P*-Element-Induced Male Recombination Can Be Produced in *Drosophila melanogaster* by Combining End-Deficient Elements in *trans

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ABSTRACT

Male recombination, not normally present in *Drosophila melanogaster*, can be produced at high rates when target *P* elements at homologous sites are combined in the presence of transposase protein. We have produced a set of elements by *in situ* deletion of a particular insertion and have found elements that have deletions stretching into either end. Elements were tested in pairs to see whether they complement each other in their ability to induce recombination. The combination of elements that are deficient for the same end produces very little recombination, but the combination of a right-end and a left-end element can generate recombination values higher than given by two complete *P*[*CaSpeR*] elements at homologous sites. This strongly suggests that "hybrid" *P* elements, containing ends from two different elements, can be recognized by transposase protein. We have also examined genotypes containing a normal and an end-deficient element and found that they yield reasonably high levels of recombination. We interpret the resultant gametes from such genotypes as showing that the majority of events in this genotype derive from the association of complementary ends from the same element, whereas the complementary ends from elements in *trans* associate in only a minority of cases.

P elements in *Drosophila* induce recombination as one manifestation of their activity (see ENGELS 1989). This activity was originally manifested in crosses involving wild-derived strains of *Drosophila melanogaster* (HIRAIZUMI 1971). Such crosses typically involve large numbers of elements, including complete and incomplete elements, and generate something of the order of 1% recombination per chromosome.

The development of genetically engineered *P* elements has allowed the introduction of much simpler systems for producing recombination (MCCARRON *et al.* 1989; SVED *et al.* 1990). The system used by SVED *et al.* (1990) uses two elements and generates around 1% recombination. A *P*[*CaSpeR*] transposon (PIRROTTA 1988) acts as a target element in this system. This is a nonautonomous element, and transposase protein is provided by the *P*[$\Delta 2-3$] (99B) element (ROBERTSON *et al.* 1988).

The two-element system was modified by SVED *et al.* (1991) by adding a second homologous target element. *P*[*CaSpeR*] insertions were combined onto a marker chromosome by female recombination in the absence of transposase protein, thereby creating a genotype in which a large part of the chromosome, including the region of the element, was homozygous. It was found that male recombination in these genotypes increased by an order of magnitude to levels of ~20%.

The mechanism whereby this recombination is produced is not currently understood nor is it clear whether the same mechanism applies in the case of the low recombination levels given by a single target element and of the higher recombination levels given by homologous target elements. However, a considerable amount is now known about the manner in which *P*-element mobilization occurs, and as a first hypothesis the possibility must be considered that recombination is a byproduct of this mobilization.

ENGELS *et al.* (1990) have hypothesized that the normal mode of *P* element increase consists of excision of the element at the four-strand stage, followed by repair of the double-stranded gap using the sister chromatid as a template and insertion of the excised element elsewhere in the genome. In this way the copy number of the element rises, creating extra burden on the host's repair machinery. This hypothesis remains unproven, because an element repaired by copying a sister element has no detectable differences from the original element. However, the process of repair against non-sister templates is well documented (*e.g.*, GLOOR *et al.* 1991; NASSIF *et al.* 1994). If repair occurs in 80–90% of cases against the sister strand, this would satisfactorily account for observed excision and insertion rates.

Excision of an element, leaving behind a double-stranded break, provides a likely means of leading to recombination. However, recent results suggest that the simplest form of the *P*-element excision-induced recombination hypothesis cannot explain the recombination induced by a single element (SVED *et al.* 1994). It has

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FIGURE 1.—Production of *P[Deleted-CaSpeR]* (50C) lines.

long been known (e.g., see SANKARANARAYANAN and SOBELS 1976) that male recombination in *D. melanogaster* is stimulated by X irradiation, which also creates double-stranded breaks. If recombination occurs between a strand from which the element has been excised and the homologous strand that does not contain an element, it is expected that recombinant chromosomes should not contain an element. Contrary to this prediction, many recombinant gametes contain a copy of the element at its original site.

One way of obtaining more information on the recombination process is to combine target elements that are modifications of the original element. JOHNSON-SCHLITZ and ENGELS (1993) have shown that such modifications can be produced *in vivo* by exposing an original element to the transposase source and selecting for derivative elements that have acquired deletions. We have applied this principle to modifying one particular insertion of the *P[CaSpeR]* element, at location 50C. *P[CaSpeR]* contains a functional copy of the *white* gene

that enables the element to be detected phenotypically in a *w* mutant background. Thus we selected for chromosomes coming from an original *P[CaSpeR]* (50C) element that were white eyed in a *w* background and that were therefore potentially deleted for part of the element. From among these deleted elements we found several that appeared to have deletions stretching into the ends of the element. It is these elements, and their recombination properties, that form the basis for the present paper.

We have fully characterized four partially deleted *P[CaSpeR]* (50C) elements deficient in either the left or right end of the element while retaining most if not all *P*-element sequences at the other end. When two left-end or two right-end elements are combined on homologous chromosomes, little if any male recombination is produced. However, the combination of a left-end element and a right-end element in *trans* results in high recombination frequency. This recombination rate is of the same order of magnitude as that produced by the combination of two complete *P[CaSpeR]* (50C) elements.

METHODS AND MATERIALS

All of the results of this paper use a single insertion of the *P[CaSpeR]* element at site 50C on chromosome 2R. The method of selecting for modification of the *P[CaSpeR]* (50C) element is shown in Figure 1. Individual males containing this element and *P[Δ2-3]* (99B) were backcrossed to white females. About 10% of G2 males with the original *P[CaSpeR]*-containing chromosome showed the white-eyed phenotype, indicating some sort of deletion, and these were crossed to a stock containing *SM1* (*al*² *Cy*) for the purpose of producing lines homozygous for the deleted element. In view of the tendency of dysgenic events to be clustered due to the premeiotic origin (ENGELS 1989), no more than two males containing deletions were used from each G1 male parent to reduce the number of lines containing deletions attributable to a single event.

Genomic DNA was extracted from single flies as described by GLOOR and ENGELS (1992) and used in studies of individual progeny from dysgenic crosses. Bulk genomic DNA from homozygous lines was prepared by grinding 100–200 flies with mortar and pestle in liquid nitrogen, placing the powder

FIGURE 2.—*P[CaSpeR]* primers (B and C) and 50C primers (A and D) for detection of the *P[CaSpeR]* (50C) element by PCR. When intact *P[CaSpeR]* (50C) element ends are present, products of 331 bp (A–B) and 435 bp (C–D) are expected. A single product of 410 bp (A–D) is expected when no element is present at this site. Primers A–D are also predicted to produce a 5401-bp fragment when an intact *P[CaSpeR]* (50C) element is present, too long to be amplified under our PCR conditions.

TABLE 1
PCR screening of putative deletion elements

Class	PCR fragments			N	Description
	A-B (331 bp)	C-D (435 bp)	A-D (410 bp)		
1	+	+	—	42	Internal deletion
2	+	—	—	6	End deleted?
3	—	+	—	6	End deleted?
4	—	—	+	3	Complete excision
5	?	?	?	7	Other
Total				64	

+, Presence of defined amplification product; —, absence of the fragment. Class 5 represents homozygous lines containing aberrant PCR products either in conjunction with defined fragments or alone.

FIGURE 3.—Southern blot of whole genomic DNA, digested with *Eco*RI restriction endonuclease, from *D. melanogaster* stocks used in this study. Fragments A–B and C–D (Figure 2) were labeled with α^{32} P-dATP and used as a probe. *al b cn bw* and *P[CaSpeR](50C)* stocks produced bands at 2.4 kb and 6.1 kb/1.3 kb, respectively, as expected. *DL1* produced a single band at 3.3 kb, predicting a deleted *P[CaSpeR]* element of ~900 bp with the *P[CaSpeR]* *Eco*RI site deleted. *DR1* produced two bands at 2.6 kb/1.3 kb, predicting a deleted *P[CaSpeR]* element of ~1.5 kb with the *P[CaSpeR]* *Eco*RI site retained. Heterozygote *DL2* produced two bands, one at 2.4 kb from the *SM1(Cy)* balancer chromosome and another band at 3.9 kb, predicting a deleted *P[CaSpeR]* element of ~1.5 kb with the *P[CaSpeR]* *Eco*RI site deleted. *DR2* produced a single band at 2.6 kb, predicting a deleted *P[CaSpeR]* element of ~200 bp with the *P[CaSpeR]* *Eco*RI site deleted.

in 2 ml TENS buffer (100 mM Tris, pH 7.6, 50 mM EDTA, pH 8.0, 100 mM NaCl, 200 mM sucrose) with 0.2% Sarkosyl and incubating at 60° for 15 min with occasional gentle inversion. Proteinase K was added to 0.1 mg/ml final concentration, and the mixture was incubated at 50° for 48 hr with gentle inversion every 12 hr. RNase was added to a final concentration of 0.1 mg/ml and incubation was continued at 37° for 2 hr. DNA was isolated by gentle phenol and chloroform extractions, ethanol precipitated and dissolved in 100 μ l TE.

Homozygous lines were screened by PCR using four primers. These primers (Figure 2) give characteristic fragments for the left end (A–B), the right end (C–D) and the empty site (A–D). Because primer pairs A–B and C–D each involve an internal and an external primer, the screen using these primers is expected to detect elements with deletions affecting the end of the element and also the flanking region.

To be able to measure recombination, elements selected for further study were recombined with the chromosome 2 markers *b* (48.5), *cn* (57.5) and *bw* (104.5). This was achieved by backcrossing females of genotype: + *b cn vg. bw* / + + + + *Ca* + to males of genotype *al b cn vg. bw*, selecting for individual double-recombinant offspring of phenotype + *b cn* + *Ca* + *bw* and producing homozygous lines. The *vg* locus is located at 67.0, whereas the 50C site is estimated to be at approximately map position 70 (LINDSLEY and ZIMM 1992), giving a significant chance that any double recombinant will not contain the *P[CaSpeR](50C)* insertion. Each recombinant line was therefore screened to confirm acquisition of the expected PCR fragments associated with particular deleted elements.

Southern hybridization was carried out using as a probe a mixture of the two radioactively labeled PCR fragments A–B and C–D (Figure 2) produced from the phage clone, 1C1, containing the *P[CaSpeR](50C)* element and surrounding region. Absence of an element in the genomic DNA gives a single 2.4-kb band when the DNA is cut with *Eco*RI and hybridized with either probe. There is a single *Eco*RI site in the *P[CaSpeR]* element, and Southern hybridization with a complete *P[CaSpeR]* element leads to 6.1- and 1.3-kb bands.

Partially deleted *P[CaSpeR]* elements were amplified in their entirety, including regions immediately flanking the insertion point. This encompassed sequences from primer A to D, including the inserted element. PCR products were purified and sequenced using the Circumvent Cycle Sequencing Kit (New England Biolabs). Resulting sequences were analyzed by alignment to the complete *P[CaSpeR](50C)* sequence.

FIGURE 4.—The structure of *P[CaSpeR]* and end-deficient derivatives of *P[CaSpeR]* (50C). E, *EcoRI* sites. Numbers of base pairs remaining on each end of the *P[CaSpeR]* element are shown. All but *DR2* have clean breaks at the deletion site, whereas *DR2* has an additional five bases added between the two breakpoints.

RESULTS

Altogether, 64 lines were produced using the procedure of Figure 1. These lines were screened using the primers of Figure 2 and gave the results shown in Table 1. Although most of the putative deletion stocks produced expected PCR fragments, some did produce novel products (class 5). Because the purpose of the

analysis was to establish deletion derivatives of the original *P[CaSpeR]* (50C) element rather than complex rearrangements, these seven lines were not included in any further study.

A feature of the results is the low number of elements in class 4. The model of ENGELS *et al.* (1990) predicts that if the homologous strand is available for repair, then complete loss of the element will occur frequently. Fortunately, use of the *CyO* balancer as the homologous chromosome appears to have reduced the frequency of this class. The inversion in this chromosome involves a break in the 50C-D region (LINDSLEY and ZIMM 1992), which presumably accounts for the low conversion frequency.

The major class of elements, class 1, showed no changes from the amplification pattern given by *P[CaSpeR]* (50C). This class is described as “internal deletion” because of the loss of the *white* phenotype but may include some cases where the phenotype has been lost for reasons other than deletion.

Attention for the remainder of the paper is focused on classes 2 and 3, in which one or other of the end fragments is missing. Because these elements may include cases where the end of an element has been deleted and/or where the flanking region has been deleted, additional data were needed to fully describe the deletion event. We have concentrated on four elements where sequencing shows that the deletion involves the end of the element itself.

Southern blots shown in Figure 3 were used to estimate the size of the deleted region and to confirm that only the single element at 50C was present in the genome. We have reconfirmed the Southern data by a series of overlapping amplification products for each of the four elements (data not shown), including in

FIGURE 5.—Genotypes of test males. Parts of chromosomes 2 and 3 are shown. (a) Combination of a left-end element with a right-end element in *trans*. (b) Combination of two left-end elements. Similar crosses were done involving two right-end elements.

FIGURE 6.—Percent recombination (vertical axis) given by various combinations of end-deficient elements.

each case the original element and the element recombined onto the *cn bw* marker chromosome.

The internal structure of the four elements as determined by sequencing is shown in Figure 4. These elements are designated as *DL1*, *DL2*, *DR1* and *DR2*, where *L* and *R* indicate the end that is *present*. The chromosome carrying the *DL2* element is lethal in homozygous condition, and this element was therefore kept in heterozygous condition against the balancer chromosome *SM1* (*Cy*). Elements are drawn approximately to scale. In each case, a large internal deletion has occurred, stretching into one end. Small pieces of the deleted end are present. However, the deletion in all cases takes out most of the 139 bp of the left end or the 163 bp of the right end required for element activity and transposase binding (O'HARE and RUBIN 1983; KAUFMAN *et al.* 1989; MULLINS *et al.* 1989).

The finding of breaks within the 31-bp repeated region is consistent with the findings of SEARLES *et al.* (1982) and TSUBOTA and SCHEDL (1986), who reported similar events in studies of reversion. JOHNSON-

SCHLITZ and ENGELS (1993) also found similar structures after *in vivo* modification of *P* elements.

Combinations of end-deficient elements: The four elements were combined in pairs, as shown in Figure 5. Single nonaged males of these genotypes were backcrossed to females of genotype *b cn bw*. All progeny were scored for the *cn* and *bw* markers. Recombination frequencies between *cn* and *bw* are shown in Figure 6. In each case, elements sharing the same end produce little or no recombination. The combination of a left element with a right element in all cases leads to recombination frequencies of the same order of magnitude as given by two complete *P*[*CaSpeR*] (50C) elements.

It should be noted that although the *DL2* chromosome was homozygous lethal on its original chromosome, the procedure of recombining the *DL2* element onto the *cn bw* chromosome allowed the production of genotypes that were homozygous in the region of the element, suggesting that the lethality lies outside of this region.

Table 2 shows the numbers observed in the different backcross classes for the four combinations of left- and right-end elements that produced the high recombination frequencies of Figure 6. Both recombinant gametes, *cn+* and *+bw*, are seen at similar frequency in all four combinations of Table 2A. The relative frequency of the two recombinant types among the few cases seen in Table 2B is not significantly different from those in Table 2A ($P = 0.26$ by Fisher's exact test). The excess among the parental gametes of *++* over *cn bw* and among the recombinant gametes of *+bw* over *cn+* are presumably due to viability differences.

Backcross progeny were classified for PCR fragment pattern using the PCR primers of Figure 2. Results are given in Table 3 for all four classes of gametes from the pair of elements *DL1/DR1*, combined in the genotype shown in Figure 5a. A representative sample is shown in Figure 7, together with PCR results from parental

TABLE 2
Recombination results from (A) left- and right-end elements combined in *trans* and (B) left-end elements only or right-end elements only combined in *trans*

Parent	<i>cn bw</i>	<i>++</i>	<i>cn +</i>	<i>+ bw</i>	Total	Rec. %
A						
<i>DL1/DR1</i>	1911	2613	733	1099	6356	28.8
<i>DL1/DR2</i>	1148	1556	520	728	3952	31.6
<i>DL2/DR1</i>	1218	1577	281	332	3408	18.0
<i>DL2/DR2</i>	611	794	194	241	1840	23.6
B						
<i>DL1/DL1</i>	859	1142	1	1	2003	0.1
<i>DL1/DL2</i>	968	1374	0	1	2343	0.0
<i>DL2/DL2</i>	549	653	2	1	1205	0.2
<i>DR1/DR1</i>	357	472	0	0	829	0.0
<i>DR1/DR2</i>	1781	2378	5	1	4165	0.1
<i>DR2/DR2</i>	1579	2114	0	1	3694	0.0

TABLE 3

PCR results from the four classes of gametes produced by the genotype with combined end-deficient elements as in Figure 4

	AB CD + +	AB CD + -	AB CD - +	AB CD - -
<i>cn bw</i>	0	57	2	4
<i>cn +</i>	0	30	46	4
<i>+ bw</i>	0	30	26	11
<i>++</i>	0	0	55	3

The expected PCR products from primer pair AD was 410 bp, as shown in Figure 2, because female parents did not contain a *P[CaSpeR]* (50C) insertion. This fragment was amplified in all gametes tested.

chromosomes. Note that the results of Table 3 are shown only for primer pairs A–B and C–D. All individuals scored were heterozygous for the chromosome from the *DL1/DR1* parent and a chromosome from the *b cn bw* parent. The *b cn bw* chromosome does not contain an element at 50C and is therefore expected to amplify the 410-bp A–D primer fragment, as shown in Figure 7. This was confirmed for all progeny classified.

Three features of these results should be noted. First, recombinant gametes are approximately evenly divided between the two parental patterns. There is no preferential tendency, for example, for the deficient end of either element to segregate with the locus with which it is linked. Second, no recombinant gamete was found having both ends present. Third, and most important, the presence of the class lacking both ends shows that there have been changes affecting the elements.

PCR fragments were also classified in the case of pairs of either two left-end or two right-end elements. Although small numbers of recombinant gametes were found in such crosses (Table 2B), no recombinant gametes were present in the subset of flies available for PCR analysis. In a total of 67 parental gametes studied, the expected parental pattern was present in all cases. This contrasts with the results from Table 3, where 24 altered PCR patterns were found in a total of 268 gametes. All those showing “--” and the two *cn bw* progeny showing “-+” PCR patterns are considered altered because the parental chromosomes cannot account for those of the progeny. Of these, ≥22 of these 24 repre-

FIGURE 7.—PCR amplification of single fly DNA using primers A–D from Figure 2. Individual examples of each of the parental stocks used in this study are shown along with a representative sample of progeny from males of the genotype shown in Figure 5a. Heterozygote *DL2* contained a 410-bp amplification product from primers A to D from the SM1 (*Cy*) balancer chromosome. The 633-bp amplification in *DR2* was caused by amplification with primers A–D, including the 223 bp of remaining *P[CaSpeR]* sequence. A positive control amplification product of 1 kb was obtained by addition of oligonucleotides from the *singed* locus 5'GCGCAACTTCGCTCGC 3' and 5'CTGGAAGTAGAGATGGTCGC 3'.

sented events from different male parents. Accepting this lower number for a conservative test, the number of altered elements is still significantly higher than that given by two left- or right-end elements ($P < 1\%$ by Fisher's exact test). Thus the PCR results are in agreement with the results from the visible markers in indicating element mobility when complementary ends are present but not when only one end is present.

Combinations of end-deficient and complete *P[CaSpeR]* elements: Genotypes were produced with an end-deficient element combined with a complete *P[CaSpeR]* (50C) element (Figure 8). Single males of these genotypes were backcrossed to females of genotype *b cn bw*. These produced less recombination than the combination of

FIGURE 8.—Genotype with end-deficient and complete *P[CaSpeR]* (50C) elements. The case of left-end elements is shown. Equivalent crosses were also done using right-end elements.

TABLE 4
Recombination results for backcross of genotypes with end-deficient
and complete *P[CaSpeR](50C)* elements

Parent	<i>cn bw</i>	++	<i>cn +</i>	+ <i>bw</i>	Total	Rec. %
<i>DL1/Ca(50C)</i>	592	809	75	78	1554	9.8
<i>DL2/Ca(50C)</i>	357	537	40	33	967	7.5
<i>DR1/Ca(50C)</i>	1901	3238	330	448	5917	13.1
<i>DR2/Ca(50C)</i>	448	634	34	65	1181	8.4

end-deficient elements (Table 4 compared with Table 2A). Both reciprocal recombinants were again found at similar frequencies.

Results of the PCR classification of the backcross progeny are shown in Table 5. This table, for convenience, shows the combined results from all left- and right-end elements. A left-end element is depicted in Figure 8. In this case, the class labeled "Parental" in Table 5 has the [+ -] PCR pattern, whereas the class labeled "New" has the [- +] pattern. These symbols are reversed for a right-end element.

A salient feature of the results in Table 5 is the homogeneity of PCR patterns associated with the chromosome carrying the end-deficient element (*cn bw* in Table 5). This indicates the stability of the end-deficient element in this genotype. Such a result is predicted by the "cut-and-paste" model of ENGELS *et al.* (1990) for a single normal element. In this model, the primary event is expected to be excision of the normal element, followed by repair against the end-deficient element. The expectation is that the opposite event, excision of the incomplete element, will be rare. This has previously been shown to occur in the context of gene conversion (JOHNSON-SCHLITZ and ENGELS 1993)

By contrast, changes in PCR fragment patterns are frequent in the other three phenotypes. This applies particularly to the recombinant chromosomes, in which there is also a strong bias toward the incomplete rather than the complete *P[CaSpeR]* element. There is ample

evidence in these genotypes for a considerable amount of mobility of the complete element, in contrast to the relative lack of mobility associated with the chromosome containing the end-deficient element. This latter result therefore suggests that in genotypes such as in Figure 8 it is rare that the complete end of an end-deficient element associates with the complementary end of the normal element.

To confirm that the parental [+ -] and [- +] PCR patterns with ++ phenotypes (Table 5, row 4) were in fact due to repair against the end-deficient element and not due to novel deletion events, we amplified 19 of the individual progeny across the deleted region (++ phenotype gametes in Figure 9). These data show that in 14 of the 19 progeny the end-deficient element replaced the complete *P[CaSpeR]* element on the ++ chromosome. The remaining five cases may be due to incomplete gap repair (GLOOR *et al.* 1991) with loss of one or both primer sites.

Combinations of complete *P[CaSpeR](50C)* elements: For comparison with the end-deficient elements, we also carried out a PCR analysis of the fragments present from crosses involving two complete *P[CaSpeR](50C)* homologous elements (Table 6). The expected pattern of PCR products in this case, in the absence of any P-element structural changes, is [++], indicating that the element is present at the original site. A high proportion of gametes (41/114) show a change from this pattern. Recombinant gametes

TABLE 5
PCR results from the four classes of gametes produced by genotypes with end-deficient
and complete *P[CaSpeR](50C)* elements such as in Figure 8

	Parental		(left) (right)	New	
	AB CD	AB CD		AB CD	AB CD
	++	+ - - +		- + + -	- -
++	55	36		3	0
+ <i>bw</i>	10	55		6	8
<i>cn +</i>	13	40		8	6
<i>cn bw</i>	1	111		1	0

Again the A-D amplification product expected from the maternal *cn bw* chromosome was confirmed for all progeny. The end-deficient element is shown here on the ++ chromosome and the complete *P[CaSpeR]* element on the *cn bw* chromosome.

FIGURE 9.—PCR amplification across putative deleted regions of ++ progeny from males of the genotype shown in Figure 8. Oligonucleotide primers used were as follows. *DL1*, primer D from Figure 2 and 5' CTGCAAAGCTGTGACTGGAG 3', which were expected to produce a fragment of 1065 bp for *DL1* sequences and 5124 bp for complete *P[CaSpeR]* (50C) elements. *DL2*, primer D and 5' ATGAAAATAAGAGCTTGAGG 3', which were expected to produce a fragment of 952 bp for *DL2* sequences and 4433 bp for complete *P[CaSpeR]* (50C) elements. *DR1*, primer A from Figure 2 and 5' AGGCAGCAAACACCCATCTG 3', which were expected to produce a fragment of 750 bp for *DR1* sequences and 4294 bp for complete *P[CaSpeR]* (50C) elements. *DR2*, primer A and 5' AACATAAGGTGGTCCCGTCG 3', which were expected to produce a fragment of 315 bp for *DR2* sequences and 5085 bp for complete *P[CaSpeR]* (50C) elements. PCR conditions did not allow for amplification of complete *P[CaSpeR]* (50C) element fragments for any of the primer pairs shown here.

show a slightly, but not significantly, higher frequency of changes ($20/46 = 0.43$) compared with nonrecombinant gametes ($21/68 = 0.31$). These results are in contrast to those of SVED *et al.* (1991), who failed to find any changes in Southern hybridization patterns in six lines arising from recombinant chromosomes. The present results show that such changes must occur moderately often.

DISCUSSION

Approximate recombination frequencies from all classes of complete and end-deficient *P[CaSpeR]* (50C) elements are summarized in Figure 10. Where a left-end element is shown, classes A, C and E, the data also include results from equivalent right-end elements. The recombination frequencies are only order of magnitude estimates, particularly because they are variable between different end-deficient elements (*e.g.*, Table 2) and between individual males, due to age and clustering effects. The recombination frequencies given by genotypes A and C, with only end-deficient elements, are close to zero. It is not clear whether the recombination frequencies in these cases are higher than those given by *P[Δ2-3]* (99B) by itself (McCARRON *et al.* 1989, 1994).

As expected from the known behavior of *P* elements

(*e.g.*, MULLINS *et al.* 1989), both a left and a right end must be present to induce *P*-element activity. Classes A and C contain only one type of end and induce little, if any, activity, whereas all the other genotypes contain both ends and induce significant activity. The novel finding from this paper comes from class D, showing that the two ends need not be on the same element or even on the same chromosome to induce male recombination.

Figure 11 shows diagrammatically the situation expected in class D genotypes. The model is based on the cut-and-paste model in which the primary event that initiates *P*-element transposition is the excision of an

TABLE 6
PCR patterns given by complete *P[CaSpeR]*(50C) homologous elements

	AB CD + +	AB CD + -	AB CD - +	AB CD - -
<i>cn bw</i>	11	6	6	3
<i>cn +</i>	14	4	7	0
<i>+ bw</i>	12	3	4	2
<i>+ +</i>	36	1	4	1

The genotype of this cross was as described for complete *P[CaSpeR]*(50C) elements in Figures 1 and 2 of SVED *et al.* (1991).

Class	A	B	C	D	E	F
Genotype						
Percent Recombination	low ≤0.2%	1%	low ≤0.2%	20 - 30%	10 - 15%	20%

FIGURE 10.—Approximate recombination frequencies for various genotypes involving end-deficient and complete *P*[*CaSpeR*] (50C) elements and *P*[$\Delta 2-3$] (99B). [Results for class B, SVED *et al.* (1990); results for class F, SVED *et al.* (1991).]

element (ENGELS *et al.* 1990). The top left panel shows the two end-deficient elements at the four-strand stage. In the top right panel, the chromosomes contort to allow association of a left and right end. This association is envisaged as being similar to what happens when the two ends of a normal element associate. In the bottom right panel, double-strand breaks are produced, in the same manner as would normally result in excision of an element, possibly accompanied by digestion back of the flanking DNA (GLOOR *et al.* 1991). In the present case, however, no element is liberated. As seen in the bottom left panel, the result is that double-stranded breaks are produced on chromatids of both homologous chromosomes. It is envisaged that these breaks lead to recombination, in a manner as yet unspecified.

Competition for end association: Accepting that left and right ends from different elements can function

together, the next issue is to what extent this mode of association occurs when the possibility of association of ends of a single element also exists. The evidence from MULLINS *et al.* (1989) is relevant here. These authors constructed a *P* element with a left end and two right ends. They showed that the left end would preferentially pair with the proximal right end in preference to the distal one. However, when the proximal end was mutated beyond recognition, the distal end was used. MULLINS *et al.* suggested that, based on their data, pairing of *P*-element ends may occur in a processive manner, whereas the results from the end-deficient elements presented here suggest that this mode of pairing is not a necessary one.

Based on the results of MULLINS *et al.*, our expectation is that association of ends from a single element, followed by cut-and-paste (ENGELS *et al.* 1990), should be the usual event and that association of ends from different elements should only occur as a secondary possibility. Two aspects of the results from class E genotypes (Table 5) support this argument. (1) Chromosomes containing the end-deficient element are much more stable than chromosomes containing the complete *P*[*CaSpeR*] element. Only 2 of 113 progeny with the parental end-deficient phenotype (*cn bw* in Table 5) show any change in PCR pattern as compared with 39 of 94 for the parental complete *P*[*CaSpeR*] element phenotype (*++* in Table 5). (2) A large number of progeny (36) with the parental complete *P*[*CaSpeR*] element phenotype show the PCR pattern expected

FIGURE 11.—Model showing “excision” of left and right ends to produce chromosome breakage. Thick lines indicate *P* elements, with left-end elements being indicated by dashed lines and right-end elements by solid lines. Flanking chromosomal DNA is shown by thin solid lines. 5' to 3' and 3' to 5' strands of both sister chromatids of the two homologous chromosomes are shown.

from gap repair after excision of a complete element. As mentioned previously, 19 of these 36 were tested, of which 14 were confirmed gap repairs (Figure 9). Thus where a right end can pair with a left end either on the same element (in *cis*) or on the homologous element (in *trans*), the evidence indicates that the *cis* pairing is chosen in most cases.

Given the conclusion that the pairing of ends from different elements is a secondary event compared with the pairing of ends in a single element, the comparison of recombination frequencies is, initially, a surprising one. In particular, class D leads to more recombination than classes E and F in which complete *P*[*CaSpeR*] elements are present. However, class D is unique in that all element-induced breaks are predicted to involve both chromosomes. Thus the lower frequency with which breaks occur in class D, as opposed to class E and F genotypes, could be compensated by the higher frequency with which such breaks lead to recombination. These arguments may be carried further to explain why recombination in classes D, E and F is an order of magnitude higher than in class B, in which no *Pe*-element sequences are available on the homologous chromosome. Association of ends in *trans* is possible in each of classes D, E and F, leading to breaks on both chromosomes, whereas in class B the only element-induced break will be on one chromosome.

Finally, it should be noted that the events postulated in this paper are closely related to what has recently been shown to occur in the production of chromosome breaks by the *Ac*-*Ds* system in tobacco and maize (ENGLISH *et al.* 1993; WEIL and WESSLER 1993). These events were detected through the production of breakage-fusion-bridge cycles rather than recombination, but the postulated association of element ends on different chromatids is very similar to the sequence of events described in Figure 11.

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