# Flanking Duplications and Deletions Associated With *P*-Induced Male Recombination in Drosophila

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Manuscript received June 17, 1996
Accepted for publication September 11, 1996

ABSTRACT

We studied *P* element-induced recombination in germline mitotic cells by examining the structure of the recombinant chromosomes. We found that most recombinants retain a mobile *P* element at the site of the recombination, usually with either a deletion or a duplication immediately adjacent to the *P* end at which the crossover occurred. The sizes of these deletions and duplications ranged from a few base pairs to well over 100 kb. These structures fit the "hybrid element insertion" (HEI) model of male recombination in which the two *P*-element copies on sister chromatids combine to form a "hybrid element" whose termini insert into a nearby position on the homologue. The data suggest that *P*-induced recombination can be used as an efficient means of generating flanking deletions in the vicinity of existing *P* elements. These deletions are easily screened using distant flanking markers, and they can be chosen to extend in a given direction depending on which reciprocal recombinant type is selected. Furthermore, the retention of a mobile *P* element allows one to extend the deletion or generate additional variability at the site by subsequent rounds of recombination.

MOBILIZATION of *P* transposable elements causes crossing over in the Drosophila male germline (HIRAIZUMI 1971; KIDWELL and KIDWELL 1976) and in somatic cells (SVED *et al.* 1990), where recombination does not normally occur at significant frequencies. Early studies of male recombination led to the discovery of the hybrid dysgenesis syndrome (KIDWELL *et al.* 1977), which in turn led to the identification of *P* elements themselves (BINGHAM *et al.* 1982; RUBIN *et al.* 1982; ENGELS 1989, 1996). Since then, *P* elements have proven to be indispensable tools for Drosophila molecular genetics (SPRADLING 1986; ENGELS 1989, 1996; SENTRY and KAISER 1992).

In the accompanying paper we showed that most male recombination occurs within 2 kb of an existing mobile *P* element (PRESTON and ENGELS 1996), as opposed to being a result of an interaction between transposase and random genomic sites (MCCARRON *et al.* 1989, 1994; DUTTAROY *et al.* 1990). Moreover, the *P* element was usually retained at its original site in the recombinant chromosomes (PRESTON and ENGELS 1996).

Retention of the *P* element is not consistent with the idea that *P*-induced recombination is a byproduct of double-strand break repair events in which the homologous chromosome serves as the template. Such repair events would not be expected to leave a *P*-element copy at the recombination site (SVED *et al.* 1995). Double-strand break repair is known to occur at high frequencies when *P* elements excise, but the process usually does not result in crossing over of flanking markers (ENGELS *et al.* 1990; GLOOR *et al.* 1991; JOHNSON-SCHLITZ and ENGELS 1993; NASSIF *et al.* 1994).

An alternative explanation for male recombination is that it occurs during an aberrant transposition attempt in which the left end of a *P* element on one DNA duplex is paired with the right end of an element on a different duplex (SVOBODA *et al.* 1995). Insertion of the resulting "hybrid element" into a new site can lead to a recombinant chromosome. Hybrid element insertion (HEI) events of this type have been demonstrated with *P* elements in which one end is missing. Heterozygotes with a left end-deleted *P* element on one homologue and a right end-deleted one on the other had high recombination frequencies, and produced the specific recombinant structures predicted by HEI (GRAY *et al.* 1996).

In this report we show that most *P*-induced male recombination events result in a deletion or duplication of the genomic sequence immediately flanking the *P* insertion. The resulting structures are those expected from the HEI model. This process can be an efficient way to produce deletion knockout mutations of nearby genes. Deletion sizes ranged from a few base pairs to >100 kb. In addition, we make use of the functional *P* element remaining at the deletion breakpoint in a second round of recombination and deletion production, thus extending the length of deletions.

## MATERIALS AND METHODS

**Drosophila crosses:** Flies were raised on standard cornmeal-molasses-agar medium and grown at room temperature

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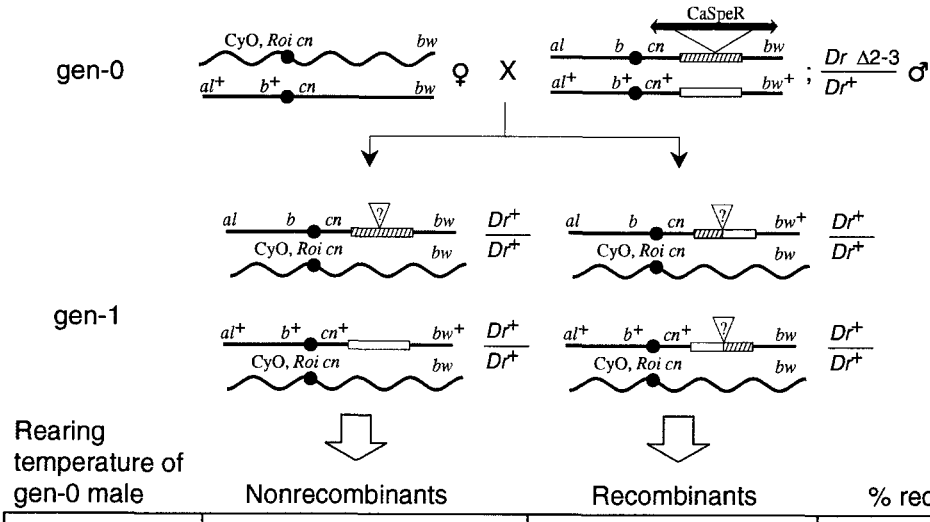



| Rearing temperature of gen-0 male | Nonrecombinants | Recombinants | % rec. |
|---|---|---|---|
| 19° | 11,008 | 33 | 0.30 |
| 24°± 1 (room temp.) | 22,945 | 243 | 1.05 |
| 25° | 8,679 | 92 | 1.05 |

FIGURE 1.—Crosses to recover male recombinants. Only the Cy Dr$^+$ progeny were scored for *cn* and *bw* in gen-1. The numbers of iso-♂ crosses for the three temperature groups were 344, 677 and 246. In approximately half of the 19° crosses and one-fourth of the 25° crosses the *bw* and *bw*$^+$ markers were reversed from the configuration shown.

(21–25°) unless otherwise noted. All genetic symbols not described in the text are in the Drosophila reference works (LINDSLEY and ZIMM 1992; FLYBASE 1996).

**Production of recombinants:** The scheme in Figure 1 (also Figure 4A of the accompanying paper, PRESTON and ENGELS 1996) was used to generate 128 recombinant lines. Recombination occurred in the germlines of males with the genotype

$$\frac{cn\ CaSpeR\ bw}{+\qquad\quad +}\ ;\ \frac{Dr\ \Delta 2\text{-}3}{+},$$

which were mated to *CyO, Roi cn bw/cn bw* females. The eye color loci *cinnabar* (*cn*) and *brown* (*bw*) are at opposite ends of chromosome arm 2R. *P{CaSpeR}*(50C) was the only mobile *P* element present (PIRROTTA 1988), and transposase was supplied by the Δ2-3(99B) element (ROBERTSON *et al.* 1988).

Most of the gen-0 males were raised at room temperature as described (PRESTON and ENGELS 1996), and the resulting recombinants were used for all subsequent experiments. In addition, we also performed similar crosses with the gen-0 males raised in more tightly controlled temperatures to determine the effect on recombination frequency. In agreement with earlier studies (KIDWELL *et al.* 1977), we found a pronounced decrease in male recombination frequency when gen-0 males were reared at lower temperatures (Figure 1).

In the room temperature group, there were 243 recombinants among 23,188 Cy Dr$^+$ progeny from the room temperature crosses. From these we selected 128 recombinants such that no more than one of each reciprocal type was taken per cross. Homozygous stocks were established two generations hence for the viable and fertile recombinant chromosomes. The others were maintained as heterozygous stocks over *CyO*.

**DNA sequencing:** All sequencing of PCR products from recombinants was done by the dye termination method (LEE *et al.* 1992; ROSENTHAL and CHARNOCK-JONES 1992) using ABI Prism model 373 and 377 automated sequencers according to the manufacturers instructions.

**PCR and restriction mapping:** All primer sequences, PCR machine settings, and other details were as described in Figure 2 and the accompanying report (PRESTON and ENGELS 1996).

## RESULTS

### Structure of rearranged recombinants

**PCR analysis of rearrangements:** In the accompanying paper (PRESTON and ENGELS 1996) we describe the generation of 128 male recombinants induced by mobilization of a *P{CaSpeR}* transposon at cytological position 50C. The recombinants were identified with the distant flanking markers *cn* and *bw*. The two parental chromosomes differed in 13 restriction fragment length polymorphism (RFLP) sites within 2 kb on either side of the *CaSpeR* element. The RFLP patterns on the *CaSpeR*-bearing chromosome are designated as C, and

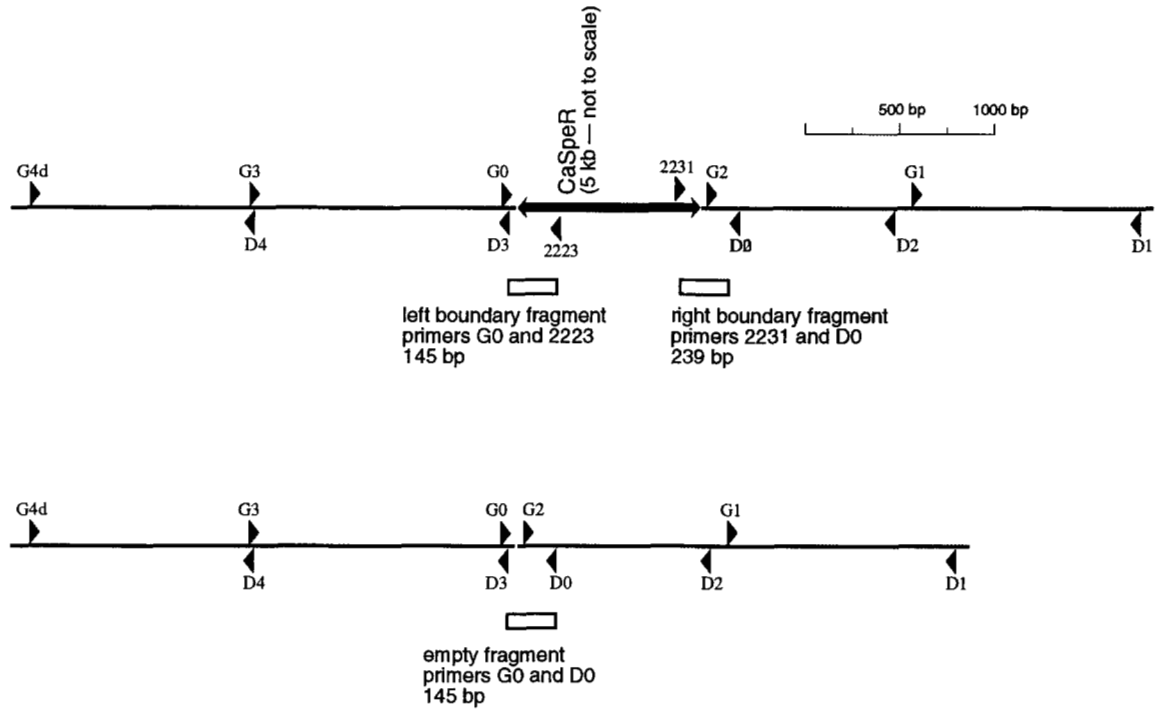



FIGURE 2.—Primer maps of 50C region. Primers are indicated by arrowheads, amplicons referred to as boundary fragments and empty fragments are shown as □. Primer sequences are given in the accompanying paper (PRESTON and ENGELS 1996). The primers G4d-D4, G3-D3, G2-D2 and G1-D1 were used to amplify fragments used in RFLP analysis, with the restriction pattern in the *CaSpeR* chromosome (top) designated C, and that of the non-*CaSpeR* homologue designated as A.

those of the non-*CaSpeR* homologue are labeled A. These sites were used to show that the crossover point was within the immediate flanking region and to determine the structure of the resulting recombinant. The preliminary analysis showed that 83 of the recombinants contained structural rearrangements as indicated by the disappearance, duplication, or alteration in size of PCR amplicons.

The first indication of common structures among the rearrangements came from PCR amplifications of the boundary fragments connecting the *CaSpeR P* ends to the flanking sequence (Figure 3). If no rearrangement has occurred, we expect either both or neither of these fragments to amplify, depending on whether the *CaSpeR* element is present. However, most of the rearranged recombinants (74/83) had one fragment but not the other, as shown in Table 1. The missing fragment either failed to amplify or yielded a size different from wild type. Furthermore, note from Table 1 that in the great majority of cases (95%), the missing or altered-size boundary fragment appeared on the side derived from the A chromosome. That is, most *cn bw*$^+$ recombinants were missing a normal right boundary fragment, and the *cn*$^+$ *bw* recombinants usually lacked the normal left boundary fragment.

One large class of rearrangements appeared to carry a duplication of the flanking sequence. In 29 cases, the RFLP analysis showed patterns of both the A and C markers in DNA of the same individual. Since the DNA came from homozygous flies, this result suggested that the amplicons were duplicated with one copy from each of the two original homologues. Duplications were also seen in an additional 10 cases by PCR and sequencing, as described below. One of these was a 9-bp duplication found among six recombinant chromosomes that appeared to be structurally normal by PCR tests. The extra nine bases were identical to the sequence on the opposite side of *CaSpeR* and duplicated in direct orientation. In each of the 39 cases, the duplicated region was on the same side of *CaSpeR* as the missing or altered boundary fragment. We interpret the missing boundary fragments in these cases as failure to amplify because of the distance between primers. As mentioned above, this missing boundary fragment was almost always on the side derived from the A chromosome. All but four of the 39 duplications fit the specific structures shown in Figure 3, but their lengths were highly variable. The shortest duplication was 9 bp, and the longest extended beyond primer G4d or D1 depending on the direction (Figure 2), implying a length >2 kb.

The other large category appeared to carry deletions of the sequence on one side or the other of *CaSpeR*. There were 36 cases in which one or more of the amplicons used for RFLP analysis failed to amplify. Many of these were also homozygous lethal, in which case the missing fragment was inferred by noting that only the A marker pattern was seen in recombinant/A heterozygotes, and only the C pattern in recombinant/C flies.

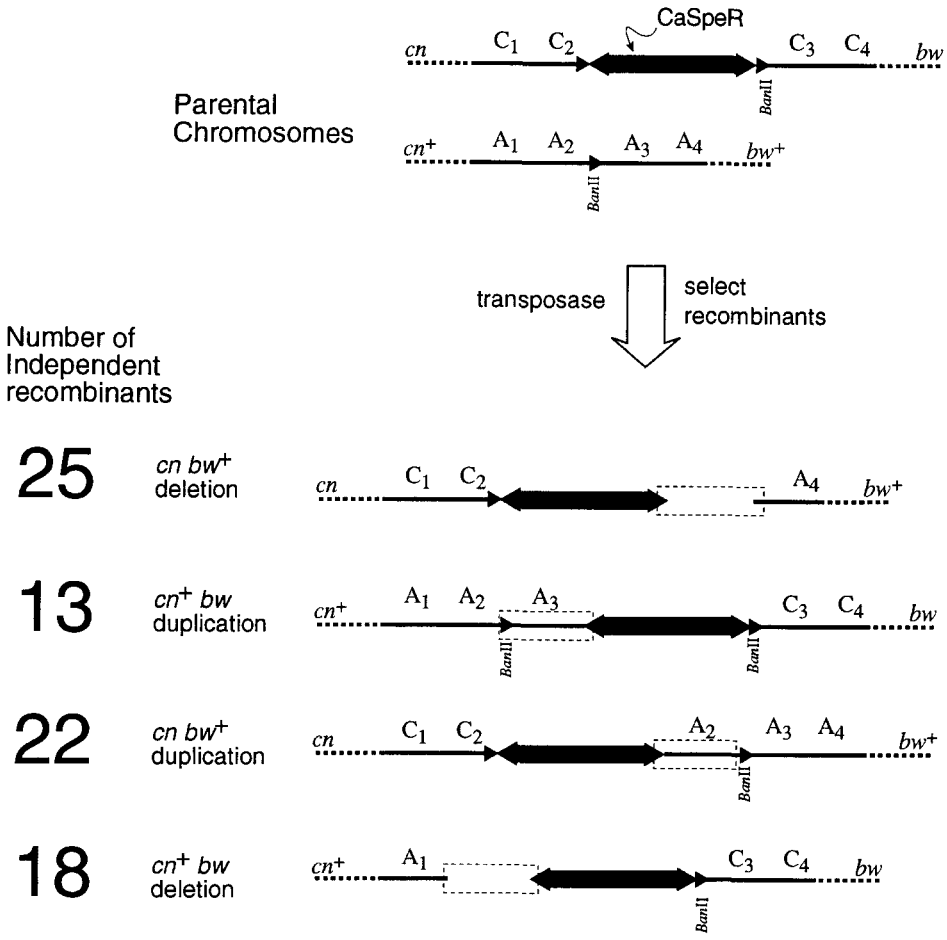



FIGURE 3.—Four generalized structures account for most of the rearranged recombinants. The symbols $C_1$, $C_2$, $C_3$ and $C_4$ stand for arbitrary segments of the parental *CaSpeR* chromosome, and $A_1$, etc., refer to its $cn^+$ $bw^+$ homologue. Corresponding A and C segments are distinguishable by RFLP markers as described (PRESTON and ENGELS 1996). Duplications and deletions are indicated by dashed boxes. The 8-bp sequence which was duplicated upon *CaSpeR* insertion is indicated by ▶. The *Bam*II site overlaps the right end of this octamer.

For six of the 36 cases, we found that an extended boundary fragment could be amplified with primers 2231 and D2 (see Figure 2) to yield a smaller-than-normal amplicon. The presence of a deletion was confirmed in these cases by DNA sequencing of this fragment (see below). An additional 11 deletions were identified by reduced size of the boundary fragments and/or DNA sequencing. Therefore, we observed a total of 47 deletions including three that had a duplication on the opposite side of *CaSpeR*. Each deletion had one endpoint at or near one boundary of *CaSpeR* and extended a variable distance into genomic sequences. Analysis of deletion sizes (see below) revealed a wide range as shown in Figures 4 and 5. For the *cn* $bw^+$ recombinants, the deletions were to the right of *CaSpeR*, and in the $cn^+$ *bw* recombinants they were to the left. Thus, as was the case for the duplications, the deletions extended toward the A-derived portion of the recombinant chromosomes. All but four of the 47 deletions had one of the two specific structures shown in Figure 3.

**The rearrangement breakpoints:** One way to account for the above observations is by postulating that a *P*-element terminus on the C chromosome is joined to a nonhomologous point in the A chromosome. Four possible structures result from such a joining, depending on which end of *CaSpeR* is involved, and on whether the nonhomologous site on the A chromosome is to the left or right. These four general structures are shown in Figure 3. Note that the size of the duplication or deletion depends only on the distance between the nonhomologous site and the original *CaSpeR* position. According to this interpretation, the missing boundary fragments can come about from duplications that make the fragment too large to amplify, or from deletions that remove either the D0 or G0 primer site. Smaller duplications or deletions will result in boundary fragments that differ from the wild-type size.

Thirteen of the duplication and deletion recombinants were selected for DNA sequence analysis. We used PCR to amplify across the junction connecting the *CaSpeR* element with the A-derived genomic sequence. Several combinations of the primers shown in Figure 2 were used for amplification depending on the size of the duplication or deletion. Sequencing was performed

**TABLE 1**

**Boundary fragments of rearrangements**

| Phenotype | Left[a] | Right[b] | No. of cases |
|---|---|---|---|
| cn bw$^+$ | Yes | Yes | 0[c] |
| cn bw$^+$ | Yes | No | 40 |
| cn bw$^+$ | No | Yes | 2 |
| cn bw$^+$ | No | No | 5 |
| cn$^+$ bw | Yes | Yes | 0[c] |
| cn$^+$ bw | Yes | No | 3 |
| cn$^+$ bw | No | Yes | 29 |
| cn$^+$ bw | No | No | 4 |

Yes indicates a fragment of wild-type size; No implies either no fragment or one that is larger or smaller than wild type.

[a] Primers G0 and 2223 (Figure 2). Expected size is 145 bp.

[b] Primers 2231 and D0 (Figure 2). Expected size is 239 bp.

[c] There were some recombinant chromosomes that had both boundary fragments intact. However, these lacked any structural changes and were classified as simple recombinants. They are described elsewhere (PRESTON and ENGELS 1996).

using either one of the amplification primers (six cases) or an internal primer (seven cases). Note that this approach limits the analysis to the shorter duplications and deletions since deletions longer than ~2 kb remove the outermost primer sites, and the very long duplications exceed the limits of PCR amplification.

We found that each of 13 sequenced rearrangements matched one of the structures shown in Figure 3. Furthermore, in each case, the novel breakpoint coincided with the *P*-element end. No bases from the *P* element were missing, and none was added. The sizes of the deletions were 14, 32, 40, 116, 116, 137, 199, 251 and 517 bp (Figure 4). The duplications were 9, 72, ~850 and ~1350. (Two of the duplication lengths are approximate because not all of the intervening bases were sequenced.)

**Alterations are usually limited to one side of the *P* element:** For most of the rearrangements, only one *P* terminus was involved. The other end appeared to remain joined to the same genomic sequence as in the original *CaSpeR* insertion. This point was confirmed by DNA sequencing for three of the deletions (lengths 14, 32, 40 bp) and one of the duplications (length 72 bp). In each case we sequenced the boundary region on the opposite end of *CaSpeR* where there was no duplication or deletion visible by PCR, and found that the *P*/50C junction was intact. One of them did have a change internal to the *CaSpeR* element in which the outermost 26 bp were tandemly duplicated, but we believe this duplication to be due to a previous insertion and unrelated to the recombination event (see Figure 4, legend). In addition, we applied a somewhat lower-resolution test to the right ends of 13 recombinants that had deletions or duplications on the left side. In each case, we amplified the right boundary fragment and digested with *Ban*II. The original *CaSpeR* chromosome has a *Ban*II recognition site occupying base positions 5–10 from the right end of the element (Figure 3). We found that all 13 left-end rearrangements retained this recognition site, suggesting that sequences to the right of *CaSpeR* were unchanged.

**Lethality complementation tests:** We found that 26 of the 128 recombinant chromosomes were lethal when homozygous (PRESTON and ENGELS 1996). These were used in complementation tests in various combinations with each other and with five known lethals from the 50CD region (FLYBASE 1996) as shown in Figure 5A. The results indicated two main complementation groups (Figure 6) accounting for 17 of the 26 lethal recombinants. The remaining nine were "unique" lethals that complemented all others and are likely to be jumps of *CaSpeR* to distant sites on chromosome *2* or other unrelated mutational events.

The two main complementation groups corresponded to the *cn bw*$^+$ and *cn*$^+$ *bw* recombination types. Furthermore, the *cn bw*$^+$ group failed to complement the known lethal mutation, *l(2)04615,* which maps to 50C20-23 by *in situ* hybridization (KARPEN and SPRADLING 1992). The other group failed to complement *l(2)04845,* which lies in 50C12-21. These results suggest that the two complementation classes represent leftward and rightward deletions. In addition, some of the rightward lethals failed to complement a newly defined gene, *l(2)50CDa,* and a subset of these failed to complement the neurogenic locus *mam,* which lies in 50C20-23 (YEDVOBNICK *et al.* 1988) and apparently to the right of *l(2)50CDa.* Lethal loci *l(2)05488* and *l(2)00248,* which were mapped by *in situ* hybridization to 50C1-2 and 50D5-7, respectively (KARPEN and SPRADLING 1992), complement all our deletions and thus serve to bracket the deleted regions.

**Sterility complementation tests:** We found that 17 of the recombinant chromosomes caused sterility in the homozygous condition, some of them being male sterile, some female sterile, and some both. Each of the sterile and lethal lines was crossed with *pea,* a previously identified male sterility mutation in the region (CASTRILLON *et al.* 1993). We also tested the male and female fertility of various other viable combinations of these recombinants with each other and with the known lethal mutations described above. The results of these tests are summarized in Figure 7.

The data are consistent with our previous interpretation of the deletions. They imply that *pea* lies between *mam* and *l(2)00248,* and also reveal two new sterility loci, *ms(2)50Ca* and *fs(2)50Ca.* According to the resulting complementation map (Figure 5A) *ms(2)50Ca* lies to the left of *CaSpeR,* and *fs(2)50Ca* overlaps the insertion point. Although the *CaSpeR* insertion itself and the shorter flanking deletions are viable and fertile, longer flanking deletions in either direction knock out *fs(2)50Ca.*

**Cytological examination of deletions:** According to the complementation tests, the two longest deletions in

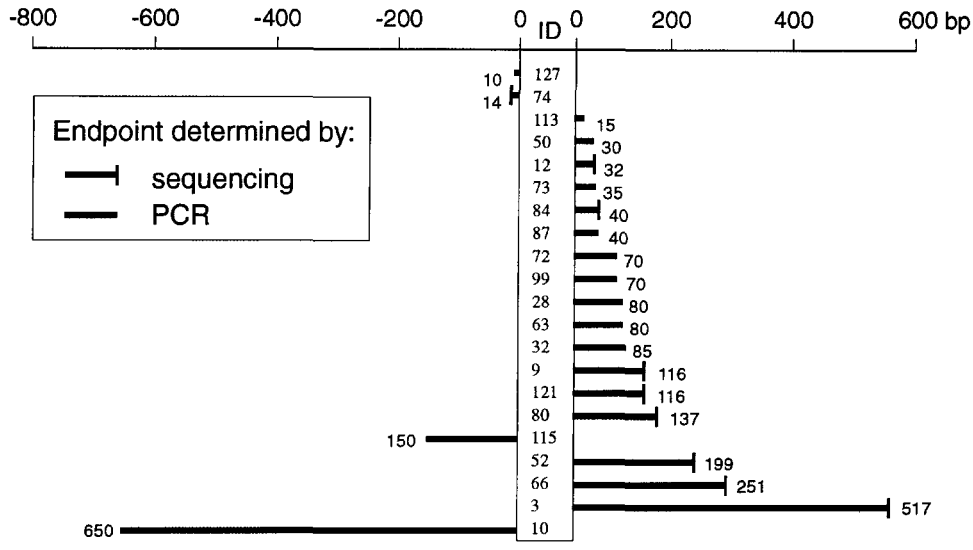



FIGURE 4.—Size distribution of short deletions. Only those deletions that were small enough to allow amplification with the primers in Figure 2 are shown. In each case an amplicon containing the deletion breakpoint was either sequenced or measured on an agarose gel as indicated. For the shortest of these deletions the amplicon used was the standard boundary fragment, but for those in which primer D0 or G0 had been deleted, then the *P* primer (2223 or 2231) was paired with one of the outer primers, G4d, G3, D2 or D1. Sequencing was also carried out on the nondeleted side of *CaSpeR* for chromosome ID 74, 12 and 84, and no change in the 50C sequence was seen there. One of them (ID 84), however, carried a 26-bp direct duplication of the *P* element 5′ terminal sequence. This structure could have come about following a previous *P*-element insertion into the internal hotspot consisting of bases 19–26 on the *P*-element 5′ end. Insertion into this site has been observed numerous times (EGGLESTON 1990), resulting in a pair of nested *P* elements. The inner element is flanked by bases 1–26 of the outer element on one side and the remainder of the outer element, beginning with base 19, on the other side. If the right end of this inner element then took part in recombination event similar to the others shown above, such as ID 87, the result would be exactly the structure we observed.

the rightward direction were #38 and #101. Figure 5B shows the polytene chromosomes of a heterozygote with #38. The deletion extends from 50C20-23 to 50D4-7. Examination of #101 showed a similar deletion. The four longest leftward deletions, 45, 102, 107 and 133, were also examined in the same way, but no aberration was seen.

**Size distribution of deletions:** An overall distribution of the deletion sizes was determined from the sequencing data, the PCR fragment sizes, the complementation tests, and the cytological examination described above. Figure 4 shows the sizes of the shorter deletions, and Figure 5A indicates what is known about the longer deletions. The longest deletions fail to complement four genetic loci, including *peanuts*, which lies further to the right than *mam*. Since *mam* alone spans 67 kb (SMOLLER *et al.* 1990), we conclude that these deletions are probably considerably longer than 100 kb.

Note that there were significantly more small deletions extending rightward than leftward (Figure 4; $P = 0.007$ by a two-tail binomial test), whereas there was no such bias in the longer deletions (Figure 5A). According to the HEI model, discussed below, effects of this kind could be explained by local differences in *P*-element insertional target site preferences.

## Tests of the HEI model

**Predicted structure according to the HEI model:** Previous work (SVOBODA *et al.* 1995) and an accompanying paper (GRAY *et al.* 1996) examine the effects of heterozygosity for a left end-deleted *P* element on one homologue opposite a right end-deleted *P* element on the other. In the presence of transposase, such heterozygotes underwent high frequencies of recombination. To explain this observation, the authors postulated the formation of a "hybrid element" composed of one end from each homologue. By the action of transposase, a double-strand break is made at each functional *P* end, followed by insertion of the hybrid element into a nearby site. Analysis of the resulting recombinant chromosomes confirmed various structures predicted by the HEI model (GRAY *et al.* 1996). In particular, one of the recombinant classes carried both copies of the postulated 8-bp duplication formed on insertion of the hybrid element ends. This observation gives strong support to the hypothesis that the recombination breakpoint was formed by a *P*-element insertion event.

Figure 8 shows how the same model can explain *P*-induced male recombination even when only one homologue carries a *P* element, and that element has both its ends intact. In this case, the hybrid element is formed between the two sister chromatids, using the left end from one *P* element copy and the right end from the

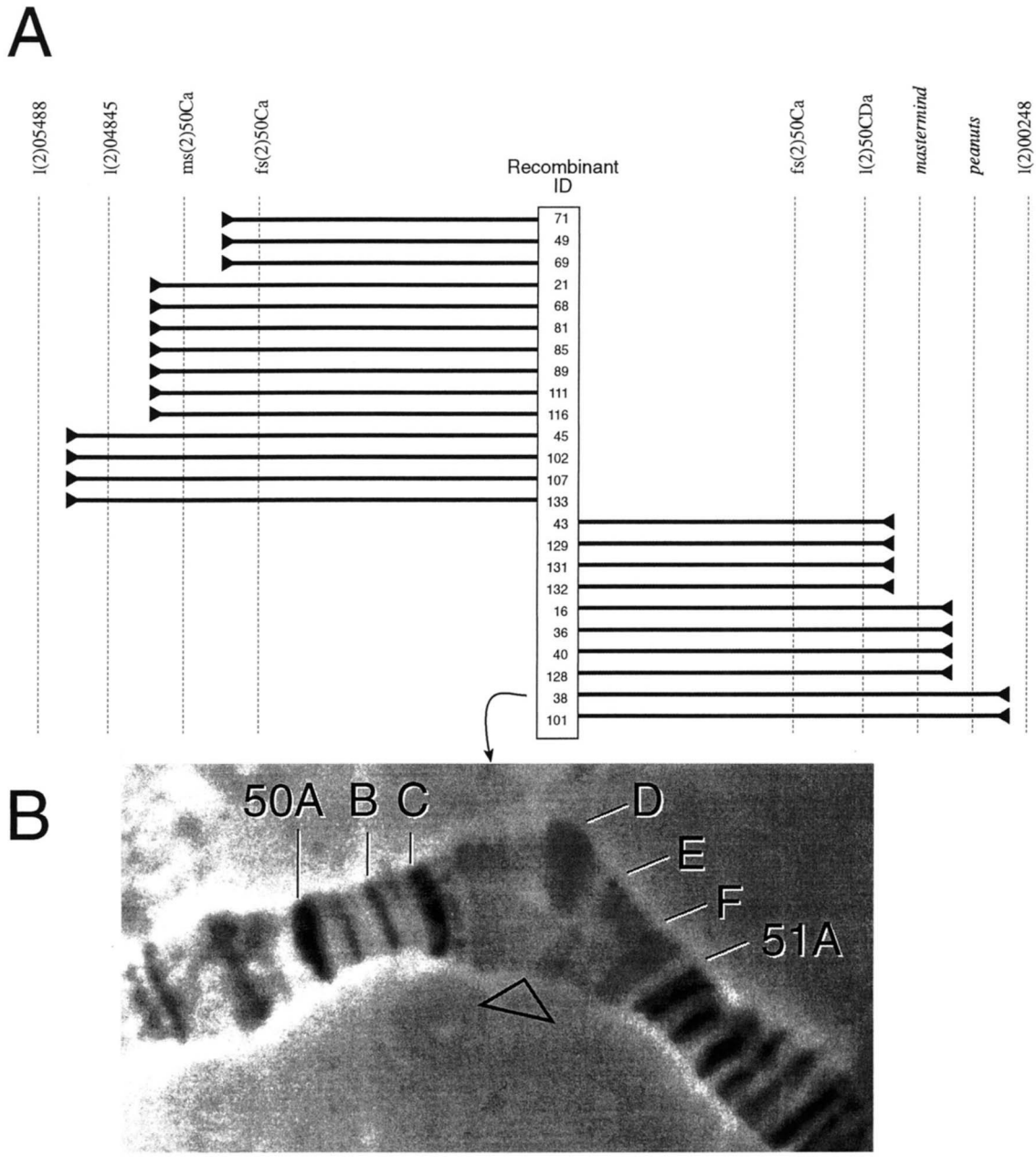



FIGURE 5.—Size distribution of long deletions. Deletions that could not be amplified with the primers in Figure 2 were determined by complementation (A) and in some cases cytological examination of polytene chromosomes (B). Complementation data are given in Figures 6 and 7. Examination of polytene chromosomes was carried out as described (ENGELS and PRESTON 1981). Note that the lethal mutation, *l(2)04615*, which was originally thought to be a point mutation because of its origin by *P*-element insertion (KARPEN and SPRADLING 1992) actually covers several neighboring loci, including *mam* and male and female sterility loci to the left. Therefore, the essential locus to the left of *mam* uncovered by *l(2)04615* will be designated *l(2)50CDa* (FLYBASE 1994).

other. Insertion of this hybrid element into a target site on one of the two homologous sister chromatids can result in recombinant chromosomes as shown. Only one of the two insertional orientations is considered in Figure 8, since the opposite orientation would yield unrecoverable dicentric and acentric products.

The recombinant products predicted by this model are similar to the four rearrangement types we observed (Figure 3). According to the model, the length of the resulting duplication or deletion depends only on where the insertion point is chosen. Figure 8 shows only the two outcomes produced when the HEI occurs to the right of the original *CaSpeR* insertion point. These correspond to the first two of the four structures shown in Figure 3. The other two would come about when the insertion is to the left.

**Breakpoints correspond to *P*-element insertion site preference:** According to the HEI model, the endpoints of recombinant duplications and deletions are actually *P*-element insertion sites. Therefore, the selec-

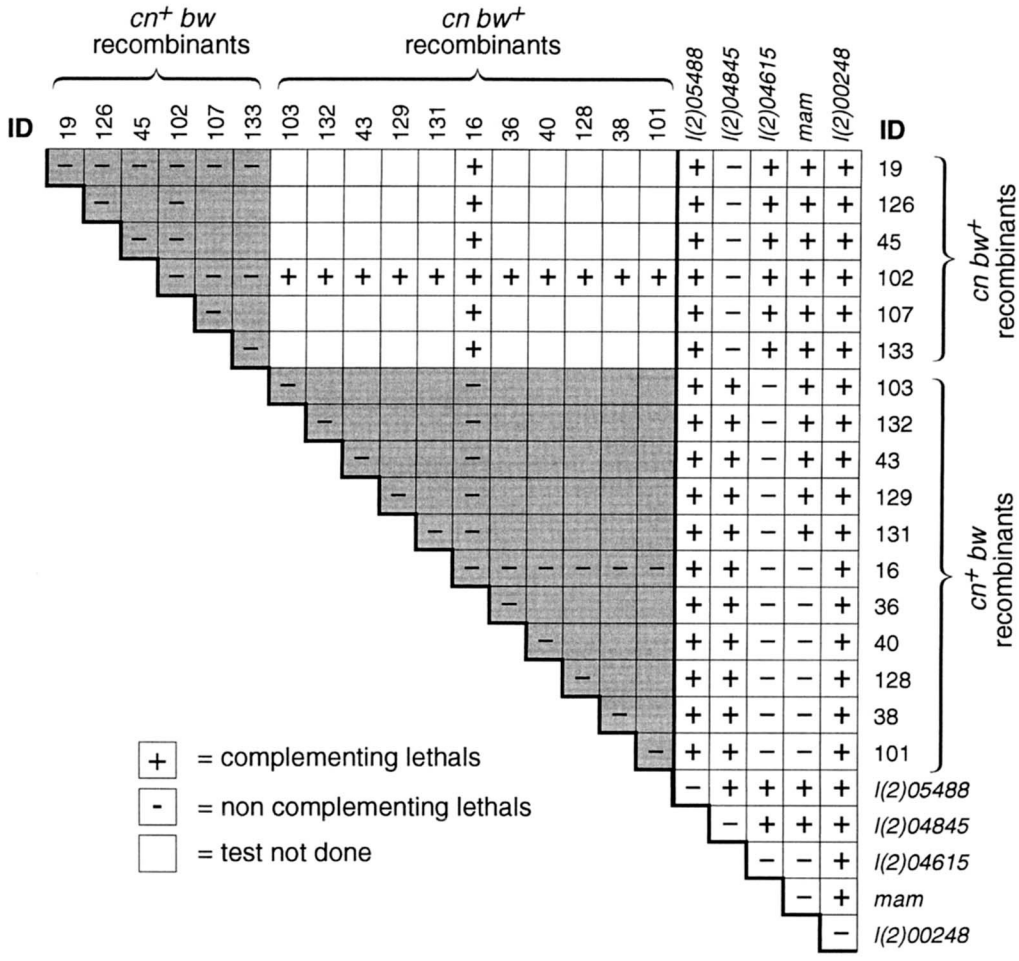



FIGURE 6.—Results of lethality complementation tests. Each test consisted of crosses between heterozygotes with the balancer *CyO, Roi cn bw* opposite the chromosomes being tested. The combination was classified as lethal if $Cy^+$ offspring constituted no more than 5% of the total, and at least 50 offspring were scored. Crosses within each of the two primary complementation groups are shaded. The results of these tests are plotted in Figure 5A. Nine other lethal recombinants were similarly tested and complemented all others.

tion of those sites should follow the same biases known to affect *P*-element insertion. There are several such biases, including preferences for euchromatin, for the 5′ ends of genes, and for target octamers similar to the sequence GGCCAGAC (O'HARE and RUBIN 1983; KELLEY *et al.* 1987; ENGELS 1989).

We made use of the target octamer preference to test the hypothesis that formation of the recombinant duplications and deletions occurred by HEI events. Figure 9 shows the expected base preferences in each position of the octamer, as derived from 61 previously published *P*-element insertion sites. We compared these frequencies to the 8 bp that had been moved adjacent to the CaSpeR end in nine of our recombinant deletions and two recombinant duplications. As expected from the HEI model, most of the octamers fit the consensus sequence reasonably well. For example, one leftward deletion breakpoint was at the octamer GGCCAACC, which matches the consensus in all positions except six and seven, where it has the second-best fitting base. Several other octamers, however, did not fit the expectation. In particular, there were three that had TA in positions four and five, making them a poor fit to the consensus, according to Figure 9, since either strand would have a T in position four.

To test the overall fit of the 11 recombinant duplications or deletions, we calculated a likelihood index measuring the goodness of fit of each octamer to the consensus pattern. The index is the product of eight frequencies, one from each column in Figure 9, according to the octamer sequence. The likelihood value was computed for both strands, and the maximum value was used for the index. The two orientations were treated equally because previous work (O'HARE and RUBIN 1983) has shown octamer preference is independent of the orientation of the *P* element. For controls, we computed the same index for each of 1000 overlapping octamers (1007 bp) from the sequence in 50C surrounding the CaSpeR insertion site. Note that this control sequence includes the sites of most of the duplication and deletion breakpoints.

The average index for the duplication and deletion

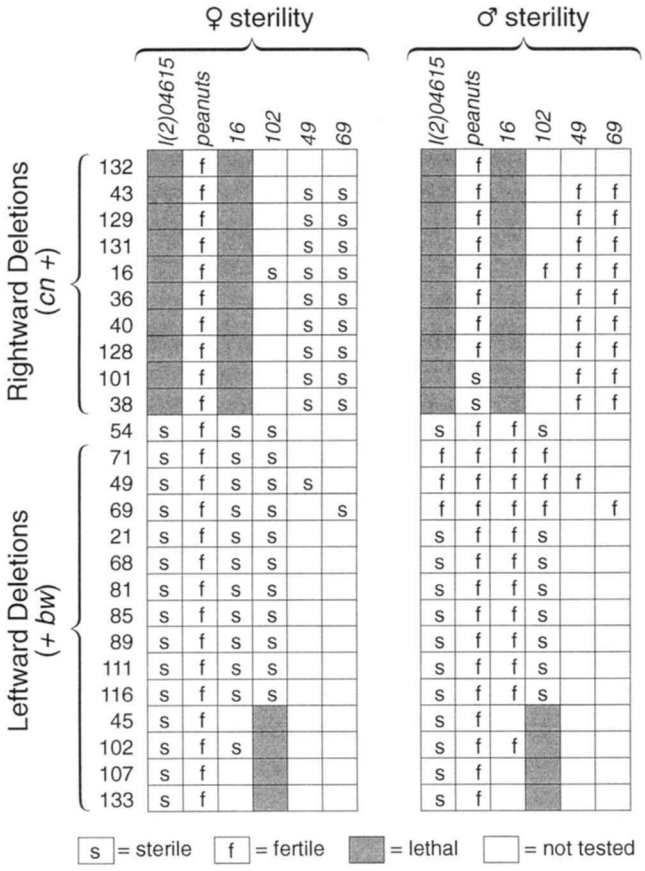



FIGURE 7.—Results of sterility complementation tests. Crosses were similar to the lethality complementation tests (Figure 6). For each cross $Cy^+$ males and females were separated and placed with flies of the opposite sex from a fertile stock. If no offspring were produced from any of five or more tested individuals after at least 10 days, the genotype was classified as sterile. The results of these tests are plotted in Figure 5A.

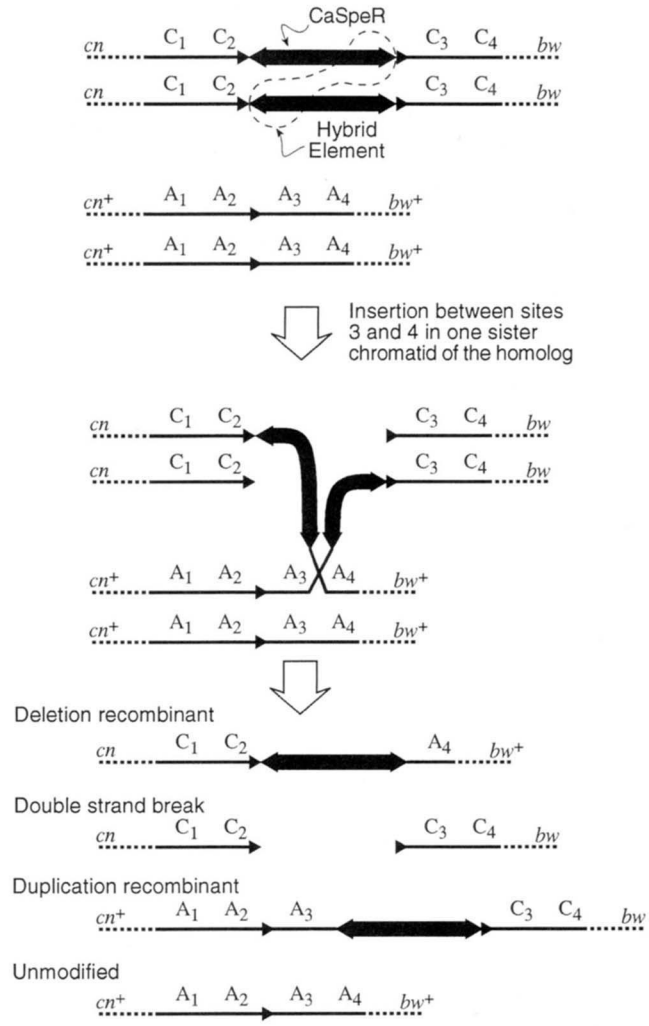



FIGURE 8.—The HEI model for male recombination. Both chromatids are shown for each homologue. The 8-bp duplication flanking *CaSpeR* is shown as a small arrowhead. In the first step, the right end of a *P* element pairs with the left *P* end of the element on the sister chromatid to form a hybrid element. This step is presumed to be mediated by *P* transposase. Double-strand breakage then occurs at each end, corresponding to the excision step of normal *P*-element transposition. These free *P* ends then integrate at a target site by the mechanism normally used for *P*-element transposition. Insertion is shown to occur in one sister chromatid of the homologue at a point to the right of the original element insertion site. Only the orientation that results in monocentric products is shown. The two free ends might be repaired to form a viable chromosome, but would be a nonrecombinant. The two *CaSpeR*-bearing products are recombinants, and would therefore be recovered by our screen. These two structures correspond to the first two observed classes shown in Figure 3. The other two observed classes would be formed if the hybrid element had inserted on the homologue to the left of the original insertion point.

octamers was 0.00045, compared to 0.00003 for the control octamers. For comparison, the 61 target site octamers from *P*-element insertions used to derive Figure 9 had an average index of 0.00078.

The statistical significance of the difference between average likelihood indices for the duplications and deletions *vs.* the controls was determined by selecting at random 11 of the 1000 control octamers, and computing whether the average index was at least as large as 0.00045. This random selection process was performed one million times, and only 2006 of the random samples fit the consensus as well as the observed group. Thus, we conclude that the duplication and deletion octamers fit the consensus better than the surrounding sequence with significance level $P = 0.002$.

**Deletions and duplications abut *P*-element ends:** Another prediction of the HEI model is that the breakpoints of the resulting deletions and duplications will correspond precisely with *P*-element ends. This is in contrast to aberrant double-strand break repair events in which the products often have extra or missing bases (TAKASU-ISHIKAWA *et al.* 1992; JOHNSON-SCHLITZ and ENGELS 1993; STAVELEY *et al.* 1995).

The HEI prediction was borne out in each of the 13 rearrangements whose sequence at the breakpoint was determined (described above). In addition, we made use of a *Ban*II restriction site that lies just to the right

| 5′ | Position | | | | | | | 3′ |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| T | 5 | 25 | 15 | 0 | 5 | 0 | 10 | 20 |
| C | 25 | 20 | **61** | **80** | 31 | 20 | 31 | **59** |
| G | **57** | **39** | 5 | 13 | 10 | **44** | 11 | 20 |
| A | 13 | 16 | 20 | 7 | **54** | 36 | **48** | 2 |

FIGURE 9.—The frequency (%) of bases at each position of previously described *P*-element insertion octamers. The consensus sequence, GGCCAGAC, is shown in bold. This table is modified from Figure 8 of O'HARE and RUBIN (1983) by the inclusion of 43 *P* insertion sites from two other sources (EGGLESTON 1990; O'HARE *et al.* 1992) in addition to the 18 sites used by O'HARE and RUBIN. For each octamer, we used the strand that best fit the consensus, following the method of O'HARE and RUBIN. All 61 insertions were independent events. There were several cases in which two or more independent insertions went to the same octamer. These were counted separately except for one extreme hotspot in the *yellow* locus where 24 insertions occurred in the same site (EGGLESTON 1990). In this case, it was thought that sequence elements external to the octamer plus selection for certain *yellow* phenotypes might have contributed to the repeated insertions (EGGLESTON 1990). Therefore, that octamer (GTCCACAG) was counted as only one of the 61 insertions.

of the *CaSpeR* insertion point to test other rearrangements. The genomic bases just to the right of the *CaSpeR* insert are GATTgggctc, with the last six bases being the *Ban*II recognition site. Note from Figure 3 that *Ban*II sites in the *cn bw*$^+$ deletions and duplications should be removed and displaced, respectively, according to the model. If the breakpoint on the *CaSpeR* chromosome were >10 bp to the right of the *P* end, the *Ban*II site would remain immediately adjacent to *CaSpeR*. In addition, the *cn*$^+$ *bw* duplications are predicted to have a new *Ban*II site to the left of *CaSpeR* if the event occurred by the HEI mechanism.

We tested 10 *cn bw*$^+$ deletions and one *cn bw*$^+$ duplication for changes in the *Ban*II site by digesting an amplicon that contains the breakpoint. As expected, the cutting site was lost in each of the deletions and displaced in the duplication by ~120 bp, the same size as the duplication. Similarly, amplicons containing the left breakpoint of six *cn*$^+$ *bw* duplications were digested with *Ban*II, and each was found to have acquired a cutting site at the expected position.

In total, the deletion or duplication breakpoint was found to coincide with the *P*-element end in each of 27 cases tested by DNA sequencing, *Ban*II digestion, or both. This result is consistent with the breakpoints being due to insertion of a hybrid element, but cannot be explained readily by models involving aberrant double-strand break repair.

**RFLP patterns of duplicated segments:** Note from Figure 8 that in the duplication recombinants, one copy of the duplicated segment is derived from the *P*-bearing chromosome and the other from its homologue. This prediction was confirmed by our initial RFLP mapping that showed both the A and C patterns in amplicons from the longer duplications. A more specific prediction is that the A pattern should be on the left side of *cn*$^+$ *bw* recombinants and the C pattern on the right side, with the reverse for the *cn bw*$^+$ recombinants. To test whether the A and C patterns were on the predicted sides, we made use of the *Ear*I restriction site polymorphism lying 30 bp to the right of *CaSpeR*, as shown in Figure 2 of the accompanying paper (PRESTON and ENGELS 1996). According to the HEI model, *cn*$^+$ *bw* duplications should be cut by *Ear*I on the right side of *CaSpeR* (derived from the C chromosome) but not on the duplicated copy to the left. We tested this prediction for 11 of the *cn*$^+$ *bw* duplications by performing *Ear*I digests separately on the G0-D0 amplicon (Figure 2), which should only amplify the left copy of the duplicated region, and 2231-D0 that should amplify only the copy to the right of *CaSpeR*. We found that all 11 of the recombinants had the C pattern on the right side and the A pattern on the left, as predicted.

## Use of recombinants for making deletions

**Approaches to making flanking deletions:** A key problem in Drosophila research is that of obtaining knockout mutations of genes known by map position and molecular data. One can sometimes start with a *P* insertion in the vicinity of the gene of interest and screen for transposase-induced flanking deletions (*e.g.*, SALZ *et al.* 1987). A commonly used method is to select among nonrecombinant chromosomes for loss of a dominant marker carried by the *P* element (*e.g.*, MELLERICK *et al.* 1992; LEE *et al.* 1993). Previous uses of this approach have often been successful, but they require large screens with many false positives. The present results suggest an improved approach is to screen among *P*-induced male-recombinant chromosomes. We find that approximately one-third of the recombinants will have deletions ranging in size from a few base pairs to at least 100 kb (Figures 4 and 5). The use of recombinants as opposed to excisions for producing flanking deletions has several practical advantages (see below).

**Recovery of longer deletions by a second round of recombination:** We selected four of the *cn bw*$^+$ recombinant chromosomes from the previous experiment to use in a second round of transposase-induced recombination. One of these chromosomes (#62) had no flanking deletion, and the others (#12, #3 and #129) had rightward deletions of 32 bp, 517 bp and >2 kb, respectively. All four retained a mobile *CaSpeR* element at the 50C site. Each of these chromosomes was made heterozygous with a *cn*$^+$ *bw* chromosome in males that also had a copy of the Δ2-3(99B) transposase source (Figure 10A). We then screened for recombinant offspring as in the previous experiments except that only *cn bw* recombinants were saved and analyzed.

Figure 10B shows the predicted outcomes according to the HEI model. These outcomes differ only in where the hybrid element insertion event takes place. Integration to the right of the deletion breakpoint (type I) results in an extended deletion, possibly knocking out the *mam* gene as shown in the figure if the extension is long enough. Types II and III occur when the HEI takes place to the left of the deletion breakpoint. The result is either a smaller deletion than the parental (type II) or a duplication (type III). Finally, types IV and V occur when the insertion occurs at a specific site, either the same target site as in the parental deletion (type IV) or the original CaSpeR insertion site (type V).

In total, 72 independent *cn bw* recombinant chromosomes were recovered and analyzed by PCR. Complementation tests as described above were applied to those that were lethal or sterile as homozygotes. The results, summarized in Table 2, showed that all five possible classes shown in Figure 10B were recovered, including the two site-specific insertion classes, IV and V. The occurrence of five recombinants of type V suggests that the site of the original *CaSpeR* insertion may represent a hotspot for *P*-element integration. Figure 11 shows the sizes of the resulting deletions. The shorter ones were determined by the sizes of amplified fragments spanning the new breakpoint, and the larger ones were classified by complementation tests.

The type I recombinants, in which the deletion was extended further rightward than the parental deletion, constituted one-fourth of all *cn bw* recombinants. This is the type most likely to be useful for generating long deletions. Of particular importance is the observation that five of the 17 recombinants from progenitor #129 had acquired longer deletions that knocked out the *mastermind* gene. Thus, the frequency of *mam* mutations among the selected recombinant class was 29% as opposed to 8% in the previous experiment where there was no preexisting deletion. The increase in *mam* mutations can be attributed to the presence of a long deletion in the progenitor chromosome that removes some but not all of the intervening sequence between *CaSpeR* and the *mam* locus. This deletion might alter the pairing between homologues such that the hybrid *P*-element ends are now closer to *mam* than they would be in a nondeleted chromosome (Figure 10). This result suggests that deletions of genes lying several hundred kb from a *P*-element insertion can be made efficiently in a stepwise process with each step moving the deletion breakpoint closer to the gene of interest but retaining a mobile *P* element.

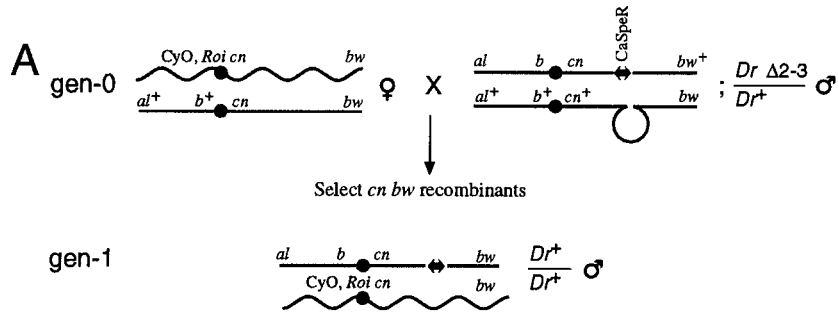
## DISCUSSION

**The mechanism of *P*-induced male recombination:** Recent work has led to three competing models for how *P*-element activity produces recombination in mitotic cells. One is that *P*-element transposase cuts random genomic sites regardless of where mobile *P* elements reside. These cuts produce recombination sites throughout the genome, and will occur in any cells where *P* transposase is expressed (DUTTAROY *et al.* 1990; MCCARRON *et al.* 1994). The second model is that male recombination is a byproduct of double-strand break repair following *P*-element excision (ENGELS *et al.* 1990; GLOOR *et al.* 1991). Finally, the HEI model sees male recombination as the result of an aberrant transposition event in which *P*-element ends on different DNA duplexes join to form a "hybrid element" (SVOBODA *et al.* 1995).

The present results along with the findings in the accompanying papers (GRAY *et al.* 1996; PRESTON and ENGELS 1996) strongly support the HEI model. The other two mechanisms may account for a small portion of the male recombination events (MCCARRON *et al.* 1994; PRESTON and ENGELS 1996), but it now seems likely that the great majority of such events are the result of formation of hybrid elements from two sister chromatids. The strongest evidence for HEI comes from the specific structures of the recombinant products that are predicted by the HEI model and no others. In particular, we find that most male recombinant chromosomes retain a *P* element at the recombination site and have a duplication or deletion flanking it. In accordance with the model, these duplications and deletions lie on the expected side of the *P* element according to which reciprocal recombinant has occurred, and their breakpoints occur precisely at the *P*-element boundaries. Furthermore, the breakpoints occur at 8-base target sites that tend to match the preferred octamers of *P*-element insertion.

The collection of deletion endpoints shown in Figures 4 and 5A can be taken as an indication of the distribution of insertion points of the hybrid elements, subject to selection for monocentric chromosomes with deletions or duplications small enough to be viable as heterozygotes. Approximately half of these insertion points were within 2 kb of the original *CaSpeR* site, indicating a preference for short-range jumps more pronounced than that reported for normal *P* transposition (ZHANG and SPRADLING 1993). The nature of hybrid elements, with each half remaining attached to one side of the donor duplexes, may place additional limitations on their insertion site selection.

Some classical work on *P*-induced recombination suggested that there was no excess of recessive lethal mutations on recombinant chromosomes (SVED 1978; ISACKSON *et al.* 1981; SINCLAIR and GRIGLIATTI 1985). Pooling data from all three papers, we see that 28 of 198 recombinant chromosomes carried recessive lethals, compared to 20 of 179 among the nonrecombinants. This result presents an apparent contradiction to our finding that recombinants are usually associated with deletions or duplications, many of which are likely to be recessive lethals. To explain this difference, note that in the earlier experiments a large number of *P* elements were mobilized from all parts of the genome. Therefore, many lethals were undoubtedly caused by simple inser-


A
gen-0
CyO, Roi cn
bw
al+ b+ cn
bw
♀ X
al b cn
CaSpeR
bw+
al+ b+ cn+
bw
; Dr Δ2-3 / Dr+ ♂
Select cn bw recombinants
gen-1
al b cn
bw
CyO, Roi cn
bw
Dr+ / Dr+ ♂

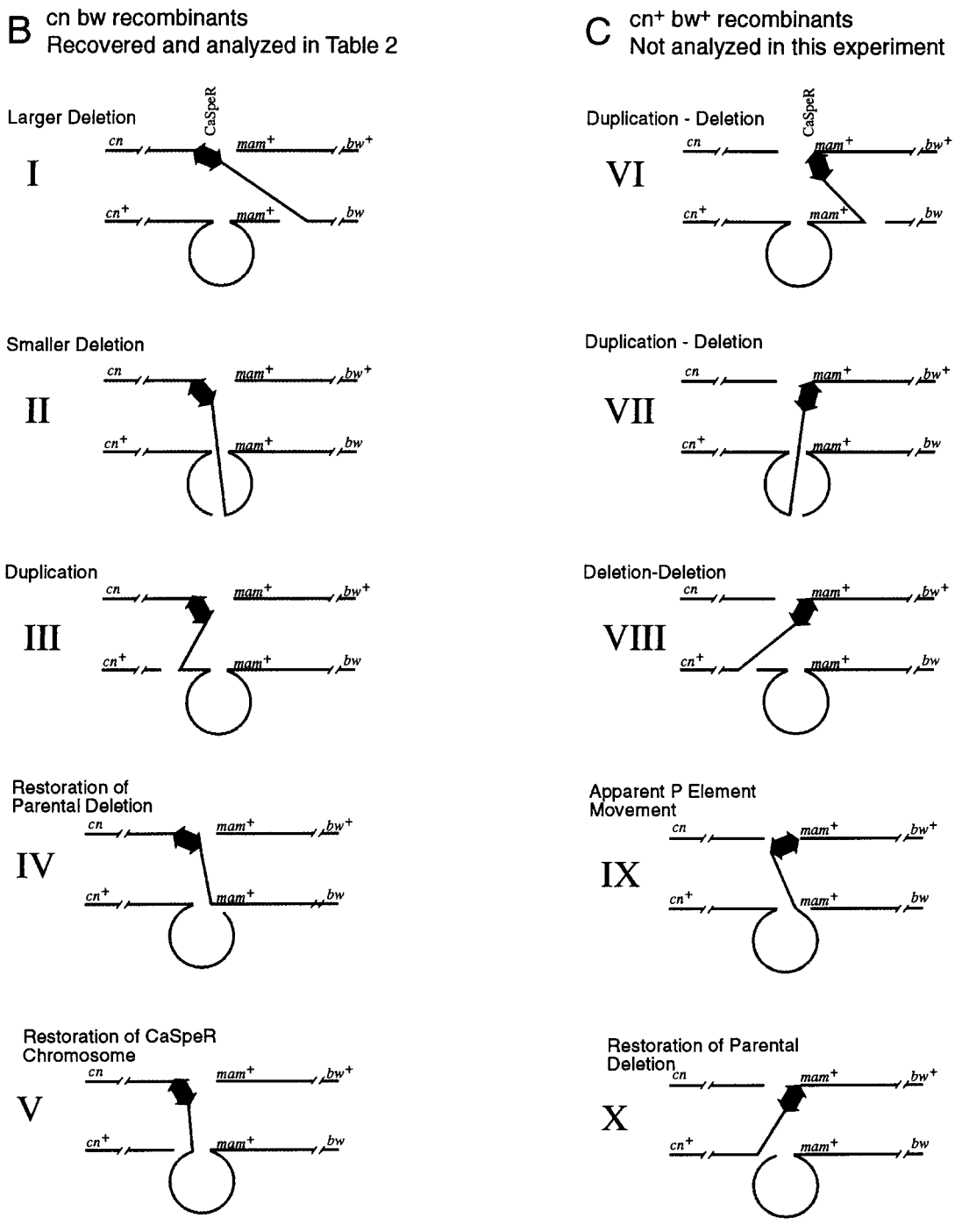

B cn bw recombinants
Recovered and analyzed in Table 2
C cn+ bw+ recombinants
Not analyzed in this experiment
Larger Deletion
I
Smaller Deletion
II
Duplication
III
Restoration of Parental Deletion
IV
Restoration of CaSpeR Chromosome
V
Duplication - Deletion
VI
Duplication - Deletion
VII
Deletion-Deletion
VIII
Apparent P Element Movement
IX
Restoration of Parental Deletion
X
cn
mam+
bw+
cn+
bw
CaSpeR

**TABLE 2**

**Classification of second-round recombinants (see Figures 10 and 11)**

| ID | Parental deletion | Independent *cn bw* recombinants analyzed | Type I (larger deletion) | Type II (smaller deletion) | Type III (duplication) | Type IV (restoration to parental) | Type V (restoration to original CaSpeR chromosome) | Estimated recombination frequency[a] | Crossovers outside 50C |
|---|---|---|---|---|---|---|---|---|---|
| 62 | None | 12 | 5 | NA | 6 | NA | NA | $\frac{51}{11642} = 0.44\%$ | 1 |
| 12 | 32 bp | 25[b] | 3 | 0 | 13 | 1 | 4 | $\frac{50}{11138} = 0.45\%$ | 1 |
| 3 | 517 bp | 18[c] | 5 | 2 | 9 | 1 | 0 | $\frac{61}{12083} = 0.50\%$ | 0 |
| 129 | Large[d] | 17 | 5 | 2 | 8 | 1[e] | 1 | $\frac{42}{10391} = 0.40\%$ | 0 |

NA, not applicable.

[a] These estimates are pooled from several experiments, not all of which contributed to the *cn bw* recombinants analyzed and described in the other columns. In most cases, both reciprocal recombinants were counted. For some groups, only *cn bw* recombinants were counted, and the reciprocal class was assumed to be the same size. There were also crosses that were used to produce recombinants but did not contribute to these counts.

[b] Includes three complex chromosomes that were not fully characterized. Each of the three had PCR results characteristic of more than one of the five types.

[c] Includes one that could not be classified unambiguously due to an internal deletion of *CaSpeR*.

[d] Parental deletion extends through *l(2)50CDa* but not *mam*, as shown in Figure 5A.

[e] The deletion breakpoint of this recombinant lies in the same complementation interval as the parental deletion, but we cannot rule out the possibility of a small change in the deletion length such that it remains within the same interval.

tions and other events not related to the observed crossover. This effect adds noise to the experimental system, making any correlation between recombination and recessive lethal production harder to detect. In addition, we expect that the production of lethal flanking deletions will be sensitive to the precise location of the *P* element. Those elements lying close to essential genes will produce lethal recombinants more readily. The early experiments employed *P* elements on chromosomes recently derived from nature, where previous natural selection over many generations is likely to bias the set of insertion sites to those that are not near essential genes. Such elements might cause fewer lethal flanking deletions than the *P{CaSpeR}* element used here. Therefore, we suggest that a correlation between recessive lethal production and male recombination might not have been detected in previous experiments even though such a correlation does exist.

Our screen was designed to recover only crossover events, but in principle the HEI process can lead to other structures depending on the selection of the insertion site. If the insertion site is on another chromosome, for example, the result can be a reciprocal translocation. If it is elsewhere on one of the same sister chromatids that participated in the formation of the hybrid element, the process could, depending on the orientation of the insertion and repair of broken ends, lead to duplication or deletion structures similar to what we have observed, or to an inversion flanked by copies of the *P* element. These structures would not be recombinant for flanking markers unless there was a second, independent, HEI event involving the homologue to produce a recombinant. In the present study, we did observe three cases in which duplications or deletions were seen on both sides of *CaSpeR*. These recombinants could be the result of two independent HEI events in different cell generations, one involving homologues and the other limited to the sister chromatids. In addition, there are indications that such intrachromosomal HEI rearrangements have been observed in previous studies. Several very large deletions that retained intact *P* elements at the breakpoints were isolated by HOWE *et al.* (1995) and might have resulted from such a process. Similarly, a deletion at the *escargot* locus with a *P* element at or near the breakpoint was reported on an apparently nonrecombinant chromosome (WHITELEY *et al.* 1992). Studies at the *Tpl* locus, in which a nearby *P* element was mobilized, yielded

FIGURE 10.—A second round of recombination to extend deletions and produce additional changes. (A) The cross used to generate second-round recombinants. The wavy line represents the balancer chromosome, CyO. A deletion to the right of *CaSpeR* is indicated by an unpaired loop in the homologue. The overall frequency of recombination was ~0.5% for each parental chromosome (Table 2) as compared to 1.0% in the previous experiment (Figure 1). This difference can be explained by a lower room temperature (~21°) in the latter experiments. (B and C) Predicted structure based on HEI model. A process similar to that shown in Figure 8 is assumed, but only one sister chromatid is shown for each homologue, since only *cn bw* recombinants were examined. For type I, the extended deletion is shown as deleting the *mam* gene to illustrate the process, but any deletion longer than the parental was classified as type I. Only one of the two reciprocal recombinant types (*cn bw*) was analyzed in this experiment, therefore no events of types VI—X were observed.

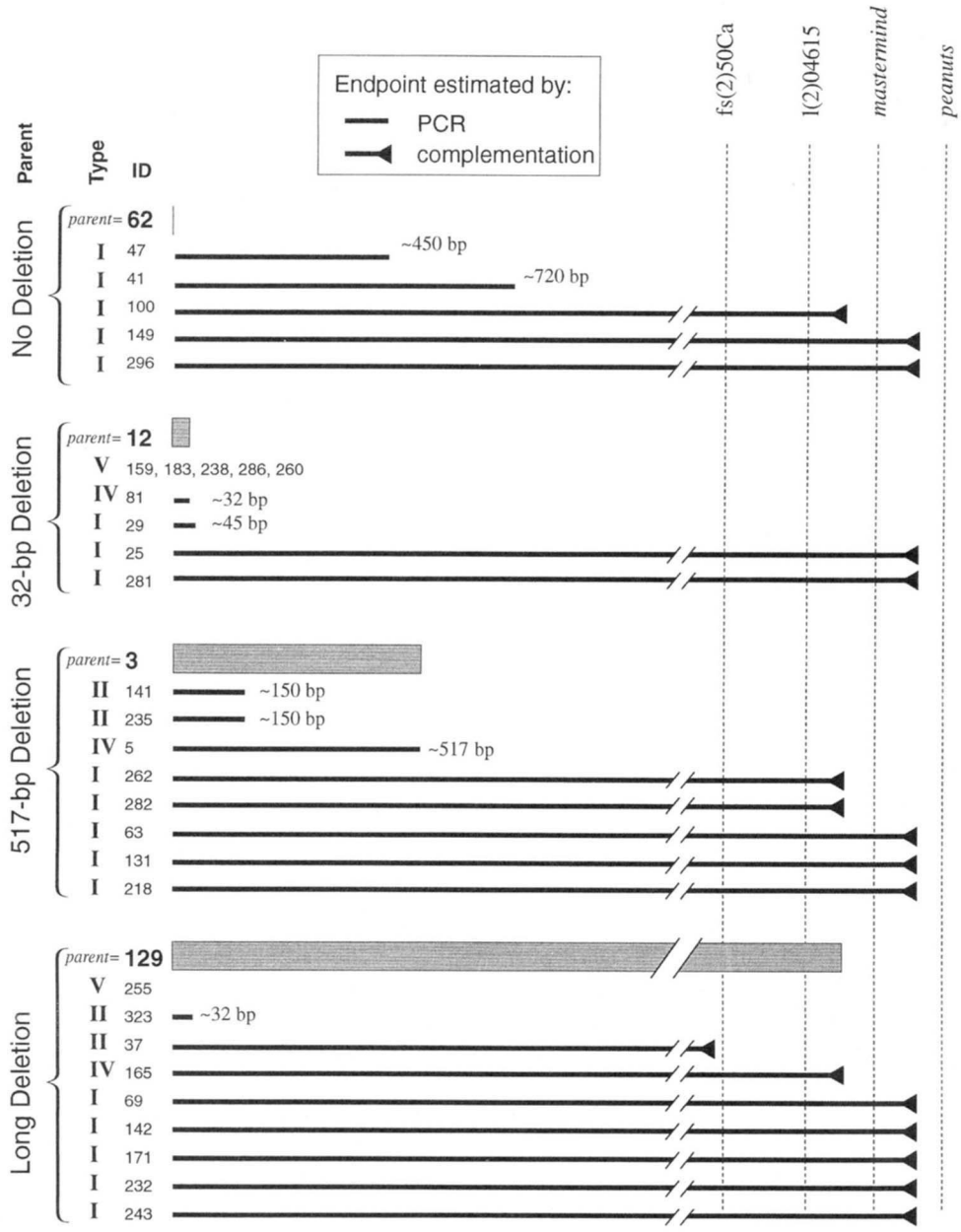



FIGURE 11.—Deletion sizes resulting from second-round recombinants. The four groups correspond to the four parental *cn bw*$^+$ chromosomes used. Types I—V correspond to the classifications in Figure 10B. For the shorter deletions, an estimate of the size was obtained from measurements of the sizes of breakpoint-spanning amplicons on agarose gels. For the longer deletions, only complementation data were available, and the physical size is not known.

both duplications and deletions of the gene in approximately equal numbers (A. CHRISTIANSEN, personal communication).

**Use of recombination for making flanking deletions:** Drosophila geneticists are increasingly able to identify genes of interest by means of expression patterns or sequence homology. Creating mutations at such loci is often the rate-limiting step in their analysis. One strategy that has been successful in some cases is to use imprecise excision of a nearby *P* element to generate flanking deletions that knock out the gene of interest. This strategy, of course, depends on the presence of a *P* element close to the gene. There is a massive effort currently underway to produce a collection of *P*-element insertion lines such that at least one will lie within 100 kb of nearly all genes (SPRADLING *et al.* 1995). Already the collection contains nearby *P* elements for the majority of genes.

We suggest that the best way to use a nearby *P* element to make flanking deletions is by selecting transposase-induced male recombination events. This approach has several advantages over screening for *P* excisions. (1) The deletions occur at reasonably high frequencies, and candidates can be identified phenotypically with flanking markers. In the present experiments, male recombination rates ranged from 0.3 to

1.0% depending on the rearing temperature of the recombinagenic males (Figure 1), and these rates are consistent with previous studies. In most cases it is not necessary to use flanking markers close to the recombination site. For example, we used *cn* and *bw*, which lie on opposite ends of 2R. Flanking markers can, therefore, be chosen solely for experimental convenience and easy identification of phenotypes. (2) The direction of the deletion can be controlled by choosing which of the two reciprocal recombination products is screened. This feature provides a significant advantage when the location of the *P* element relative to the target gene is known. (3) The deletion products usually retain a mobile *P* element that can be used to generate further variability. For example, we made use of a second round of recombination to increase the frequency of *mam* knockouts (Figure 11). In fact, this technique can be used to generate a variety of alterations that both increase and decrease the size of the first-round deletion, as illustrated in Figure 10B. In addition, one could screen in the second round for recombination in the opposite direction to generate changes on the other side of the *P* element. For example, we used the scheme in Figure 10A to select *cn bw* recombinants, many of which restored part of the original deletion on the right side (designated type II in Figure 10B). Had we selected $cn^+$ $bw^+$ recombinants instead, many of them would have been partial restorations of the deletion from the left side as shown in Figure 10C. This process could be used efficiently for a functional analysis of a local region.

In our experiments, the recombination events occurred in the male germline. There is evidence that *P*-element mobilization also causes recombination in the female germline (KIDWELL 1977), but it is harder to detect because ordinary meiotic recombination tends to obscure it. It should, therefore, be possible to use *P*-induced recombination to make deletions on the *X* chromosome by selecting for recombination in females. To minimize false positives due to meiotic recombination, it would be preferable to use closely linked flanking markers in the screen.

Two additional considerations should be kept in mind by researchers attempting to make deletions with this approach. First, since the deletions are expected to retain a mobile *P* element at the site, it is preferable to recover the recombinants in a genotype lacking the transposase source. This was accomplished in the present work by utilizing only the $Dr^+$ recombinants shown in Figure 1 since the Δ2-3(99B) transposase source was closely linked to *Dr* (ROBERTSON *et al.* 1988). However, a complication arises if the gene of interest lies on chromosome *3 and* the *P* element is situated to the left of the gene. In such cases, if Δ2-3(99B) is used, the transposase source will be together with the mobile *P* element in the desired class of recombinants. This undesirable situation cannot be avoided by starting Δ2-3 on the same chromosome as the mobile *P* element, because that would risk unwanted mobilization in the previous generation. Although not ideal, one additional generation of *P* mobilization is probably an acceptable nuisance. Alternatively, one could use a transposase source on another chromosome (COOLEY *et al.* 1988).

Second, not all recombinants will be deletions, and not all deletions will be large. We found that approximately one-third of the recombinants carried flanking deletions, and the rest were approximately evenly divided between duplications and simple crossovers. The size distribution shown in Figures 4 and 5A includes all the deletions whose structures we were able to determine. Therefore, it can be considered an unbiased sample, and other loci might be similar. Approximately half of the deletions, those shown in Figure 5A, were classified as "large", meaning they extended outside the 4-kb region defined by our primers and had to be mapped by complementation.

Thus, to estimate the frequency of useful deletions, we can use the product of the recombination rate (1%), the fraction of the desired reciprocal class (1/2), and those lacking the transposase source (1/2), to obtain 1/400 as the expected proportion of offspring with the desired phenotype. These would then have to be screened with a more specific technique, such as a PCR test or a cross to a known mutant, to find the ones with a long deletion knocking out the gene of interest. Our data suggest 1/3 of them will have a deletion, of which approximately half will be large. Therefore, approximately 1/6 of the phenotypically selected flies will have a deletion of the desired type. This compares favorably to the alternative method of screening for imprecise (flanking) excisions, most of which are not large and in the right direction. Perhaps more importantly, the deletions occurring by recombination are more useful for generating further variability by additional rounds of recombination.

Helpful comments were provided by DENA JOHNSON-SCHLITZ, CARLOS FLORES, JOHNG LIM, ALAN CHRISTIANSEN, DIRK LANKENAU and GREG GLOOR. This is paper 3466 from the University of Wisconsin Laboratory of Genetics (http://www.wisc.edu/genetics) supported by National Institutes of Health grant GM-30948.

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Communicating editor: R. S. HAWLEY