Genet. Res., Camb. (1989), **53**, *pp.* 163–171 *With 3 text-figures Printed in Great Britain*

Structure, frequency and distribution of P elements in relation to P–M hybrid dysgenic male recombination in *Drosophila melanogaster*

K. A. EXLEY AND P. EGGLESTON

Department of Genetics and Microbiology, University of Liverpool, P.O. Box 147, Liverpool L69 3BX

(*Received 25 May 1988 and in revised form 21 November 1988*)

Summary

The frequency and distribution of P elements were investigated in the third chromosomes of two wild-type strains of *Drosophila melanogaster* using *in situ* hybridization of biotinylated probes to the polytene chromosomes. The relationship between these data and the extent of hybrid dysgenesis was determined through assays of egg production, egg hatchability (F_2 embryo lethality), sn^w destabilization and male recombination along the third chromosome. The results suggest that P-element distribution, frequency and structure are all contributory factors in the regulation of hybrid dysgenesis. Texas 6 was shown consistently to be a stronger P strain than Texas 1, eliciting greater reductions in fertility, more extensive sn^w destabilization and higher frequencies of male recombination. Clustering of male recombination events, arising from pre-meiotic crossing over, was evident among the dysgenic progeny of each strain. Male recombination and sn^w destabilization were independently distributed among the dysgenic males studied, suggesting that these traits represent separate P-mediated functions. The third chromosome male recombination maps produced by the two strains differed significantly from each other and from the published female meiotic and polytene chromosome maps. Male recombination breakpoints were associated with the original distribution of P sequences in the two strains and the results suggest that this relationship may be closer for potentially complete P factors than for P sequences in general. An analysis of sub-lines derived from individual recombinant males revealed that chromosomal breakpoints could also be associated with novel insertions following P-element transposition.

1. Introduction

Hybrid dysgenesis is the collective title given to a group of germ line abnormalities observed in the progeny of certain interstrain crosses of *Drosophila melanogaster*. The genetic aberrations observed include reduced male and female fertility, illicit male recombination and enhanced female recombination, increased rates of mutation and chromosomal rearrangement and segregation distortion (Kidwell, Kidwell & Sved, 1977). These traits arise from the activity of transposable elements which move in the germ line but not the soma of the interstrain hybrids.

Two independent transposable element families (P and I) have been shown to give rise to dysgenic phenomena. Of these the best characterized are the P elements which are found in certain *Drosophila melanogaster* strains (P strains) but absent in others (M strains). Dysgenic traits most commonly occur when a P-strain male fertilizes an M-strain female. They can also be generated whenever functional transposase is produced in the absence of normal P-strain regulatory factors, for example during the transformation of an M-cytotype embryo. Characteristically, both male and female progeny are affected (Kidwell, 1979; Engels, 1983).

The P-element family consists of intact 2·9 kb P factors (which encode a *trans*-acting transposase capable of eliciting P-element mobility) and smaller P elements which appear to be derived from the P factor by internal deletion (O'Hare & Rubin, 1983). The P factor has four open reading frames and all are required for the production of a functional transposase. The appropriate splicing events are only carried out in germ line tissues, resulting in the translation of an 87000 Da protein which is thought to be the functional transposase (Rio, Laski & Rubin, 1986). In the soma, failure to splice out the intron between ORF2 and ORF3 results in the translation of a truncated 66000 Da protein which is unable to

mobilize P elements. All P elements possess 31 bp inverted terminal repeats which are thought to be the transposase recognition sites. Thus, both P factors and internally deleted P elements are capable of germ line transposition in the presence of functional transposase. Certain strains of *Drosophila melanogaster* possess only defective P elements. When females of these strains are crossed to P-strain males the resulting hybrid dysgenesis is generally at a reduced level when compared to a standard dysgenic cross. Because of this attenuation such strains are referred to as pseudo-M or M′.

P elements are dispersed among all of the major chromosomes of P strains and tend to show preferential sites of insertion (Engels & Preston, 1980; Bingham *et al.* 1982). The nature of these preferred sites is not fully understood although a degree of sequence specificity has been found for the 8 bp direct duplication of the target DNA at the site of P-element integration (O'Hare & Rubin, 1983). It has also been suggested that the spatial organization of chromosomes within the nucleus might play a part in site specificity (Sved, 1976). Given that *Drosophila* chromosomes exist in non-random configurations within the nuclear envelope (Gruenbaum *et al.* 1984) it could be that insertion sites represent areas of chromosomes which are held in 'visible' or otherwise amenable orientations for transposable element insertion. There is also evidence that transcriptional start sites of genes represent 'hot spots' due to the variation in chromosome structure when they are activated or expressed (Kelley *et al.* 1987).

Although much progress has been made in the characterization of P elements at the molecular level, relatively little is known of the way in which they exert their biological effects. It is not clear whether variation in the extent of dysgenesis is determined quantitatively (by the copy number of P factors or elements), qualitatively (through differences in structure or location) or by a combination of the two. Surveys of a variety of strains indicate that P-sequence copy numbers are highly variable (Engels, 1984; Ronsseray & Anxolabehere, 1986) and there is some evidence that copy numbers may be highest in those strains which generate the more extreme dysgenesis when crossed to M-strain females. It has been suggested that P-element excision and integration results in chromosome breakage which would account for the observed effects on recombination and chromosomal rearrangement (Engels & Preston, 1981). It is also known that P-element insertion mutations can disrupt normal gene expression although the precise mechanisms by which reduced fertility and segregation distortion occur, for example, are not known.

Here we report our investigations into the distribution of P element sequences along the third chromosomes of two wild-type inbred strains of *Drosophila melanogaster*. The gross molecular structure of these elements, as revealed by the use of both intact P-factor and internal P-fragment DNA probes, is related to the production of P–M dysgenic traits among the inter-strain hybrid progeny with particular reference to the frequency and distribution of male recombination events.

2. Materials and methods

(i) *Strains used*

Texas 1 and Texas 6: two long-established wild-type inbred P strains from the Texas population of *Drosophila melanogaster*. This population originated from inseminated females captured in Austin, Texas in 1965 (Linney, *et al.* 1971). The inbred lines were established at that time by repeated single-pair matings and have subsequently been maintained as mass-mated cultures which are subjected to single pair inbreeding every few generations. The extent of inbreeding within these lines is evident from genetic analyses of a range of quantitative characters and isoenzyme loci (Kearsey & Barnes, 1970); Birley *et al.* 1981). *ru h th st cu sr e^s ca:* a standard laboratory stock, classified as M and carrying the following recessive visible markers; *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*, stripe (3–62·0); e^s, ebony-sooty (3–70·7); *ca*, claret (3–100·7). *y; bw; st* (A): an M strain carrying the following recessive visible mutations; *y*, yellow (X–0·0); *bw*, brown (2–104·5); *st*, scarlet (3–44·0). *y sn^w; bw; st/y^+ Y*: an attached Y strain, classified as M′ and kindly donated by M. J. Simmons. In addition to markers mentioned above it carries sn^w, singed-weak (1–21·0) an allele of the *singed* bristle locus used in the sn^w destabilization assay (Engels, 1979; 1984). The y^+ allele carried on the attached *Y* chromosome confers a wild-type body colour on the males of this strain. *y sn^w; bw; ru h th st cu sr e^s ca:* a further M′ strain created by introducing the standard *rucuca* marker chromosome (described above) into the strain *y sn^w; bw; st/y^+ Y*. *y sn^3 v:* a true M strain carrying the visible mutations *y*, yellow (1–0·0); *v*, vermilion (1–33·0) and sn^3 (1–21·0) an independent extreme allele of the *singed* bristle locus. The dominance relationships at this locus are $sn^{(+)} > sn^w > sn^3 = sn^e$ and thus all destabilizations at this locus can be distinguished in heterozygotes with sn^3. 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 hybridization of P-element probes to total genomic DNA. All strains were maintained and experimental crosses raised on a sucrose, yeast, agar medium in either $\frac{1}{3}$ pint milk bottles or $3'' \times 1''$ vials. Fresh yeast suspension was added as a food source and nipagin (15 ml per litre of a 10% solution in ethanol) was added to all cultures to inhibit fungal contamination. All flies were raised at 25 ± 1 °C unless otherwise stated.

(ii) *Measurement of female fertility*

Both reduced egg production and reduced egg hatchability (F_2 embryo lethality) were measured at a developmental temperature of 25 °C. The former estimates the average egg production of groups of dysgenic females and is related to the GD sterility assay of gonadal dysgenesis (Kidwell & Novy, 1979; Eggleston & Kearsey, 1980). Estimates of reduced egg hatchability (embryo lethality) provide a compound measure of lowered F_1 fertility since the females are mated with their brothers and the fertility of these male sibs may also be compromised within the P–M system.

Ten replicate cultures were initiated for both the dysgenic and control crosses at 25 °C. The F_1 progeny were mated together for three days at 25 °C and transferred to fresh culture vials containing plastic partitions coated with charcoal blackened starch paste. The females were allowed to lay overnight to eliminate eggs accumulated *in utero* and egg production measurements were taken by transferring each group of flies (25 ♀♀ and 25 ♂♂) to fresh starch-coated partitions for two consecutive hours at 25 °C. These measurements were routinely taken at four days of age and finished around mid-day. Egg production was recorded in terms of the mean number of eggs laid per female per hour. The egg hatchability of these females was recorded both before and after the measurements of egg production (at 3 and 5 days of age). For each group of females 50 eggs were chosen at random and transferred to charcoal blackened 2% agar in 50 mm shallow form Petri dishes. The mean percentage hatchability was recorded after 48 h.

(iii) *Measurement of male recombination*

Following Eggleston (1984) male recombination was assayed among the progeny of 100 individual dysgenic males using the crossing scheme in Fig. 1. Each male was mated to two broods of 20 virgin females at a developmental temperature of 25 °C. The presence of a recombinant genotype among the progeny of a family was taken to represent a single recombination event. The occurrence of a particular recombinant (or its reciprocal) in more than one individual of a family was treated as a cluster and taken to be derived pre-meiotically from a single independent recombination event.

(iv) *Measurement of sn^w destabilization*

This was carried out essentially as described by Kocur *et al.* (1986). 100 dysgenic males were produced as shown in Fig. 1 and crossed individually to virgin $y\,sn^3\,v$ ♀♀ at a developmental temperature of 25 °C. The female progeny were scored for the presence of the sn^e phenotype. Given that sn^w is assumed to revert to sn^e and $sn^{(+)}$ with equal frequency under dysgenic conditions, such a measure will estimate half of the true mutation rate. Destabilization was recorded as a percentage of the total number of females scored.

(v) *In situ hybridization*

Salivary gland polytene chromosome squashes were prepared from third instar larvae raised in uncrowded conditions at 18 °C. The technique was a modification of that of Pardue & Gall (1975) and Lim & Snyder (1968). The larvae were dissected in Insect Ringer's solution, the salivary glands extracted and the fat bodies carefully removed. The glands were then transferred to a drop of 45% glacial acetic acid and heated at 45 °C for 10 min to soften the nuclear membrane. A Denhardt's slide (Engels *et al.* 1986) was lowered onto the drop and gently tapped from side to side to spread the chromosome arms. The preparation was then frozen over liquid N_2, fixed in ice cold 3:1 (ethanol:glacial acetic acid), dehydrated in 95% ethanol at −20 °C and allowed to warm up to room temperature before air drying. Hybridization was overnight at 37 °C to 20 μl of a solution containing denatured probe DNA labelled by random priming (Feinberg & Vogelstein, 1983) to an incorporation of at least 35% with biotin (Bio-11 dUTP). The hybridization solution also contained deionized formamide and dextran sulphate (Engels *et al.* 1986). The sites of hybridization to polytene bands were visualized using the Vectastain ABC kit (Vector Laboratories, UK) following Hsu, Raine & Fanger (1981) and the chromosomes were stained in 4% phosphate buffered Giemsa. The P-element distribution maps presented here were based on the analysis of approximately 20 third chromosomes for each Texas strain. We found no evidence for polymorphism of P-element insertion sites during this investigation. In addition, the female fertility and sn^w destabilization patterns of the two strains remained stable during the period of approximately 10 months which separated the preparation of the maps and the measurement of male recombination.

(vi) *The construction of pπ Pvu* II

The 2·9 kb P factor, contained within the construct pπ25·1 (Rubin *et al.* 1982) has three *Pvu* II restriction sites, two of which occur within the P factor, separated by 875 bp and spanning parts of ORF1 and ORF2. This fragment was cloned using standard techniques (Maniatis *et al.* 1982) into the *Pvu* II site of the vector pBR328 and the construct (denoted pπ *Pvu* II) used to transform *E. coli* MC1061. Many P elements have deletions spanning this central transposase region and the *Pvu* II fragment has been used previously in attempts to differentiate between intact P factors and internally deleted P elements.

https://doi.org/10.1017/S0016672300028123 Published online by Cambridge University Press

(vii) *The construction of th-st male recombinant sublines*

Recombinant males were generated in a dysgenic cross derived from the P strain Texas 1 as described in section 2(iii) above. Three males which carried breakpoints between the markers *th* and *st* were isolated and individually backcrossed to *ru h th st cu sr* e^s *ca* marker females. Male and female progeny of the appropriate phenotype were mated together and their progeny were subjected to single pair inbreeding with selection to stabilize the recombinant genotype. A restrictive developmental temperature of 18 °C was used throughout to prevent, or at least to minimize, subsequent P-element mobility and recombination in the male germ line. True breeding lines of each genotype ($ru^+ h^+ th^+ st\ cu\ sr\ e^s\ ca$; $ru^+ h^+ th^+ st\ cu\ sr\ e^s\ ca^+$ and $ru^+ h^+ th^+ st\ cu\ sr\ e^{s+}\ ca^+$) were obtained after four generations.

3. Results

Texas 1 and Texas 6 were crossed reciprocally to the M′ strain $y\ sn^w$; *bw*; *st*/y^+ *Y* to determine the ability of each wild type line to elicit P–M hybrid dysgenesis. The egg production of the female F_1 progeny was measured as an assay of female fertility and the hatchability of these eggs (F_2 embryo lethality) recorded. The ability of the F_1 males to destabilize the sn^w allele was also determined. The results of these experiments are shown in Table 1 and it is clear that both Texas 1 and Texas 6 are able to elicit P–M hybrid dysgenesis, although to differing extents. Texas 6 is a stronger P strain than Texas 1, producing higher levels of sn^w destabilization, more embryo lethality and a greater reduction in female fertility.

Following the procedure described by Eggleston (1984) and utilizing the crossing scheme in Fig. 1, dysgenic males were produced in which P elements were restricted to the third chromosome. The ability of these third chromosomes to destabilize the sn^w allele was determined and as expected from the preliminary experiments Texas 6 displayed higher levels of P-element activity than Texas 1. Indeed, the sn^w destabilization recorded for Texas 6 (7·03%) was almost twice that for Texas 1 (3·75%) with each measurement based on samples of around 5000 flies.

The gross levels of male recombination resulting from the P elements on these third chromosomes reveal a similar trend for the two lines (Table 2). Texas 6 is once again the stronger P strain although its male recombination frequency (3·04%) is not quite twice that of Texas 1 (1·84%). Table 2 reveals the effects of clustering which is a feature of P–M dysgenic male recombination (Hiraizumi, 1979; Eggleston, 1984).

Table 1. *Extent of hybrid dysgenesis elicited by the Texas lines*

	Texas 1	Texas 6
Egg hatchability		
Cross A	59·51 ± 11·09***	36·32 ± 13·85***
Cross B	79·72 ± 6·72	80·84 ± 5·89
Egg production		
Cross A	2·15 ± 1·37 N.S.	0·75 ± 0·81***
Cross B	2·29 ± 1·50	2·76 ± 1·50
sn^w destabilization		
Cross A	5·42%	46·80%
Cross B	0·00%	0·00%

Mean percentage egg hatchability (transformed to angles) and mean egg production (eggs per female per hour, transformed to square roots) at 25 °C, for Texas 1 and Texas 6 when crossed reciprocally to the M′ strain $y\ sn^w$; *bw*; *st*;/y^+ *Y*. In each case the dysgenic cross (Texas male parent) is referred to as Cross A and the normal reciprocal as Cross B. F_1 males from each cross were subsequently tested for their ability to destabilize the sn^w allele (see text for details). The results of an analysis of variance testing the differences between the Cross A and Cross B data are given as follows: N.S., $P > 0{\cdot}05$; *$0{\cdot}05 > P > 0{\cdot}01$; **$0{\cdot}01 > P > 0{\cdot}001$; *** $P < 0{\cdot}001$.

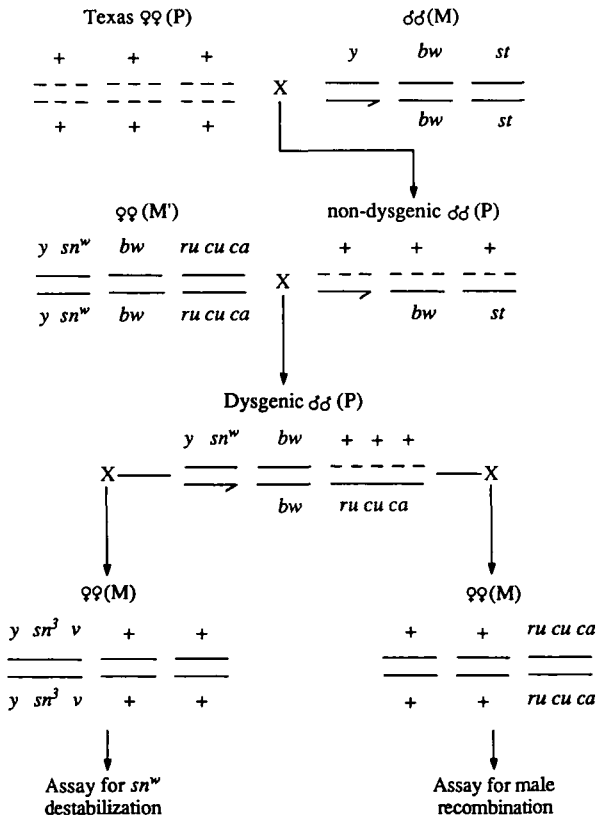

Fig. 1. Crossing scheme for the production of P–M dysgenic males in which all P sequences (mobilized by the cross to M′ cytotype females) are restricted to the third chromosome of the wild-type inbred strains Texas 1 and Texas 6. The fourth chromosomes of these strains are devoid of P elements. Chromosomes carrying P elements derived from the Texas strains are represented by broken lines. The third chromosome of the marker strain carrying the recessive mutations *ru h th st cu sr* e^s and *ca* [see Section 2(i)] is abbreviated to *ru cu ca*. Appropriate test crosses for the assays of male recombination and sn^w destabilization are shown.

https://doi.org/10.1017/S0016672300028123 Published online by Cambridge University Press

Table 2. *P–M dysgenic male recombination frequencies*

	Texas 1	Texas 6
Total number of progeny scored	18802	13295
Total recombination events	348	406
Total independent recombination events	242	279
Recombination frequency (gross)	1·84%	3·04%
Recombination frequency (corrected)	1·28%	2·08%

Male recombination frequencies arising from P-element arrays on the third chromosomes of Texas 1 and Texas 6. Pre-meiotically derived clusters among the progeny of individual males are treated as a single independent recombination event in the calculation of corrected frequencies. See text for further details.

The number of independent male recombination events [section 2(iii)] can be employed to take such clusters into account, yielding recombination frequencies of 2·08% for Texas 6 and 1·28% for Texas 1. These figures represent reductions of 32 and 30% from the gross unadjusted frequencies for Texas 6 and Texas 1 respectively. Both strains therefore have the ability to elicit pre-meiotic crossing over, resulting in recombination events which are amplified through mitotic divisions and consequently highly represented in the sperm pool after meiosis. This is confirmed by a t test comparing the difference between the mean number of recombination events ($\bar{x}_1$) and the mean number of independent recombination events ($\bar{x}_2$) for each strain. For Texas 1 we obtain $\bar{x}_1 = 3{\cdot}94$, $\bar{x}_2 = 2{\cdot}71$, $t = 2{\cdot}54$, $0{\cdot}05 > P > 0{\cdot}01$* and for Texas 6 $\bar{x}_1 = 4{\cdot}38$, $\bar{x}_2 = 2{\cdot}94$, $t = 3{\cdot}67$, $P < 0{\cdot}001$***. There was no evidence for a significant difference in the mean cluster sizes of the two strains (2·38 and 2·73 for Texas 6 and Texas 1 respectively; $t = 1{\cdot}22$, $P > 0{\cdot}05$). However, three Texas 1 families produced unusually large clusters, involving 7, 7 and 8 recombinants, which were not apparent among the Texas 6 families.

Measurements of sn^w destabilization and male recombination were obtained independently for each of the dysgenic males tested. For Texas 1 (and Texas 6; figures in parentheses) 79 (75) dysgenic males, bearing P elements on the wild-type third chromosome, were analysed. Of these, 8 (4) induced neither trait, 26 (10) induced male recombination only, 19 (12) induced sn^w destabilization only and 26 (49) induced both traits. The males in the latter group were ranked with respect to the number of independent male recombination events and the percentage sn^w destabilization they produced. No significant correlation was found between these rankings ($r_s = 0{\cdot}016$, $P > 0{\cdot}05$ and $r_s = 0{\cdot}091$, $P > 0{\cdot}05$ for Texas 6 and Texas 1 respectively) suggesting independence in the regulation of these two measures of P-element transposition.

The distribution of male recombination events along the third chromosome of each strain is shown in Fig. 2 by means of the proportion of independent events occurring between adjacent pairs of markers. The male recombination maps for Texas 1 (Fig. 2*c*) and Texas 6 (Fig. 2*d*) differ significantly from the standard maps (Fig. 2*a*, *b*) and from each other, particularly for the left chromosome arm. It is clear that the number of recombination events does not depend solely on the physical distance between markers. The polytene chromosome map distance between the *roughoid* and *hairy* markers, for example, is approximately ten times greater than the Texas 1 male recombination map distance for the same region (Fig. 2*b*, *c*). This chromosome interval and, indeed, the whole of the left arm of the Texas 1 third chromosome appears to be refractory to P-mediated male recombination. There is no evidence from the analysis of polytene preparations that this low recombination frequency is due to an inversion or other gross chromosomal defect.

The relationship between male recombination breakpoints and the distribution of P elements was investigated by *in situ* hybridization (Fig. 3). The pπ25·1 probe, which includes the intact 2·9 kb P factor, will hybridize to all P-homologous sequences and is therefore unable to distinguish between complete P factors and deleted P elements. The pπ*Pvu* II

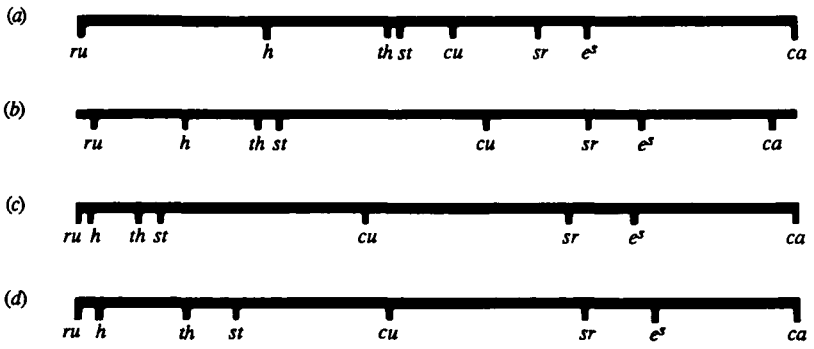

Fig. 2. Maps of the third chromosome based on the proportion of independent male recombination events occurring between adjacent markers for Texas 1 (*c*) and Texas 6 (*d*). Comparable maps resulting from female meiotic recombination (*a*) and cytological preparations of polytene chromosomes (*b*) are included for comparison.

https://doi.org/10.1017/S0016672300028123 Published online by Cambridge University Press

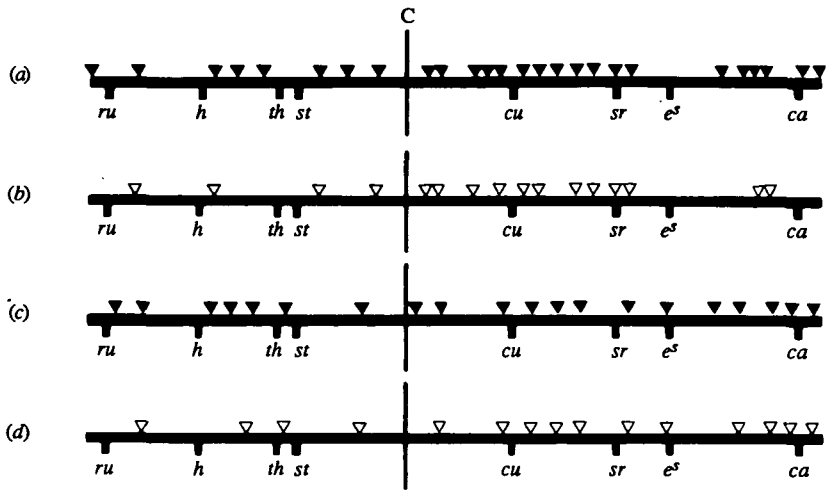

Fig. 3. Diagrammatical representation of P-element distributions along the third chromosome superimposed on the standard polytene chromosome map. (*a*) Texas 1 probed with pπ25·1; (*b*) Texas 1 probed with pπ*Pvu* II; (*c*) Texas 6 probed with pπ25·1 and (*d*) Texas 6 probed with pπ*Pvu* II. Sites of homology to pπ25·1 and pπ*Pvu* II are represented by solid and open triangles respectively. The centromere is shown by the letter C.

probe, however, will not hybridize to P elements with deletions spanning the central transposase coding region from which it was derived. Since this region is deleted in many P elements, the use of these two probes can distinguish to a certain extent between P factors and P elements. However, those elements with deletions which do not entirely exclude the *Pvu* II region will hybridize to both probes.

Although Texas 6 has been shown to be a stronger P strain than Texas 1 the data in Fig. 3 indicate that dysgenic activity is not simply related to the number of P element sites. The third chromosome of Texas 1, for example, has 26 sites of homology to pπ25·1 (Fig. 3*a*) compared with 20 for that of Texas 6 (Fig. 3*c*). Moreover, although as expected the number of sites detected by pπ*Pvu* II is less than that for pπ25·1 the difference between the two strains in the number of putative P factors is negligible with 16 and 15 for Texas 1 (Fig. 3*b*) and Texas 6 (Fig. 3*d*) respectively. However, there does appear to be a relationship

Table 3. *Analysis of P-element distribution in relation to third chromosome maps*

Chromosome interval	Number of P elements (A)				Male recombination events (B)		Female meiotic map (C)	Polytene chromosome map (D)
	pπ25·1		pπ*Pvu* II					
	Texas 1	Texas 6	Texas 1	Texas 6	Texas 1	Texas 6		
ru-h	1 (6)	2 (5·5)	1 (5·5)	1 (6)	1·76 (7)	2·86 (7)	26·0 (2)	14·3 (3)
h-th	3 (4)	3 (3·5)	1 (5·5)	1 (6)	6·69 (5)	12·70 (4)	17·5 (3)	12·9 (4)
th-st	0 (7)	1 (7)	0 (7)	1 (6)	3·17 (6)	6·98 (6)	1·0 (7)	2·6 (7)
st-cu	8 (1)	4 (1·5)	6 (1)	3 (2)	28·17 (2)	21·59 (2)	7·0 (6)	38·1 (1)
cu-sr	5/6 (2)	3 (3·5)	4/5 (2)	3 (2)	28·87 (1)	27·94 (1)	12·0 (4)	10·7 (5)
sr-e^s	1/2 (5)	2 (5·5)	1/2 (4)	2 (4)	9·86 (4)	9·52 (5)	7·5 (5)	6·9 (6)
e^s-ca	4 (3)	4 (1·5)	2 (3)	3 (4)	21·48 (3)	18·41 (3)	29·0 (1)	14·5 (2)
r_s (A–B)	0·893**	0·771*	0·901**	0·849*				
r_s (A–C)	0·107 N.S.	0·385 N.S.	0·054 N.S.	0·077 N.S.				
r_s (A–D)	0·642 N.S.	0·826*	0·595 N.S.	0·463 N.S.				

The number of P elements and putative P factors (as defined by the probes pπ25·1 and pπ*Pvu* II respectively) and the proportion of independent male recombination events found for each chromosome interval in the third chromosomes of Texas 1 and Texas 6. The figures in parentheses refer to the rank of each observation. The relationships between these variables are illustrated by rank correlation coefficients, each for 5 degrees of freedom, such that r_s (A–B) represent the rank correlation between the number of P elements and the proportion of independent male recombination events found for a given chromosomal region in a given strain. Similar coefficients reveal the correlation of P element distribution with proportional female meiotic map distance (r_s, A–C) and proportional polytene chromosome map distance (r_s, A–D). Statistical tests of significance are given as in Table 1. The ambiguity over the number of P-element sites recorded for the (*cu-sr*) and (*sr-e^s*) regions of the Texas 1 third chromosome results from the detection of P sequence homology within the cytologically defined *sr* locus itself.

https://doi.org/10.1017/S0016672300028123 Published online by Cambridge University Press

between P-element location and the distribution of male recombination breakpoints. The regions with the most sites of P homology are also those where most male recombination occurs. This can be verified statistically using Spearman's rank correlation test (Snedecor & Cochran, 1980) the results of which are shown in Table 3. All of the coefficients obtained in this way are statistically significant, indicating that the observed relationships between P element location and male recombination breakpoint are not due to chance. Furthermore, it can be seen that higher rank correlations are obtained in each case for sites of pπ*Pvu* II homology in comparison with that for pπ25·1 (Table 3). Although these differences are not statistically significant the trend suggests that putative P factors identified by pπ*Pvu* II are more closely associated with the distribution of male recombination events than the gross sites of P-element homology detected by pπ25·1. Similar rank correlation coefficients between P-element distribution and female meiotic or polytene preparation map distances are not significant in all but one case (Table 3).

The data show clearly that male recombination breakpoints can occur at sites originally devoid of P-element homology. For example, low levels of male recombination were recorded between the markers *thread* and *scarlet* for Texas 1 and yet no P-sequence homology was detected in this region. In order to investigate this phenomenon a series of sub-lines were established from individual recombinant males derived from Texas 1 and displaying breakpoints between the markers *thread* and *scarlet*. One of these ($ru^+ h^+ th^+ st\, cu\, sr\, e^s\, ca^+$) was subjected to an *in situ* analysis of the third polytene chromosome using the probe pπ25·1. This revealed the presence of a novel site located between the markers *thread* and *scarlet* which must have arisen through transposition following P-element mobilization in the dysgenic cross. This novel insertion event provides strong evidence that P element transposition was involved in the generation of male recombination breakpoints in this sub-line.

4. Discussion

The experiments presented here describe the relationships between the frequency and distribution of P elements and male recombination events along the third chromosomes of Texas 1 and Texas 6. The inbred lines were first tested for their ability to elicit P–M hybrid dysgenesis, acting as paternal strains in a dysgenic cross. Texas 6 was shown to be a stronger P strain than Texas 1, producing greater reductions in fertility and more pronounced destabilization of the sn^w allele. Using a mating scheme to limit P elements to the third chromosome resulted in the same superiority of Texas 6 although the overall levels of dysgenesis were less than those observed for the whole genome. The P elements on the third chromosome of Texas 6 gave rise to higher frequencies of male recombination and sn^w destabilization than those on the third chromosome of Texas 1. The male recombination maps produced by the two strains differed significantly from each other and from the standard third chromosome maps derived from female meiotic recombination and cytological preparations of polytene chromosomes. Pre-meiotically derived clusters of male recombinants were evident among the dysgenic progeny of each strain and there was some evidence that Texas 1 was able to produce a limited number of unusually large clusters. This may reflect differences in the timing of male recombination events, mediated by variations in the P-sequence arrays of the two strains.

No relationship could be found between the extent of sn^w destabilization and the number of independent male recombination events elicited by individual dysgenic males. In many cases a single male would induce one trait to the exclusion of the other and no significant rank correlation was evident among those males which induced both traits. Male recombination and sn^w destabilization are both used as assays of P-element transposition and the results presented here suggest that they are separately regulated and provide measures of different P-mediated functions.

The distribution of male recombination events is not primarily determined by the physical distance between marker loci, but is affected by the frequency and distribution of P sequences within the genome. *In situ* hybridization techniques have been used to study sites of P-element homology and have revealed quite different distributions for Texas 1 and Texas 6. These have both quantitative and qualitative roles in the regulation of dysgenic traits. Texas 6, for example, is a stronger P strain than Texas 1 and yet has fewer P elements on the third chromosome and in the genome as a whole. Texas 1 has 55 sites of P homology per haploid genome compared with 52 for Texas 6 (Exley & Eggleston, unpublished data). However, a quantitative effect was evident from the reduction in the extent of dysgenesis when P elements were limited to the third chromosome. The data suggest that the number, location and structure of P elements are all contributory factors in the regulation of dysgenesis. A similar conclusion was made by Engels *et al.* (1987) with respect to P–M dysgenic pupal lethality.

The probe pπ*Pvu* II was used in an attempt to distinguish between complete and deleted P elements. For Texas 1, 38% of the pπ25·1 sites did not hybridize to pπ*Pvu* II compared with 25% for Texas 6. This suggests that a greater proportion of the P elements in the genome of Texas 1 are defective and is consistent with the hypothesis of transposase titration by non autonomous P elements (Simmons *et al.* 1987). The reduced levels of P–M dysgenesis in Texas 1 would then be consistent with a reduction in the availability of functional transposase following binding to the inverted terminal repeats of the defective elements.

The results presented in this paper suggest that chromosome breakage resulting in male recombina-

https://doi.org/10.1017/S0016672300028123 Published online by Cambridge University Press

tion might be the product of a number of different P mediated processes. Transposition is an obvious candidate although spontaneous breaks could also arise from altered stresses placed on the DNA helix or higher order chromatin structure by the presence of a P element. It has been suggested (Engels & Preston, 1984) that the obvious site for chromosome breakage would be at the end of a P element, or at both ends, allowing excision of the element in addition to chromosomal rearrangement. We have found a clear relationship between the original distribution of P sequences in the Texas strains and that of the male recombination breakpoints subsequently generated. However, our experiments are unable to locate chromosome breakage precisely at the ends of the P element. The detection of a novel P-element site at or near to the Texas 1 *th-st* male recombination breakpoint lends strong support to the involvement of P-element transposition in the generation of chromosome breaks. Such transposition events would involve both excision and integration of P sequences, either of which could generate chromosome breakage and lead to a recombination event. Although the novel *th-st* P-element site provides direct evidence for transposition we are unable to determine whether this represents a conservative or replicative process. The total number of P-element sites detected in the *th-st* sub-line was approximately the same as in the original Texas strain from which it was derived. Many of these sites were shared with the progenitor strain but the apparent loss of certain sites and the gain of at least one novel site suggests a largely conservative transposition mechanism. However, this analysis was based on a relatively small sample and a more detailed investigation of this and similar lines is required.

The rank correlation coefficients presented in Table 3 suggest that there may be a closer relationship between the distribution of male recombination events and sites of pπ*Pvu* II homology than that found for pπ25·1. This difference was not statistically significant for either strain but if it is found to represent a consistent trend than it would suggest that putative intact P factors are more closely associated with male recombination breakpoints than are P sequences in general.

We gratefully acknowledge the financial support of the SERC (Studentship to K. A. S.) and the Lister Institute of Preventive Medicine (Research Fellowship to P. E.). Thanks are due to Andy Leigh-Brown and Tony Shrimpton (Department of Genetics, Edinburgh) for invaluable advice on *in situ* techniques and the use of biotinylated probes. We are also grateful to Joachim Rodrigues de Miranda for helpful discussion, Lynda Mitchell for technical assistance and Philip McIntrye for photographic expertise.

References

Bingham, P. M., Kidwell, M. G. & Rubin, G. M. (1982). The molecular basis of P–M hybrid dysgenesis: the role of the P element, a P strain specific transposon family. *Cell* **29**, 995–1004.

Birley, A. J., Couch, P. A. & Marson, A. (1981). Genetical variation for enzyme activity in a population of *Drosophila melanogaster*. VI. Molecular variation in the control of alcohol dehydrogenase (Adh) activity. *Heredity* **47**, 185–196.

Caligari, P. D. S. & Mather, K. (1980). Dominance, allele frequency and selection in a population of *Drosophila melanogaster*. *Proceedings of the Royal Society of London* **B 208**, 163–187.

Eggleston, P. & Kearsey, M. J. (1980). Hybrid dysgenesis in *Drosophila*: correlation between dysgenic traits. *Heredity* **44**, 237–249.

Eggleston, P. (1984). Hybrid dysgenesis in *Drosophila melanogaster*: the frequency and distribution of male recombination events. *Heredity* **52**, 189–202.

Engels, W. R. (1979). Extrachromosomal control of mutability in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences, USA* **76**, 4011–4015.

Engels, W. R. (1983). The P family of transposable elements in *Drosophila*. *Annual Review of Genetics* **17**, 315–344.

Engels, W. R. (1984). A *trans*-acting product needed for P-factor transposition in *Drosophila*. *Science* **226**, 1194–1196.

Engels, W. R., Benz, W. K., Preston, C. R., Graham, P. L., Phillis, R. W. & Robertson, H. M. (1987). Somatic effects of P element activity in *Drosophila melanogaster*: pupal lethality. *Genetics* **117**, 745–757.

Engels, W. R. & Preston, C. R. (1980). Components of hybrid dysgenesis in a wild population of *Drosophila melanogaster*. *Genetics* **95**, 111–128.

Engels, W. R. & Preston, C. R. (1981). Identifying P factors in *Drosophila* by means of chromosome breakage hotspots. *Cell* **26**, 421–428.

Engels, W. R. & Preston, C. R. (1984). Formation of chromosome rearrangements by P factors in *Drosophila*. *Genetics* **107**, 657–678.

Engels, W. R., Preston, C. R., Thompson, P. & Eggleston, W. R. (1986). *In situ* hybridisation to *Drosophila* salivary chromosomes with biotinylated DNA probes and alkaline phosphatase. *BRL Focus* **8**(1), 6–8.

Feinberg, A. P. & Vogelstein, B. (1983). A technique for radio-labelling DNA restriction and endonuclease fragments to high specific activity. *Analytical Biochemistry* **137**, 266–267.

Gruenbaum, Y., Hochstrasser, M., Mathog, D., Saumweber, H., Agard, D. A. & Sedat, J. W. (1984). Spatial organisation of the *Drosophila* nucleus: a three dimensional cytogenetic study. In: *Higher Order Structure in the Nucleus* (ed. P. R. Cook and R. A. Laskey). *Journal of Cell Science*, Supplement 1, 223–234.

Hiraizumi, Y. (1979). A new method to distinguish between meiotic and premeiotic recombinational events in *Drosophila melanogaster*. *Genetics* **92**, 543–554.

Hsu, S. M., Raine, L. & Fanger, H. (1981). The use of avidin–biotin–peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between (ABC) and unlabelled antibody (PAP) procedures. *Journal of Histochemistry and Cytochemistry* **29**, 577–581.

Kelley, M. R., Kidd, S., Berg, R. L. & Young, M. W. (1987). Restriction of P element insertions at the *notch* locus of *Drosophila melanogaster*. *Molecular and Cellular Biology* **7**, 1545–1548.

Kidwell, M. G. (1979). Hybrid dysgenesis in *Drosophila melanogaster*: the relationship between the P–M and I–R interaction systems. *Genetical Research* **33**, 205–217.

Kidwell, M. G., Kidwell, J. F. & Sved, J. A. (1977). Hybrid dysgenesis in *Drosophila melanogaster*: a syndrome of aberrant traits including mutation, sterility and male recombination. *Genetics* **86**, 813–833.

Kidwell, M. G. & Novy, J. B. (1979). Hybrid dysgenesis in *Drosophila melanogaster*: sterility resulting from gonadal dysgenesis in the P–M system. *Genetics* **92**, 1127–1140.

Kocur, G. J., Drier, E. A. & Simmons, M. J. (1986). Sterility and hypermutability in the P–M system of hybrid dysgenesis in *Drosophila melanogaster*. *Genetics* **114**, 1147–1163.

Lim, J. K. & Snyder, L. A. (1968). The mutagenic effects of two monofunctional alkylating chemicals on natural spermatozoa of *Drosophila*. *Mutation Research* **6**, 127–137.

Lindsley, D. L. & Grell, E. H. (1968). Genetic variations of *Drosophila melanogaster*. *Carnegie Institution of Washington*, Publication 627.

Linney, R., Barnes, B. W. & Kearsey, M. J. (1971). Variation for metrical characters in *Drosophila* populations. *Heredity* **27**, 163–174.

Manniatis, T., Fritch, E. F. & Sambrook, J. (1982). *Molecular Cloning: A Laboratory Manual*. New York: Cold Spring Harbor Laboratory.

O'Hare, K. & Rubin, G. M. (1983). Structures of P transposable elements and their sites of insertion and excision in the *Drosophila melanogaster* genome. *Cell* **34**, 25–35.

Pardue, M. L. & Gall, J. G. (1975). Nucleic acid hybridization to the DNA of cytological preparations. *Methods in Cell Biology* **10**, 1–17.

Rio, D. C., Laski, F. A. & Rubin, G. M. (1986). Identification and immunochemical analysis of biologically active *Drosophila* P element transposase. *Cell* **44**, 21–32.

Ronsseray, S. & Anxolabehere, D. (1986). Chromosomal distribution of P and I transposable elements in a natural population of *Drosophila melanogaster*. *Chromosoma* **94**, 433–440.

Rubin, G. M., Kidwell, M. G. & Bingham, P. M. (1982). The molecular basis of P–M hybrid dysgenesis: the nature of induced mutations. *Cell* **29**, 987–994.

Simmons, M. J., Raymond, J. D., Boedigheimer, M. J. & Zunt, J. R. (1987). The influence of non-autonomous P elements on hybrid dysgenesis in *Drosophila melanogaster*. *Genetics* **117**, 671–685.

Snedecor, G. W. & Cochran, W. G. (1980). *Statistical Methods*, 7th edidion, pp. 191–193. Iowa State University Press.

Sved, J. A. (1976). Hybrid dysgenesis in *Drosophila melanogaster*: a possible explanation in terms of spatial organisation of chromosomes. *Australian Journal of Biological Sciences* **29**, 375–388.