P-Element-Induced Recombination in *Drosophila melanogaster*: Hybrid Element Insertion

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

ABSTRACT

It has previously been shown that the combination of two deleted *P* elements in *trans*, one containing the left functional end and the second element the right functional end, can lead to high levels of male recombination. This finding strongly suggests that *P*-element ends from different chromosomes can become associated, followed by "pseudo-excision." We show that two different processes are involved in resolving the pseudo-excision event: (1) the excised *P*-element ends continue to function as a single unit (Hybrid Element) and insert at a nearby site in the chromosome or into the element itself [Hybrid Element Insertion (HEI)] and (2) free ends that do not contain *P* elements repair and rejoin [(Hybrid Excision and Repair (HER)]. Both types of resolution can lead to recombination, and this paper concentrates on the HEI class. One type of HEI event predicts the exact reverse complementary duplication of an 8-bp target site, and we have confirmed the existence of such a structure in six independently derived recombinant chromosomes. There is also a high tendency for insertion events to occur within a few bases of the original 8-bp target site, including six apparent cases of insertion into the exact site.

HIRAIZUMI (1971) showed that male recombination in *Drosophila melanogaster,* which had previously been found only at very low levels, can occur at frequencies of up to 1% in crosses involving recently collected wild-type strains. This recombination has been shown to be associated with *P* elements (BINGHAM *et al.* 1982; MCCARRON *et al.* 1989; SVED *et al.* 1990).

Using a system in which a single *P*{CaSpeR} element (PIRROTTA 1988) is mobilized by a transposase source, *P*{Δ2-3}(99B) (ROBERTSON *et al.* 1988), SVED *et al.* (1990) showed that levels of ~0.5–1.0% recombination can be produced. SVED *et al.* (1991) found that the rate of recombination rises by an order of magnitude, to ~20%, if two *P*{CaSpeR} elements are present at homologous sites on the chromosome. SVOBODA *et al.* (1995) then showed that these two elements could function to produce even higher rates of recombination, 30% or more, if one had only a functional left end and the other only a functional right end.

The high rate of recombination produced by end-deleted elements can be understood in terms of the "cut-and-paste" model of ENGELS *et al.* (1990). Under this model, it is postulated that the normal method of *P*-element propagation consists of excision of an element at the four-strand stage of division, followed by repair using the sister *P* element and insertion of the excised element at a new site on the chromosome. Although the details of how *P* elements excise are not clear, it seems likely that this is achieved by an association of left and right ends. The result of SVOBODA *et al.* (1995) strongly suggests that this association need not involve a left and right end from the same element.

Association of opposite *P*-element ends at the four strand stage, followed by "excision," can be pictured as in Figure 1 (*cf.* SVOBODA *et al.* 1995, Figure 11). The diagram shows the two DNA strands of both sister chromatids of both chromosomes.

There are several possibilities for the resolution of the breaks shown in panel 3 of Figure 1. The first is that the two ends with *P* elements (e_1 and e_2) remain associated as a "hybrid element." This element could then insert elsewhere in the genome. This event is labeled as Hybrid Element Insertion (HEI), and evidence is presented in this paper for its regular occurrence. The second possibility is that there is template repair followed by rejoining of two of the four ends [Hybrid Excision and Repair (HER)]. In cases where the *P* element-containing ends retain their association, the only ends available for such repair are n_1 and n_2, and evidence is presented that these regularly rejoin. The possibility of rejoining events involving ends other than n_1 and n_2 is also discussed.

MATERIALS AND METHODS

Stocks and procedures used in this paper are as outlined in SVOBODA *et al.* (1995). Sequencing was carried out using the Circumvent Cycle Sequencing Kit (New England Biolabs).

All results in the paper are from crosses of males that contain deleted elements derived from an initial insertion of *P*{CaSpeR} in the 50C region of chromosome 2. Test males

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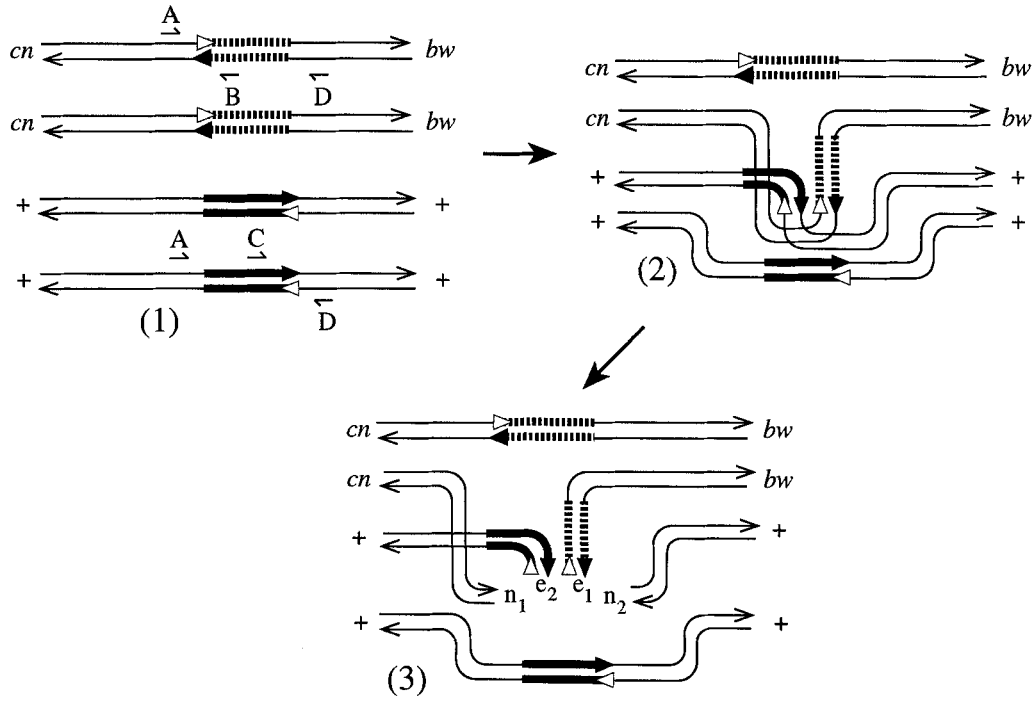

FIGURE 1.—Pseudo-excision of a hybrid element. The first panel shows the left- and right-end elements after DNA replication has occurred. Arrowheads represent DNA 3′ ends. The *P[Δ2-3]* transposase source is present but not shown. Primers A and D were used to detect the presence of ends are shown in this panel, for convenience only on the outer two chromatids. The second panel shows the association of one left and one right end to make a hybrid element. The third panel shows the situation after the hybrid element has "excised," with four broken ends numbered for later reference, ends labeled *e* containing elements and ends labeled *n* without elements. Originally ends n_1 and e_1 were joined and likewise ends e_2 and n_2.

are heterozygous for a left-end element, either *DL1* or *DL2*, and a right-end element, either *DR1* or *DR2*, together with distant flanking markers *cn* and *bw*, and contain the transposase source *P*{Δ2-3}(99B) (see SVOBODA *et al.* 1995, Figure 5a). Single males were crossed to *cn bw* females, and two males, where available, were selected from each of the four classes *cn bw*, *cn* +, + *bw* and + + for analysis by PCR. Primers external to the insertion site and facing inward (A and D) and primers facing outward at the element ends (B and C) are as described in SVOBODA *et al.* (1995). An additional primer was designed to screen for tandem duplication events (primer E, see Figure 6). The sequence of this primer (5′ GCATGTCCGTGGGGTTTGAAT 3′) is close to the right-hand end of the element, proximal to the right-end internal primer C. When used in conjunction with primer C, this is expected to detect a fragment only from tandem duplications of a right-end element.

MODELS FOR HYBRID ELEMENT EXCISION, INSERTION AND REPAIR

Because of the complexity of some of the models and their use in interpreting the data, it is convenient to present the models before presenting evidence for their validity. HEI can produce a great variety of outcomes, depending on the position and orientation of the insertion event. It is convenient to summarize the different types of outcomes in a single figure (Figure 2, after TANAKA 1994). This figure assumes, as in Figure 1, that the HEI events occur after DNA replication in mitotic cells before meiosis in the germ-line. The figure shows the expected chromosome patterns in metaphase. Normal disjunction of sister centromeres is expected to occur at anaphase, leading to diploid products.

Insertion: Eight possible insertion regions are distinguished, depending on which of the four chromatids contains the target site and whether the new insertion is proximal or distal to the original insertion. Figure 2 does not consider the intermediate case of insertion directly into one or another element, although this possibility will be considered later.

Each insertion site has two possible outcomes, depending on the orientation of the insertion. Alternative orientations at site 1, for example, are labeled as 1 or 1^- depending on whether the orientation of left and right ends is in the same or opposite direction to the original insertion. With normal element insertion, such differences in orientation do not lead to structural differences. However with hybrid elements, the consequences of each type of insertion need to be followed in detail. This has been done in Figure 2, which shows the expected chromosome types and the genotypes of flanking markers.

All events shown in Figure 2 show the repair and rejoining of strands that do not contain an element end (ends n_1 and n_2). The rejoining of these strands is pictured with a dotted line, and no attempt has been made here to consider the details of this event, including the likely possibility that one or other element will

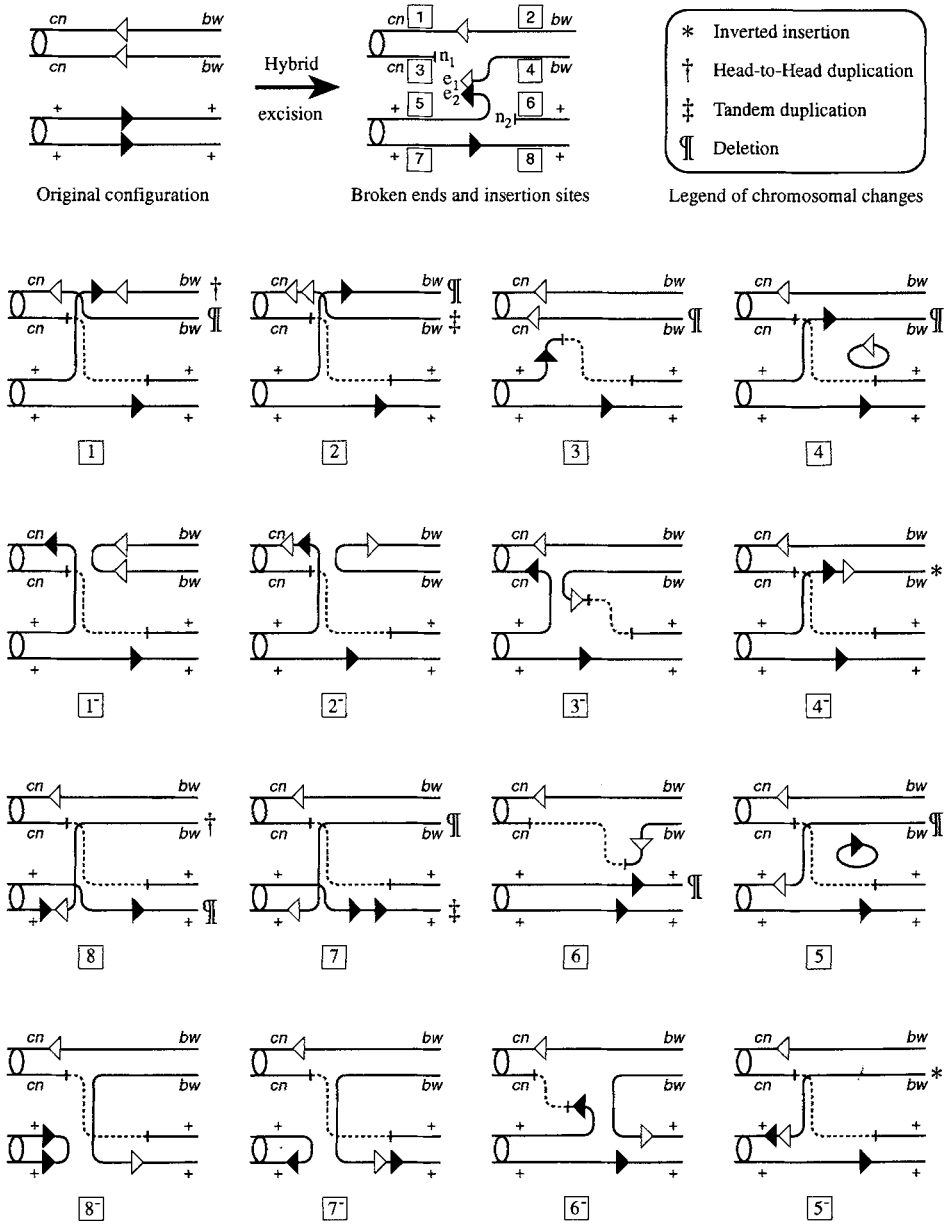

FIGURE 2.—A classification of possible HEI events. For simplification, chromatids are pictured as single rather than double as in Figure 1. The striped left elements of Figure 1 are replaced by ◁ in Figure 2, and black right elements are replaced by ▶. The top two panels of Figure 2 correspond to panels 1 and 3 of Figure 1, with equivalent numbering of broken ends. Eight possible integration sites are shown in the second panel, and two possible outcomes from each depending on the orientation (see text) are shown in the remainder of the figure. Because of the symmetry of the model, events 1 and 8, 2 and 7, 3 and 6, 4 and 5 share many properties, and events in the last two rows of the figure are shown in reverse order to reveal this symmetry. Normal monocentric chromosomes with structural changes are indicated using the symbols *, †, ‡ and ¶. These altered chromosomes are considered in detail in the text. ---, the expected repair of chromatid breaks where *P*-element ends are not present (n_1 and n_2). The possibility of copying of elements on to this newly synthesized DNA is not shown.

be copied onto the newly synthesized strands. Such possibilities are discussed at the end of this section.

Examination of Figure 2 leads to several predictions. First, several structures containing both left and right elements can be produced, classes 1 and 8 and classes 4^- and 5^-. These dual element structures are all expected to occur in recombinants of genotype + *bw*. Tandem repeats of both original elements are also found, in gametes of the respective parental genotypes (classes 2 and 7). Note that because it is always the complete end of an element that inserts, none of these element pairs can be of a "tail to tail" structure like that of a normal *P* element.

The model is asymmetrical in its predictions for the recombinant genotypes in the progeny. With the genotypes as shown in Figure 2, all recombinants produced by HEI are of genotype + *bw* rather than *cn* +. This is counterbalanced by the fact that all HER recombinants

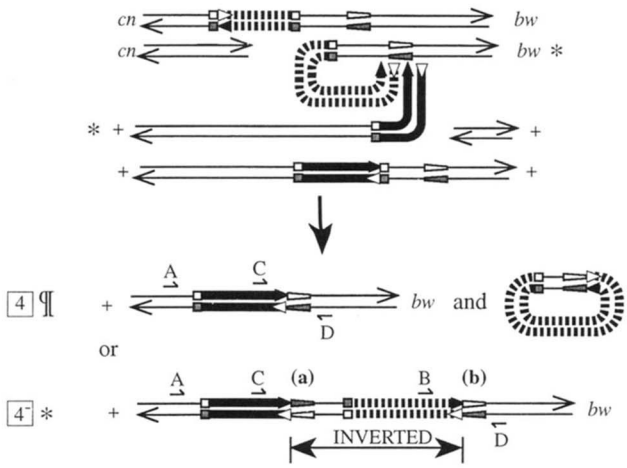

FIGURE 3.—Insertion into a chromatid that is involved in the hybrid element. Depending on the orientation of the insertion, this produces either a deletion and a small ring (class 4) or an inverted insertion (class 4^-). The original target site is indicated by a rectangle, and a new 8-bp target site is shown using a trapezoidal symbol that allows the polarity to be seen. Two copies of the target site, (a) and (b), are present in class 4^-.

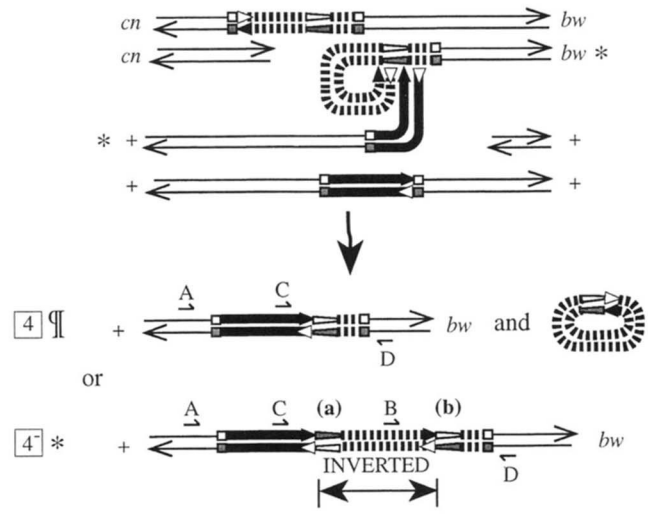

FIGURE 4.—Insertion of the hybrid element into one of its constituent elements. Symbols are as in Figure 3.

produced by repair of broken strands are expected to be of genotype *cn* +.

Inverted insertions: Referring to the duplicated elements of classes 4^- and 5^-, an important prediction can be made. This prediction may be seen by considering a more detailed double-stranded representation (Figure 3) of the events involved in class 4. Only two of the four chromatids are involved in the insertion event in this case, the ends of which are marked by asterisks. This type of insertion is unusual in giving rise to monocentric gametes regardless of the direction of insertion. If insertion occurs in the same orientation as the original insertion, corresponding to class 4, a gamete is produced containing a deletion, plus a small circular element that would presumably be lost.

Insertion in the opposite orientation, 4^-, leads to a gamete with precisely a single copy of each of the two elements, and of their flanking regions. It contains also a duplication of the 8-bp target site. The left-end element and its right flanking region are inverted and placed to the right of the right-end element. As a result, the duplicated copies of the target site, marked (a) and (b) in Figures 3 and 4, are present in complementary reverse polarity relative to each other.

The target site in Figure 3 is shown to the right of the element. An equivalent result is produced if the target site lies within the element (Figure 4). It can be seen that the inverted insertion class contains the same material as the equivalent gamete from Figure 3, except for the bases at the 8-bp target site. The inverted region in this case contains only material from the *P* element, with the 8-bp target sequence present in inverted form as previously.

The structures in Figures 3 and 4 lead to predictions of the expected PCR fragment patterns, using the four primers diagrammed in the figures. Provided that the insertion event occurs inside the right primer D of Figure 1, as is the case in both Figures 3 and 4, a novel fragment is expected from primers B and D, which are normally not expected to produce a PCR product (Figure 1). Furthermore, it is predicted that this occurrence of the B-D fragment will be accompanied by an increase in the size of the C-D fragment. The C-D primer pair initially gives a fragment from the right end of the element, and the increase in fragment size is predicted to equal the size of the left element plus the 8-bp target sequence, regardless of whether insertion is into the element or to the right of it. The C-D and B-D fragments provide fortuitous sequencing primer sites to confirm the expected structure of the 8-bp target sequence.

Class 5^- produces a product that is very similar to that of class 4^- (Figure 2). The orientation of the two elements is reversed in this case, however, with the right-end element inverted and inserted at the incomplete end of the left-end element. The flanking marker genotypes are the same in the class 4^- and class 5^- inverted insertion genotypes.

Head-to-head duplications: Figure 5 shows the head-to-head structure, plus an associated duplication, in which the insertion event is at the complete end of an element, on a chromatid not involved in the original association. Figure 5 shows the structure for class 8, while class 1 produces a similar outcome. There are several differences between this class and the one shown in Figure 3. First, as with most HEI events, only one insertion orientation produces monocentric chromosomes. Second, two different products are formed in this case, one of which involves crossing over between homologous chromatids and one sister-strand recombination. Last, although there is again a duplication of the 8-bp target sequence, in this case the two copies

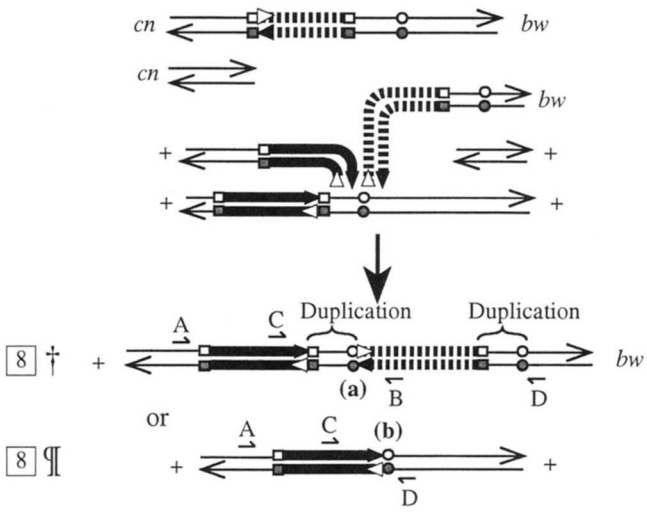

FIGURE 5.—Reciprocal products produced by insertion into region 8 of Figure 2. The insertion site is indicated by a small circle, which is proximal to primer D. The result is either a combination of left and right elements with a duplication or a deleted region. Figure 5 differs from Figures 3 and 4 in showing the products of only one of the two possible insertion orientations. The opposite orientation, 8^{-}, leads to inviable dicentric and acentric products. The two products of Figure 5 are due to the fact that, unlike Figures 3 and 4, the insertion event is into a third chromatid, not involved in the formation of the hybrid element.

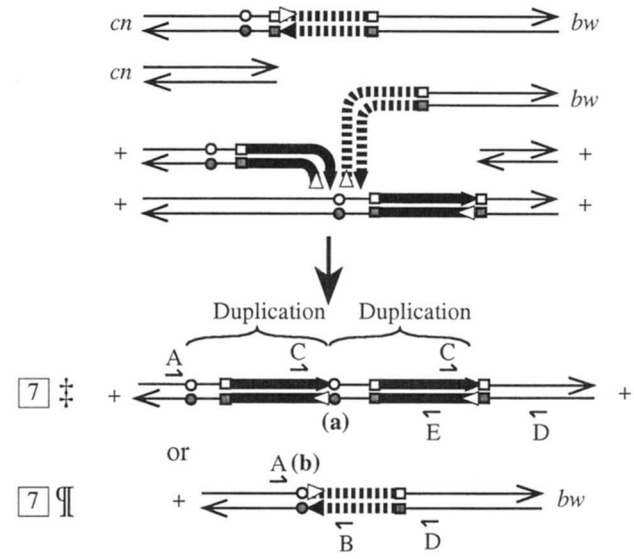

FIGURE 6.—Reciprocal products, including a tandem duplication, produced by insertion into site 7 of Figure 2. The position of primer E, used with primer C to detect tandem duplications, is shown.

of the duplicated sequence are in chromatids that will ultimately go into different gametes. The 8-bp target region is, in fact, duplicated in one of the resultant chromosomes, but as part of a larger duplication. This sequence, except for the 8-bp target site, is deleted from the reciprocal recombinant product.

Tandem duplications: True tandem duplications are produced (classes 2 and 7) when insertion occurs at the deficient end of an element on a chromatid not involved in the hybrid element association. The recombination event in these cases involves sister chromatids, so that the tandem duplication occurs on a parental rather than a recombinant gamete (Figure 6).

Deletions: The last class of structurally altered chromosomes contains those that involve deletions. Each of the rearrangements considered thus far is accompanied by a reciprocal deletion class. In two additional classes, 3 and 6, the only viable rearrangement due to the insertion event is a deletion. A corresponding duplication event may arise from repair of the broken chromosomes.

Under the HEI model, deletions are necessarily at the complete end of the element. Chromosomes containing deletions are a mixture of recombinant and parental genotypes, such that in no case is it possible to infer the exact class of insertion event from a knowledge of the deletion plus the flanking marker genotype. However, insertions into equivalent sister chromatid regions produce identical deletions, such that classes 1 and 3 (*cn bw*), 2 and 4 (+ *bw*), 5 and 7 (+ *bw*), 6 and 8 (+ +) lead to identical deletion phenotypes.

Repair: The simplest model consistent with current data for Hybrid Excision and Repair (HER) is based on the Synthesis Dependent Strand Annealing (SDSA) model (see NASSIF *et al.* 1994). The situation is closely analogous to the repair following normal *P*-element excision, where excision leaves two free chromosome ends that are on the same chromosome. In the present case, HEI, which does not involve true excision, leaves two free ends that are on homologous chromosomes.

Figure 7 shows the expected outcome if one of the free ends initiates synthesis by copying its sister chromatid. After synthesis has occurred past the end of the element, annealing of complementary strands occurs, followed by synthesis at the 3′ end of each of the annealed strands and ligation. Other models are possible, *e.g.*, if both free ends synthesize past their respective element ends, followed by annealing, synthesis, and mismatch-repair in the region of the element. An alternative possibility is that synthesis could occur on a nonsister template.

RESULTS

Large-scale screen: Males containing opposite end-deleted elements and heterozygous for the flanking markers *cn* and *bw* were crossed to females homozygous for *cn* and *bw*. Table 1 summarizes the results from PCR screening of the progeny of such crosses for the presence of the left- and right-end fragments indicative of the two parental elements. All progeny screened were heterozygous for *P* element-containing chromosomes and for a chromosome with an empty insertion site. Absence of a particular parental fragment means either absence of a fragment or a change in size. It could indicate either loss of the end or, as predicted from the

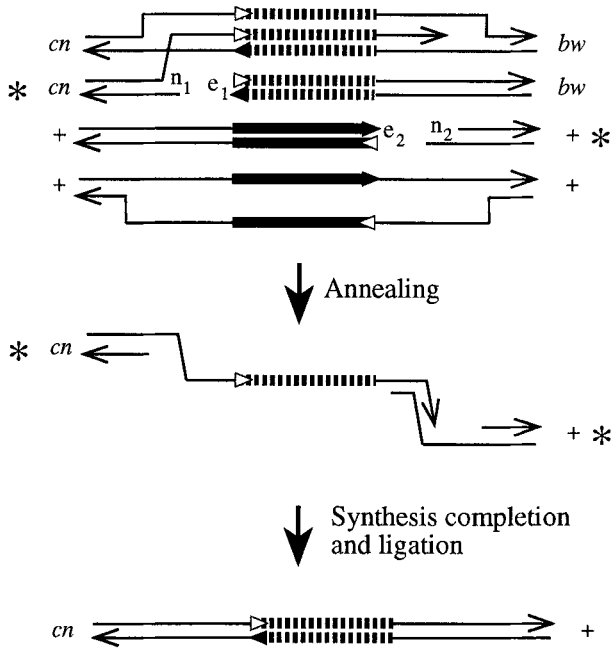

FIGURE 7.—Synthesis according to the SDSA model to repair a double-stranded break for chromatids without *P*-element ends. Numbering of the broken chromatids is as in Figure 1.

HEI model, movement of the element end to a different chromosomal location. Note that the total numbers for the four classes + +, + *bw*, *cn* + and *cn bw* do not reflect the observed numbers in these classes, since only a fixed number from each class, one or two per progeny group, was saved for analysis by PCR.

The most striking feature of the results, in agreement with predictions from the HEI model, is the high frequency of recombinants of + *bw* genotype that have neither parental fragment. More than one-third of such genotypes (132/320) fall into this category. The remaining three genotypes show much lower frequencies of parental fragment loss.

Numerous novel fragments were produced in the screen. Each fly producing a novel fragment was rescreened using all six possible single primer pairs, with the results shown in Table 2. The first three columns show results from primer pairs in which a band is normally expected, but where the band is of altered size. The last three columns show bands where none is normally expected. All occurrences of bands in the last three columns are from independent male parents.

Table 2 shows that novel bands were largely associated with the + *bw* recombinant class, reinforcing the conclusions from Table 1. It should be noted, however, that results in the first two columns are, in the main, a subset of the results in Table 1, representing cases where the original band was replaced by another of different size.

The remainder of this section involves further analysis of the results of Table 2 in terms of the expectations from the HEI model.

Inverted insertions: Six individuals, all from independent crosses, were found with PCR fragments in agreement with the predictions of the model (Figures 3 and 4). These had a novel band from the B, D or A, C primer pairs, accompanied, respectively, by an increase in the size of the C, D or A, B band that was approximately equal to the size of an inserted element. The final two columns of Table 2 include these six cases, as well as one additional case that is considered later (Figure 8) in which the increase in the size of the C, D fragment was not sufficient to include the insertion of the end-deficient element involved. Of the six cases, four were from the expected recombinant class, one from the opposite recombinant class and one from a parental chromosome.

We sequenced the novel band as well as the increased C, D and A, B fragment in all six cases to establish the structure in the region of the element end (Table 3). All confirmed the duplication of the 8-bp target sequence. The first five cases shown in Table 3 involve insertion into the sequence of the element itself (Figure 4), and the remaining case involves insertion into the region flanking the element (Figure 3). We confirmed the expectations from Figures 3 and 4 that the two copies of the target site should be present not as direct repeats, as normally expected from *P*-element insertion (O'HARE and RUBIN 1983), but as reverse complements of each other.

Head-to-head duplication: Classes 1 and 8 of Figure 2 are expected to give rise to this type of rearrangement. As shown in Figure 5, the class is detected by the presence of a B, C PCR fragment. As shown in Table 2, there were altogether 17 cases of this type, of which 14 were of the expected + *bw* genotype. Lengths of the PCR fragment were variable, as expected from the model.

Sequencing of 14 of the B, C fragments was carried out. In all cases the structures were precisely as predicted from Figure 5, with no addition of any extraneous bases. The range of sizes of the regions between the two elements is shown in Table 4. In some cases the values are negative, indicating that an insertion occurred directly into an element, leading to one element being truncated at its previously functional end. These cases are, strictly speaking, not head-to-head structures and do not involve any duplication of the surrounding DNA.

The results show that there are six cases with exactly 8 bp between the two ends. Under the model of Figure 2, these must represent cases where the hybrid element has inserted exactly into the original 8-bp target site, now adjacent to the element. This 8 bp is clearly a "hot spot" of insertion.

Occurrence of tandem duplications: We attempted to answer the question of whether tandem duplications occur as frequently as head-to-head duplications. This would be expected if insertion occurs equally frequently at the incomplete end (classes 2 and 7 of Figure 2) as

TABLE 1

Classification by PCR for the presence of unaltered left- and right- element parental fragments

Parent type	Progeny genotype	PCR fragment present				Total
		Both	Left	Right	Neither	
DL1/DR1	+ +	2	9	103	12	126
	+ *bw*	1	44	27	60	132
	cn +	3	46	51	11	111
	cn bw	0	116	4	5	125
DL1/DR2	+ +	0	6	44	4	54
	+ *bw*	0	18	13	21	52
	cn +	0	13	30	3	46
	cn bw	0	39	8	3	50
DL2/DR1	+ +	0	1	87	8	96
	+ *bw*	2	24	34	29	89
	cn +	1	25	54	7	87
	cn bw	0	88	6	2	96
DL2/DR2	+ +	0	1	43	5	49
	+ *bw*	0	9	16	22	47
	cn +	0	7	36	6	49
	cn bw	0	39	3	7	49
Combined	+ +	2	17	277	29	325
	+ *bw*	3	95	90	132	320
	cn +	4	91	171	27	293
	cn bw	0	282	21	17	320

Parent types such as DL1/DR1 indicate the combination of the left-end element DL1 and the right-end element DR1. The last four lines show the totals from all parent types. The tables include results from genotypes in which the left-end element was introduced on the ++ chromosome rather than the *cn bw* chromosome as shown in Figures 1 and 2. For these crosses, progeny numbers have been included with the complementary genotypes, *i.e.*, ++ and *cn bw* symbols have been reversed, and similarly + *bw* and *cn* +.

at the complete end of an element (classes 1 and 8). Tandem structures would not be detected by the primers used in the PCR screen detailed above. We therefore estimated the frequency of tandem duplications in a second experiment. This experiment used the shorter right-end element DR2, which is 223 bp in length, and a new primer E (MATERIALS AND METHODS) that would give a fragment between two tandem DR2 elements (Figure 6).

Under the model, tandem duplications should be produced only in the parental chromosome containing the original element (Figure 2). This may lead to a bias in the comparison with head-to-head structures, since chromosomes that have been involved in an insertion event and that therefore potentially contain a tandem duplication are phenotypically indistinguishable from chromosomes that have not been involved in any event (see DISCUSSION).

All four classes were screened (Table 5). One duplication was found. The element involved in this case, DR2, was present originally on the + + chromosome. As expected, the progeny contained this genotype. The structure of the duplication was confirmed by sequencing.

Deletions: Many cases were found in which the pa-

TABLE 2

Number of occurrences of novel bands associated with particular primer pairs

Progeny genotype	Primer pair					
	A, B	C, D	A, D	B, C	A, C	B, D
+ +	2	5	5	2	0	1
+ *bw*	18	28	18	14	1	4
cn +	2	4	7	0	0	1
cn bw	2	1	2	1	0	0

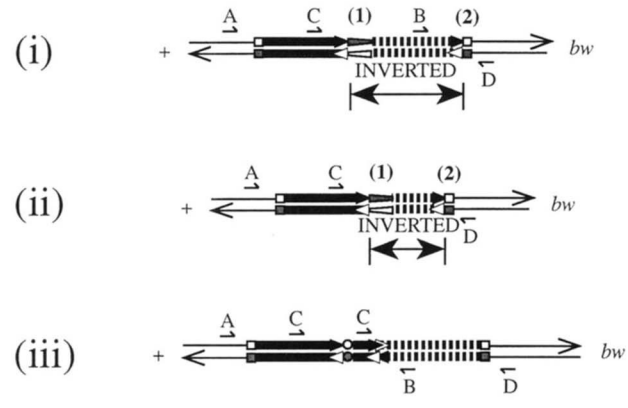

FIGURE 8.—Three cases that cannot be explained by a single HEI event.

TABLE 3

Structure of six apparent inverted insertion events

Parental genotype	Target sequence (a)	Target sequence (b)	Insertion site	Progeny genotype
+ DR1 +/*cn* DL2 *bw*	5′GTCGTTAG′3 3′CAGCAATC′5	5′CTAACGAC′3 3′GATTGCTG′5	942–949 bp inside DL2 (949 bp inverted)	+ // DR1 → DL2 → // *bw*
+ DR1 +/*cn* DL2 *bw*	5′CACTTAAC′3 3′GTGAATTG′5	5′GTTAAGTG′3 3′CAATTCAC′5	1526–1533 bp inside DL2 (1533 bp inverted)	+ // DR1 → DL2 → // *bw*
+ DR1 +/*cn* DL2 *bw*	5′AGTTGCAC′3 3′TCAACGTG′5	5′GTGCAACT′3 3′CACGTTGA′5	590–597 bp inside DR1 (597 bp inverted)	+ // ← DR1 ← DL2 // *bw*
+ DL2 +/*cn* DR1 *bw*	5′CATTCACC′3 3′GTAAGTGG′5	5′GGTGAATG′3 3′CCACTTAC′5	976–983 bp inside DL2 (983 bp inverted)	+/?/ DR1 → DL2 → /?/ *bw* (expect *cn* +)
+ DL2 +/*cn* DR1 *bw*	5′GGCCCGAC′3 3′CCGGGCTG′5	5′GTCGGGCC′3 3′CAGCCCGG′5	1362–1369 bp inside DL2 (1369 bp inverted)	*cn* // DR1 → DL2 → /?/ *bw* (expect *cn* +)
+ DR1 +/*cn* DL1 *bw*	5′GTGGTGGT′3 3′CACCACCA′5	5′ACCACCAC′3 3′TGGTGGTG′5	135–142 bp to the right of DL1 (1052 bp inverted)	+ // DR1 → DL1 → // *bw*

Two left-end elements (DL1, DL2) and one right-end element (DR1) were used. Target sequences correspond to (a) and (b) in Figures 3 and 4. Internal insertion sites (first five events) are described relative to the complete end of the inverted element. Progeny genotypes show the inferred mode of insertion using arrows, include genotypes of flanking markers and ? where genotypes are not in agreement with predictions from a single HEI event.

rental fragments were reduced in size, indicating a deletion event. Four of these were sequenced. In each case they showed, as predicted from the model, that the deletions occurred precisely at the end of the element.

Complex events: The main aim of our study was to demonstrate the existence of products predicted from the HEI model. Not all products could, however, be explained by a single HEI event. This finding does not invalidate the model. For example, there are at least five cases where a second HEI or repair event can be invoked to explain the finding. In two of the cases analyzed in Table 3, one or both outside markers are not as expected. Either of these cases could be explained if a second, later, excision event occurred, followed by copying of the inverted insertion structure into a new chromosome type.

The remaining three complex cases that could be explained by a second event are diagrammed in Figure 8. The first two share a common structure, with different 8-bp target sites at either end, labeled (1) and (2), the second of which is the original target site. The structures are similar to those of Figure 4 but require a second HEI event of the left-end element into the exact target site. The results from the head-to-head duplication classes (Table 4) indicate that such an event is not implausible, although the probability of two identical such events would seem small. Any alternative model would, however, need to explain the fact that the element ends have been "swapped" around, exactly joining a left element end with the chromosome region to the right of the original element and preserving the 8-bp target site.

TABLE 4

Distances between the ultimate *P*-element bases in head-to-head duplications

Base pairs between elements	No. of occurrences
232	1
21	1
13	1
8	6
0	1
−1	1
−9	1
−14	1
−81	1

DISCUSSION

Confirmation of the HEI model: The HEI model predicts that one class of recombinants, with phenotype + *bw*, will have an elevated frequency of structural changes. Our results confirm this expectation. Furthermore, demonstration of the 8-bp duplication in the "inverted insertion" class of recombinant chromosomes shows unequivocally that a *P*-element insertion event is involved in the production of recombinant chromosomes. Other models for the induction of recombination, typically involving chromosome breakage followed by repair, would not

TABLE 5

Number of occurrences of tandem duplications

Progeny genotype	Duplications	Total screened
+ +	1	142
+ *bw*	0	127
cn +	0	127
cn bw	0	140

predict such a precise duplication. Since the overall structure of the inverted insertion chromosomes agrees in all details with that expected under the HEI model, we conclude that the insertion event must come following an association of elements on different chromosomes. The fact that complementary element ends are present only in different elements on different chromosomes in this system would appear to rule out any other type of end association.

The 8-bp duplications are present as reverse complements of each other, rather than as direct repeats as normally found at element ends. This is exactly as expected in the model, in which the maintenance of 5′ to 3′ polarity in the insertion event ensures that the bases, as well as being in reverse order due to the insertion, are also complementary to each other.

HER events: Confirmation of the HER (hybrid excision and repair) class of events is more difficult than for HEI events. An HER event, unlike an HEI event, is not expected to leave a detectable rearrangement. However there is strong circumstantial evidence that the HER class of event occurs at high frequency. Only one class of recombinant chromosomes is produced by HEI events, the + *bw* chromosome in the case of a starting genotype as in Figure 2. The reciprocal *cn* + chromosome can, under the model outlined in Figure 2, only be produced by an HER event. The fact that there is no overall deficiency of the *cn* + class, and in fact even a small excess (M. TANAKA, X. LIANG, Y. GRAY and J. SVED, unpublished results) argues that repair occurs regularly. Such repair could be identical to the repair that is known to occur for normal *P*-element excision (JOHNSON-SCHLITZ and ENGELS 1993; NASSIF *et al.* 1994).

The prediction from Figure 2 is that all chromosomes of + *bw* genotype will inherit some chromosomal change. However a majority of the chromosomes of genotype + *bw* involve no detectable chromosomal change using the screen for element ends (185/320; Table 1). There are several possibilities for explaining such results by HEI, which we cannot exclude at present. Some insertions may be exactly into the original 8-bp target site, or produce deletions or duplications too small to be detected by PCR. Second, head-to-head insertions may occur in which the distance between the elements' ends is too great to permit PCR amplification, leading to a normal PCR pattern. Finally, some recombination events may occur in regions other than the 50C insertion site (PRESTON and ENGELS 1996). As an alternative to such explanations, however, it is possible that HER may be invoked to explain the rejoining of strands containing element ends. This would imply that the association of element ends is lost before insertion can occur.

It is difficult to deduce whether or not HER events occur for the other combination of chromosome ends, one involving an element and one not. It can be seen from Figure 1 that one combination, n_1 with e_1 and n_2 with e_2, simply restores the original configuration. If repair occurs against the sister chromatid, no detectable changes are expected. The second combination, n_1 with e_2 and n_2 with e_1, leads to dicentric and acentric chromosomes that are not expected to lead to viable products.

One aspect of the results that throws some light on the HER process is the constitution of element ends in the + *cn* recombinants. Looking at the results from Table 1, it can be seen that more of such offspring possess the right-end element compared to the left-end element. The disparity between these two classes is particularly marked in the cases involving the DR2 element. This element is only 223 bases long, as compared to 910, 1577 and 1482 for DL1, DL2 and DR1 (SVOBODA *et al.* 1995). The DL2/DR2 combination, which involves the greatest mismatch of sizes, results in the largest deviation from a 1:1 ratio, *i.e.*, 7:36. Under the model, this result can be explained if both free 3′ ends initiate DNA synthesis, and synthesis beyond the short element is completed before synthesis beyond the long element, leading to preferential reannealing of the chromatid involving the shorter element.

Tandem duplications: The low frequency with which we were able to detect tandem duplications, compared to the head-to-head duplications, deserves some comment. The two events that lead to these two structures involve insertion at the incomplete end of an element, and at the complete end, respectively. There is even the possibility of insertion at the same 8-bp target site adjacent to the incomplete end, an event that appears to have occurred several times at the complete end (Table 4).

While we only detected a single tandem duplication, the deficiency of this class may still be open to question. We found one event out of 142 + + chromosomes analyzed. By contrast, we found 14 head-to-head structures out of 150 + *bw* chromosomes analyzed. This deficiency of tandem duplications is significant at the 0.1% level by Fisher's exact test. However, as mentioned previously, there is a bias in the expected detection frequency, since + *bw* chromosomes have necessarily been involved in some recombination-causing event, an HEI event under the model, whereas + + chromosomes are a mixture of chromosomes that may or may not have been involved in an event. An unbiased test would use unselected progeny rather than selecting fixed numbers of parental and recombinant progeny. However a calculation based on the outcomes of Figure 2 (M. TANAKA, X. LIANG, Y. GRAY and J. SVED, unpublished results) shows that the observed recombination levels of ~30% (SVOBODA *et al.* 1995) are consistent with the notion that slightly more than one-half of the + + chromosomes have come from an HEI event. Thus the figure of 142 should be reduced to around half this value to validate the comparison between the head-to-head and tandem duplications. A calculation based on

this value still gives an excess of head-to-head events that is significant at the 5% level based on Fisher's test.

Insertion of *P* elements into other *P* elements, or in their vicinity, has been reported on many occasions (*e.g.*, EGGLESTON 1990; O'HARE *et al.* 1992; TOWER *et al.* 1993; ZHANG and SPRADLING 1993; GOLIC 1994). One possibility suggested by this finding is that there is a direct interaction between the end(s) of the incoming element and the resident element. Such events normally involve *P* elements with both ends present. Numerous occurrences of insertion close to the complete element end have been found in the present study (Table 4). Since the hybrid element remains covalently bonded to its constituent chromosomes, the fact that insertion occurs nearby is perhaps not surprising. However any deficiency of the class of insertions close to the incomplete end would argue for the existence of a direct interaction of element ends.

Other elements: The last point that we wish to consider concerns the applicability of the HEI model to normal *P* elements and to transposons other than the *P* element. Although the results of the present paper are derived using a system with two end-deleted elements, results from the accompanying paper (PRESTON *et al.* 1996) indicate that the HEI model is equally applicable to normal *P* elements.

Models related to ours have been put forward to explain the occurrence of chromosome rearrangements, most specifically for the Tn10/IS10 element in *Escherichia coli* (ROBERTS *et al.* 1991), the Ac/Ds element in tobacco and maize (ENGLISH *et al.* 1993; WEIL and WESSLER 1993), and the Tam3 element in Antirrhinum (LISTER *et al.* 1993). The present study, using the *P*-element system, adds two critical lines of evidence for the HEI model. First, the two ends involved in the association in our case could only come from different elements, thereby eliminating other possible associations. Second, the demonstration of the 8-bp target duplication shows unequivocally that a typical transposon insertion event is involved in the production of the chromosomal rearrangements. We believe that the HEI model may be valid in a wide variety of transposable element systems.

We are grateful to XIUMEI LIANG for assistance with the fly counting, and to BILL ENGELS and CHRISTINE PRESTON for comments on the manuscript. The work was supported by Grant A09530096 from the Australian Research Council.

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