Genet. Res., Camb. (1992), **60**, *pp.* 165–174 *With 2 text-figures*

Chromosome interactions in P-M dysgenic male recombination of *Drosophila melanogaster*

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(*Received 13 May 1992 and in revised form 23 June 1992*)

Summary

The frequency, distribution and structure of P elements on the second and third chromosomes of Texas 1, a wild-type inbred strain of *Drosophila melanogaster*, were investigated by *in situ* hybridization. These autosomes were isolated individually and used as P-element donors to study the frequency and distribution of male recombination events generated on recipient chromosomes which were originally devoid of P sequences. The P-element array of chromosome 2 was shown to generate higher male recombination frequencies on chromosome 3 than vice versa, despite having fewer P factors and fewer P elements in general. This is likely to be due to the presence and distribution of specific P-deletion derivatives, which vary in their ability to repress P mobility. The male recombination generated on recipient chromosomes is associated with the insertion of donated P sequences, but only in a small minority of cases could a novel P-element site be detected at, or near, the recombination breakpoint. The majority of such breakpoints appear to be associated either with unsuccessful P insertion, or with the action of P transposase attracted by P elements newly inserted elsewhere on the recipient chromosome. Recent evidence also suggests that a small proportion of the breakpoints may be associated with the action of P transposase alone. Male recombination breakpoints appear to be distributed effectively at random along the recipient autosomes, and their frequency of occurrence was shown to correlate with the physical length of DNA available between markers, as revealed by the polytene map distance.

1. Introduction

There are at least three families of transposable element found in the *Drosophila melanogaster* genome which, when activated in certain non-reciprocal interstrain crosses, can give rise to germ-line abnormalities. The genetic aberrations have been collectively referred to as 'hybrid dysgenesis' by Sved (1976). Three such systems have been associated with transposition of the P, I and *hobo* families of mobile element. Although these transposons function independently at the molecular level, they share the ability to induce increased mutation and sterility when mobilized in a dysgenic cross.

The P-M system of hybrid dysgenesis, recently reviewed by Engels (1989) is the best characterized of the three. P elements, present in some *D. melanogaster* strains (P strains) but absent from others (M strains), exist in two forms. P factors are complete, 2·9 kb transposons capable of producing an 87 kDa transposase protein which can mobilize both themselves and smaller, internally deleted P elements by binding to DNA sequences at the element termini (Kaufman *et al.* 1989). Thus, the shorter internally deleted elements yield a subset of non-autonomous P elements ranging in size from 0·6 to 2·9 kb (O'Hare & Rubin, 1983). When P strain males mate with females which lack P elements (M strain) their F_1 progeny suffer the effects of P-element mobilization, namely reduced fertility and sterility (gonadal dysgenesis), increased mutation rates, illicit male recombination and segregation distortion (Kidwell et al. 1977). Hybrid dysgenesis may also be induced by transforming P factors into M-strain embryos by micro-injection. Characteristically, there is no hybrid dysgenesis in the reciprocal cross (M strain males by P strain females), nor does it occur in crosses between P strains or between M strains. Thus P elements can only be mobilized in the cytoplasmic background of an M-

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strain embryo (M cytotype) and not in the cytoplasm contributed by P-strain mothers (P cytotype) (Engels, 1979).

P elements move very rarely in somatic cells (Brookfield & Lewis, 1989), and the basis of this germline specificity is now understood. The P factor has four open reading frames (ORFs 0, 1, 2 and 3) and the final intron (between ORFs 2 and 3) is retained in somatic tissue. Thus the most abundant message found in the soma translates into a 66 kD truncated protein, which is unable to mobilize P elements (Karess & Rubin, 1984). Only in the germ line is the third intron correctly spliced out to yield the functional 87 kD transposase.

In the germ line, the high frequency of P-element movement is now thought to occur by a non-replicative 'cut and paste' transposition mechanism (Engels *et al.* 1990; Craig, 1990). The model proposed by Engels *et al.* (1990) suggests that when a P element jumps from a donor site, the resulting gap is repaired by double-strand gap repair. The consequences of this repair depend upon whether or not the homologue used as a repair template also carries a P element. If it does, double-strand gap repair will produce a chromosome identical to the donor chromosome before transposition. If the template lacks a P element, however, the repair will yield a wild-type (i.e. non-P-element) chromosome, which would appear to be a reversion resulting from P-element excision.

Studying the precise relationship between P elements and the dysgenesis they cause when active has proved to be difficult. In the case of male recombination, the first aberrant trait to be associated with P-M hybrid dysgenesis (Hiraizumi, 1971; Kidwell, 1977), there does not seem to be a simple relationship. Indeed, homologous male recombination which occurs, at least in part, pre-meiotically appears to have a multi-causal basis (Duttaroy *et al.* 1990). The distribution of recombination sites and the frequency of recombination depend upon the P strain used as the male parent (Exley & Eggleston, 1989). Recent investigations using two P strains, Texas 1 and Texas 6, considered the dysgenic effects attributable to individually isolated autosomes (Exley and Eggleston, 1989). From this investigation a close, but incomplete, relationship between original P-element location and male recombination site emerged. This was taken to indicate that P-element excision gave rise to the chromosome breakage necessary for the majority of male recombination events to occur. Sved *et al.* (1990) reported that elevated frequencies of male recombination required the presence of transposase (*in trans*) and resident P elements in the interval of recombination (*in cis* requirement). However, they also detected a low frequency of male recombination events occurring outside P element-containing intervals. McCarron *et al.* (1989) reported an increase in male recombination in the presence of P ($\Delta 2$–3, ry^+) (99B), a non-mobile but transposase-producing P element, indicating that P-element transposase, in the absence of P targets or mobile P elements, can induce a significant rate of crossing-over in dysgenic *Drosophila* males.

Here we detail a series of experiments designed to investigate the frequency and distribution of those male recombination events apparently unassociated with the original locations of P elements on the chromosomes. Specifically, we investigated whether P elements can produce chromosome breaks, at sites distant from their original location, which result in male recombination either through attempted or successful P-element insertion. The implications of these findings with respect to the relationship between P elements and male recombination breakpoints are discussed.

2. Materials and methods

(i) *Drosophila melanogaster strains used*

Texas 1: a long-established inbred P strain produced from a wild-type population originally collected in Austin, Texas in 1965. Inbred lines were created by repeated single-pair matings and have since been maintained as mass sib-mated cultures (Linney *et al.* 1971). The extent of the inbreeding within Texas 1 is evident from genetic analyses of a range of quantitative characters and isoenzyme loci (Birley *et al.* 1981).

Canton S: a wild-type laboratory M strain.

Harwich: a wild-type laboratory P strain.

ru h th st cu sr e^s *ca*: a standard laboratory third-chromosome marker stock, classified as M-cytotype and carrying the following recessive mutations; *ru*, roughoid (3–0·0); *h*, hairy (3–26·5); *th*, thread (3–43·2); *st*, scarlet (3–44·0); *cu*, curled (3–50·0); *sr*, striped (3–62·0); e^s, ebony-sooty (3–70·7) and *ca*, claret (3–100·7). Obtained from Bowling Green Stock Center, USA.

al dp b pr c px sp: a standard laboratory second-chromosome marker stock, classified as M-cytotype and carrying the following recessive mutations; *al*, aristaless (2–0·01); *dp*, dumpy (2–13·0); *b*, black (2–48·0); *pr*, purple (2–54·5); *c*, curved (2–75·5); *px*, plexus (2–100·5) and *sp*, speck (2–107·5). Obtained from Bowling Green Stock Center, USA.

C23(a): a balancer strain for chromosomes 2 and 3 obtained from the University of Birmingham, UK. It has multiple inversions on both autosomes to minimize recombination and has the following genotype; In(2L+2R) *Cy*; *Cy* cn^2/In(2LR) *Pm*, al^4, ds^{33k}, l_t, bw^{v1} and In(3LR) *Dcx F*; *D*/*Sb*. Each autosome therefore carries one of two dominant markers; *Cy*, Curly (2–6·1) or *Pm*, Plum (2–104·5) and *D*, Dichaete (3–40·7) or *Sb*, Stubble (3–58·2). This strain has been classified as M-cytotype.

dp, dumpy (2–13.0): a single recessive marker stock classified as M-cytotype.

y, sn^w; *bw*, *st*/y^+ *Y*. This strain, kindly supplied by Mike Simmons, has an attached Y chromosome

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carrying the wild-type allele from the *yellow* locus. Males therefore have a wild-type body colour, whereas females express the *yellow* mutation. The strain carries the following recessive mutations; *y* yellow (X–0·0); sn^w, singed-weak (X–21·0); *bw*, brown (2–104·5) and *st*, scarlet (3–44·0). The singed-weak (sn^w) mutation results from the tandem insertion of two non-autonomous P elements, in opposite orientations, and this strain is therefore classified as M′. See Lindsley & Grell (1968) for further details of all markers used.

All dysgenic classifications were determined both genetically (using the appropriate biological assays) and molecularly, by hydrization of P-element probes to total genomic DNA. All strains were maintained and experimental crosses raised on either (sucrose/yeast/agar) or (maize/molasses) media at 25 ± 1 °C unless otherwise stated. Fresh yeast suspension was added as a food source, and nipagin (15 ml l^{-1} of a 10% solution in ethanol) was added to all cultures to inhibit fungal contamination.

(ii) *Construct production*

dp; *st*: Virgin *dp* females were mass mated to *y*, sn^w; *bw*; st/y^+ *Y* males at 25 °C. The progeny were allowed to mate *inter se* and males which had both scarlet and dumpy phenotypes were selected from the F_2 progeny. These males segregate for the *y*, sn^w and wild-type X chromosomes. In order to stabilize the genotype, they were individually mated to their (non *y*, sn^w) virgin *dp*; *st* female sibs. Individuals, wild type for body colour, were selected from among the progeny. This strain is classified as M-cytotype.

al, *dp*, *b*, *pr*, *c*, *px*, *sp*; *st*: Virgin *al*, *dp*, *b*, *pr*, *c*, *px*, *sp* females were mass mated to *y*, sn^w; *bw*; st/y^+ *Y* males at 25 °C. The F_1 progeny were mated *inter se* and males which were homozygous for all the second-chromosome mutations and for scarlet were selected from the F_2 progeny. These males segregated for the wild type and *y*, sn^w X chromosomes and were therefore mated individually to virgin female sibs with the same autosomal constitution to produce a stable line expressing *al*, *dp*, *b*, *pr*, *c*, *px*, *sp* and *st*. The eye-colour phenotype in this case is orange, resulting from expression of both scarlet and purple mutations. This strain is classified as M-cytotype.

(iii) *Measurement of male recombination*

To measure the extent of male recombination on chromosome 2 resulting from the P-element array of chromosome 3, the following crossing scheme was employed. Virgin Texas 1 females were mated to *dp*; *st* males, and the non-dysgenic male progeny, heterozygous for dumpy and scarlet, were crossed to virgin females of the M strain *al*, *dp*, *b*, *pr*, *c*, *px*, *sp*; *st*. The resulting dysgenic male progeny with the dumpy phenotype were designated 3-P to indicate the sole possible source of P elements on the Texas 1 third chromosome. These males were individually backcrossed at 25 °C to two broods of 20 virgin *al*, *dp*, *b*, *pr*, *c*, *px*, *sp* marker females to measure the frequency and distribution of recombination events among the male gametes. A similar design was employed to measure the extent of male recombination on the third chromosome, resulting from the P-element array on chromosome 2. Virgin Texas 1 females were mated to C23(a) males and the non-dysgenic male progeny, heterozygous for the dominant mutations *Pm* and *Sb*, were crossed to virgin females of the marker M strain, *ru*, *h*, *th*, *st*, *cu*, *sr*, e^s, *ca*. The resulting dysgenic male progeny, with the Stubble phenotype, were designated 2-P to indicate the sole possible source of P elements on the Texas 1 second chromosome. These males were individually backcrossed to two broods of 20 virgin *ru*, *h*, *th*, *st*, *cu*, *sr*, e^s, *ca* females to measure the frequency and distribution of recombination events among the male gametes. For both 2-P and 3-P males the presence of a recombinant genotype in the progeny of a family was taken to represent a single recombination event. If a male recombination event occurs pre-meiotically, clusters of individuals carrying the same recombinant genotype, or its reciprocal, can be found within a family. Therefore, the occurrence of a particular recombinant type more than once in a family was treated as a cluster and taken to be derived from a single 'independent recombination event'. Any resulting bias will therefore tend to underestimate male recombination frequency, since a given recombinant type could have arisen more than once, independently, within a single family.

The design of the crossing scheme to assess male recombination on the second chromosome requires comment. All progeny of the final backcross must express the dumpy (*dp*) phenotype. As such, it is not possible to quantify recombination events between the markers (*al* and *dp*) or (*dp* and *b*). We have chosen to consider the *al* to *b* region as a single interval for the measurement of male recombination. This design will still record a small proportion of double recombination events as *al*, *dp*, *b* or +, *dp*, + parentals, although our experience suggests that any such bias will be small.

(iv) *The construction of third-chromosome recombinant sub-lines*

Individual third-chromosome recombinant males were backcrossed to virgin females from the *ru*, *h*, *th*, *st*, *cu*, *sr*, e^s, *ca* marker stock. Recombinant progeny were subjected to at least three successive rounds of single-pair inbreeding in order to yield stable recombinant genotypes. Throughout this procedure, a restrictive temperature of 18 °C was employed to minimize further P-element movement in the male germ line. True breeding lines with the following genotypes were isolated in this way: (*ru*, *h*, *th*, st^+, cu^+, sr^+, e^{s+}, ca^+); (*ru*, *h*, *th*, *st*, cu^+, sr^+, e^{s+}, ca^+); (*ru*, *h*, *th*, *st*, *cu*, sr^+, e^{s+},

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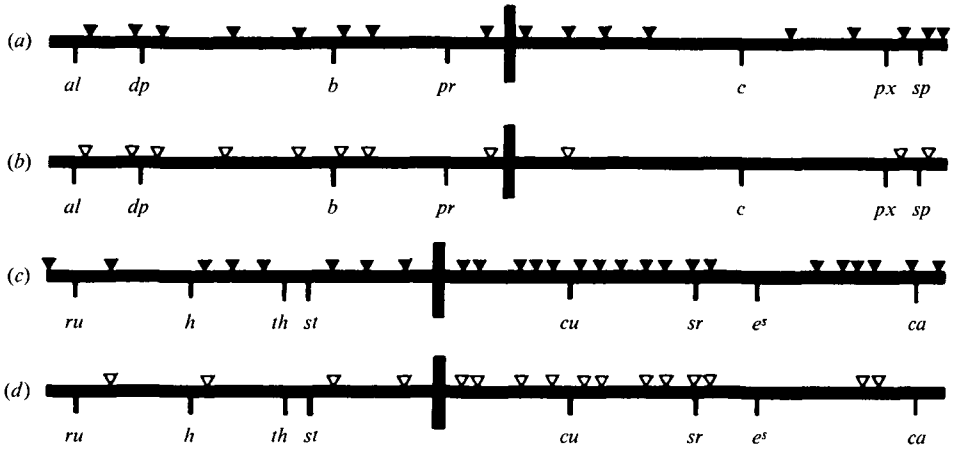

Figure 1. Diagrammatic representation of P-element distributions along the second and third chromosomes of Texas 1 (as revealed by *in situ* hybridization to the salivary gland polytene chromosomes) superimposed on the standard polytene chromosome maps (Lindsley & Grell, 1968). (*a*) Second chromosome probed with pπ25·1; (b) Second chromosome probed with pπPvull; (c) Third chromosome probed with pπ25·1: (d) Third chromosome probed with pπPvull. All maps are based on the analysis of at least twenty independent preparations, and no polymorphism for P-element sites was evident within this strain.

ca^+); (*ru, h, th, st, cu, sr,* e^{s+}, ca^+) *and* (*ru, h, th, st, cu, sr,* e^s, ca^+).

(v) In situ *hybridization*

Salivary gland polytene chromosome squashes were prepared as described by Exley & Eggleston (1989) from large third-instar larvae. Preparations were made from Texas 1, Canton S, Harwich and the recombinant sub-lines described in section (iv). Hybridizations were carried out overnight at 45 °C using 20 μl of a solution containing denatured probe DNA, labelled by nick translation to give over 30% incorporation of Bio-16-UTP (Rigby *et al.* 1977). The hybridization mix also contained deionized formamide, Denhardt's solution, dextran sulphate and carrier DNA (Engels *et al.* 1986). Sites of hybridization were visualized using a streptavidin-horseradish peroxidase kit from ENZO Biochemicals (Detek 1-hrp) and the peroxidase substrate 3–3′ Diaminobenzidine tetrachloride (DAB), following Ashburner (1989). Subsequently, the chromosomes were stained with 5% phosphate-buffered Giemsa. The P-element distribution maps presented here were based on the analysis of at least twenty independent preparations. We found no evidence for polymorphism of P-element insertion sites in Texas 1 during this investigation.

Two DNA probes were used for *in situ* hybridization. The first, pπ25·1, carries the intact P factor and will therefore hybridize to all P homologous sequences. The second, pπPvull, carries an 875 bp Pvull fragment from the P element which spans most of ORF1 and the beginning of ORF2 in the central transposase coding region. Since this internal fragment has been lost in the majority of deleted, non-autonomous P elements, these two probes can be used to distinguish between intact P factors and most of their deleted derivatives. For each probe, appropriate restriction endonucleases were used to separate insert from plasmid vector sequences, and the inserts were purified prior to labelling.

3. Results

The ability of Texas 1 to elicit P-M hybrid dysgenesis has previously been verified using assays of egg production, sn^w destabilization and male recombination (Exley & Eggleston, 1989). The complement of P elements, complete and internally deleted, residing in the genome of Texas 1 has been studied using P-element DNA probes hybridized *in situ* to polytene chromosomes prepared from the salivary glands of Texas 1 larvae (Exley & Eggleston, 1989). The distribution of P elements on the second and third chromosomes of Texas 1, detected using the probes pπ25·1 and pπPvull, is shown in Figure 1. As described in Materials and methods, the second and third chromosomes of Texas 1 were isolated individually in genetic backgrounds otherwise lacking P-element homology. Dysgenic males were produced which carried either an intact Texas 1 second chromosome (2-P males) or an intact Texas 1 third chromosome (3-P males). Recombination in these chromosomes during their isolation was prevented by transmitting them through the male line and by avoiding crosses which would elicit hybrid dysgenesis. The experiment was designed so that 2-P males also carried a third chromosome marked with a series of recessive mutations. Thus, male recombination events resulting from the array of P elements on chromosome 2 could be detected on chromosome 3. Similarly, 3-P males carried a marker second chromosome so that male recombination resulting from the activity of P elements on the third chromosome could be detected on chromosome 2. In each case, the only source of P element sequences is the wild-type second or third

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Table 1. *Frequency of male recombination events on recipient chromosome*

	Recombination on 3 (P elements on 2)	Recombination on 2 (P elements on 3)
Total number of progeny scored	25528	13262
Total number of recombination events	234	51
Total number of independent recombination events	113	41
Gross recombination frequency	0·92%	0·38%
Corrected recombination frequency	0·44%	0·31%

Male recombination frequencies for chromosome 3 arising from the activity of P elements originally located on chromosome 2 and male recombination frequencies for chromosome 2 arising from the activity of P elements originally located on chromosome 3. Pre-meiotically derived clusters of recombinants among the progeny of a single family are treated as a single independent male recombination event in the calculation of corrected frequencies.

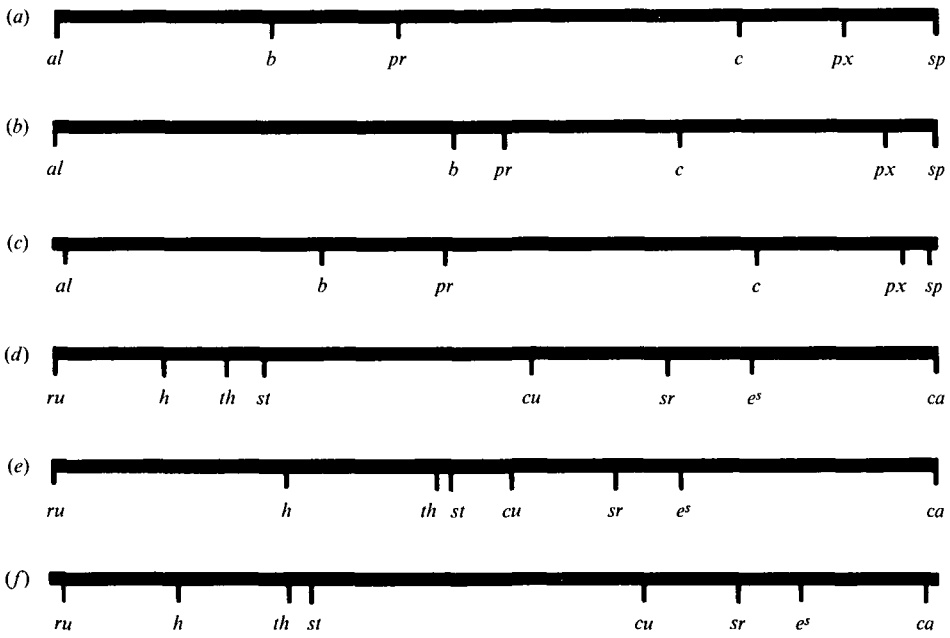

Figure 2. Maps of the second and third chromosomes, based on the proportion of independent male recombination events occurring between adjacent markers. Comparative maps arising from female meiotic recombination and cytological preparations of the polytene chromosomes are also given. (*a*) Distribution of male recombination events on chromosome 2 arising from the activity of P elements originally situated on chromosome 3; (*b*) map of the second chromosome resulting from normal female meiotic recombination; (*c*) map of the second chromosome as revealed in cytological preparations of the polytene chromosomes; (*d*) distribution of male recombination events on chromosome 3 arising from the activity of P elements originally situated on chromosome 2; (*e*) map of the third chromosome resulting from normal female meiotic recombination; (*f*) map of the third chromosome as revealed in cytological preparations of the polytene chromosomes.

chromosome derived from Texas 1. The small Texas 1 fourth chromosome, which was uncontrolled throughout these crossing schemes, does not carry P sequences.

The frequencies of male recombination observed on each autosome are given in Table 1. Recombination frequencies on chromosome 3, resulting from P elements originally situated on chromosome 2, are higher than those on chromosome 2, which resulted from P elements originally located on chromosome 3 (gross frequencies 0·94% *v.* 0·38%; corrected frequencies 0·44% *v.* 0·31% respectively). This is an interesting result, since there are significantly more P elements on the wild-type third chromosome than on the wild-type second chromosome (26 P elements *v.* 17 P elements respectively). The lower number of P sequences on the second chromosome is therefore responsible for a higher frequency of male recombination on chromosome 3 than vice versa.

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Table 2. *Distribution of male recombination events on recipient chromosome*

Chromosome interval	Independent male recombination events (%) (A)	Proportional female meiotic map (%) (B)	Proportional polytene chromosome map (%) (C)
(i)			
ru–h	11·5 (4)	26·0 (2)	14·3 (3)
h–th	7·1 (6)	17·5 (3)	12·9 (4)
th–st	4·4 (7)	1·0 (7)	2·6 (7)
st–cu	30·1 (1)	7·0 (6)	38·0 (1)
cu–sr	15·9 (3)	12·0 (4)	10·7 (5)
sr–e^s	9·8 (5)	7·5 (5)	6·9 (6)
e^s–ca	21·2 (2)	29·0 (1)	14·6 (2)
		$r_s(A\text{-}B) = 0{\cdot}286^{NS}$	$r_s(A\text{-}C) = 0{\cdot}821$*
(ii)			
al–b	24·4 (2)	45·3 (1)	29·7 (2)
b–pr	14·6 (3)	5·6 (5)	14·1 (4)
pr–c	39·0 (1)	19·6 (3)	35·9 (1)
c–px	12·2 (4)	23·4 (2)	17·2 (3)
px–sp	9·8 (5)	6·1 (4)	3·1 (5)
		$r_s(A\text{-}B) = 0{\cdot}3$	$r_s(A\text{-}C) = 0{\cdot}9$

The proportion of independent male recombination events (A) and the equivalent proportional female meiotic map (B) and polytene map (C) distances for each defined interval of the third chromosome (i) and the second chromosome (ii). The figures in parentheses refer to the rank of each observation, and the relationships between these variables are illustrated by rank correlation coefficients with sample sizes of $N = 7$ for the third chromosome [Table 2 (i)] and $N = 5$ for the second chromosome [Table 2 (ii)]. Thus, for example, $r_s(A\text{-}B)$ represents the rank correlation between the proportion of independent male recombination events and the proportional female meiotic map distance. Tests of statistical significance are given as NS, $P > 0{\cdot}05$ and *, $0{\cdot}05 > P > 0{\cdot}01$ for the third chromosome, but a robust test for the second chromosome is not available as the number of ranked intervals is less than 6.

Overall male recombination frequencies tell us nothing about the distribution of recombination events. If P elements, or the action of P transposase, from one chromosome can generate 'distance effects' on another chromosome, where (in the recipient chromosome) do these 'distant' male recombination events occur? In order to investigate this distribution, the chromosomes may be subdivided into regions flanked by recessive markers, and the proportion of recombination events occurring in each interval assessed. The distribution of male recombination events along the second and third chromosomes, produced by the activity of P elements on chromosomes 3 and 2 respectively, are shown in Fig. 2. It is clear that the distribution of male recombination events is very different from that produced by normal female meiotic recombination; compare Fig. 2 (*a*, *b*) and (*d*, *e*). See also Eggleston (1984) and Exley & Eggleston (1989). There does appear, however, to be a relationship between the physical length of target DNA between markers, as revealed by the polytene map distance, and the proportion or frequency of male recombination events observed. This can be verified statistically for the third chromosome using Spearman's rank correlation test (Snedecor & Cochran, 1980), the results of which are given in Table 2(i). For chromosome 3 the rank correlation between male recombination events and polytene map distance is significant ($r_s(A\text{-}C) = 0{\cdot}821$; $0{\cdot}05 > P > 0{\cdot}01$), whereas that between male recombination and female meiotic map distance is not ($r_s(A\text{-}B) = 0{\cdot}286$; $P > 0{\cdot}05$). Similar analyses are not available for the second chromosome, as the number of maker intervals which can be ranked (without the use of dumpy (*dp*); see Materials and methods) is too low to generate a valid test of significance. However, visual inspection of Table 2 (ii) shows the high degree of rank correlation between male recombination and polytene map distance ($r_s(A\text{-}C) = 0{\cdot}9$), although once again agreement between male recombination and female meiotic map distance is poor ($r_s(A\text{-}B) = 0{\cdot}3$).

One explanation of these data is that P elements originally situated on a donor chromosome can transpose to another chromosome and insert at apparently random sites on the recipient. During this transposition, chromosome breaks would be introduced which ultimately give rise to hybrid dysgenic male recombination. In order to approach the testing of this hypothesis, a number of recombinant third-chromosome sub-lines were produced (see Materials

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and methods) and subjected to an *in situ* hybridization analysis of the third polytene chromosome using the pπ25·1 probe. Of the five recombinant sub-lines examined, only one (*ru*, *h*, *th*, *st*, *cu*, *sr*, e^s, ca^+) revealed the presence of a novel P-element site at, or near, the recombination break point, which must have arisen through transposition following P-element mobilization from the donor second chromosome. Four of the five recombinant sub-lines, however, did not reveal novel P-element sites in the region of the recombinant breakpoint. The implications of these findings, in relation to recently proposed hypotheses, are discussed below.

4. Discussion

The experiments presented here attempt to determine the frequency and distribution of male recombination events arising, during P-M dysgenic transposition, on autosomes which were originally devoid of P sequences. As such, they yield information on the relative importance of 'local' and 'distant' effects of P-element mobilization. Local effects primarily concern events surrounding excision, whether precise or imprecise, of existing P sequences. Distance effects, however, are interpreted to involve the ability of P transposase to catalyse double-strand breaks in genomic DNA which lacks P sequence homology, as well as those events downstream of any initial excision events, namely attempted or successful insertion. It is important to note, however, that excision and transposition are not necessarily correlated events, but they may have common steps which are dependent on transposase (Yoshihara *et al.* 1991).

Texas 1 is a P strain for which the complement of P elements, intact and internally deleted, has been mapped using DNA probes and *in situ* hybridization assays. A close association between male recombination breakpoints and the original distribution of P sequences on both the second and third chromosomes of this strain has previously been reported (Exley & Eggleston, 1989; Exley, 1989). This was interpreted to suggest that the majority of chromosome breakage occurred at sites occupied by P elements and resulted from the excision, or attempted excision of those elements (whether precise or imprecise) under conditions of hybrid dysgenesis. These results also suggested that a small proportion of the observed male recombination resulted from P elements, originally situated on the third chromosome, transposing to novel sites on the same chromosome. Thus the mobilisation of these P sequences provides a pool of transposable elements which are capable of insertion at novel genomic locations. The distribution of such novel insertions can be determined by *in situ* hybridization to the polytene chromosomes and related to putative male recombination breakpoints.

To investigate this phenomenon further, Texas 1 chromosomes carrying P elements were individually isolated in *D. melanogaster* lines otherwise devoid of P sequences. In this way the ability of P elements situated on one chromosome to induce hybrid dysgenic male recombination events on a different chromosome could be tested and compared with the intra-chromosome effects (Exley, 1989; Exley & Eggleston, 1989). These results show that the P-element array of the Texas 1 third chromosome is able to generate male recombination frequencies of 1·28% on chromosome 3 and 0·31% on chromosome 2. Similarly, the P-element array of the Texas 1 second chromosome is able to generate male recombination frequencies of 1·87% on chromosome 2 and 0·44% on chromosome 3. In both cases, these figures are based on the numbers of independent male recombination events.

The total number of P elements on the Texas 1 third chromosome is 26, of which 10 are known to be internally deleted, whereas the second chromosome has 17 P sequences, 6 of which are known to be internally deleted. Thus the wild-type third chromosome, despite having more P sequences in total and more intact P factors, generates less intra-chromosome and inter-chromosome male recombination than does the wild-type second chromosome. It has been suggested that various classes of internally deleted P element are able to repress P element mobility (Black *et al.* 1987), perhaps through competition for transposase binding sites, although the precise mechanism of repression is not known. There is clearly a qualitative difference between internally deleted P elements in their ability to repress transposition, with some – for example the KP element – being particularly effective. It may be that the presence (or distribution) of particular deletion derivatives is responsible for the effect we describe above. For example, the P-element array of the Texas 1 third chromosome may include more effective repressors of transposition than that of the second chromosome. The proportion of internally deleted P elements on chromosomes 2 and 3 is very similar (35·3% *v.* 38·5%, respectively) and suggests that there is no straightforward quantitative relationship between deleted elements and the repression of P mobility.

It is evident from Table 2 that a relationship exists between male recombination frequency and the physical length of target DNA (as revealed by the polytene map distance) available between markers on the recipient chromosome. This relationship has also been described previously for intra-chromosome effects (Eggleston, 1984; Exley & Eggleston, 1989). Our interpretation of these results is that the majority of male recombination events arise from excision of existing P-element sequences. In this respect it does not matter whether the excision is precise or imprecise; the procedure will inevitably introduce chromosome breakage, and recombination events can arise from errors in the repair of these breaks. A proportion of male recombination events, however, arise at sites which are distant from the original P-element lo-

cations. These can be at novel sites on the same chromosome, or even at sites on a different chromosome. The mode of action of these 'distance effects' is unclear, but the process must introduce chromosome breaks which are subject to resolution as male recombination events. Such effects appear to occur at effectively random sites on the recipient chromosome, and the greater the physical length of target DNA the higher the frequency of chromosome breakage that results. This finding is not in disagreement with recent hypotheses in support of 'hot spots' for P-element insertion, for example at the transcriptional start sites of genes expressed in the germ line (Bownes, 1990) or at (or near) genomic DNA sequences which attract P transposase by chance similarity to the P element (Sved *et al.* 1990). It is a matter of scale (the data presented here cover both of the major autosomes) and the need for a simple assumption that such 'hot spots' are comparatively evenly distributed throughout the genome.

The actual cause of the chromosome breakage is open to question, but a number of hypotheses may be advanced. P elements which are mobilized from a donor chromosome form a pool with the potential to insert at novel genomic locations. The attempted or successful insertion of such elements into a site previously devoid of P sequences would be expected to generate chromosome breakage and therefore the potential for male recombination. Successful insertion could, of course, be detected by *in situ* hybridization of P-element probes to the polytene chromosomes. However, an attempted (but unsuccessful) insertion event might introduce staggered nicks in the target DNA which could be resolved as a male recombination event. In this case, there would be no detectable P sequence 'footprint' at the male recombination breakpoint. Similar arguments for unsuccessful insertion have been advanced previously by Engels *et al.* (1990), who suggest that P elements may transpose through a 'cut-and-paste' mechanism to a novel location, and that during this process the DNA strands recombine. Then, in spite of attempts to stabilize the recombinant autosome, the newly inserted P element excises from the recombination site, presumably relocating to a distant site. The gap left in the 'donor' strand is then repaired, using a wild-type DNA strand as a repair template, leaving a recombination breakpoint and no P-element sequence.

The confirmation that P elements from a donor chromosome are able to generate male recombination events by transposing to novel sites on a recipient chromosome came from an *in situ* polytene hybridization analysis of several third-chromosome recombinant sub-lines. In one of these lines, which carried a recombination event between the markers e^s (ebony-sooty) and *ca* (claret), a novel P-element site was detected at, or near, the male recombination breakpoint. The only possible source of this novel site was the P-element array of the wild-type Texas 1 second chromosome. This is in agreement with an earlier study of the third chromosome (Exley, 1989), where a P element was shown to have transposed to a novel location on the same chromosome, and this novel site was associated with a male recombination breakpoint between the markers *th* (thread) and *st* (scarlet).

The majority of the recombinant sub-lines, however, did not show evidence of novel P-element sites associated with the male recombination breakpoints. This is in agreement with the results of Duttaroy *et al.* (1990), who also found that male recombination sites appeared to correlate with the physical map but were not commonly associated with novel P elements. Sved *et al.* (1990) suggest that male recombination requires both the supply of transposase (*in-trans*) and the presence of P elements (*in-cis*), although they acknowledge that a low frequency of male recombination can occur outside P-containing intervals. However, we have described male recombination breakpoints on autosomes which were originally devoid of P elements. This suggests either that the *in-cis* P element requirement is weak or that it is supplied by P elements which have transposed from donor to recipient chromosome, but at sites removed from the actual breakpoint. Alternatively, the *in-cis* requirement (which presumably involves the resident P element acting as a focus for transposase activity) could arise through close physical juxtaposition of chromosome arms within the nucleus. Thus a recipient chromosome could, through close proximity to a donor chromosome, come within the sphere of influence of transposase attracted by P elements on the donor.

There is now good evidence that genomic DNA can interact with transposase alone, in the absence of P targets or mobile P elements, to generate male recombination breakpoints (McCarron *et al.* 1989). Furthermore, Rio *et al.* (1988) have shown that certain P elements can encode a transposase which acts *in-trans* at specific chromosomal locations, generating double-strand breaks and hence the potential for male recombination. This is supported by the work of Kaufman *et al.* (1989), who report that P transposase protein, as well as binding to an internal 10 bp sequence at P-element termini, also shows an exceptionally high non-specific affinity for DNA. Such binding could be involved both in determining P-element insertion sites and in the generation of chromosome breakage in the absence of an inserting P element.

Although it is difficult to make direct comparisons between alternative studies, the frequencies of male recombination generated by the action of transposase alone appear to be much lower than those we describe here. McCarron *et al.* (1989) showed that transposase from the non-mobile element P ($\Delta 2$–3, ry^+) (99B) was able to generate male recombination frequencies of around 0·04% (approximately twenty times higher

than the background frequency of 0·002%) over an interval corresponding to about half of the third chromosome. A comparable male recombination frequency for the same region in our experiments (where P elements were mobilized from the second chromosome) would be 0·2%, a fivefold increase over that seen for transposase alone.

We conclude that the majority of male recombination breakpoints in P-M dysgenic crosses arise at, or near, existing P-element sites. Such sites act as a focus for P transposase activity, which is able to generate chromosome breakage and thus the potential for excision and male recombination. There is now good evidence that resident P elements can act in this way as foci for local dysgenic effects. Such effects include localized excision and transposition, deletions, chromosomal rearrangements and recombination (A. Chovnick, personal communication; Eggleston, 1990). This phenomenon largely accounts for the positive correlation between the distributions of male recombination breakpoints and original P-element arrays observed here and in other studies (Exley, 1989; Exley & Eggleston, 1989; Duttaroy *et al.* 1990). Recent research indicates that the majority of secondary insertions following P mobilization occur within 50 bp of the primary element, most integrating into the 5′ terminal repeat or the 8 bp target site (A. Chovnick, personal communication; Eggleston, 1990). This observation perhaps serves as a reminder that P-element frequencies may well be underestimated by *in situ* hybridization to the polytene chromosomes, given that multiple intragenic insertions may be beyond the resolution of the technique. However, such underestimates do not alter the general interpretation of distance effects presented here.

A proportion of male recombination events presumably arise from the attempted or successful insertion of P elements which have been mobilized from other genomic locations. The experiments reported here do not allow us to determine how frequent an occurrence this might be, since attempted insertion would not be detected. However, our data and that of Duttaroy *et al.* (1990) indicate that distant male recombination breakpoints associated with a novel P-element site are in the minority.

Finally, there are those male recombination events which arise from the action of P transposase alone, even in the absence of P targets or mobile elements (McCarron *et al.* 1989; Sved *et al.* 1990, 1991). Recently, evidence has been accumulated showing that non-mobile P-element transposase sources may have an additive effect on distant male recombination (A. Chovnick, personal communication). In the experiments described here, P elements on one autosome are mobilized by hybrid dysgenesis, and distant male recombination (on the other major autosome) assessed. Given the numbers of putative autonomous P factors involved in this study (11 on chromosome 2 and 16 on chromosome 3), this additive effect could be substantial and may, in fact, form the dominant 'distant' process.

Taking these pieces of evidence together, we can derive crude estimates of the relative importance of the processes involved in the generation of male recombination events when P-element arrays are mobilized. For example, we might expect male recombination frequencies on a typical autosome (generated by the P-element array on that autosome) of around 1·5–2%, the vast majority of which (1–1·5%) might involve local effects, namely the focusing of transposase activity on resident P sequences. The remainder (perhaps 0·5%) presumably involve effects which take place at some distance, including those due to distantly located P transposase sources and the attempted/successful insertion of P elements mobilized from distant sites. Given that a single, non-mobile transposase source can generate distant male recombination frequencies of around 0·1% (McCarron *et al.* 1989) and that such sources appear to behave additively (Chovnick, personal communication), and given the number of potential transposase sources involved in this study (Fig. 1), the action of transposase alone may be sufficient to account for the majority of the distant male recombination events we observe.

These figures, of course, relate to P elements which are located on only one of the two homologous chromosomes for any particular site. It has recently been shown (Sved *et al.* 1991) that homologous P elements (one element on each homologue at a specific site) are able to generate male recombination frequencies an order of magnitude higher than those observed in standard dysgenic crosses. Presumably, this is because of the lengths of the double-strand gaps introduced (Engels *et al.* 1990), which must be at least as long as the P element involved. This enhancement, however, is presumably limited to 'local' effects at the site of the homologous P element and would not be found at more distant sites.

We are grateful for the financial support of the SERC, Lister Institute of Preventive Medicine, University of Nottingham and the Royal Society. Paul Eggleston is a Lister Institute Research Fellow. Thanks are due to John Brookfield for helpful discussion, Joyce Ellis for technical assistance and Art Chovnick for valuable comments and access to unpublished data.

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