWC: The plasmid pπ25.7BWC ("both wings clipped"), which was used to identify the *P* element head and tail regions of *P(lArB)*, was constructed by Kevin O'Hare from pπ25.7WC (Karess and Rubin 1984).

pSalI 12.5 kb: Genomic DNA contiguous to the 3′ end of *P(lArB)* was cloned directly by plasmid rescue (Wilson *et al.* 1989). The clone carries the 3.5-kb fragment from the Bluescript *M13*$^{+}$ KS vector, the 3′ *P* element end and 9 kb of genomic DNA contiguous to the 3′ end of *P(lArB)*.

pEcoRI 8 kb: This is the 8-kb *Eco*RI restriction fragment in *87C9* into which *P(lArB)* was inserted.

Cytology: Larval salivary gland chromosomes were prepared for squashes and for *in situ* hybridization from mature third instar larvae grown at 18°. Probes were prepared by standard nick translation in the presence of Biotin-11-dUTP (Enzo Biochemicals). Hybridization conditions are described in Engels *et al.* (1986), and sites of hybridization were identified with the streptavadin-alkaline phosphatase staining reaction.

EXPERIMENTAL DESIGN

Flanking lethal crossover selective screen: A flanking lethal crossover selective screen (Figure 1) was used to allow recovery only of rare male recombinant exceptions from a large experimental progeny (McCarron

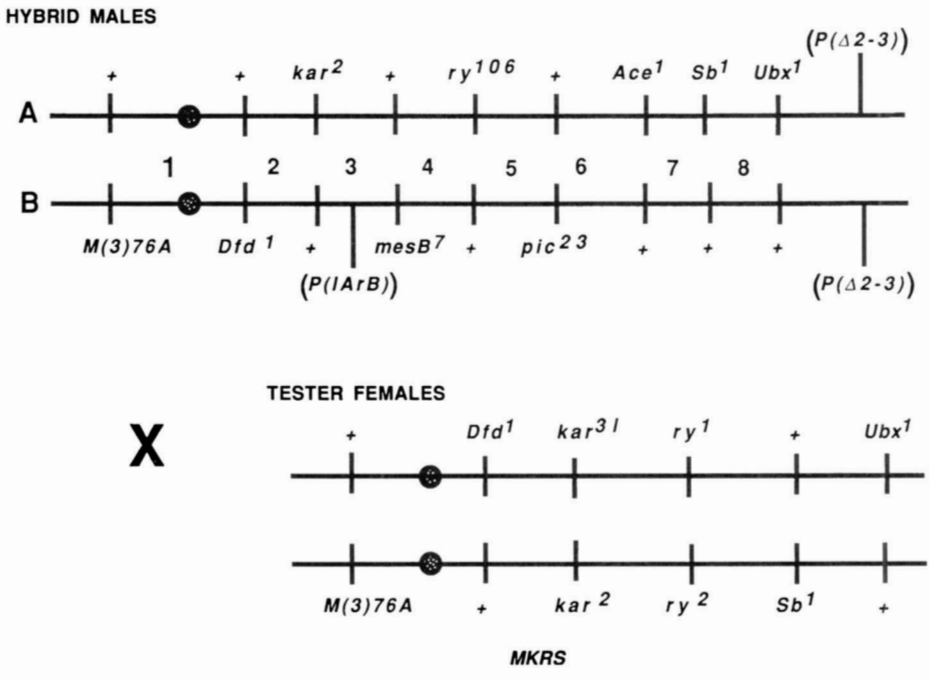

FIGURE 1.—The third chromosomes of a hybrid male parent are shown with genetic markers delineating the crossover regions, which are numbered. Optional markers are shown in parenthesis. The tester female parent is shown. The chromosomal distribution of lethals in the male parent and tester female constitutes a flanking lethal crossover selective system. This mating scheme kills parental male chromosomes but permits recovery of crossovers between *M(3)76A* and *Ubx* in one direction only. Survivors carry crossovers consisting of that portion of the A chromosome to the left of the event and that portion of the B chromosome to the right of the event.

et al. 1989; DUTTAROY *et al.* 1990). A male parent heterozygous for the recessive lethal marked third chromosomes, *M(3)76A Dfd ++/++ Sb Ubx* is mated to tester females carrying the recessive lethal marked third chromosomes *Tp(3;3)MKRS, M(3)76A + Sb +/+ Dfd + Ubx*. In the absence of recombination in the region from *M(3)76A* to *Ubx*, all developing zygotes die due to homozygosity for one or another of the lethals, *M(3)76A*, *Dfd*, *Sb* and *Ubx*. The tester females carry the multiple break rearrangement *Tp(3;3)MKRS*. Since crossovers within this region in such females have never been found, the lethal arrangement in the tester females is secure. Hence, the only zygotes expected to survive and complete development are those that receive one or another of the several classes of single crossovers that might occur in the male parent. We recover one-half of the exchanges between *Dfd* and *Sb*. One exchange product is free of lethals and survives over either tester chromosome, whereas the reciprocal product carries all four selective lethals and dies over either tester. Therefore, in estimating total events in this region the observed events are multiplied by two. Multiple exceptions of a single crossover class from the same male parent are treated as a cluster. Each recognized cluster is treated as a single recombination event and multiplied by two in all regions when estimating total events. We recover one-fourth of the exchanges in the regions *M(3)76A* to *Dfd* and *Sb* to *Ubx*. The retrievable exchange products live over only one tester chromosome and the reciprocal products die over either tester. Therefore, surviving single recombinants are multiplied by four to calculate the frequency of crossing over. However, a premeiotic event that produces a cluster of meiocytes bearing the viable product (*+ Dfd ++*) has multiple opportunity to be detected by fertilizing an MKRS-bearing egg. Each cluster, counted as a single event, is multiplied by two rather than four in calculating total events. The male parent is also heterozygous for a series of five recessive markers of the region between *Dfd* and *Sb* (*kar*, *mesB*, *ry*, *pic*, *Ace*) that are not selected against in the cross. These allow assignment of exchange events to specific intervals within the region bounded by the selective lethal markers. Moreover, they serve to verify the legitimacy of the surviving exceptions. Note that the surviving crossovers do not carry the $P(\Delta 2\text{-}3ry^{+})$ transposase source except in cross 3 which is homozygous for $P(\Delta 2\text{-}3)$.

Little information is available on possible gonial cell lethality consequent upon homozygosis of the *Dfd*, *mesB*, *Ace* and *Sb* markers following premeiotic crossover. Homozygous *Dfd* and *Ace* are lethal in late embryogenesis; homozygous *Sb* is lethal in early larval development. *MesB* is semilethal and produces a distinctive survivor class when homozygous. *Pic* is a recessive lethal whose time of action is unknown, but occasional homozygotes survive with a small bristle phenotype. Should any of these genes have vital function in gonial cells, mutant homozygosis following a premeiotic crossover would result in cell death. A to B chromosome crossovers (Figure 1), which will be the only ones to survive over the tester chromosomes in the zygotes, are expected to occur in four gonial cell contexts, three of which carry no homozygous lethals. The fourth class carries one to three lethals. Premeiotic A to B crossovers will pair with either the reciprocal crossover strand or the parental strand due to equational assortment of the centromeres. Cells with crossovers between *M(3)76A* and the centromere will carry no homozygous lethals in either pairing. Cells with premeiotic crossovers to the

TABLE 1

Crossing over occurring in males of the indicated genotypes crossed to tester females

Male parent	Single male matings	Observed exceptions (singles, clusters)	Independent events		Progeny estimate	Recombination mean[a] $\times 10^5$
			Minimum	Extrapolated		
1[a,b] 1a. A/B	99	0	0	0	46,431	
1b. B/A	135	1	1	2	69,525	
Control cross sum: A/B plus B/A	234	1	1	2	115,956	1.72
2[c] A, *P(Δ 2-3)*/B	259[d]	27 (8S, 5C)	13	36	81,478	44.2
3 A, *P(Δ 2-3)*/B, *P(Δ 2-3)*	326[e]	246 (8S,25C)	33	80	101,131	79.1

[a] In the absence of *P* transposase, there is no crossing over expected in Drosophila males. The maximum value of the 95% Poisson confidence interval for a null event is three events in a large sample (STEVENS 1942).

[b] These data were reported previously in MCCARRON *et al.* (1989).

[c] A portion of these data was reported previously in MCCARRON *et al.* (1989).

[d] Twelve matings produced crossovers.

[e] Thirty-two matings produced crossovers.

right of the centromere, when paired with the parent B strand, may carry as many as three such lethals. Crossovers further to the right would carry fewer lethals thereby providing a possible advantage to region 2–8 crossovers.

All surviving crossovers carry *M(3) 76A*$^+$; those region 1 crossovers to the left of the centromere, which pair with the parental A strand will be M^+/M^+ cells which are reported to proliferate more rapidly in somatic tissue than M/M^+ cells (MORATA and RIPOLL 1975). Perhaps they might form larger gonial clusters. Premeiotic crossovers to the right of the centromere do not form M^+/M^+ cells.

RESULTS

Male recombination and P(Δ2-3) dosage variation: In previous experiments, using the flanking lethal selective system, we demonstrated that, in the presence of *P(Δ 2-3)* as a source of transposase, and in the complete absence of *P* element targets, male recombination occurred in chromosome regions clearly distinct from the *P(Δ 2-3)* site. The male recombination rate for the monitored region *M(3) 76A* to *Ubx* was shown to be twenty three times the background rate seen in the absence of a transposase source (MCCARRON *et al.* 1989). We have repeated this experiment in order to amplify the data and have added the results to those published earlier. The control data from MCCARRON *et al.* (1989) is also reviewed here to provide a more coherent background for the present series of experiments. Cross 1 (Table 1, rows 1–3) is that control, and demonstrates the rate of male crossing over in the selective lethal system without a *P* element transposase source. In this experiment, which is composed of two smaller experiments, heterozygous males are mated to tester females carrying appropriate markers, and heterozygous for multiple break rearrangements that totally suppress crossing over in the female in the region covered by the genetic markers. The male parental chromsomes (Figure 1) are lethal in progeny receiving either of the female tester chromsomes due to homozygosity of *Sb*, *Ubx*, *M(3) 76A* or *Dfd*. In the first test (row 1), no exceptions were found in a screen that sampled 46,431 progeny. Upon reversing the direction of the cross, introducing chromosome A into the hybrid male through the female parent (row 2), we recovered only one exception, a kar^2 ry$^+$ crossover in region 3 (Figure 1), in a progeny sample estimated at 69,525. Since the reciprocal crossover is lost to the selective lethal system, we estimate a recovery of two exceptions. Since these results are not significantly different (see Table 1, note *a*), we pooled these data in Table 1, row 3. The resulting pooled male recombination data (row 3, column 7) are used as a base line for further tests.

Cross 2 (Table 1, row 4) describes the male recombination induced by a single transposase source, *P(Δ 2-3)(99B)*, introduced into the same lethal selective system. In a screen that sampled 81,478 zygotes we recovered 27 exceptions. These 27 exceptions were recovered as crossovers in the intervals between *M(3) 76A* and *Ubx*, a region physically separated from the *P(Δ 2-3)* transposase source at *99B*. Only the first exception of any genetic class arising from a single mating is counted as an independent event in order to eliminate possible premeiotic clusters. Thus, of the 27 observed exceptions, 13 are considered to be the minimum number of observed, independent crossover events (Table 1, column 4). From this minimum number of independent events we estimate the total number of independent events (Table 1, column 5) using multipliers that correct for zygote lethality (see EXPERIMENTAL DESIGN). The 36 extrapolated independent events in a progeny count of 81,428 give a male recombination mean of 44.2×10^{-5} (Table 1, column 7). This represents the male recombination rate induced by a single transposase source in a genetically defined region separated from the transposase source and without *P* element targets. The upper limit of the 95% Poisson confidence interval (STEVENS 1942) constructed about the frequency of minimum independent events in the summed controls (4.80×10^{-5})

TABLE 2

Distribution of male recombinants in cross 2

Exchange interval		Observed recombinants (singles, clusters)	Independent events		Percent of total independent events	Recombination mean $\times 10^5$
Genetic	Cytological[a]		Minimum	Extrapolated		
1. *M(3)76A-Dfd*	*76A3/B2-84A4/5*	21 (5S,4C[b])	9	28	77.8	34.4
2. *Dfd-kar*	*84A4/5-87C8*	1	1	2	5.6	2.45
3. *kar-mesB*	*87C8-87D7/10*	1	1	2	5.6	2.45
4.–5. *mesB-pic*	*87D7/10-87D11/14*	0	0	0	0	0
6. *pic-Ace*	*87D11/14-87E3*	0	0	0	0	0
7. *Ace-Sb*	*87E3-89B9/10*	4 (1S.1C[b])	2	4	11.0	4.90
8. *Sb-Ubx*	*89B9/10-89E1/2*	0	0	0	0	0
Totals	*76A3/B2-89E1/2*	27 (8S,5C[b])	13	36	100	44.2

[a] LINDSLEY and ZIMM (1992), DUTTAROY *et al.* (1990).

[b] Observed cluster sizes—Region 1: one each of 7, 4, 3, 2; Region 7: one of 3. Mean cluster size (Region 1 cluster sizes are multiplied by 2): $\bar{X} = 7.0 \pm 5.0$.

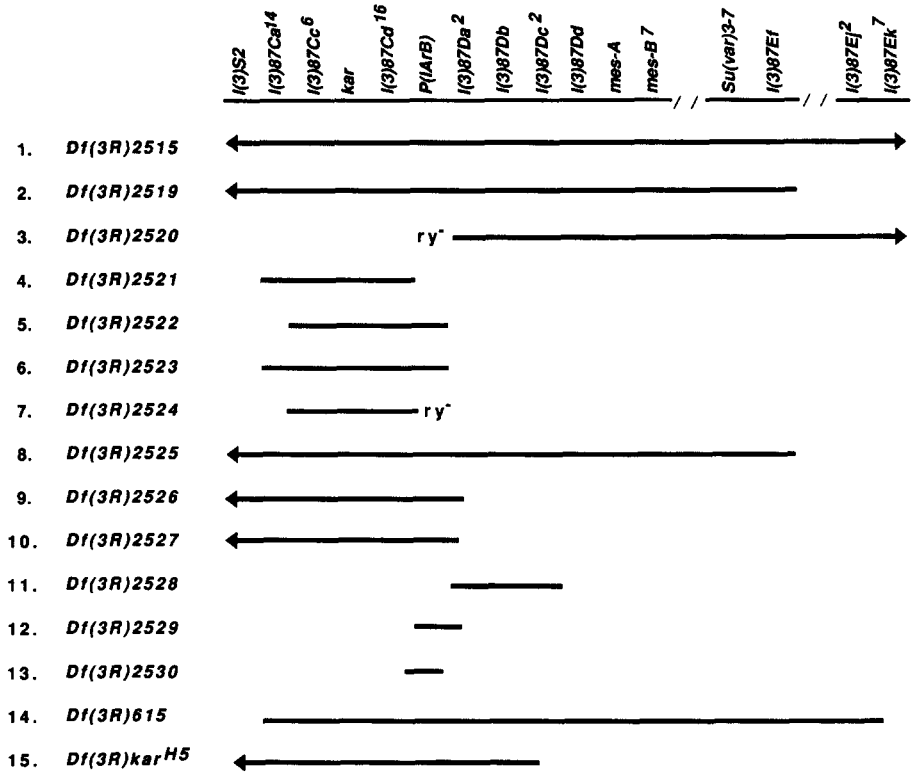

FIGURE 2.—Complementation maps of deletions. *Df(3R)2515* was recovered from cross 2 (Table 1, row 4). *Df(3R)2519* was recovered from cross 3 (Table 1, row 5). Deletions in rows 3 through 8 were recovered from cross 5 (Table 4, row 2). Deletions in rows 9 through 13 were recovered from cross 7 (Table 5, row 2). Absence of *P(lArB)*, as indicated by a line extending through the region, was determined by Southern blots and *in situ* hybridization. The loss of the ry phenotype is noted in two instances where part of the transposon is retained: *Df(3R)2520* (row 3) and *Df(3R)2524* (row 7). *Df(3R)615* (row 14) was induced with ethyl methanesulfonate on a ry^{+6} background and was used as a tester for deletions in cross 5. $Df(3R)kar^{H5}$, x-ray-induced (HENIKOFF 1979), was used as a deletion tester in cross 7.

is less than the lower limit of the 95% Poisson Confidence Interval for the independent events in cross 2 (8.49×10^{-5}). This indicates a highly significant difference between these two crosses, whose only difference is the addition of *P(Δ2-3)* to cross 2. We attribute this difference to *P* element transposase induction of crossovers in cross 2.

Cross 3 (Table 1, row 5) demonstrates the effect of two doses of *P(Δ2-3)(99B)* on male recombination in the same genetic intervals monitored in crosses 1 and 2 (Table 1, rows 1–4). A total of 246 fertile exceptions were recovered from a screen that sampled an estimated 101,131 zygotes. The recombination mean, based on 80 extrapolated independent events (Table 1, row 5, column 5) is 79.1×10^{-5}. The cross 3 recombination mean of minimum independent events is 3.26×10^{-4}. This mean is greater than the upper limit of the 95% Poisson confidence interval for the cross 2 mean of independent events indicating a significant difference between these crosses, which we attribute to the addition of a second dose of *P(Δ2-3)*. The recombination means of independent events of cross 2 and 3 are consistent with a dose dependent effect: 1.60×10^{-4} for one dose of *P(Δ2-3)*, 3.26×10^{-4} for two doses of *P(Δ2-3)*.

The recovered exceptions of cross 2 (Table 1, row 4), are detailed in Table 2. Exchange classes 4 and 5 have been pooled in Tables 2, 3, 6 and 7 since no exchanges were recovered in the intervals between *mesB-ry-pic*. All

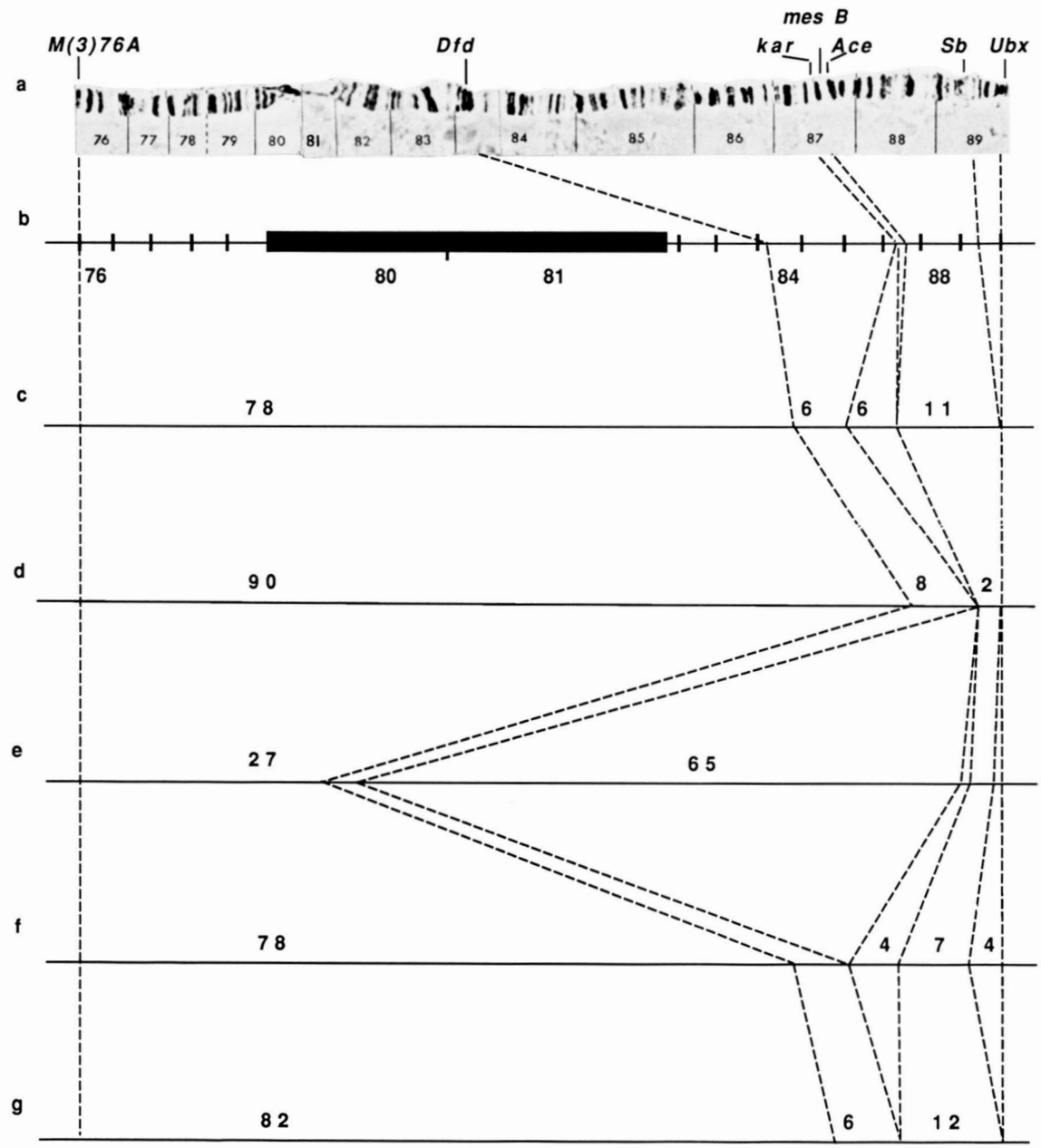

FIGURE 3.—A comparison of relative map distances (in percent) between genomic markers on seven chromosome maps. (**a**) Polytene salivary gland chromosome (LEFEVRE 1976). (**b**) Cytological map showing position of markers on the metaphase chromosome (BECKER 1976). The heavy line represents heterochromatin. (**c**) Male recombination map from cross 2 (Table 2, column 6) with heterozygous *P(Δ2-3)(99B)*. (**d**) Male recombination map from cross 3 (Table 3, column 5) with homozygous *P(Δ2-3)(99B)*. (**e**) Male recombination map from cross 7 (Table 6, column 5) with heterozygous *P(lArB)(87C9)* and heterozygous *P(Δ2-3)(99B)*. (**f**) Cross 7 without region 3 [containing *P(lArB)*]. (**g**) Cross 2 without region 3.

members of a cluster of three crossovers in region 7 (Table 2, row 7) were found to be associated with a large deletion, *Df(3R)2515* (Figure 2, row 1). Each member of the cluster was tested against an array of deletions and lethals (Figure 2, top row) and all three showed the same deletion extending from *l(3)S2* through *l(3)87Ek*^{7}. The larval salivary gland squash showed a deletion from *86E7/8* to *87F1/2*.

Figure 3, row c, provides a comparison of the events of cross 2, represented as percent of total independent events (Table 2, column 6), with the larval salivary gland map (LEFEVRE 1976) and with the metaphase map of the region (BECKER 1976). The distribution of events in cross 2 closely approximates BECKER's metaphase map of the region, where region 1 (*M(3)76A-Dfd*) is three times the size of regions 2–8 (*Dfd-Ubx*) combined. In order to compare the data for these regions on the metaphase map length basis, we must further adjust any events in regions 1 and 8 where only half of the single events survive due to the lethal selection system (see EXPERIMENTAL DESIGN). In the observed recombinant listing of single and cluster events (Table 2, column 3) we see region 1

TABLE 3

Distribution of male recombinants in cross 3

Genetic exchange interval	Observed recombinants (singles, clusters)	Independent events		Percent of total independent events	Recombination mean $\times 10^5$
		Minimum	Extrapolated		
1. *M(3)76A-Dfd*	233 (7S,22C[a])	29	72	90.0	71.2
2. *Dfd-kar*	8 (1S, 2C[a])	3	6	7.5	5.93
3. *kar-mesB*	0	0	0	0	0
4.–5. *mesB-pic*	0	0	0	0	0
6. *pic-Ace*	0	0	0	0	0
7. *Ace-Sb*	5 (1C[a])	1	2	2.5	1.98
8. *Sb-Ubx*	0	0	0	0	0
Totals	246 (8S, 25C[a])	33	80	100	79.1

Comparison of mean event size in cross 2 ($\bar{X} = 2.67 \pm 1.72$) and cross 3 ($\bar{X} = 11.98 \pm 7.30$): $t = 2.5130$, $n = 56$, $P \sim 0.016$

Comparison of mean event size in the size limit range from 1 through 14 (7×2) in cross 2 ($\bar{X} = 2.67 \pm 1.72$) and cross 3 ($\bar{X} = 5.21 \pm 1.60$: $t = 2.2360$, $n = 50$, $P \sim 0.031$

[a] Observed cluster sizes regions 1: 64, 37, 16, 14, 11, 9, 7(2), 6(4), 5(2), 4(4), 3(3), 2; region 2: 4, 3; region 7: 5. Mean cluster size (region 1 cluster sizes are multiplied by 2): $\bar{X} = 18.6 \pm 11.1$.

gave 10 singles (5×2) and 4 clusters, or 14 total events. The events per metaphase map length for region 1 are then 4.67 (14/3) and for regions 2–8 are 4.00, consistent with the notion of a broad uniformity of male recombination activity across the monitored region from *M(3)76A* to *Ubx*.

Table 3 presents the distribution of crossover events of cross 3 (Table 1, row 5). Of the total independent events (Table 3, column 5), 90% occur in the *M(3)76A-Dfd* region. Figure 3, row d, compares this distribution of events with the cross 2 distribution and the metaphase chromosome map. A cluster of five region 7 crossovers (Table 3, row 7) was found to be associated with a deletion, *Df(3R)2519*, extending from *l(3)S2* through *l(3)87Ef* (Figure 2, row 2). The cluster members were each tested using the same protocol described for the deletion in cross 2.

Comparison of the results of cross 3 (Table 3) with those of cross 2 (Table 2) reveals several features of interest. First note that all of the increase in minimum independent events has occurred in region 1 (29 compared with 9 in cross 2) while regions 2–8 produced four independent events in both cross 2 and cross 3. Thus, the region 1 crossover mean of cross 3 (2.87×10^{-4}) lies outside of and is greater than the 95% Poisson confidence interval for the crossover mean of cross 2 (5.06×10^{-5} to 2.10×10^{-4}), whereas the means for regions 2–8 in crosses 2 and 3 are not significantly different. A further feature of the results of crosses 2 and 3 is seen in the large increase in number and size of cluster events in cross 3 compared to cross 2. We infer from this observation that the increased availability of transposase leads to an increased likelihood of recombination earlier in gonial development in cross 3 and hence the difference in cluster size and number. Since members of a cluster class are treated as a single crossover event, when, in fact, they may result from more than one event, our estimate of crossover frequency is less than the real value. For cross 3, then, there is a greater underestimate of crossover frequency than for cross 2.

***P(lArB)* destabilization by P(Δ2-3):** We now consider the effect on male crossing over of the presence of a mobilizable *P* element which would serve as a target for the *P(Δ2-3)* transposase action. In this experiment, we use a chromosome carrying *P(lArB) A53.1M3* located at *87C9* on chromosome *3*. This chromosome is homozygous viable. It carries a visible ry^+ marker which has been completely stable in the original homozygous stock as well as in other balanced stocks. Cross 4 (Table 4, row 1) serves as a control to demonstrate the stability of the *P(lArB)* transposon as well as the absence of crossing over in males in the absence of a source of transposase. In this cross, progeny receiving the male parental chromosome A die with either of the female tester chromosomes due to homozygosity for *Sb* or *Ubx*. Progeny receiving the *P(lArB)* chromosome survive with either female tester chromosome. All of these were kar^+ ry^+ in phenotype reflecting the stability of the transposon carrying a ry^+ gene. Crossovers eliminating *Sb* and *Ubx* will survive and such crossovers occurring to the right of the *kar* marker are recognized by the kar phenotype. No crossovers were observed in cross 4.

Cross 5 (Table 4, row 2) demonstrates the impact of *P(Δ2-3)* transposase upon both the stability of *P(lArB)* and crossing over in the male parent. Thirty-five of the male parents yielded crossovers recognizable by the kar phenotype. From the surviving progeny of these exceptional individuals, we were able to confirm a minimum of 21 independent crossover events. The appearance of kar^+ ry progeny in cross 5 indicates either loss of the ry^+ marker in *P(lArB)* or loss of the transposon, and is used as a measure of *P(lArB)* destabilization. All 69 of the single male matings of cross 5 show some *P(lArB)* destabilization yielding kar^+ ry progeny ranging from 22 to 98% of total offspring for each mating. Total kar^+ ry progeny scored was 7,992 (Table 4, row 2), or 71.9% of all non-crossover progeny. Crossover progeny may be considered separately since some crossovers may occur to the right of *P(lArB)* and thus not be indicators of

TABLE 4

P(lArB)* excision events and crossing over occurring in males heterozygous for *P(lArB)(87C9)rosy^{506}* crossed to tester females of the genotype *P18,kar ry Ubx e^{4}/MKRS,M(3)76A kar ry^{2} Sb

Male parent	Single male matings	Progeny scored	Observed phenotypes: kar$^+$ ry$^+$	kar$^+$ ry	Crossovers: kar ry$^+$	Crossovers: kar ry	Total ry progeny	Percent ry progeny
4. A/*P(lArB) rosy^{506}*	40	6,515	6,515	0	0	0	0	0
5. A, *P(Δ2-3)/P(lArB)rosy^{506}*	69	11,370	3,119	7,992	101	158	8,150	72

P(lArB) destabilization. Among the crossover progeny, 61% were rosy. Total rosy progeny scored, including kar ry crossovers, was 8150, or 71.7% of the surviving progeny. It should be emphasized that loss of the ry$^+$ phenotype does not indicate a loss of the entire transposon. Nor does the ry$^-$ phenotype always indicate an absolute loss of the *ry*$^+$ gene. We have recovered three kar$^+$ ry position effect exceptions from cross 5 in which *P(lArB)* has apparently been transposed from *87C9* and inserted into or near heterochromatin. Progeny of these exceptions show a restored ry$^+$ phenotype when crossed to *Df(3R)615* (Figure 2, row 14). The ry mutant phenotype in these exceptions is suppressed by the reduced dosage of *Su(var) 3-7* (Figure 2), which functions as a dominant suppressor of heterochromatic position effect (REUTER *et al.* 1990).

Genomic deletions place *P(lArB)* between *l(3)87Cd^{16}* and *l(3)87Da^{2}*: In a screen for new deletions in the *P(lArB)* region, all kar ry$^+$ and kar ry crossovers were crossed to *Df(3R) 615/MKRS* (Figure 2, row 14) in order to expose lethals in the region. The *P(lArB)* chromosome itself is both homozygous viable and viable over *Df(3R)615*. We also tested 460 randomly chosen non-crossover exceptions (90 ry$^+$ and 370 ry). Six new deletions were recovered. Each deletion was tested against an array of deletions and lethals to identify deleted areas in the region from *l(3)S2* to *l(3)87Ek^{7}* (Figure 2). Deletions which extended to the right or left limit of this region were examined in salivary gland squashes. *In situ* hybridization and Southern blots, serially reprobed, were used to determine the presence of *P(lArB)*. We did not clone the 19-kb insert, but did clone by plasmid rescue the 3.5-kb 3′ end of the insert with the adjacent 9 kb of genomic material (see METHODS AND MATERIALS). This 12.5-kb *pSalI* clone was useful in identifying both the 3′ end of the insert and the genomic sequence into which *P(lArB)* is inserted. We also cloned the 8-kb *Eco*RI fragment from *87C9* into which *P(lArB)* was inserted. This is a *P* element-free clone recognizing genomic sequences surrounding the insert. The plasmid pπ25.7BWC was also used. *P* element fragments identified on Southern blots, which were also recognized by the genomic probe *pEco*RI 8 kb, were considered to be in *87C9*. This was confirmed by *in situ* hybridization. In cross 5, excision of *P(lArB)* or partial deletion of the *ry*$^+$ in *P(lArB)* will result in a rosy phenotype. The results are presented in Figure 2, rows 3–8. Of the six deletions, only *Df(3R)2521* (Figure 2, row 4) has retained the ry$^+$ phenotype associated with *P(lArB)*. Two of the deletions lost the ry$^+$ phenotype due to internal deletions in the transposon: *Df(3R)2520* (row 3) and *Df(3R)2524* (row 7). Although *Df(3R)615*, which was used in the initial identification of the new deletions, extends from *l(3)87Ca^{14}* through *l(3)87Ej^{2}*, no new deletions were recovered in which the deleted sequences were clearly distant from *P(lArB)(87C9)* (Figure 2, rows 3–8).

P(lArB)(87C9) is placed between *l(3)87Cd^{16}* and *l(3)87Da^{2}* by the following observations: (1) *Df(3R) 2520* (Figure 2, row 3) extends from *l(3)87Da^{2}* through *l(3)87Ek^{7}*. It does not remove *P(lArB)* or *l(3)87Cd^{16}*. (2) *Df(3R) 2521* (row 4) extends from *l(3)87Ca^{14}* through *l(3)87Cd^{16}*. It does not remove *P(lArB)* or *l(3)87Da^{2}*. (3) Confirming this placement is *Df(3R) 2522* (row 5) which extends from *l(3)87Cc^{6}* through *l(3)87Cd^{16}* and removes *P(lArB)* but not *l(3)87Da^{2}*.

***P(lArB)* impact on male recombination:** Table 5 presents the results of experiments designed to examine the effect of *P(lArB) A53·1M3* on *P(Δ2-3)* induced male crossing over utilizing the complete crossover selective system (Figure 1). Cross 6 (Table 5, row 1) serves as a control establishing a background rate for male crossing over in the absence of transposase but containing *P(lArB)*. Heterozygous *P(lArB)* males without *P(Δ2-3)* were mated individually to six tester females of the constitution *Dfd ry^{1} Ubx/MRS*. One crossover was recovered in the region *M(3)76A* to *Dfd* in an estimated zygotic sample of 14,400. This observation is consistent with male recombination in the absence of a source of *P* transposase (see Table 1) and the 95% Poisson confidence interval for cross 6 is consistent with that for control cross 1.

Cross 7 (Table 5, row 2) examines crossing over in the presence of *P(Δ2-3)* transposase. Cross 7 yielded 195 fertile survivors in an estimated progeny of 72,491. Correcting for clusters, the 195 observed exceptions represent a minimum of 70 independent crossover events. The 70 minimum independent events extrapolate, due to homozygotic lethality, to 156 events, giving a male recombination mean of 215×10^{-5}.

TABLE 5

Crossing over occurring in males heterozygous for *P(lArB)(87C9)* crossed to tester females of the genotype *Dfd ry[1] Ubx/MRS, M(3)76A ry[2] Sb*

Male parent	Single male matings	Observed exceptions (singles, clusters)	Independent events		Progeny estimate	Recombination mean $\times 10^5$
			Minimum	Extrapolated		
6. A/B, *P(lArB)*	40	1	1	4	14,400	
7. A, *P(Δ 2-3)*/B, *P(lArb)*	193[a]	195 (33S,16C)	70	156	72,491	215

[a] Sixty-two matings produced crossovers.

TABLE 6

Distribution of male recombinants in cross 7

Genetic exchange interval	Observed recombinants (singles, clusters)	Independent events		Percent of total independent events	Recombination mean $\times 10^5$
		Minimum	Extrapolated		
1. *M(3)76A-Dfd*	74 (8S, 5C[a])	13	42	26.9	57.9
2. *Dfd-kar*	2 (2S)	2	4	2.57	5.52
3. *kar-P(lArB)-mesB*	91 (33S, 18C[a])	51	102	65.4	141
4.–5. *mesB-pic*	0	0	0	0.0	0.0
6. *pic-Ace*	1	1	2	1.28	2.76
7. *Ace-Sb*	25 (1S, 1C[a])	2	4	2.57	5.52
8. *Sb-Ubx*	2 (1C[a])	1	2	1.28	2.76
Totals	195 (45S, 25C[a])	70	156	100	215

[a] Observed cluster sizes region 1: 27, 19, 11, 5, 4; region 3: 10, 6, 5, 4(2), 3(2), 2(11); region 7: 24; region 8: 2. mean cluster size: 8.68 ± 5.30.

The 195 exceptions from cross 7 were individually mated to *Df(3R)kar^{H5}* (Figure 2, row 15) to test for deletion of vital genes adjacent to *P(lArB)*. Three new deletions were recovered (Figure 2, rows 9–11) and fully tested as described for crosses 2, 3 and 5. Two additional smaller deletions, which do not extend into adjacent vital genes, were discovered by the molecular analysis of the *P(lArB)* region in the crossovers. These deletions are shown in Figure 2, rows 12 and 13.

Southern blots were prepared from DNA from each independent event. *In situ* hybridizations were prepared from third instar larvae. We found 30 crossovers with *P* transpositions in the same genetic interval, and 38 with none. The mean number of *P* transpositions per crossover (30/68) for cross 7 is then $\bar{X} = 0.44 \pm 0.12$. A molecular analysis will be the subject of a separate report.

Distribution of exchange events: Table 6 presents the distribution of the crossovers of cross 7. The presence of *P(Δ 2-3)* is shown to induce crossovers in all monitored regions from *M(3)76A* to *Ubx*, except the very small *mesB-pic* region (Table 6, column 2). The *kar-mesB* region, which marks the position of *P(lArB)*, exhibits the largest increase in crossovers (58-fold) when compared with the same region without *P(lArB)* (Table 2, row 3). The failure to find crossovers between *mesB* and *pic* (Table 6, row 4), is consistent with the observations of DUTTAROY *et al.* (1990) and MCCARRON *et al.* (1989). Figure 3, row e, compares the distribution of events in cross 7 with the metaphase chromosome map and with the distribution of events in crosses 2 and 3. Row f shows the cross 7 distribution without Region 3 (*kar-mesB*), which contains *P(lArB)*, and should be compared with Figure 3, row g, which shows cross 2 also without the *kar-mesB* region. The cross 7 mean of minimum independent events excluding region 3 is significantly greater than that of cross 2 exclusive of region 3. The mean for either cross lies outside the 95% Poisson confidence interval for the other cross.

Cross 7 gave 25 clusters whose observed sizes are given in note a to Table 6. The mean cluster size (region 1 cluster sizes are multiplied by two) is 8.68 ± 5.30. This mean, however, represents two different regional means. The 18 clusters of region 3 (*kar-P(lArB)-mesB*) have a mean of 3.17 ± 0.99, whereas the mean for the seven clusters outside the *P(lArB)* target region is 22.57 ± 16.41. A comparison of these means finds them significantly different: $t = 2.7898$, $P \sim 0.0106$. In contrast, comparison of the Cross 7 cluster size omitting Region 3 with the cluster sizes for Cross 2, which also carried a single dose of *P(Δ 2-3)*, finds the means not significantly different ($t = 2.1607$, $P \sim 0.058$).

DISCUSSION

Target-free male recombination distribution: In an earlier study of male recombination with multiple *P* elements in the genome (DUTTAROY *et al.*, 1990), we noted the lack of correspondence of the crossover distribution pattern with the distribution of *P* element targets or mobilization events. The analysis was complex due to: (1) the multiple sources of transposase as well as the (2) multiple *P* element target recipients of transposase. In the present report, we have reexamined the distribution

pattern while controlling these influences: (1) The monitored region is examined without a transposase source, (2) a stable *P* element source of transposase (one or two doses) is introduced outside the monitored recombination region, and (3) we provide either one *P* element target site, or none.

The data of Table 1 compare the effects of zero, one and two doses of a transposase source on male recombination in the absence of a *P* element target. These data demonstrate that *P* element transposase is able to produce crossovers in the Drosophila genome in a dose-dependent fashion. Moreover, these results are consistent with the observations of KAUFMAN *et al.* (1989), who have shown that *P* element transposase has a high affinity for Drosophila DNA. Thus, transposase is able to initiate the crossover event without the mediation of a *P* element target. The distribution pattern in these target-free experiments (Figure 3, rows c and d), however, shows no correspondence with either the standard female meiotic recombination map or the polytene chromosome map. The distribution more nearly approximates that seen in radiation-induced mitotic crossing over and shows a correspondence with the cytological map of the metaphase chromosome (see comparative maps in BECKER 1974, 1976; SLATKO 1978; SINCLAIR and GRIGLIATTI 1985). The presence of multiple *P* element targets by virtue of their possession of sequences with even higher affinity for transposase than Drosophila DNA (KAUFMAN *et al.* 1989) may then be viewed as producing concentrations of activity which serve to mask that distribution which may be related, overall, to the DNA content of the chromosomes.

The question of precisely where in the pericentric region the crossovers are occurring, whether in euchromatin or in α- or β-heterochromatin, cannot be resolved with markers as widely placed around the centromere as are *M(3)76A* and *Dfd* (Figure 1). SINCLAIR and GRIGLIATTI (1985) have clearly demonstrated transposase induced male recombination between *light* and *rolled* in the centromeric heterochromatin of chromosome *2*. They observed a small, but most important, rate of crossing over between these markers using MR-h12, the "least potent" of their male recombination strains.

Effect of a *P* element target: The effect of a *P* element target is best demonstrated by comparison of zero and one target. Cross 2 (Table 1, row 4), with a single transposase source and without a *P* element target, exhibited male recombination of 44.2×10^{-5}. Cross 7 (Table 5, row 2), utilizing the same single transposase source as cross 2, but, with the addition of the *P(lArB)* target in *87C9*, achieves male recombination of 215×10^{-5}. Most of the increase in crossing over occurs in region 3, which harbors *P(lArB)*, and where crossing over was 141×10^{-5} as compared with 2.5×10^{-5} in cross 2. This represents an intense transposase action in the region carrying *P(lArB)* and utilizing a single transposase source.

In DUTTAROY *et al.* (1990) we suggested that the effect of a *P* element target on crossing over extends beyond the immediate target site rather than being limited to the immediate region of *P* element sequences. In cross 7 this effect is seen to extend left and right of the target site to the limits of the genetic regions monitored, generating crossovers even in regions 6 and 8 where none had been observed in crosses 2 and 3. The overall increase in cross 7 exclusive of region 3 results in a crossover mean of minimum independent events which is significantly greater than that for cross 2 exclusive of region 3.

Rearrangements and *P* element targets: Previous reports have documented the association of rearrangement breakpoints with the sites of parental *P* elements and novel *P* element sites, as well as non-*P* element sites in dysgenic individuals (ENGELS and PRESTON 1981, 1984; BERG *et al.* 1980; YANNOPOULOS *et al.* 1983; DUTTAROY *et al.* 1990). We also find rearrangements (Figure 2) in experiments where there are no *P* element targets. Therefore, neither *P* element targets nor *P* element excision or insertion events are required to produce rearrangements: transposase alone is sufficient to induce rearrangements.

Prior studies of *P* element-induced male recombination were consistent with the notion that the exchange events were symmetrical (DUTTAROY *et al.* 1990; ISACKSON *et al.* 1981; SINCLAIR and GRIGLIATTI 1985; SVED 1978; VOELKER 1974). In the present report, we have noted that transposase induced male recombination in both the presence and absence of *P* element targets produced several deletions suggesting the occurrence of some asymmetrical exchange events.

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