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Localization of P elements, copy number regulation, and cytotype determination in *Drosophila melanogaster*

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Summary

Seventeen highly-inbred lines of *Drosophila melanogaster* extracted from an M′ strain (in the P/M system of hybrid dysgenesis) were studied for their cytotype and the number and chromosomal location of complete and defective P elements. While most lines were of M cytotype, three presented a P cytotype (the condition that represses P-element activity) and one was intermediate between M and P. All lines were found to possess KP elements and only eight to bear full-sized P elements. Only the lines with full-sized P elements showed detectable changes in their P-insertion pattern over generations; their rates of gain and of loss of P-element sites were equal to 0·12 and 0·09 per genome, per generation, respectively. There was no correlation between these two rates within lines, suggesting independent transpositions and excisions in the inbred genomes. The results of both Southern blot analysis and *in situ* hybridization of probes made from left and right sides of the P element strongly suggested the presence of a putative complete P element in region 1A of the X chromosome in the three lines with a P cytotype; the absence of P copy in this 1A region in lines with an M cytotype, favours the hypothesis that the P element inserted in 1A could play a major role in the P-cytotype determination. Insertion of a defective 2 kb P element was also observed in region 93F in 9 of the 13 M lines. The regulation of the P-element copy number in our lines appeared not to be associated with the ratio of full-length and defective P elements.

1. Introduction

A knowledge of the mechanisms that regulate mobile element copy number in the genome is of considerable importance for evolutionary considerations, but these mechanisms are not yet fully understood. Recently it was shown in *Drosophila melanogaster* that the retrovirus-like transposable elements copia (Emori *et al.* 1985) and mdg-1 (Ilyin *et al.* 1980; Tchurikov *et al.* 1981), and the I element (Fawcett *et al.* 1986; Di Nocera & Casari, 1987), which all have a DNA sequence for reverse transcriptase, differ in their copy number distribution (Biémont, 1986; Ronsseray & Anxolabéhère, 1986; Ronsseray *et al.* 1989; Leigh Brown & Moss, 1987; Biémont & Gautier, 1988) and regulation from the transposons P and hobo which code for a transposase (O'Hare & Rubin, 1983; Streck, MacGaffey & Beckendorf, 1986). The copy number of the former elements is submitted to a genomic control, i.e. there is a compensatory effect among chromosome arms for the number of insertions (if one or more chromosome arms have many copies of an element, other chromosomes have few copies) (Biémont & Gautier, 1988); and a correlation between the number of copies of these families was observed. With the P and hobo elements, however, lines with high or low number of insertions on all the chromosome arms have been observed, and contrary to the retrovirus-like elements, these two elements appeared to be independently regulated (Biémont, Gautier & Heizmann, 1988). One aspect of P-element regulation is, however, that this regulation involves non-autonomous defective elements derived from complete elements by internal deletions (Simmons & Bucholz, 1985; Simmons *et al.* 1987; Black *et al.* 1987), suggesting that total P-copy number in a genome may depend on a combination of full-sized and defective P elements (Black *et al.* 1987; Boussy *et al.* 1988; Kidwell, Kimura & Black, 1988).

P-transposable elements are involved in the so-called P–M hybrid dysgenesis phenomenon (Kidwell *et al.* 1977; Brégliano & Kidwell, 1983; for a review see Engels, 1989), which results from crossing a male of a P strain (paternally contributing), having a number of complete P elements which may be

dispersed over all major chromosome arms, with a female of an M strain (maternally contributing). The dysgenesis syndrome consists of several germline abnormalities arising from P-element activity, including mutations, chromosomal rearrangements, and sterility due to gonadal dysgenesis. All P strains manifest a condition called the P cytotype that brings about repression of P-element activity. M strains manifest the M cytotype (they lack regulatory ability). The term 'cytotype' refers to a particular cellular state that can be passed on through the maternally derived cytoplasm and whose long-term determinants are the P elements themselves (Engels, 1979; Engels & Preston, 1979).

The vast majority of *Drosophila melanogaster* populations found in the wild in Europe, Asia and South East Australia have an M cytotype (Anxolabéhère *et al.* 1985; Boussy *et al.* 1988). These populations contain sequences homologous to P elements, a few of which may be full-sized, but most of which are heterogeneous in size because of internal deletions (Todo *et al.* 1984; Anxolabéhère *et al.* 1985; Black *et al.* 1987). These strains are called pseudo-M or M′.

In the present work we have analysed 17 highly-inbred lines (sib crossed) of *Drosophila melanogaster* for their cytotype and for their number and chromosomal location of full-sized and defective P elements estimated by *in situ* hybridization and Southern blots.

2. Materials and methods

(i) *Reference population and inbred lines*

Harwich. A strong P inbred strain, originating from Kidwell & Novy (1979).

Canton-S. a long-established laboratory strain that is devoid of P elements and is classified as true M.

The Lerik population and inbred lines. A laboratory population of *Drosophila melanogaster* was established with about 50 flies captured in Lerik (Azerbaïdjan, USSR) at the end of 1983. The population so formed was maintained en masse in the laboratory at 25 °C before 17 inbred lines were established in October 1984. The 17 initial pairs of flies were randomly taken from the population. The lines were then maintained by one sib pair every generation; six sib pairs were individually isolated for each line, every generation, and for a given line, the sib pair that was considered for obtaining the following generation was taken at random among those that were fertile. To avoid losing some of the lines with very low viability, these lines were maintained en masse (ten couples per generation) after the 100th generation. At generations 84–89 and 116 each inbred line was analysed for the number and location of the P elements in its genome by *in situ* hybridization, and the insertions patterns were compared to those obtained previously at generation 52 (Biémont & Gautier, 1988). The kinds of P elements (complete and incomplete) were determined by Southern blots at generations 89 and 116.

(ii) *Gonadal dysgenesis (GD) tests*

The phenotypic characteristics of each line were determined at generations 62 and 117 by the standard crosses as proposed in Kidwell (1983).

Cross A: 30 females from the true M strain Canton-S were crossed en masse with 30 males from the line tested. This cross determined P activity potential, i.e. the ability of the P elements of the tested strain to induce dysgenesis.

*Cross A**: 30 males from the strong P-strain Harwich were crossed en masse with 30 females of the line tested. This cross determined the ability of the line to regulate P-element activity.

Progenies from both crosses were allowed to develop at 29 °C, and gonadal sterility was measured directly by dissecting around 50 2-day-old F1 females. Dissected ovaries were scored as unilateral (S1 type) or bilateral (S0 type) dysgenic ovaries. The frequency of dysgenic ovaries was thus calculated as % GD = % S0 + $\frac{1}{2}$% S1.

A strain was classified as P cytotype if it produced less than 5% sterility in the cross-A* progeny. A strain was classified as M cytotype if it produced less than 5% sterility in cross-A progeny but greater than 5% in cross-A* progeny. In this A* cross, true M strains showed 100% sterility whilst the M′ strains showed varying degrees of sterility from 5 to 100% (Kidwell, 1985; Anxolabéhère, Kidwell & Périquet, 1988).

(iii) *DNA preparation, restriction enzyme digestion and Southern blot analysis*

At generations 89 and 116, DNA was extracted from 100 flies per line using the method described by Junakovic *et al.* (1984). Restriction enzyme digestion of about 5 μg of DNA was performed according to supplier's instructions. After gel electrophoresis bi-directional transfers were done on nylon filters (Biodyne, Pall) which were then submitted to successive hybridization-dehybridization rounds. Filters were prehybridized 2 h at 65 °C in solution containing 5× Denhart, 5× SSPE (20× SSPE is 3·6 M-NaCl, 0·2 M-Na phosphate pH 8·3, 0·02 M-EDTA), 0·2% SDS and 500 μg/ml denatured salmon sperm. They were then hybridized overnight at 65 °C in similar mixture in which ^{32}P-labelled DNA probe was added. Two post hybridization washes of 30 min each were done in 5 mM-Na phosphate pH 7, 1 mM-EDTA, 0·2% SDS. The filters were autoradiographed in presence of two intensifying screens with Kodak XAR film.

At generation 89 the probes consisted of restriction fragments of pπ 25·7 Bwc (Fig. 1) eluted from agarose

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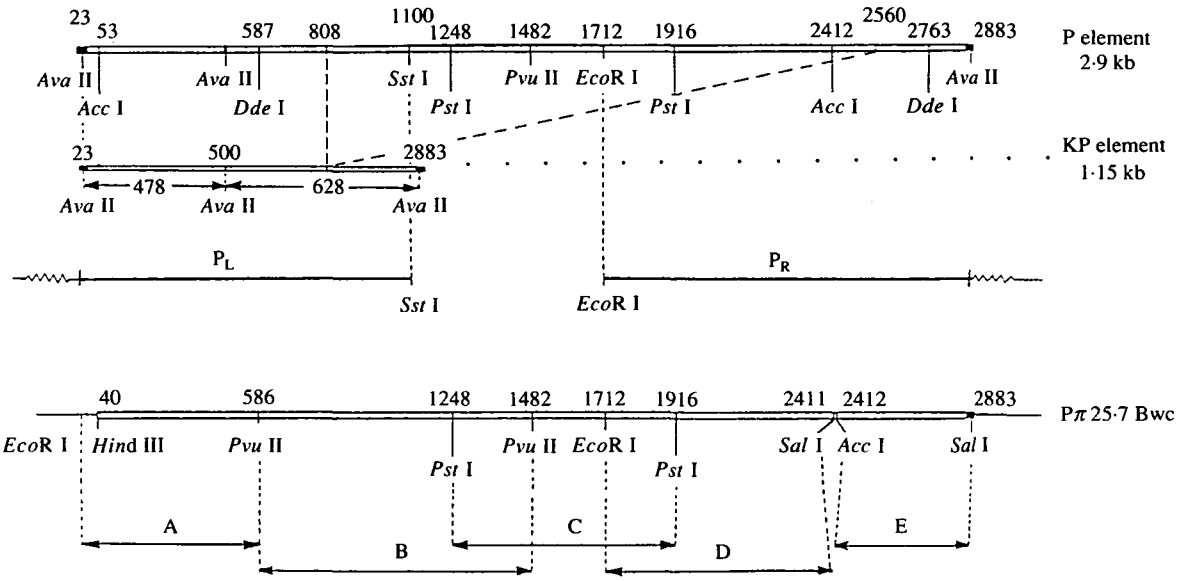

Fig. 1. Structures of P elements in plasmids pπ 25·1 and pπ 25·7 Bwc, and of probes used in *in situ* hybridization and in Southern blots. The plasmid pπ 25·7 Bwc ('both wings clipped') contains a P element that lacks 39 bp from its left end, 23 bp from its right end, and has no flanking genomic DNA; this plasmid was constructed by O'Hare. We present the KP element with its *Ava* II sites and the restriction fragments generated by them (Black *et al.* 1987). Dotted lines indicate the internal deletion that distinguishes the KP elements from a complete P element. The probes A, B, C, D and E were used in the Southern blot analysis and labelled with ^{32}P (the A probe includes 29 bp of pBR322 DNA). The P_L and P_R probes were labelled with biotin and used in the *in situ* hybridization. The P_L DNA sequence includes the left part of the P element (thick bar) plus about 1 kb of genomic DNA (zigzag line) and around 4 kb of pBR322 (thin bar). The P_R probe includes the right part of the P element (thick bar) plus around 900 bp of genomic DNA (zigzag line) and 375 bp of pBR322 (thin bar). The pBR322 DNA was cut with *Eco*R I.

gel by the squeeze freeze technique (Tautz & Renz, 1983) and purified by gene clean (BIO 101). At generation 116 *Acc*I digests of genomic DNA were probed with the plasmid pπ 25·1 as in Boussy *et al.* (1988).

(iv) In situ *hybridization*

Polytene chromosome spreads from salivary glands of third instar larvae taken directly from the inbred lines were prepared and treated with nick-translated, biotinylated DNA probes, as previously described (Biémont, 1986). Two to six larvae were analysed per inbred line (we analysed a high number of larvae when a high rate of changes in the insertion pattern of the P elements was detected). We used pπ 25·1 (O'Hare & Rubin, 1983) to detect all kinds of P elements, and a left part and a right part obtained by a *Sst* I–*Eco*R I digest to detect defective P elements (see Fig. 1). The genomic DNA, of around 1 kb long, associated with each probe, hybridized to the 17C region on the X chromosome (O'Hare & Rubin, 1983); a signal at this 17C region was thus used as positive control indicator of hybridization.

3. Results

(i) *Gonadal dysgenesis (GD) in lines*

As seen in Table 1, at generation 62 the lines 10, 14 and 18, which gave 0% sterility in cross-A* progeny, were classified as P cytotype; all the other lines except no. 7 gave nearly 100% sterility in this cross and were therefore of M cytotype. The F1 females from the crosses involving line 7 showed great variation in the frequency of sterility, indicating that the line 7 had a cytotype intermediate between M and P. The sterility frequencies with cross A GD were all very low (under 5%) as expected for M′ strains, showing thus the absence of a significant P activity potential. Similar results were obtained at generation 117, indicating thus that the cytotype characteristics of the lines had not changed significantly between the beginning (generation 62) and the end (generation 117) of the experiment.

(ii) *Types of P elements revealed by Southern blots*

We have investigated the composition of the P-element population in the 17 lines by genomic restriction analysis. Because the autonomous P elements and the KP elements have been implicated in the suppression of P-induced hybrid dysgenesis, we searched for their presence in a qualitative way. Two sets of genomic DNA were separately digested with *Acc* I and *Dde* I endonucleases. After electrophoresis, each DNA set was submitted to a bidirectional transfer. The twin filters corresponding to the *Acc* I digest were separately probed with probes A and B (Fig. 1) and rehybridized with probes C and D. Similarly, the twin filters corresponding to the *Dde* I

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Table 1. *Cytotype determination of the 17 inbred lines, and types of P elements revealed by Southern blot analysis. The values correspond to the percentage of dysgenic ovaries in F1 females from crosses A and A*. See the Material and Methods section for meaning of crosses A and A**

Lines	Crosses				Cytotype		Types of elements		
	A^*_{62}	A^*_{117}	A_{62}	A_{117}	62	117	Full-sized P element	KP element	'2 kb' element
Lerik[a]	99	100	1	0	M	M			
2	100	100	—	2	M	M	−	+	−
3	100	100	0	4	M	M	−	+	+
4	100	100	0	2	M	M	+	+	−
5	98	100	2	0	M	M	+	+	−
6	100–98[b]	97	0	0	M	M	−	+	+
7	67–42[b]	69	2	0	inter	inter	+	+	−
8	94	95	0	0	M	M	+	+	+
9	100	100	0	0	M	M	+	+	−
10	0–0[b]	1	3	0	P	P	+	+	−
11	98	100	3	0	M	M	−	+	+
12	100	100	2	1	M	M	−	+	+
13	100	100	0	4	M	M	−	+	−
14	0–0[b]	2	0	1	P	P	+	+	−
15	100	100	2	0	M	M	−	+	+
16	97	93	0	0	M	M	−	+	+
17	100	100	2	3	M	M	−	+	+
18	0–0[b]	1	2	0	P	P	+	+	−

[a] Lerik is the initial population from which the inbred lines originated.
[b] The cross A* test was done on two samples in these lines. The numbers 62 and 117 correspond to the generations at which the lines were analysed.
'inter' means that the cytotype was intermediate between P and M.
+ and − denote presence and absence of the expected band, respectively.

digest were probed with probes B and C and rehybridized with probes D and E.

Full-sized P elements. *Acc* I enzyme cuts a full-length P element at sites 53 and 2412, generating a single 2·4 kb internal fragment which hybridized with probes A, B, C and D. *Dde* I enzyme cuts a full-sized P element at the sites 587 and 2763, generating a 2·2 kb internal fragment which hybridized with probes B, C, D, and E. The presence in a line of both the *Acc* I and *Dde* I fragments argues in favour of putative complete P elements in this line. Hence, lines 4, 5, 7, 8, 9, 10, 14 and 18 were found to possess at least one putative complete P element, as seen in Fig. 2 in some lines. Lines with complete P elements presented either

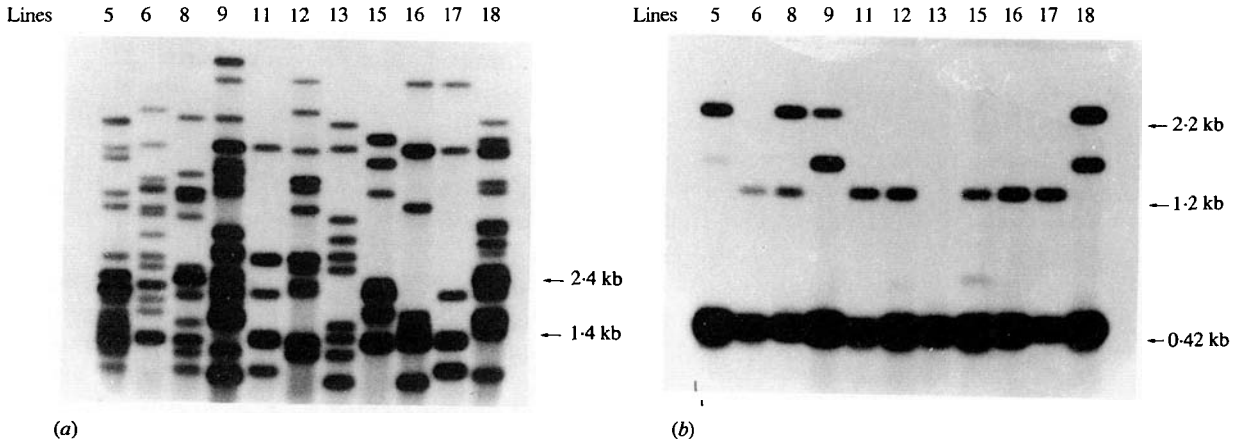

Fig. 2. Examples of Southern blot analysis of the whole genome of a set of lines. In each line 5 μg of DNA were digested with *Acc* I (*a*) or with *Dde* I (*b*) and probed with the *Pvu* II restriction fragment of the full-sized P element. In *a* the bands at 2·4 and 1·4 kb correspond to the expected restriction fragments of full-sized P element and the '2 kb' P element, respectively. In *b*, the bands at 2·2, 1·2 and 0·42 kb correspond to the expected restriction fragments of the full-sized P element, the '2 kb' P element, and the KP element, respectively.

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Table 2. *Rates of gain (transpositions) and of loss (excisions) of P-element sites per haploid genome, per generation in the 17 inbred lines, calculated from the 52nd to the 84–89th generations and from the 84–89th to the 116th generations*

Lines...	2	3	4[a]	5[a]	6	7[a]	8[a]	9[a]	10[a]	11	12	13	14[a]	15	16	17	18[a]
	52nd to 84–89th generation period																
	Gain of sites																
Total per genome	4	0	9	4	1	4	2	7	2	0	1	0	3	0	2	1	0
Total per generation	0·13	0·00	0·26	0·13	0·03	0·13	0·05	0·22	0·06	0·00	0·03	0·00	0·09	0·00	0·06	0·03	0·00
	Loss of sites																
Total per genome	3	0	1	0	3	3	6	4	2	2	1	0	3	0	1	0	4
Total per generation	0·1	0·00	0·03	0·00	0·09	0·09	0·16	0·13	0·06	0·06	0·03	0·00	0·09	0·00	0·03	0·00	0·13
No. of larvae analysed	3	2	2	3	3	3	2	3	3	2	2	3	2	2	3	3	3
	84–89th to 116th generation period																
	Gain of sites																
Total per genome	0	0	6	3·75	0	1·8	0	5·3	1	0	0	0	4	0	0	0	2
Total per generation	0	0	0·19	0·12	0	0·06	0	0·17	0·04	0	0	0	0·14	0	0	0	0·06
	Loss of sites																
Total per genome	0	0	3	0·8	0	5	0	1	1	0	0	0	3	0	0	0	1
Total per generation	0	0	0·10	0·03	0	0·17	0	0·03	0·04	0	0	0	0·10	0	0	0	0·03
No. of larvae analysed	3	2	3	4	2	3	3	6	2	2	2	2	2	2	2	2	3

Because of the strong inbreeding in the lines due to the single sib pair mating system used every generation, no P insertion polymorphism was detected among the two to three larvae analysed per inbred lines at the 84–89th generations.
At generation 116, in the lines that showed P-element movements, a slight polymorphism was detected between larvae for their P-insertion patterns as a result of the new mating system used (see text). Hence, the numbers of gained and lost sites were averaged over the number of larvae analysed per line.
[a] Denotes presence of putative complete P elements detected by the Southern blot analysis done at generations 89 and 116.

an M or a P cytotype, but all lines with a P cytotype possessed at least one complete P element (Table 1). To test again the inbred lines for presence or absence of full-sized P element at the end of the experiment, DNA was extracted from flies at generation 116, cut with *Acc* I, and probed with the plasmid pπ 25·1. A 2·4 kb *Acc* I fragment expected from a complete P element (see Fig. 1 and Boussy *et al.* 1988) was observed in lines 4, 5, 7, 8, 9, 10, 14 and 18 (data not shown); this strongly suggests that these lines had conserved their putative full-sized P elements throughout the 89–116 generation period.

KP elements. The KP elements, a distinct class of deletion-derivative P elements (Black *et al.* 1987), is cut at two sites by *Dde* I endonuclease enzyme, generating an internal fragment of 422 bp. All lines were shown to possess several KP elements (see Fig. 2); thus no correlation was detected between the presence of KP elements and the cytotype.

Defective 2 kb P elements. The comparison of the hybridization patterns obtained with *Acc* I digests probed with A, B, C and D with the patterns obtained with *Dde* I digests probed with B, C, D, and E, indicates that a band of about 1·4 kb (with *Acc* I digest) and a band of about 1·2 kb (with the *Dde* I digest) have identical patterns of occurrence and density among the lines, as illustrated in Fig. 2 for some lines. These bands were present in 9 of our lines (Table 1). Figure 1 shows that a deletion of about 1 kb from a complete P element, somewhere between the *Dde* I site at position 587 and the *Pst* I site at position 1916, would generate a 2 kb P element that should manifest bands of size 1·4 and 1·2 kb by the *Acc* I and the *Dde* I digests, respectively.

(iii) *Movements of P elements over generations*

The numbers of insertion sites of P elements and their localization on chromosome arms in the 17 inbred lines studied at the 52nd generation of sib crosses, have been published elsewhere (Biémont & Gautier, 1988). These lines were again analysed at generations 84–89. The rates of gain of new P-element sites and of loss of sites per generation and per haploid genome during this 32–37 generation period, are summarized in Table 2. On average, the rates of gain and of loss of P elements were equal to 0·072 and 0·059 per haploid genome, per generation, respectively. Inequality between these two rates was tested by the Wilcoxon signed-rank statistic making a paired comparison of gain and loss rates in each line; the normal approximation of this statistic in our data is 0·63 (the 5% threshold is 1·96), indicating absence of a significant difference between the two rate values. Three lines appeared stable over time whilst the others showed various rates of gain of sites or of excisions.

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There was no correlation between these two rates among lines (Spearman rank correlation coefficient equal 0·35; $P = 0·20$). Movements of P elements were detected in both M and P cytotype lines, the highest values of gain of sites being observed in lines 4, 5 and 9 (see Table 2), which had putative complete elements as seen above by Southern blots. The average rates of gain and of loss of sites were equal to 0·12 and 0·09, respectively, in the lines with complete P elements. Because the first blot analysis was done a long time after the detection of P-insertion patterns by *in situ* hybridization at generation 52, we cannot eliminate the hypothesis that some changes had occurred in the lines through generations of inbreeding. For example, some lines noted M and found to be devoid of complete P elements, but in which some changes in P-insertion pattern were observed, may actually have lost their complete P elements between generations 52 and 89, and may have, therefore, lost their ability to mobilize P elements. To test this possibility, we analysed again the 17 lines at generation 116, by both *in situ* hybridization and Southern blots. Note that after the 100th generation the lines were maintained by small mass mating (around 10 couples per generation) instead of the single brother–sister matings. This mating system could generate some insertion polymorphism increasing thus the number of new sites detected as heterozygotes and still not fixed by inbreeding (as they were with the sib mating crosses), and decreasing the number of excisions since they were not noticed in heterozygotes.

The Southern blots have revealed that the lines with putative complete P elements at generation 116 were those which had them 30 generations before. No inbred lines had lost their complete P elements during the additional generations. The pictures observed for the rates of gain and loss of sites were, however, different. Indeed, as summarized in Table 2, and contrary to what was reported in the generation interval 52–89, only the lines with complete P elements showed significant changes in their P-insertion pattern; again, the highest values of rates of gain of sites were observed in the M lines 4, 5 and 9, which had complete P elements. This rate of gain of sites was lower in lines 10, 14 and 18, which had the P cytotype. The other M-cytotype lines, which had no complete P element, had no change at all in their P-insertion patterns. Line 8 was unusual in that it had an M cytotype and an apparently complete P element as detected by the Southern blots but showed no change in P-insertion pattern between generations 89 and 116. This may mean that the P element, although apparently of full-length, was inactivated either by a point mutation, a very small deletion, or by a location in a heterochromatic region of the chromosomes, this latter hypothesis being in agreement with the fact that no complete P element was detected by *in situ* hybridization as reported below.

Note that the rates of gain and of loss of P elements in lines with putative complete P elements (lines 4, 5, 7, 9, 10, 14 and 18), calculated during the 52–89 generation period (0·13 for gain and 0·076 for loss rates), did not differ from those calculated during the 89–116 generation period (0·11 and 0·071, respectively). This similarity indicates that the way the inbred lines were maintained during the 89–116 generation period (mass mating of around 10 couples per generation) had not increased the amount of detected P movements.

(iv) *Localization of complete and defective P elements by* in situ *hybridization*

As shown in Fig. 1, the P_L probe (from the left part of the P element), which corresponded to the DNA sequence contained between the *Sst* I site of the P element and the *Eco*R I site of pBR322, could hybridize with complete P elements and any P elements defective for either a central region or the right part of the sequence. This probe could then detect KP elements which were characterized by a deletion of 1753 bp removing nucleotides 808-2560 (Black *et al.* 1987). The P_R probe (from the right part of the P element) which corresponded to the DNA sequence between the *Eco*R I site of the P element and the *Eco*R I site of pBR322, was used in the *in situ* hybridization to detect complete P elements and any elements defective for a left part of the P sequence. It is indeed usually admitted that *in situ* hybridization can detect DNA sequences of no less than 500 base pairs. Hence, our P_R probe would not detect elements like KP which possessed only 200 base pairs from the right part of the P sequence (see Fig. 1). The use of the three probes (P, P_L and P_R) allowed precise localization of some of the potentially full-sized and defective P elements previously shown by Southern blots to be present in the lines. To be sure of the detective power of the probes, however, we used as a control three strains known for the presence of only one full-sized P element in their genome, and two lines containing many KP elements (these strains were given by D. Higuet); the P_R probe detected all the expected full-sized P elements but not the KP elements which were detected, however, with the $p\pi$ 25·1 and P_L probes.

Table 3 summarizes the number of hybridization sites revealed by these three probes at generations 84–89. In all the lines the number of insertions revealed by the P_L probe was high and equal to the number of insertions revealed by the complete P sequence. The P_R probe, however, revealed only a low number of insertions, and in many lines only one insert was detected. This means that the lines had a high number of defective elements; these elements were mostly of the KP kind as revealed by the genomic blot analysis. When a P_R-revealed P element existed, it was localized in region 1A in the P cytotype (lines 10, 14, 18) and in region 93F in the M cytotype,

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Table 3. *Numbers of insertions revealed by* in situ *hybridization with the probes pπ 25·1 (noted P on the table), P_L and P_R on the chromosome arms of the 17 inbred lines*

		Chromosome arms																	
		X			2L			2R			3L			3R			Total		
Lines	C	P	P_L	P_R	P	P_L	P_R	P	P_L	P_R	P	P_L	P_R	P	P_L	P_R	P	P_L	P_R
2	M	3	3	0	3	3	0	4	4	0	2	2	0	1	1	0	13	13	0
3	M	3	1	0	4	4	0	2	2	0	1	1	0	5	5	1 (93F)	15	15	1
4	M	10	7	1 (9B)	5	5	0	6	4	0	3	1	0	6	5	1 (93F)	27	22	2
5	M	4	4	1 (9B)	3	3	0	6	4	0	5	5	0	5	5	0	23	21	1
6	M	2	2	0	3	3	0	3	3	0	3	3	0	6	6	1 (93F)	17	17	1
7	P/M	8	8	8	5	5	3	11	11	11	7	5	7	12	10	10	43	39	39
8	M	4	4	0	3	3	0	2	2	0	1	1	0	5	4	1 (93F)	15	14	1
9	M	4	2	0	4	4	0	6	5	0	5	5	0	8	7	0	27	23	0
10	P	6	6	1 (1A)	6	6	0	3	3	0	2	2	0	1	1	0	18	18	1
11	M	0	0	0	3	3	0	0	0	0	3	3	0	2	1	1 (93F)	8	7	1
12	M	4	4	0	1	1	0	4	4	0	3	3	0	4	4	1 (93F)	16	16	1
13	M	3	3	0	3	2	1	1	1	0	2	2	1	3	1	2	12	9	4
14	P	8	5	1 (1A)	3	1	0	0	0	0	1	0	0	6	6	0	18	12	1
15	M	3	3	0	2	2	0	2	2	0	3	3	0	4	4	1 (93F)	14	14	1
16	M	1	1	0	4	3	0	5	5	0	2	1	0	1	1	1 (93F)	13	11	1
17	M	0	0	0	3	3	0	2	0	3	3	0	1	1	1	1 (93F)	9	4	1
18	P	6	6	2 (1A)[a]	0	0	0	2	2	1	2	2	1	6	6	2	16	16	6

Two to three larvae were analysed per line. C: cytotype state.
[a] This line had insertions in regions 1A and 1B on the X chromosome.

although two M lines (4 and 5) had a P_R insertion in region 9B.

Comparison of blots and *in situ* hybridizations allows in some cases to estimate the type of elements inserted in some particular chromosomal sites. For example, the lines 5, 10, 14, have been shown by Southern blots to possess complete P elements. Since these lines exhibited only one *in situ* hybridization site with the P_R probe (in 9B, 1A and 1A, respectively) we conclude that a complete P element was inserted in these sites. Because lines 4 and 18 have respectively 2 (in 9B and 93F) and 6 (one in 1A) P_R-revealed insertions and show evidence of complete P elements by Southern blots, it is likely that a complete P element was inserted in the 9B and 1A regions of these lines (maybe because of identity by descent). Note that the M line 2, which did not show any P_R-detected insertion by *in situ* hybridization, was also found to be devoid of complete P element by Southern blots. Line 13 showed 4 P_R-detected insertions, three of which were not detected by the P_L probe; hence, this line 13 might possess P elements defective for a left part of the P sequence.

The lines 3, 6, 11, 12, 15, 16, and 17, which have no complete P elements as seen by Southern blots, have been shown, however, to possess the 2 kb defective P element defined above. In all these lines only one site of hybridization was detected with the P_R probe: it was localized in the 93F chromosomal region (Table 3). Although it could be that the 93F region actually bore multiple insertions of a small deleted P elements detected thus by the P_R probe, the above strong correlation between presence of a 2 kb P element (revealed by Southern blots) and presence of an hybridization signal at 93F strongly suggests insertion of a least one 2 kb P element in the 93F region. Note that line 4, which according to the Southern blot analysis possessed the complete and the 2 kb P elements, presented two P_R-detected chromosomal hybridization sites, one in the 93F region, the other in the 9B region. It is thus tempting to conclude that a full-sized P element is inserted in 9B, and a 2 kb P element is inserted in 93F (as seen above for the lines 3, 6, 11, 12, 15, 16 and 17). The P_R-detected insertion in the 9B region in line 5 may thus also correspond to a putative complete P element.

One problem persists for lines 8 and 9. Indeed, the Southern blot analysis revealed that these two lines possessed at least one complete P element, line 8 having in addition the '2 kb' P element. But, the *in situ* hybridization detected only one site in 93F in line 8 (this site may correspond to the 2 kb P element insertion) and no insertion site at all in line 9. *In situ* hybridization done in generation 116 confirmed this result. Although one possibility is to suppose that the complete P elements were inserted either in a heterochromatic region (in which DNA may be underreplicated and thus not detectable by *in situ* hybridization) or in the 17C region (where we always observed a hybridization signal due to the genomic sequence contained in the Pπ 25·1, P_L and P_R probes used for the *in situ* hybridization and which served as control for the sensitivity of the method), it is clear that these lines need further investigation.

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Line 7 is of particular interest because it had the highest number of detected P insertions of all the lines (Table 3) and because its cytotype is intermediate between P and M (Table 1). This line had no insertion in either the 1A or the 93F regions, and most of its insertions were revealed with both the P_L and the P_R probes. To test the possibility that the intermediate cytotype was due to a high polymorphism of insertion in this line, we analysed 6 larvae with the P probe, 2 with P_L, and 2 with P_R. No significant polymorphism of insertions was detected among the 6 larvae analysed; this very low level of polymorphism was confirmed by the insertion pattern revealed by the four additional larvae analysed with the P_L and P_R probes. Many complete or quasi-complete P elements together with KP elements existed in this line, as revealed by the Southern blot analysis. Note, moreover, that three insertions (in regions 75A, 79A and 84F) were revealed with the P_R probe but not with P_L, suggesting the presence of an element defective for a left part of the P sequence as in line 13.

(v) *Control of total, complete and defective P-element copy numbers*

To test for the possible regulation in P copy numbers within the genome, we have worked with the variances of the number of copies per inbred line, and with the variances of the number of copies per chromosome arm, for either the total P elements or the defective ones. Our statistical approach is based on the consideration that if there is no genomic control, then the five variables 'number of element copies in each chromosome arm' must be statistically independent. This implies: (1) that the observed variance of the total copy number (on all the chromosome arms) equals the sum of the variances of copy numbers in each arm, and (2) that permutations among the lines of the copy numbers on chromosome arms do not modify the variance of total copy number per line. Table 4 shows that for the total number of detected P elements, the observed variance is greater than the sum of the variances for each arm. This indicates that no compensatory mechanism (which should have produced an observed variance smaller than the sum of variances per arm) exists between chromosome arms for total P copy number in generations 84–89, a result already reported for earlier generations (Biémont & Gautier, 1988). To test the influence on the statistical test of the high copy number of P elements in line 7, we eliminated this value and did the test again; the calculated and observed variances become then equal for the total number of P elements indicating independency among the chromosomal arms for insertions of 'total' P elements. To apply the test to the defective elements, we used the number of insertions detected by the P_L probe and not detected simultaneously by the P_R probe. So the data do not correspond exactly to those summarized in Table 3.

Table 4. *Observed variance in copy number per line, and sum of variances of copy number in each chromosome arm, for the elements revealed by the probes pπ 25·1 and P_L*

	Probes		
	pπ 25·1	pπ 25·1*	P_L
Observed variance	69·70	24·56	29·05
Sum of variances per arm	26·75	19·90	14·88
Mean of 1000 estimated variances	26·70	20·30	14·77
F_{sup}	0	0·24	4×10^{-3}
F_{inf}	1	0·76	0·99

F_{sup}, frequency of simulated (1000 sets of permutations) variance values superior or equal to that observed.
F_{inf}, frequency of simulated variance values inferior or equal to that observed.
P_L, elements detected with the P_L probe but not detected simultaneously with the P_R probe.
pπ 25·1*, the line 7 with its high number of insertion is excluded from the calculations.

Only the comparison of the two insertion patterns lead to a decision about which insertions were of defective elements. This comparison gave the data in Table 5 in which only the number of defective P elements is reported. Again, as seen in Table 4, the chromosome arms are independent for copy number of defective P elements. A test using 1000 sets of permutations demonstrates that this structure is highly significant. Note that the kind of 'non-parametric variance analysis' we have used to test the relationships between the chromosome arms for number of P-element insertions, insures the absence of bias as could result in other tests from a particular genomic

Table 5. *Number of defective elements (KP and KP-like) detected by the P_L probe but not by the P_R probe*

	Chromosome arms					
Line	X	2L	2R	3L	3R	Whole genome
2	3	3	4	2	1	13
3	1	4	2	1	4	12
4	6	5	4	1	4	20
5	3	3	4	5	5	20
6	2	3	3	3	5	16
7	0	2	0	0	2	4
8	4	3	2	1	3	13
9	2	4	5	5	7	23
10	5	6	3	2	1	17
11	0	3	0	3	0	6
12	4	1	4	3	3	15
13	3	2	1	2	1	9
14	4	1	0	0	6	11
15	3	2	2	3	3	13
16	1	3	5	1	0	10
17	0	3	0	0	0	3
18	4	0	1	1	4	10

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structure in the initial population. This absence of bias results from the fact that the observed data are compared with simulated data obtained with the same chromosomes which are only assorted in a different way (by random choice). For example, a high level of inbreeding in the initial population from which the inbred line originated, should change the observed and simulated results in the same extent, and should thus not modify the significance of our test.

4. Discussion

(i) *Movements of P elements*

It is well documented that P elements move greatly under conditions of hybrid dysgenesis. The rates of transposition per X chromosome per generation has been estimated to be around 0·8 when an M-derived chromosome is the recipient of transposition from P-derived autosomes (Bingham, Kidwell & Rubin, 1983; Engels, 1983). In the P cytotype in which the autonomous elements are essentially quiescent because of immunity conferred by this cellular state, the transposition rate is much lower and was estimated to be 0·29 per X chromosome per generation while the excision rate was 0·0015; the difference in value of the two rates suggested that the population studied was not in equilibrium (Engels, 1983; Preston & Engels, 1984).

In our inbred lines originating from an initial M′ population, we find a lower, but still appreciable, rate of transposition and a rate of excision higher than those reported above in P cytotype; and the average values of transposition and excision rates averaged over all the lines are similar (0·014 and 0·012 per X chromosome per generation, respectively and 0·072 and 0·059 for the whole genome, for the 52–89 generation period).

It is not yet clear what kind of cellular mechanism controls the movements of transposons in a *Drosophila* genome, and this question is particularly crucial since all the elements under test or only some of them may be mobilized depending on the genetic system used (Gerasimova *et al.* 1984; Biémont *et al.* 1987; Woodruff *et al.* 1987; Eggleston *et al.* 1988).

It may be objected that the apparent P element movements detected in our lines by *in situ* hybridization, simply reflect pre-existing polymorphisms in the parent stock. Because our lines were maintained by one sib pair every generation, it is untenable to assert that, after more than 50 generations, a residual heterozygosity within the lines still remained. Indeed, a theoretical probability of heterozygosity at any unselected locus of the lines is already around 7×10^{-3} at the 20th generation of sib matings; it is equal to 2×10^{-22} at the 100th generation. We accept, however, the idea that when a gain or a loss of element is observed in a line over generations, it may reflect either a change in pattern arrived during the generation period under analysis, or the reflection of the heterogeneity in the lines prior to this period. According to the above argument of a negligible residual heterozygosity in the lines, this hypothesis actually means that the elements had moved in the line (this is exactly what we were looking for) and the sib mating system had not had time to fix the change in all the larvae. Another strong argument against the idea of polymorphism is the absence of any kind of P-element movement in lines devoid of full-sized P elements. If pre-existing polymorphism accounted for apparent movements of P elements, such movements should also have been observed in these lines; and this argument holds also against the hypothesis of maintenance of heterozygosity by a balanced lethal system.

(ii) *Regulation of P copy number*

In recent studies of our inbred lines in earlier generations (Biémont & Gautier, 1988; Biémont, Gautier & Heizmann, 1988), we detected a genomic control of copy number (a compensatory effect among the chromosomes) for the elements copia, mdg-1 (a copia-like element) and I (three elements with a putative reverse transcriptase: Emori *et al.* 1985, for copia; Arkhipova *et al.* 1986, for mdg-1; Fawcett *et al.* 1986 and Di Nocera & Casari, 1987, for I) but not for P and hobo (two elements with a putative transposase: Spradling & Rubin, 1982, for P; Streck *et al.* 1986, for hobo), which showed, moreover, a high variance in copy number per line. Hence, no compensatory mechanism existed between chromosome arms for P and hobo copy numbers, but some lines showed low or high copy number on each chromosome arm. This experiment, however, dealt with total number of defective and complete P insertions; but it may be that the regulation concerned only the complete P elements or some kinds of defective elements. Here we show that the inbred lines had mostly defective elements and only a few, if any, full-sized elements. Again, we did not find any compensatory mechanism between chromosome arms for the defective KP-like elements; instead, the chromosome arms behaved independently for their element number.

(iii) *Determinants of cytotype*

In a genetic analysis of a natural population from Mont Carmel, Kidwell (1981) showed that some determinants of the P cytotype were located in the distal part of the X chromosome. In more recent studies, Engels (1989) suggested that insertion of P elements in particular genomic positions might play a role in the determinism of susceptibility to P elements, and Jackson *et al.* (1988) proposed that the genomic locations in which the defective P elements, KP, reside may be important in determining their repression ability. We show here that the P cytotype of inbred

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lines is associated with a P-element insertion in the region 1A of the X chromosome, while no insertion is reported in this region for M-cytotype lines. The *in situ* hybridization in the 1A region with both the P_L and P_R probes, and the existence of full-sized P elements in these lines as revealed by Southern blots, greatly suggest that a full-sized P element may be inserted in this region. Such elements may play the role of determinants of the P cytotype. It may be that the region of the chromosomes in which the elements are inserted is more important than the elements themselves, as seen in lines 4 and 5, which possess full-sized P elements and are of M cytotype. Note that the site 1A was previously found to be a hot-spot of insertion for P elements in natural populations (Ajioka & Eanes, 1989) and that the frequency of occurrence at that site was found to be higher in populations of P cytotype than in populations of M cytotype (Ronsseray, Lehmann & Anxolabéhère, 1989).

A striking result is that in 9 of the 13 lines with a M cytotype, we observed an insertion of a 2 kb defective P element in region 93F. Four lines of M cytotype had, however, no insertion in 93F and some of these 4 lines had complete P elements in region 9B of the X chromosome (line 5) or the autosomes (line 13). Notice that we did not observe lines with insertions in both the 1A and the 93F regions, and the line 7, which is intermediate in cytotype between M and P, had no insertion in either the 1A or the 93F regions. We thus favour the hypothesis of a fortuitous event between presence of the 2 kb P element in 93F and the M cytotype. In addition, a previous study on the 17 inbred lines reported a high scatter of P insertions along the chromosomes, and that only the band 93F appeared as a 'hot spot' of insertion (Biémont & Gautier, 1988). This may mean that either the 93F region is attractive for the 2 kb P element, or that this region has the same type of insertion as a result of identity by descent; in this case the element must have a low rate of excision from the 93F region.

Many data suggest that certain incomplete or mutated P elements may play a direct role in copy number regulation. For example, Sakoyama *et al.* (1985) showed that a Japanese strain with no cross-A* GD (strong P regulation) completely lacked full-sized P elements, implying that incomplete P elements may play a direct role in P regulation. The structure of such incomplete P elements have been described; they carry mutations in the 2–3 intron splicing region or mutations that put open reading frame 3 out of frame or remove most of ORF 3 (Laski, Rio & Rubin, 1986; Nitasaka *et al.* 1987; Robertson & Engels, 1989). The KP element (Black *et al.* 1987; Jackson *et al.* 1988) was found quite different structurally from the above mutated elements, and because it was frequently observed in M′ populations with intermediate level of P regulation, it was considered to represent another class of potential regulatory P element. In our study, however, there was no evident relationship between presence of KP elements and regulatory ability, suggesting thus that, in our lines, KP elements have nothing to do with cytotype or P-element regulation.

Our results are more in favour of a role of some apparent full-sized elements located on the 1A region of the X chromosome. According to this point of view, it may be hypothesized that chromosomal regions could control or affect transcription level which could then have some secondary effect on splicing of the third intron, which is necessary for the production by the normal P element of a functional transposase. It is indeed known that the failure of this splice results in the production of a truncated transposase (a 66-kDa polypeptide repressor; Laski *et al.* 1986) often postulated to be related to the P cytotype. The production of the repressor may thus be achieved by defective or full-sized P elements according to their chromosomal region of insertion. The region 1A of the X chromosome appears, therefore, to be of great importance to understand the way the P cytotype is established and maintained. The distal part of an X chromosome with P insertions in regions 1A and 1B has recently been isolated by adequate crosses and put into the genetic background of an M strain, which is devoid of P element; presence of these two insertion sites was shown to be sufficient to bring the P cytotype about. Cloning and molecular analysis of the P element inserted at the 1A site and of the genomic flanking sequences will thus be necessary to elucidate the mechanisms involved in the P regulation observed here.

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References

Ajioka, J. W. & Eanes, W. F. (1989). The accumulation of P-elements on the tip of the X chromosome in populations of *Drosophila melanogaster*. *Genetical Research* **53**, 1–6.

Anxolabéhère, D., Nouaud, D., Périquet, G. & Tchen, P. (1985). P-element distribution in Eurasian populations of *Drosophila melanogaster*: a genetic and molecular analysis. *Proceedings of the National Academy of Sciences, USA* **82**, 5418–5422.

Anxolabéhère, D., Kidwell, M. G. & Périquet, G. (1988). Molecular characteristics of diverse populations are consistent with the hypothesis of a recent invasion of *Drosophila melanogaster* by mobile P elements. *Molecular Biology and Evolution* **5**, 252–269.

Arkhipova, I. R., Mazo, A. M., Cherkasova, V. A., Gorelova, T. V., Schuppe, N. G. & Ilyin, Y. V. (1986). The steps of reverse transcription of *Drosophila* mobile dispersed genetic elements and U3-R-U5 structure of their LTRs. *Cell* **44**, 555–563.

Biémont, C. (1986). Polymorphism of the mdg-1 and I mobile elements in *Drosophila melanogaster*. *Chromosoma* **93**, 393–397.

Biémont, C. & Aouar, A. (1987). Copy-number dependent transpositions and excisions of the mdg-1 mobile element in inbred lines of *Drosophila melanogaster*. *Heredity* **58**, 39–47.

Biémont, C., Aouar, A. & Arnault, C. (1987). Genome reshuffling of the copia element in a *Drosophila melanogaster* inbred line. *Nature* **329**, 742–744.

Biémont, C. & Gautier, C. (1988). Localisation polymorphism of mdg-1, copia, I and P mobile elements in genomes of *Drosophila melanogaster*, from data of inbred lines. *Heredity* **60**, 335–346.

Biémont, C., Gautier, C. & Heizmann, A. (1988). Independent regulation of mobile element copy number in *Drosophila melanogaster* inbred lines. *Chromosoma* **96**, 291–294.

Bingham, P. M., Kidwell, M. G. & Rubin, G. M. (1983). The molecular basis of P–M hybrid dysgenesis: the role of the P element, a P-strain specific transposon family. *Cell* **29**, 995–1004.

Black, D. M., Jackson, M. S., Kidwell, M. G. & Dover, G. A. (1987). KP elements repress P-induced hybrid dysgenesis in *Drosophila melanogaster*. *EMBO Journal* **6**, 4125–4135.

Boussy, I. A., Healy, M. J., Oakeshott, J. G. & Kidwell, M. G. (1988). Molecular analysis of the P–M gonadal dysgenesis cline in Eastern Australian *Drosophila melanogaster*. *Genetics* **119**, 889–902.

Brégliano, J. C. & Kidwell, M. G. (1983). Hybrid dysgenesis determinants. In *Mobile Genetic Elements* (ed. J. A. Shapiro). Academic Press.

Di Nocera, P. P. & Casari, G. (1987). Related polypeptides are encoded by *Drosophila* F elements, I factors, and mammalian L1 sequences. *Proceedings of the National Academy of Sciences, USA* **84**, 5843–5847.

Eggleston, W. B., Johnson-Schlitz, D. M. & Engels, W. R. (1988). P–M hybrid dysgenesis does not mobilize other transposable element families in *Drosophila melanogaster*. *Nature* **331**, 368–370.

Emori, Y., Shiba, T., Kanaya, S., Inouye, S., Yuki, S. & Saigo, K. (1985). The nucleotide sequences of Copia-related RNA in *Drosophila* virus-like particles. *Nature* **315**, 773–776.

Engels, W. R. (1979). Hybrid dysgenesis in *Drosophila melanogaster*: rules of inheritance of female sterility. *Genetical Research* **33**, 219–236.

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

Engels, W. R. (1989). P elements in *Drosophila*. In *Mobile DNA* (ed. D. Berg and M. Howe). ASM Publications.

Engels, W. R. & Preston, C. R. (1979). Hybrid dysgenesis in *Drosophila melanogaster*: the biology of male and female sterility. *Genetics* **92**, 161–175.

Fawcett, D. H., Lister, C. K., Kellet, E. & Finnegan, D. J. (1986). Transposable elements controlling I-R hybrid dysgenesis in *D. melanogaster* are similar to mammalian LINEs. *Cell* **47**, 1007–1015.

Gerasimova, T. I., Mizrokhi, L. J. & Georgiev, G. P. (1984). Transposition bursts in genetically unstable *Drosophila melanogaster*. *Nature* **309**, 714–716.

Ilyin, Y. V., Chmeliauskaite, V. G. & Georgiev, G. P. (1980). Double-stranded sequences in RNA of *Drosophila melanogaster*: relation to mobile dispersed genes. *Nucleic Acid Research* **8**, 3439–3457.

Jackson, M. S., Black, D. M. & Dover, G. A. (1988). Amplification of KP elements associated with the expression of hybrid dysgenesis in *Drosophila melanogaster*. *Genetics* **120**, 1003–1013.

Junakovic, N., Cavena, R. & Ballario, P. (1984). Genomic distribution of copia-like elements in laboratory stocks of *Drosophila melanogaster*. *Chromosoma* **90**, 378–382.

Kidwell, M. G. (1981). Hybrid dysgenesis in *Drosophila melanogaster*: the genetics of cytotype determination in a neutral strain. *Genetics* **98**, 275–290.

Kidwell, M. G. (1983). Evolution of hybrid dysgenesis determinants in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences, USA* **80**, 1655–1659.

Kidwell, M. G. (1985). Hybrid dysgenesis in *Drosophila melanogaster*: nature and inheritance of P-element regulation. *Genetics* **111**, 337–350.

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* **36**, 813–833.

Kidwell, M. G., Kimura, K. & Black, D. M. (1988). Evolution of hybrid dysgenesis potential following P element contamination in *Drosophila melanogaster*. *Genetics* **119**, 815–828.

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.

Laski, F. A., Rio, D. C. & Rubin, G. M. (1986). The tissue specificity of *Drosophila* P element transposition is regulated at the level of mRNA splicing. *Cell* **44**, 7–19.

Leigh Brown, A. J. & Moss, J. K. (1987). Transposition of the I element and copia in a natural population of *Drosophila melanogaser*. *Genetical Research* **49**, 121–128.

Nitasaka, E., Mukaï, T. & Yamazaki, T. (1987). Repressor of P elements in *Drosophila melanogaster*: cytotype determination by a defective P element carrying only open reading frames 0 through 2. *Proceedings of the National Academy of Sciences, USA* **84**, 7605–7608.

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.

Preston, C. R. & Engels, W. R. (1984). Movement of P elements within a P strain. *Drosophila Information Service* **60**, 169–170.

Robertson, H. M. & Engels, W. R. (1989). Modified P elements that mimic the P cytotype in *Drosophila melanogaster*. *Genetics* **123**, 815–824.

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

Ronsseray, S., Lehmann, M. & Anxolabéhère, D. (1989). Distribution of P and I mobile elements copy number in *Drosophila melanogaster* populations. *Chromosoma* **98**, 207–214.

Sakoyama, Y., Todo, T., Ishiwa-Chigusa, S., Honjo, T. & Kondo, S. (1985). Structures of defective P transposable elements prevalent in natural Q and Q-derived M strains of *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences USA* **82**, 6236–6239.

Simmons, M. J. & Buchold, L. M. (1985). Transposase titration in *Drosophila melanogaster*: a model for cytotype in the P–M system of hybrid dysgenesis. *Proceedings of the National Academy of Sciences, USA* **82**, 8119–8123.

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

Spradling, A. C. & Rubin, G. M. (1982). Transposition of cloned P elements into *Drosophila* germ line chromosomes. *Science* **218**, 341–347.

Streck, R. D., MacGaffey, J. E. & Beckendorf, S. K. (1986). The structure of hobo transposable elements and their insertion sites. *EMBO Journal* **5**, 3615–3623.

Tautz, D. & Renz, M. (1983). An optimized freeze-squeeze method for the recovery of DNA from agarose gels. *Analytical Biochemistry* **132**, 14–19.

Tchurikov, N. A., Ilyin, Y. V., Skryabin, K. G., Ananiev,

E. V., Bayev, A. A., Krayev, A. S., Zelentsova, E. S., Kulguskin, V. V., Lyubomirskaya, N. V. & Georgiev, G. P. (1981). General properties of mobile dispersed genetic elements in *Drosophila*. *Cold Spring Harbor Symposium on quantitative Biology* **45**, 655–665.

Todo, T., Sakoyama, Y., Chigusa, S. I., Funukaga, A., Honjo, T. & Kondo, S. (1984). Polymorphism in distribution and structure of P elements in natural populations of *Drosophila melanogaster* in and around Japan. *Japanese Journal of Genetics* **59**, 441–451.

Woodruff, R. C., Blount, J. L. & Thompson, J. N. Jr. (1987). Hybrid dysgenesis in *Drosophila melanogaster* is not a general release mechanism for DNA transpositions. *Science* **237**, 1206–1207.

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