Heredity (2009) 102, 342–348

www.nature.com/hdy

ORIGINAL ARTICLE

Properties of re-arranged P elements in *Drosophila melanogaster*

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P elements, both complete and incomplete, contain a left and right end, normally depicted as pointing away from each other. Here, we examine the properties of P elements that may be described as 're-arranged elements' or 'inside-out elements', containing inverted ends. Two such structures exist, having either ends pointing towards each other, 'head-to-head or H–H', or ends pointing in the same direction 'head-to-tail or H–T'. We show that both structures are unstable in the presence of P element transposase. For the H–H element there is a high frequency of deletion of the intervening material and almost exact rejoining of element ends with the 4 bp CATG palindromic end sequence shared by the two element ends. This result is predicted by the Beall and Rio model of P element excision. For the H–T element there is a high frequency of exact excision of the entire inverted right-end, a result again predicted by the Beall and Rio model. Both structures lead to recombination in the way expected from a normal element. The rates of recombination are, however, much lower than might be expected from the organization of ends, a result that can be explained in terms of the low likelihood of insertion into a chromosomal region lacking another P element end. We also investigate the properties of combinations of re-arranged and normal elements, and show that there is a directionality property when left and right ends are combined in *trans* that can be explained in terms of strand repair.
Heredity (2009) **102,** 342–348; doi:10.1038/hdy.2009.1; published online 28 January 2009

Keywords: *Drosophila melanogaster*; *P* elements; recombination; hybrid elements; re-arranged elements

Introduction

The complete P element is a structure of 2907 bp containing 31 bp complementary ends pointing in opposite directions (O'Hare and Rubin, 1983). Most of the intervening DNA codes for a single transposase protein. Although the ends are identical for 31 bp, Mullins *et al*. (1989) showed that the adjacent 100 or so bases are different for the two ends. They also showed that elements must contain both a full left end and a full right end to transpose normally.

Svoboda *et al*. (1995) described the production of integrated P elements that carried only one functional end, either a complete left end or a complete right end. These elements were produced by internal deletion from a P[CaSpeR] element, an incomplete P element containing complete left and right ends, but where much of the intervening DNA has been replaced by a white minigene (Qian and Pirrotta, 1995). Transposase protein was provided by the P[Δ2–3 (99B)] transposon (Robertson *et al*., 1988). Various deletions were induced in one instance of a P[CaSpeR] element integrated at a site in 50C on chromosome 2R. These deletions were isolated and maintained in lines in homozygous condition in the absence of transposase protein. Among this pool of deleted elements, some were found to lack functional parts of either the left or right end. The process by which these single-ended structures were produced is indicated in the top line of Figure 1.

Most of the end-deleted elements were found to have deletions extending into the 31 bp end region, but leaving the final 15 or 16 bases. The determination by Beall and Rio (1997) of the action of P element transposase provides a simple explanation for the preponderance of this type of deletion (Liang and Sved, 2009).

As expected from the results of Mullins *et al*. (1989), neither left-end nor right-end elements led to any mobility in the absence of the other. However, the combination of a left-end and a right-end element on homologous chromosomes, in the presence of a transposase source, led to levels of male recombination of the order of 30% or more; whereas recombination is normally absent in male *Drosophila*. This outcome was attributed to the formation of 'hybrid elements' containing the left end from one chromosome associated with the right end from the homologous chromosome. True excision of an element is not possible in this case. However, assuming that the same processes that govern excision of normal elements apply, the attempt at excision is expected to lead to structures such as those shown in Figure 1.

Under the cut-copy-paste model of P element mobility (Engels *et al*., 1990), excision is expected to be associated with integration into the chromosome, possibly at a nearby site. This combination of excision and integration of hybrid elements was confirmed by Gray *et al*. (1996).

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Received 5 March 2008; accepted 28 December 2008; published online 28 January 2009

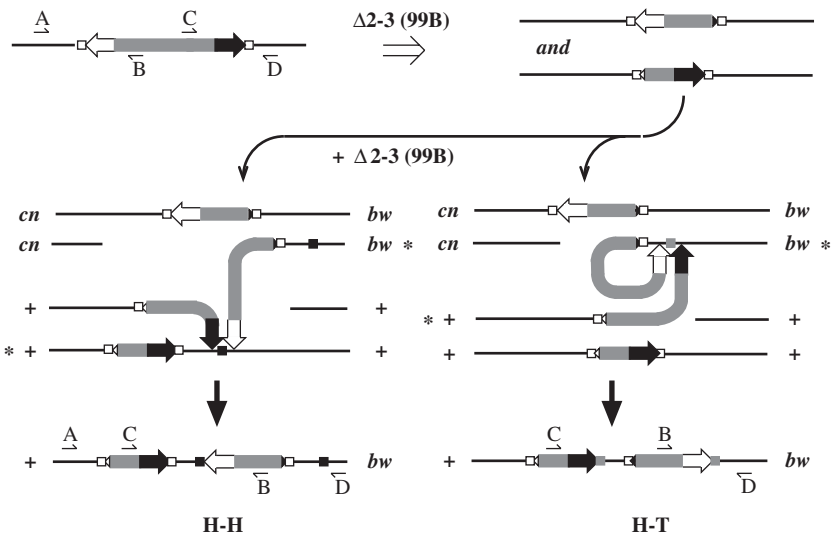

Figure 1 The production of head-to-head and head-to-tail 'elements'. The top line shows a P[CaSpeR](50C) element with primer sites, and the production of end-deleted elements (Svoboda *et al*., 1995). The open squares indicate a duplicated 8 bp target site, The following lines show ends from homologous elements combining to produce a hybrid element, followed by two different modes of integration (Gray *et al*., 1996), to produce re-arranged elements that are the subject of the present paper. In each case, asterisks mark the chromosome end leading to the progeny type of interest.

Among many normal and deleted recombinant types, two classes of progeny were found that are of interest for the present study, H–H and H–T in Figure 1. In each case these progeny contained both a left-end and a right-end element integrated nearby on the same chromosome. The left- and right-hand side show two different ways in which the hybrid element can be integrated back into one of the chromatids: in one case this leads to the H–H arrangement, in the other to the H–T. The progeny of interest are created in the case when the chromatid ends marked with asterisks become joined.

The H–H element can be described as 'head-to-head', since the head of the original right-end element is inserted near the head of the original left-end element. Elements of the H–T type may similarly be described as 'head-to-tail'. By contrast, the original P element is a 'tail-to-tail' element. In H–H elements, the region bounded by 8 bp repeats (shown as the region between the white and black squares in Figure 1) is duplicated around the element (white arrow and associated grey region) lying on the right of the final H–H arrangement. The H–T element contains an inversion of the region involving the original left-end element, but contains only an extra 8 bp of genetic material of the region adjacent to the original element.

The interest in the two types of elements is that they contain, on one chromosome, the essential ingredients of a transposable element, viz a complete left-end and a complete right end. The elements can be genetically manipulated in stable condition in the absence of P element transposase. The point of interest is to see what happens when they are brought into the same individual as a transposase source. The elements can also be combined with each other, or with left- and right-elements etc. There are altogether 11 different combinations to be investigated.

Materials and methods

PCR primers and conditions are as given in Svoboda *et al*. (1995). Positions of the primer pairs (A, B) and (C, D) for establishing the original inserted left and right-hand ends, and their use for establishing re-arranged structures, are shown in Figure 1.

Production of re-arranged elements

Gray *et al*. (1996) estimated that 4–5% of recombinants from crosses involving opposite end-deleted elements were of type H–H and 1–2% of type H–T. However the insertion points were such that most type H–H recombinants were produced by insertion either exactly into the 8 bp target site lying to the right of the original right-end element, or nearby. Such elements therefore had little or no intervening DNA between the two ends. Similarly with type H–T recombinants, most cases were found to be inserted into the left-end element rather than proximal to it, producing a structure with a right-end element inserted into a left-end element. A large screen was therefore carried out to identify new H–H and H–T elements with substantial intervening segments, and to produce lines containing such elements.

A number of crosses were set up of males containing opposite end-deleted elements and P[Δ2–3](99B) crossed to *cn bw* females. Three hundred male progeny of the required HEI recombinant type (+*bw* in Figure 1) were individually crossed to three *SM1*, *Cy al*^{2} *cn*^{2}/*Pm* females. After 3 days, each male was screened by PCR to locate individuals of type H–H or H–T. Progeny groups of the required type were then intercrossed to produce homozygous lines, and lines were sequenced to determine the exact nature of the element. This process produced many H–H lines, including two with intervening segments greater than a few base pairs, H–H1 and H–H2, having

respectively 124 and 232 bp between the two ends. With respect to the H–T type, two lines, H–T1 and H–T2, were produced with respectively 8 and 167 bp between the two ends. The element with the higher insert size, H–T2, was lethal in a homozygous condition. The mapping carried out by Gray *et al*. (1998) shows that the lesion causing lethality probably lies well outside the region containing the element. However, high levels of lethality and infertility associated with crosses involving this element led us to use mainly crosses involving the H–T1 element. The small insert size is not as critical for the H–T element, as the element heads are separated by some 800 bp, the length of the left-end element.

Stability of elements

Male flies containing a single re-arranged element balanced over *CyO*, and containing the P[Δ2–3](99B) transposase source, were crossed to females containing a deletion of the 50C region. Progeny were analyzed using combinations of the four primers shown in Figure 1. As the female parent carried a deletion, all bands could be attributed to events in the male parent under test.

Recombination induced by elements and element combinations

Recombination was measured between *cn* and *bw* in males containing a single re-arranged element and P[Δ2–3](99B). Elements were also tested in combination with each other, with end-deleted elements and with complete CaSpeR elements. Altogether 11 different element combinations are possible (see Figure 4). The production of some of these genotypes involves a difficulty, as the transposase source, P[Δ2–3](99B) needs to be introduced from one or other parent. In six of the eleven cases the P[Δ2–3](99B) element was introduced through the parent carrying either no element or a single end-deleted element which is unaffected by the transposase (Svoboda *et al*., 1995). In the remaining five cases, one or other of the elements had to be exposed to transposase for two generations.

Results

Recombination in single elements

Both H–H and H–T elements gave low recombination frequencies (Table 1). The table shows a range of H–H type elements, including two with longer inserts and two with short inserts. All recombination frequencies are low. The crossing programs used a variety of double heterozygote genotypes, making it difficult to summarize results other than by recombination frequency.

The low frequency of recombination led us to investigate whether the process of recombination is predictable from the HEI model. Figure 2 shows the expectation of the HEI model for the case of H–H elements. The prediction made from the model is that left-end elements should be associated with one type of recombinant (*cn bw* in Figure 2) and right-end elements with the opposite type.

Recombinant progeny were screened by PCR for agreement with these expectations using the E, D and A, F primer pairs shown in Figure 2. No cases were found where the element-end present was inconsistent with the recombinant genotype. However, 6/23 *cn bw* and 2/58 + + offspring gave no band using either primer pair, presumably indicating some deletion. Using primer pairs A, B and C, D (Figure 1), it was found that 22/81 recombinants were attributable to insertion within the A–D region. The remaining 59 presumably represent insertion outside of this region.

Table 1 Recombination induced by single A and B elements

Stock	*No. of progeny*	*Recombination frequency %*
H–H1	806	0.49
H–H2	907	0.33
H–H3	491	0.41
H–H4	417	0.48
H–T1	466	0.43

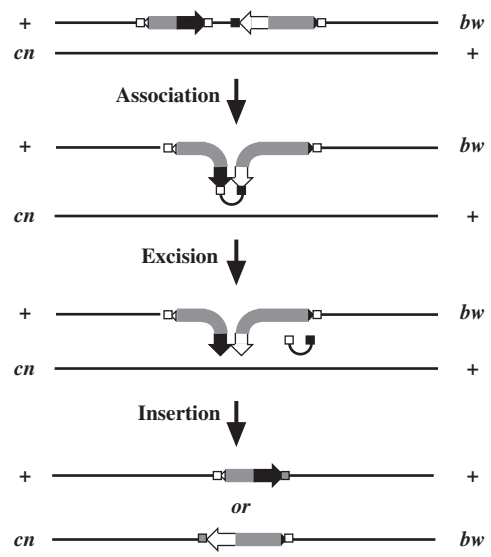

Figure 2 Recombinants expected from hybrid element insertion from a single H–H element.

Table 2 PCR analysis for 'Head-to-Head' non-recombinant progeny using B–C primers

Stock	*Distance between two ends (bp)*	*Total no.*	*B–C band same as parental*		*B–C 360 bp ± 20*		*Other sizes of B–C band*		*Others without B–C band*	
H–H1	123	160	65	40.6%	38	23.8%	7	3.4%	50	31.3%
H–H2	232	166	43	25.9%	66	39.8%	8	3.8%	49	29.5%

The stability of elements in non-recombinant progeny

Head-to-head elements: Table 2 summarizes the results of PCR analysis from 326 progeny of the H–H1 and H–H2 elements. There are three major classes of approximately equal frequency, including the unaltered class. Of the two altered classes, the first appeared to have deletions approximating to expectation if the intervening DNA between the two element ends had been excised. The second class, with no B-C band, appeared to indicate a larger deletion or a different chromosomal change.

The class with apparent excision of the intervening DNA was further examined by sequencing 17 of the B–C

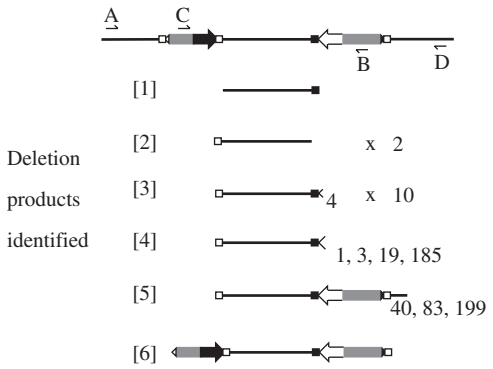

Figure 3 Six classes of deletions identified in 21 offspring from a Head-to-Head element. The most frequent class, [3], with exact deletion of the intervening DNA plus one copy of the CATG palindrome at the element end, occurs in nearly 50% of cases.

fragments. All turned out to have the two 31 bp ends in close proximity, indicating deletion of the intervening DNA (Figure 3, classes [1]–[4]). None had the 31 bp ends exactly joined. The major class, with 10 individuals, had a 4 bp deletion of the DNA at the tip, including one individual with a C–G substitution. Three other individuals had one or the other of the 8 bp target sites remaining between the two 31 bp ends (classes [1], [2]). The remaining four sequences had deletions extending into the left end element (class [3]).

The class with the missing B–C band was further investigated by amplification with the A, D primers. Of 30 individuals analyzed in this way, 19 gave band sizes indicating large deletions and 11 failed to amplify. Four of the amplification products were subject to sequencing. Three had deletions taking out the whole of the left element end (class [5]), whereas one deletion took out the entire region, including both element ends (class [6]).

Head-to-Tail elements: Results are shown in Table 3. More than 50% of such progeny, 145/227, showed no evidence of change in structure. Among the remaining progeny, the majority, 65/227, gave a PCR band with the PCR primers C and D (Figure 1). All fragments appeared to be of the same size, indicative of loss of the inverted insertion containing the left-end element. Sequences were obtained from four of the fragments, and in each case the excision of the left-end element plus the intervening DNA was exact.

Recombination induced by element combinations

Recombination frequencies are given in Figure 4. These come from a variety of crosses involving Head-to-Head and Head-to-Tail elements. The top line (1), (2) shows results of single elements, some of which have been discussed in Table 1. Then follow combinations of the two element types, (3)–(5), combinations with end-deleted elements (6)–(9) and combinations with a complete *CaSpeR* element (10), (11). Complementary ends from different elements are labeled (see Discussion).

Discussion

Both H–H and H–T elements are unstable in the presence of P transposase. Around 50% of elements, slightly more in the case of the H–H element, have undergone a deletion in one generation. However, the types of deletions are different for the two elements (Table 2 and Figure 3 vs Table 3). The major class for the H–H element consists of a deletion of the intervening DNA between the two elements, usually with an additional 4 bp deletion of one or the other end. For the H–T elements, the major class consists of a complete excision of the left-end (T) element.

There is evidence that three different mechanisms are involved, particularly for the Head-to-Head elements. The three mechanisms that will be considered in turn are:

(1) Direct rejoining under the model of Beall and Rio (1997).
(2) Repair under the model of Engels *et al*. (1990).
(3) Sister-strand recombination under the Hybrid Element Insertion (HEI) model of Gray *et al*. (1996).

Beall and Rio (1997) have shown that P element transposase acts as an endonuclease, cutting at the element end and creating a 17 bp 3′ overhang. The process is pictured for the H–H structure in Figure 5.

The situation depicted in Figure 5 is in one sense the opposite of that following a normal P element excision. Normally, following endonuclease digestion, each strand of chromosomal DNA is attached to just 17 bp of P element DNA. The 17 bp ends contain only small random stretches of complementarity, leading to a series of ligation/repair outcomes (O'Brochta *et al*., 1991; Takasu-Ishikawa *et al*., 1992; Staveley *et al*., 1995; Gloor

Table 3 PCR analysis for 'Head-to-Tail' elements

Stock	*Distance between two ends (bp)*	*Total*	*With B–D band*		*DL1 element excision*		*Without both B–D and C–D bands*	
H–T1	8	96	77	80.2%	12	12.5%	7	7.3%
H–T2	167	131	71	53.2%	53	40.5%	7	5.3%

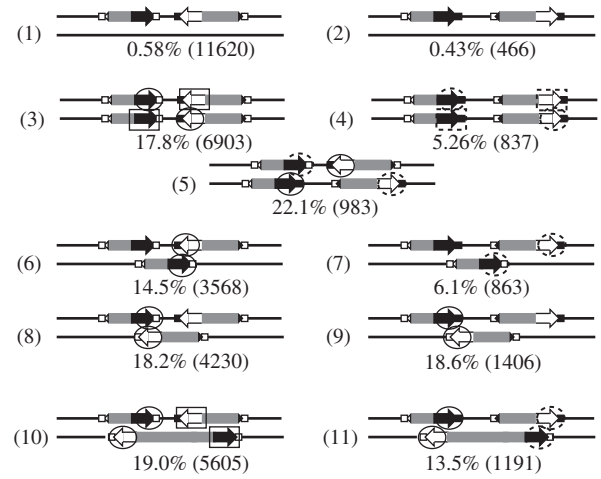

Figure 4 Recombination percentages and number of offsprings (in parentheses) induced by various combinations of re-arranged, end-deleted and non-deleted elements. Homologous element ends are outlined, with those cases where the ends point in the same direction indicated by dotted lines.

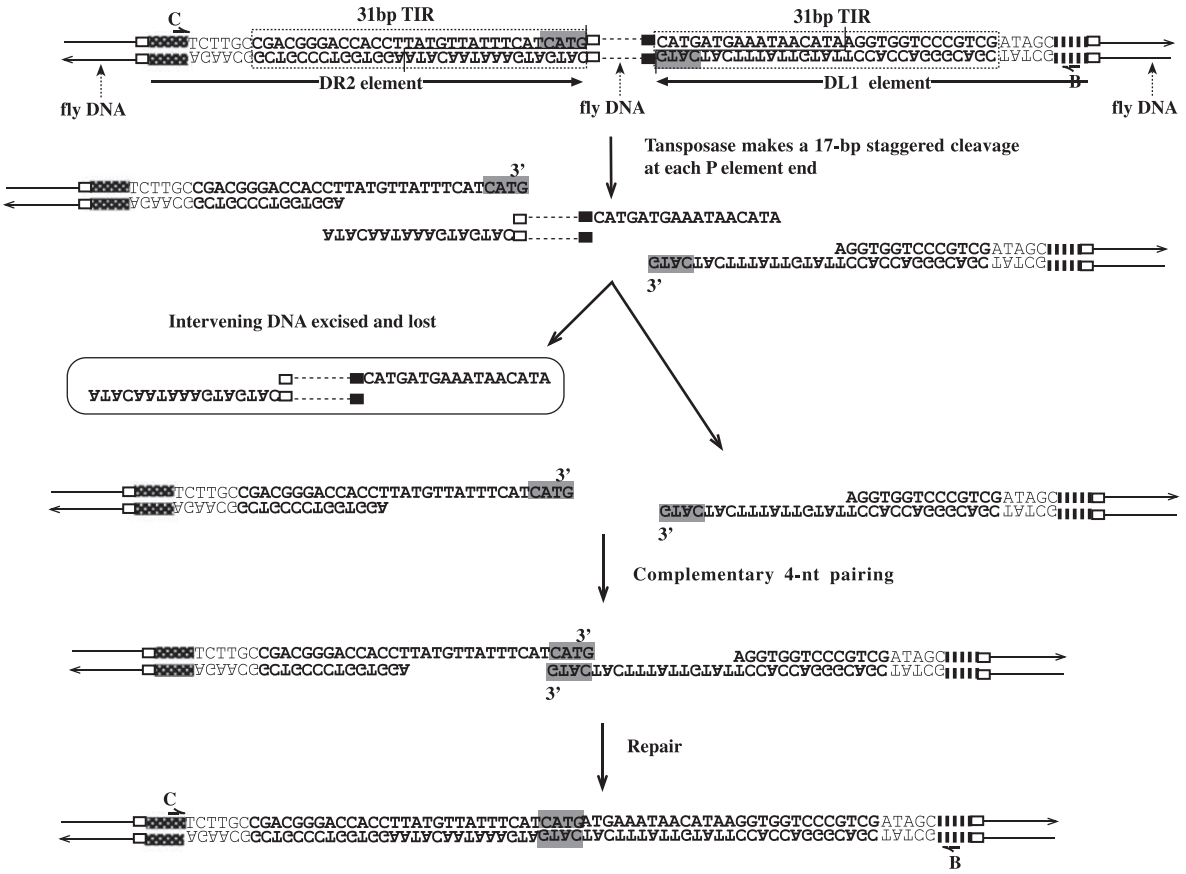

Figure 5 One form of resolution of P element 'excision' expected for the case of Head-to-Head elements under the Beall and Rio model.

et al., 2000). These outcomes are of little consequence in the case of P element excision when repair against the sister chromatid restores the original element structure (Engels *et al*., 1990).

In this case, these 17 bp ends are contained on a small piece of DNA, which is presumably lost. The free ends attached to chromosomal DNA contain P elements ending with the complementary 17 bp. The 4 bp at the tips in this case are the palindromic sequence CATG. This leads to the possibility shown in Figure 5 in which the 17 bp strands pair initially at the 4 bp ends, followed by filling in and ligation to restore a double-stranded structure in which the original P element ends are ligated with the loss of 4 bp. The size of class [6] of Figure 3 indicates that this outcome occurs frequently (10 out of 21 cases in which the intervening DNA is excised). It is unclear whether the palindromic sequence at the element end is of functional significance in the normal P element integration process. However, other transposons with short inverted repeat ends (*Tam*3, *Hobo*, *mariner*) do not appear to share this feature, making a functional role less likely.

The resolution of the Head-to-Tail structure is also as expected under the Beall and Rio model. In this case, the unusual situation arises in which the 17 bp ends attached to chromosomal DNA after excision are complementary to each other. The expected result in this case is a simple association of the two ends, resulting in exact excision of the inverted right-end element. The results of Table 3 and the associated sequencing indicate that this event occurs with high frequency (65 out of 79 cases in which there is some alteration of sequence at the element site).

A second possible mechanism for the observed Head-to-Head offspring is repair against the sister chromatid, restoring the original element structure. A total of 108 out of 326 offsprings are unchanged in structure. However, it seems likely that most of these are cases in which no event has occurred. The one case of exact excision of both P element ends (product [6] in Figure 3) clearly indicates the possibility of repair of the region against the homologous chromosome, which in this case does not contain a copy of the element.

The remaining classes of elements in Figure 3, particularly classes [1] and [2], cannot easily be explained by rejoining and repair. A more likely mechanism in this case is that of sister-strand recombination. Figure 2 shows the HEI process for recombination between homologues. Sister strands are not pictured in this case. However, insertion of the hybrid element into the sister chromatid could explain the above classes. Exact insertion into the 8 bp target sites would explain classes [1] and [2]. Such exact insertion into previous 8 bp target sites is consistent with the HEI results of Gray *et al*. (1996).

Classes [4] and [5] can be explained by insertion into the right element or distal to it. The reciprocal products for such insertion would not easily be seen as they would result in either small or no changes in fragment sizes for

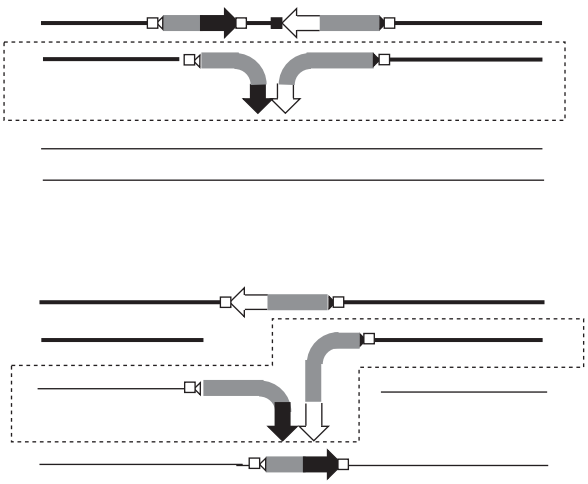

Figure 6 Hybrid element formed from Head-to-Head element compared to left and right-elements *in trans*, showing the similarity of the two types of hybrid elements (hybrid elements outlined by dashed lines).

the B–C fragment (Figure 3). The fact that all recombination occurs to the right of the element is surprising under this model, but such directionality of insertion is also a feature of the HEI studied by Gray *et al*. (1996).

Several aspects of the recombination results are of interest. First, the low rate of recombination induced by single elements (Table 1 and Figure 4) needs some examination. Both single elements induce recombination at a rate of around 0.5%, which is comparable to that given by a normal element. The expectation, particularly for Head-to-Head elements (Figure 2), would suggest that the rate of recombination should be higher. The situation for a left and right-end element on the same chromatid appears comparable to that in which left and right-elements are present on homologous chromosomes. The rate of recombination is 30% or higher in this latter case. Gray *et al*. (1996) argued that end association forces the formation of a hybrid element, and that integration of such an element would lead to high rates of recombination. The high rate of excision for the Head-to-Head structure indicates that end association is also occurring, but is not being accompanied by a high rate of integration.

The difference in structure in the two cases is that in one the hybrid element connects ends from two different chromatids whereas in the other it connects ends from the same chromatid (Figure 6). In one case the hybrid element frequently resolves by integration (Gray *et al*., 1996), although also by repair of P element ends (Liang and Sved, 2009). In the other, the present case, the resolution is almost entirely through repair.

It is noteworthy that recombination in the latter (repair) case involves insertion in the vicinity of an element, whereas in the former (integration) case there is no element available. There is substantial evidence that insertion occurs preferentially in the vicinity of existing elements (Eggleston, 1990; Sved and Liang, 2006). A corollary of this explanation is that in the Head-to-Head case, insertion should preferentially occur in the sister chromatid. As discussed above, there is evidence for a substantial rate of sister-strand recombination leading to deletions in the non-recombinant gametes.

The results from combinations of elements (classes (3)–(11) of Figure 4) reveal that with two exceptions, the rates of recombination are in the range 13–20%, comparable to that given by homologous unaltered elements (Sved *et al*., 1991). The exceptions, classes (4) and (7), are in the range 5–6%. Figure 4 shows the associations of left and right-elements on homologous chromosomes, and it is notable that these two classes only allow association of ends that point in the same direction. A feature of the hybrid elements produced in these cases is that only insertion can lead to viable gametes. Joining of ends not containing elements, a major source of recombinants in the case of HEI studied by Gray *et al*. (1996), leads to inviable fragments and bridges. We postulate that the decreased rate of recombination in flies containing such co-oriented element ends is due to the absence of viable repair products.

Finally, we note that although this is the first case that we know of in which simple re-arranged elements have been studied, the results are similar to those in which combinations of elements on the same chromosome have been studied. Golovnin *et al*. (2002) found numerous insertions and inversions in a region containing three P elements. Comparable events have also been analysed in other eukaryotes, including *Ac* in maize (Zhang and Peterson, 1999) and a *Tc*1-*mariner* element, *impala*, in the fungus Fusarium (Hua-Van *et al*., 2002).

Acknowledgements

Comments from Bill Engels and Christine Preston are acknowledged. The work was supported by grants from the Australian Research Council.

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