Amplification of *KP* Elements Associated With the Repression of Hybrid Dysgenesis in *Drosophila melanogaster*

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ABSTRACT

Mobile *P* elements in *Drosophila melanogaster* cause hybrid dysgenesis if their mobility is not repressed. One type of repression, termed P cytotype, is a complex interaction between chromosomes carrying *P* elements and cytoplasm and is transmitted through the cytoplasm only of females. Another type of repression is found in worldwide M' strains that contain approximately 30 copies per individual of one particular *P* element deletion-derivative termed the *KP* element. This repression is transmitted equally through both sexes. In the present study we show that biparentally transmitted repression increases in magnitude together with a rapid increase in *KP* copy-number in genotypes starting with one or a few *KP* elements and no other deletion-derivatives. Such correlated increases in repression and *KP* number per genome occur only in the presence of complete *P* elements, supporting the interpretation that they are probably a consequence of the selective advantage enjoyed by flies carrying the highest numbers of *KP* elements. Analysis of Q strains also reveals the presence of qualitative differences in the way the repression of dysgenesis is transmitted. In general, Q strains not containing *KP* elements have the P cytotype mode of repression, whereas Q strains with *KP* elements transmit repression through both sexes. This difference among Q strains further supports the existence of at least two types of repression of P-induced hybrid dysgenesis in natural populations of *D. melanogaster*.

P-M hybrid dysgenesis in *Drosophila melanogaster* is a syndrome of correlated germ-line abnormalities which are caused by the *P* element family of mobile genetic elements (for recent review, see ENGELS 1988).

The functionally complete *P* element has been cloned and sequenced. It is 2.9 kb in length, has 31-bp terminal inverted repeats and creates an 8-bp target site duplication on insertion (O'HARE and RUBIN 1983). All four exons of the *P* element are required for the production of a P transposase (KARESS and RUBIN 1984). It is transcribed in both somatic and germ cells. However, splicing of the third intron does not occur in somatic cells, leading to the germ-line specificity of *P* element transposition (LASKI, RIO and RUBIN 1986). In addition to the complete 2.9-kb *P* elements, smaller deletion derivatives are found, which are heterogeneous in size (O'HARE and RUBIN 1983). These elements are mobile in the presence of P transposase if they retain the sequences required in *cis* for transposition.

P element transposition and the associated phenotypic traits of hybrid dysgenesis usually occur only at high frequencies in the progeny of crosses between P strain males and M strain females, with levels of transposition in reciprocal crosses and intrastain matings being low or absent. Thus, *P* elements exist in a repressed or unrepressed state, and ENGELS (1979)

has termed the former the P cytotype and the latter the M cytotype (found in M strains). P cytotype arises by a complex interaction between chromosomes carrying complete *P* elements and the cytoplasm. Once established it is transmitted through the cytoplasm of the female line only.

D. melanogaster strains differ in their ability to induce the traits of hybrid dysgenesis and to repress them. On the basis of this variation strains are classified phenotypically into three types; P, Q and M. P strains possess the P cytotype and have the potential to cause all the traits associated with hybrid dysgenesis when crossed to M strain females. A strong P strain has been shown to possess 40–50 dispersed *P* elements per genome of which about a third are the complete 2.9-kb element, the rest being deletion-derivatives (O'HARE and RUBIN 1983). Some Q strains possess a similar number of genomic elements (SAKOYAMA *et al.* 1985, BLACK *et al.* 1987) and possess the P cytotype, but differ from P strains in that, although they may carry complete *P* elements, they do not cause gonadal sterility when outcrossed to M females (KIDWELL 1981; ENGELS and PRESTON 1981). M strains can be divided into two types. True M strains possess the M cytotype and contain no *P* element sequences in their genomes. These are currently rare or absent in nature. The only known true M strains are long-established laboratory stocks (BINGHAM, KIDWELL and

RUBIN 1982). M' (or pseudo M) strains are currently common in nature and are found in Europe, Asia, North Africa, and S.E. Australia (ANXOLABEHRE *et al.* 1984; BLACK *et al.* 1987; BOUSSY 1987). Many of these strains contain complete *P* elements (BLACK *et al.* 1987; JONGEWARD, SIMONS and HEATH 1987), together with many deletion derivatives (ANXOLABEHRE *et al.* 1985, BLACK *et al.* 1987). Most M' strains are able to partially repress hybrid dysgenesis. This partial repression has been shown to be distinct from P cytotype in that it is inherited through both sexes (KIDWELL 1985; BLACK *et al.* 1987).

We have shown that all tested naturally occurring M' strains, and some Q strains, contain up to 30 copies per genome of one particular deletion-derivative termed the *KP* element (BLACK *et al.* 1987). The presence of one deletion-derivative in such high copy number, and over a wide geographic range, suggests that the *KP* element is responsible for the biparentally transmitted repression seen in M' strains. Variation in the ability of flies to repress the deleterious effects of active *P* elements, due to variation in *KP* element repression, could lead to the selection of flies with relative high numbers of *KP* elements at each generation and hence to the striking distribution of this element in natural populations.

Experimental evidence of the involvement of *KP* elements in repression of dysgenesis was demonstrated when individual chromosomes from the Cambridge (A1) strain (which contains approximately 10 *KP* elements per autosome) reduced levels of P activity and intrastrain sterility in experimental lines containing chromosomes from the strong P strain Harwich. These lines evolved to stable M' states. They could transmit their ability to repress dysgenesis through both sexes, and the copy number of the *KP* element increased (BLACK *et al.* 1987). In contrast, control lines which only inherited *P* elements from Harwich chromosomes evolved to become phenotypically P with maternally inherited repression.

In order to examine the effects of known numbers of *KP* elements on repression and to exclude the possible confounding effects of other deletion-derivatives, we report here the phenotypic and molecular characteristics of a specially constructed set of lines containing one to five *KP* elements derived from Cambridge chromosomes, and their interaction in crosses with Harwich chromosomes containing complete *P* elements. We find that such lines evolve to either M' or to weak P with repression transmitted bisexually and that the copy number of the *KP* element is greatly amplified over a small number of generations. One line evolved to a strong P strain and was found to possess many fewer *KP* elements than the others.

We have also analyzed the inheritance of repression

in naturally occurring Q lines which contain *KP* elements and show that this is chromosomally based in being transmitted through both sexes. Q strains which do not contain *KP* elements transmit their potential to repress dysgenesis maternally (with one exception). This result suggests that there are qualitative differences in Q strains in the type of repression they contain, which parallels the distinction between P cytotype and the *KP* modes of repression.

MATERIALS AND METHODS

Strains employed: For descriptions of mutants and balancers, see LINDSLEY and GRELL (1968).

H-41: *Basc*, *w^aB*; *In(2LR)bw^{v1}*, *ds^{33k}dp p bw^{v1}/SM1*, *al² Cy cn² sp²*; *Sb/TM2*, *Ubx¹³⁰ e^s*; *spa^{pol}*: A multiply marked balancer stock which completely lacks *P* element homology (BINGHAM, KIDWELL and RUBIN 1982).

w¹¹¹⁸: A mutant stock isolated by Dr. R. Levis as a spontaneous white eyed derivative of *w^{DZL}.w^{DZL}* arose from the long established laboratory stock Oregon-R (BINGHAM 1980), and contains no *P* elements.

Canton-S: A laboratory strain which contains no *P* elements and is routinely used as the M reference strain in P-M dysgenesis test crosses (KIDWELL, KIDWELL and SVED 1977).

Harwich *w*: white-eyed derivative of strong P strain isolated by M. G. KIDWELL and routinely used as the reference P strain in P-M dysgenesis test crosses (KIDWELL, KIDWELL and SVED 1977).

Cambridge (strain A1 in BLACK *et al.* 1987): A highly inbred line derived from a small number of individuals caught near Cambridge, England, in September 1984. It has behaved as an M' strain in the P-M system since its date of capture.

DY: This line was constructed by mating 15 *w¹¹¹⁸* males to females containing the Cambridge third chromosome in a *w¹¹¹⁸* background. It has been maintained by mass transfer for seven generations to allow recombination between the Cambridge and *w¹¹¹⁸* derived third chromosomes. It behaves as an M' strain in the P-M system of hybrid dysgenesis. The only *P* elements it contains are deletion-derivatives from the Cambridge third chromosome (BLACK *et al.* 1987).

Synthesis of mixed genotypes containing Cambridge (KP) and Harwich (complete P) chromosomes: Lines containing different known numbers of *KP* elements in combination with zero, one or two chromosomes from the strong P strain Harwich were synthesized. Two separate breeding schemes were employed with males derived from one scheme being mated to females derived from the other. To obtain males with known numbers of *KP* elements, DY males of the genotype *w/Y*; *+/+*; *+/+* were mated to H-41 females *en masse* at 21°. Sixty isofemale lines were set up by individually mating F₁ *Basc/w*; *Cy/+*; *Ubx/+* females to *w¹¹¹⁸* males. After 8 days of laying, the parents were removed and the DNA from each F₁ female was examined by Southern analysis to ascertain the number of *P* elements each had inherited from her DY father. Ten of the isofemale lines were selected on the basis of the number of *P* elements present and the number of progeny produced, and emerging males which possessed a DY second and third chromosome (*i.e.*, were not *Cy* and *Ubx*) were collected. To obtain females with one or two Harwich chromosomes, Harwich *w* males were mated *en masse* to H-41 females at 21°. F₁ males of the genotype *Basc/Y*; *Cy/+*; *Ubx/+* were then mated *en masse* to *w¹¹¹⁸*

females and F₂ females with Harwich chromosome two (*Basc/w*; +/+; *Ubx*/+) and Harwich chromosomes two and three (*Basc/w*; +/+; +/+) were collected. In both breeding schemes the small fourth chromosome was ignored. In our experiments, the continued use of markers and balancers, after the initial crosses, would not seem to be appropriate in that it would be highly artificial, inhibit recombination and, hence, the spread of elements throughout the genome, (see also experimental design in BLACK *et al.* 1987).

Three sets of lines termed A, C and I were established as follows using the individuals collected as described above.

A lines (one Harwich chromosome, variable numbers of KP elements): Five males from each of the DY-derived isofemale lines were mated to ten Harwich-derived *Basc/w*; +/+; *Ubx*/+ females. In addition four control lines were set up by mating *w*¹¹¹⁸ males to females of the above genotype.

C lines (two Harwich chromosomes, variable numbers of KP elements): Five males from each of the DY-derived isofemale lines were mated to ten Harwich derived *Basc/w*; +/+; +/+ females. Four control lines were set up by mating *w*¹¹¹⁸ males to females of the above genotype.

I lines (no Harwich chromosomes, variable number of KP elements): Five males from each of the DY-derived isofemale lines were mated to ten *w*¹¹¹⁸ females.

For the purposes of discussion, the progeny from each of these matings were called the F₁. All lines were maintained at 21° ± 1° by mass transfer of nonoverlapping generations on standard yeast/glucose medium supplemented with ampicillin and tetracycline to a final concentration of 10 mg/liter.

Phenotypic monitoring of strains: The frequency of ovarian dysgenesis in the F₁ females from three standard crosses was used to monitor the phenotypic P-M characteristics of the lines. Two of the crosses (designated A and A*) are standard assays used to determine the P activity and P susceptibility, respectively, of an unknown line. The A cross involves crossing the unknown males to true M (Canton-S) females while the A* cross involves mating P strain males (Harwich) to the unknown females. Both crosses are performed at 29° and the F₁ daughters of these crosses are dissected to determine the frequency of undeveloped ovaries (SCHAEFER, KIDWELL and FAUSTO-STIRLING 1979). The third test involves monitoring levels of ovarian dysgenesis within the lines either at stock room temperature (21°) or at 29°. DANIELS *et al.* (1987) and BLACK *et al.* (1987) have found significant levels of intrastrain sterility at both temperatures in lines in which P elements have been introduced. All crosses (A, A* and intrastrain) were performed by mass mating 50 males to 50 females at the appropriate temperature. A minimum of 25 F₁ females were initially dissected and scored from each cross for the presence or absence of fully developed ovaries. If the level of ovarian dysgenesis (defined as the percentage of sterile ovaries of a group of females) was found to be between 10% and 90% a further 25 females were dissected. In addition the following controls were tested with each set of crosses at 29°: Harwich males × Canton-S females and Harwich males × Harwich females. These crosses consistently gave 100% and 0% ovarian dysgenesis (GD sterility), respectively. For details of the crosses and GD sterility assay see BREGLIANO and KIDWELL (1983).

Molecular analysis: DNA was extracted either from 20 adult flies selected at random or from individual adult females as described in COEN, THODAY and DOVER (1982). DNA fragments for nick translation were extracted from agarose gels by the freeze-squeeze technique of TAUTZ and RENZ (1983). All Southern blots were washed at high stringency (0.1 × SSC, 0.5% SDS at 65°). All other standard

molecular techniques such as gel electrophoresis, Southern blotting and filter hybridization were performed as described in MANIATIS, FRITSCH and SAMBROOK (1982) [see also BLACK *et al.* (1987) for further details].

RESULTS

Characterization of experimental lines: We constructed an experimental line (DY) which contained a Cambridge third chromosome in an otherwise *w*¹¹¹⁸ background. After seven generations, DY males were mated to H-41 females and the F₁ females from this cross were mated to *w*¹¹¹⁸ males, to establish lines with different numbers of KP elements. These lines have been called the I lines (see MATERIALS AND METHODS). After mating, DNA was isolated from individual F₁ females to ascertain how many P elements they had received from their fathers. Genomic DNA was digested with *Eco*RI and probed with the 0.7-kb *Hind*III-*Xho*I fragment of the P factor. This combination of digest and probe yields one band of hybridization from each P element present in the genome (Figure 1). Figure 2 shows the P element restriction patterns of 35 F₁ females. The number of P elements present in each female ranges from zero to nine, and the distribution of elements is not random, presumably reflecting different linkage distances between them.

The 10 females indicated in Figure 2 were used to construct lines with varying numbers of P elements from the Cambridge strain in combination with Harwich chromosomes. To determine the type of P elements present in each line, and to check that no contamination had occurred, the I lines were examined further at the third generation. DNA from the lines was digested with *Eco*RI and probed with the 0.7-kb *Hind*III/*Xho*I fragment of the P factor. The results are shown in Figure 3A and demonstrate that each I line contains only the expected *Eco*RI fragment seen in Figure 2. Figure 3B shows that all the lines, with the exception of line 19 (lane 1) have only a single band of hybridization at 0.42 kb when digested with *Dde*I and probed with the 0.9-kb *Pvu*II fragment of the P factor. This corresponds to the fragment size expected from the KP element (Figure 1). Line 19 also contains a band of hybridization at 0.7 kb which corresponds to an approximately 1.5-kb P element from the Cambridge third chromosome. We were unable to isolate a line containing a 3rd chromosome with this element in the absence of KP elements. Reprobing the *Eco*RI digests (Figure 3A) with the probe used in Figure 3B revealed that all the fragments seen in Figure 3A are homologous to it (data not shown). This demonstrates that at least six of the P elements on the Cambridge third chromosome (those present in lines I, 1–5) are KP elements, and that the only P

FIGURE 1.—Structure of the 2.9 kb P factor in $p\pi$ 25.1, and the 1.15 kb KP element in pKP3. The deletion endpoints of the KP element are shown, as are all relevant restriction sites. There are two *DdeI* sites at positions 2796 and 2814 which, for simplicity, are not shown. Key: D = *DdeI*; E = *EcoRI*; H = *HindIII*; P = *PvuII*; X = *XhoI*.

FIGURE 2.—P element restriction fragment pattern of 35 individual females containing recombinant third chromosomes from DY in otherwise pure M backgrounds. Each lane contains *EcoRI* digested total genomic DNA from a single female, with the exception of the lane marked DY which contains 1 μ g of DNA extracted from DY. The filter was probed with the 0.7-kb *HindIII/XhoI* fragment of the P factor (Figure 1). The lanes containing DNA from the ten females used to construct the competition lines are labeled.

FIGURE 3.—Molecular analysis of I lines containing only DY derived P elements. A, *EcoRI* digests of total genomic DNA, probed with the 0.7-kb *HindIII/XhoI* fragment of the P factor. Approximately 5 μ g of DNA were loaded per lane. The order of the DNA samples in lanes 1–11 is as follows: I9, I5, I4.2, I4.1, I3, I2, I1.1, I1.4, I1.3, I1.2 and A1 (Cambridge). B, *DdeI* digests of total genomic DNA, probed with the 0.9-kb *PvuII* fragment of the P factor. Approximately 5 μ g of DNA was loaded per lane. The order of the DNA samples in lanes 1–11 is the same as in Figure 3A.

elements present in the recombinant lines (with the exception of I9) are *KP* elements.

Effect of *KP* elements on hybrid dysgenesis: To monitor the effects of the *KP* element on hybrid dysgenesis we maintained the experimental lines for 14 generations and performed the A, A* and intra-strain crosses every second generation. The A and A* crosses test the ability of the strains to induce or repress dysgenesis, respectively (see MATERIALS AND METHODS). The results for the lines that inherited one Harwich autosome (chromosome 2) and varying numbers of *KP* elements are shown in Table 1.

The A cross (P activity) results show that all 10 lines with *KP* elements gave low levels of P activity at generation three. By generation nine only lines which inherited one *KP* element (A1.1–1.4) gave more than 2% GD sterility and by generation 13 only line A1.4 gave sterile progeny (3%) in the A cross. In contrast the four control lines (AV1–4) showed significant levels of P activity at all generations with only one line (AV4) giving less than 10% GD sterility (5% at F₁₂). The sterility in the other controls remained above 20% throughout and at generations 12 and 13 levels of P activity were high in these lines (47%, 78% and 100%).

The A* (P repression ability) results (Table 1) are similar to those obtained by BLACK *et al.* (1987) using complete Cambridge chromosomes. The 10 lines with *KP* elements all gave some fertile progeny in A* crosses at every generation and by generation 11 intermediate levels of repression were present in all lines. The four controls produced no fertile offspring in A* crosses between generations 3 and 8 but by generations 12 and 13 three of the four controls (AV1–3) possessed intermediate levels of repression (20–60% GD sterility). The control line AV4 (which lost its initial P activity as assayed by the A test) showed no evidence of P repression (100% sterility).

The results of the intrastrain matings are also given in Table 1. In the matings performed at 29° lines A1–A9 initially gave intermediate levels of sterility which rapidly fell, and only low levels of sterility were detected at generation 12. The controls (no *KP* elements) gave initially high levels of sterility (>80%) which fell to intermediate levels (20–55%) by generations 12 and 13. The intrastrain matings performed at 21° gave results comparable to those at 29°, with sterility levels in the *KP* element-containing lines consistently being lower than the controls. The numbers of sterile offspring recorded at 21° were, however, much lower than the numbers recorded at 29°. Thus, clear differences were found between the ten lines with *KP* elements and the four control lines in all the phenotypic assays.

The results for the C lines, which inherited two Harwich autosomes (two and three) and different numbers of *KP* elements, are given in Table 2. In the

A cross, the ten *KP* element containing lines gave much lower levels of P activity than the controls, with the levels of GD sterility falling to below 10% by F₁₂ for seven out of the ten lines. However, in one line (C1.4) sterility increased from 27% at generation 3 to 80% at generation 13 (see later) and three other lines (C1.1, 1.3 and 3) consistently gave intermediate levels of sterility throughout the experiment. In the A* crosses all 10 lines with *KP* elements gave high initial levels of GD sterility (80–100% at F₃) which fell over 10 generations to between 0% and 60%. In the 29° intrastrain matings (Table 2) lines with *KP* elements initially gave high levels of GD sterility which fell over the course of the experiment, with only one line (C1.4), giving more than 10% sterility at generation 12. The 21° results follow the same pattern as the 29° intrastrain results but GD sterility levels were again lower, 14% being the highest figure recorded in lines with *KP* elements.

The C lines controls (CV1–CV4) behaved like two lines constructed in the same way which were studied by BLACK *et al.* (1987). P activity, as measured by the A test, increased from 30 to 50% at generations three and four to 80–100% at generations 12 and 13. No P repression was initially seen in these lines (100% GD sterility in the A* cross) but by generations 12 and 13 all four controls gave less than 15% GD sterility in the A* cross. In the intrastrain matings the initial high levels of sterility (80–100% GD sterility at 29° and 30–50% at 21°) dropped to 10% or under by generations 11 and 12, with the exception of CV2 which gave 26% GD sterility at 29° at generation 12.

Transmission of repression in the experimental lines: To determine if the repression in the mixed lines is transmitted through both sexes or maternally only (P cytotype), the 14 C lines were crossed reciprocally to Canton-S at generation 14. The female progeny of these crosses were tested for their P repression potential when crossed to Harwich males at 29°. The results of three replicate crosses are given in Table 3. The repression seen in the four control lines (CV1–CV4), which are phenotypically P, is largely inherited maternally, with only three nondysgenic ovaries out of a total of 1200 scores in cross 1 (Table 3), a result consistent with the P cytotype mode of repression (ENGELS 1979). Five of the 10 lines with *KP* elements (C1.3, 4.1, 4.2, 5 and 9) transmit their repression equally through both sexes, with no significant differences being found between their performances in cross 1 and cross 2. This mode of repression transmission is typical of M' strains (KIDWELL 1985, BLACK *et al.* 1987). The other five lines (C1.1, 1, 2, 1.4, 2 and 3) consistently give nondysgenic progeny in both crosses but significantly more are found in cross 2 than in cross 1 ($P < 0.05$ for C1.2, $P < 0.001$ each for the other four lines by a χ^2 analysis). We believe this shows that these lines possess both bipar-

TABLE 1

Percentage of F₁ gonadal sterility in the A, A* and intrastrain crosses at selected generations in the A lines (1 Harwich chromosome, variable numbers of KP elements)

Line name	No. of <i>KP</i> elements	A cross and generation no.:											A* cross and generation no.:										
		3	4	5	6	7	8	9	10	11	12	13	3	4	5	6	7	8	9	10	11	12	13
AV1	0	20		36		35		43		21		47	100		100		100		96		82		48
AV2	0	22		37		43		83		61		78	100		100		100		70		57		28
AV3	0		22		32		70		90		100			100		100		100		95		54	
AV4	0		14		35		12		10		5			100		100		100		100		100	
A1.1	1	10		5		5		8		0		0	96		96	97			73		67		77
A1.2	1	10		10		17		10		0		0	98		86	83			84		45		68
A1.3	1	18		12		5		8		0		0	93		96	92			94		82		78
A1.4	1	18		14		17		7		6		3	96		94	90			94		80		63
A2	2	8		4		3		0		0		0	92		88	90			85		75		78
A3	3	14		0		5		0		2		0	92		92	87			88		88		63
A4.1	4	14		2		0		2		0		0	90		92	92			87		80		86
A4.2	4	8		0		0		2		0		0	84		82	77			73		43		70
A5	5	8		0		0		0		0		0	84		90	96			85		73		82
A9	9	2		0		0		0		0		0	92		88	86			90		67		85

TABLE 2

Percentage of F₁ gonadal sterility in the A, A*, and intrastrain crosses at selected generations in the C lines (2 Harwich chromosomes, variable numbers of KP elements)

Line name	No. of KP elements	A cross and generation no.:											A* cross and generation no.:										
		3	4	5	6	7	8	9	10	11	12	13	3	4	5	6	7	8	9	10	11	12	13
CV1	0	33		68		90		93		80		84	100		100		62		38		20		6
CV2	0	35		73		84		85		83		85	100		100		76		58		20		3
CV3	0		52		70		90		89		100			100		72		18		13		0	
CV4	0		47		68		64		80		90			100		88		84		46		13	
C1.1	1	31		50		20		29		10		23	100		96		68		66		45		40
C1.2	1	27		29		47		7		8		10	94		96		88		68		63		30
C1.3	1	25		21		8		7		0		0	92		87		93		85		50		60
C1.4	1	27		26		60		33		78		80	98		94		100		51		18		0
C2	2	23		16		0		3		3		3	98		84		60		38		40		18
C3	3	35		36		27		25		13		22	93		80		60		50		40		22
C4.1	4	30		27		8		14		7		0	96		87		68		60		35		17
C4.2	4	17		10		0		0		0		0	95		95		73		47		57		57
C5	5	20		0		0		2		0		0	84		93		83		70		58		60
C9	9	7		0		0		0		0		0	90		87		62		70		30		22

ental and maternal only transmitted components in their repression. Interestingly, all four of the C lines (1.1, 1.2, 1.4 and 3) that contain KP elements and retained some P activity (as assayed by the A test) appear to contain both types of repression.

Molecular analysis of experimental lines: To correlate changes in the copy number of P elements in the experimental lines with the phenotypic variation seen in Tables 1 and 2, Southern analyses were performed on total genomic DNA extracted from 24 of the lines at generations 2 and 14. The results are shown in Figure 4, A–F. The DNAs were digested with *DdeI* as this enzyme gives conserved internal fragments of 2.2 kb and 0.42 kb, from the complete P element and the KP element, respectively, when probed with the 0.9 kb *PvuII* fragment of the complete P element (Figure 1). Thus it is possible to analyze relative copy number changes both within and between lines with this single digest.

Figure 4, A and B, shows the results from the A and C lines, respectively. It is clear that the most dramatic and consistent temporal change which has occurred within the lines is the increase in intensity of the 0.42-kb band. As expected, this band is not seen in the four control lines (Figure 4, A and B, lanes 1 and 2) which did not inherit KP elements. In 19 of the 20 KP-containing lines a 0.42-kb band is visible in the F₁₄ but not the F₂ samples (B lanes), in line C1.4 however, the one line with KP elements which evolved to a P strain, this band is not visible at either generation (Figure 4B, lanes 3A and 3B).

A longer exposure of the filters (Fig. 4, C and D) reveals the full extent of this increase in intensity with faint bands of hybridization at 0.42 kb being visible in some of the F₂ lanes (A lanes in Figure 4). It is also apparent from these exposures that the 0.42-kb band has increased in intensity in line C1.4 (Figure 4D, lane 3, A and B) but less dramatically than in other KP-

Intrastrain cross 29° and generation no.												Intrastrain cross 21° and generation no.:											
2	3	4	5	6	7	8	9	10	11	12	3	4	5	6	7	8	9	10	11	12			
89		76		65		89		67		49	16		25		21		23		19				
86		89		75		92		50		37	14		26		26		15		12				
	90		92		100		87		52			18		18		28		37		14			
	94		72		48		20		22			13		23		26		5		3			
81		61		52		37		13		4			11		3		7		0				
66		60		32		28		8		3			2		3		6		0				
67		66		35		31		7		0			6		1		4		0				
63		83		83	50	22		12		8			5		2		3		2				
63		40		30		7		8		0			0		4		0		0				
41		23		23		2		2		2			2		0		0		0				
11		14		5		7		4		0			2		0		0		0				
38		22		15		10		2		0			4		0		0		0				
18		30		5		3		0		0			0		0		0		0				
28		8		0		2		0		0			0		0		0		0				

Intrastrain cross 29° and generation no.:												Intrastrain cross 21° and generation no.:											
2	3	4	5	6	7	8	9	10	11	12	3	4	5	6	7	8	9	10	11	12			
96		100		90		38		23		8	25		48		34		15		2				
88		100		94		55		27		26	24		40		36		17		6				
	98		84		380		22		0			28		42		5		6		0			
	96		86		87		48		10			32		37		28		17		5			
90		79		75		50		8		8	11		11		3		3		0				
69		76		57		32		25		8	11		4		6		8		3				
78		64		33		9		2		0	12		13		10		0		2				
77		84		86		52		29		21	14		16		6		17		5				
56		42		18		8		4		0	7		3		3		0		0				
79		46		35		15		10		2	9		4		3		5		6				
70		53		42		14		13		3	10		4		2		0		0				
48		60		27		24		5		0	11		2		3		0		0				
37		50		30		10		2		0	7		0		0		0		0				
39		23		10		8		0		0	3		0		0		0		0				

containing lines. The 0.7-kb band present in A9 and C9 (Figure 4, C, line 12, and D, line 12) has also increased in intensity although both this fragment and the 0.42-kb fragment are clearly visible in the F₂ lanes of these lines demonstrating that copy number changes have been less extreme in these lines. The conserved 2.2-kb fragment from the complete *P* element (Figure 4, A and B) shows temporal changes which are minor in comparison to those of the 0.42-kb band. Although the intensity of this band has increased in the four control lines (Figure 4, A and B, lines 1 and 2) and in the C experimental lines (Figure 4B, lines 3–12), the differences in intensity of this band are largely due to differences in the amount of DNA loaded which can be seen when the filters are washed and re-probed with a *D. melanogaster* actin gene, (Figure 4, E and F). The same is true for the internal *DdeI* fragments from two Harwich-specific deletion-derivatives which are also seen in Figure 4,

A and B, below the 2.2-kb fragment (approximately 2.1 and 1.8 kb). In some lines the intensity of one or both of these fragments has increased over time, but these increases do not correlate with phenotype as they have occurred in two of the control lines and in some, but not all, of the lines with *KP* elements.

Inheritance of repression in naturally occurring Q and M' strains: A strain is normally defined as Q if it gives less than 5%, (ANXOLABEHERE *et al.* 1985) or 10% (KIDWELL, FRYDRYK and NOVY 1983) sterile daughters in both the A and A* crosses. Analysis of the Q strain, $\nu 6$ (ENGELS and PRESTON 1981) showed that it transmitted its repression maternally. However, the discovery of *KP* elements in some Q strains (BLACK *et al.* 1987) suggested that this may not be the case for all Q strains.

We have studied the transmission of repression in 16 Q lines and 4 M' lines. The *DdeI* digests of these lines (Figure 5) shows that only 5 do not contain *KP*

TABLE 3
Inheritance of repression in the C lines

Line name and no. of <i>KP</i> elements from Cambridge 3rd chromosome		Cross 1		Cross 2	
		Line ♀ × Canton-S ♂ F ₁ ♀ × Harwich ♂		Canton-S ♀ × Line ♂ F ₁ ♀ × Harwich ♂	
CV1	0	54, 41, 42***		100, 100, 100	
CV2	0	52, 45, 38***		100, 100, 100	
CV3	0	24, 20, 29***		100, 98, 100	
CV4	0	42, 36, 45***		100, 100, 99	
C1.1	1	57, 60, 52***		84, 84, 80	
C1.2	1	70, 65, 77*		86, 77, 73	
C1.3	1	79, 84, 75		82, 78, 73	
C1.4	1	45, 41, 29***		97, 95, 98	
C2	2	41, 51, 46***		70, 65, 59	
C3	3	28, 36, 36***		84, 81, 80	
C4.1	4	74, 78, 69		74, 67, 80	
C4.2	4	70, 57, 59		72, 59, 67	
C5	5	80, 85, 72		76, 76, 85	
C9	9	65, 73, 70		74, 66, 77	

The results of three replicate experiments performed at generation 14 are shown. Numbers are percentages of gonadal sterility seen in A* crosses performed on females derived from crosses 1 and 2. Crosses and scoring were performed as in *Materials and Methods* except that 50 females scored from every cross. A χ^2 analysis, performed on 3 × 2 contingency tables, found no significant heterogeneity between the replicates in any one cross. Data from the replicates was therefore pooled to look for between cross heterogeneity using a χ^2 analysis. (* = $P < 0.05$, *** = $P < 0.001$, all other comparisons being nonsignificant at the 5% level.)

elements (lines 1, 2, 3, 16 and 20). The classification of the lines using the A and A* tests, their country of origin, and the results of a reciprocal cross analysis are given in Table 4. All four M' lines (17–20) show no significant difference between their performances in cross 1 and 2. However, Muller-5 Birmingham gave 100% GD sterility in both crosses despite giving only 63% in an A* cross. Variation exists in the performances of the Q lines in crosses 1 and 2. Using a χ^2 analysis only 5 Q lines (8, 10, 11, 14 and 15) showed no significant difference in the number of dysgenic and nondysgenic ovaries between the two crosses. The other 11 Q lines gave significantly more dysgenic ovaries in cross 1 (see Table 3) demonstrating the existence of a maternal component to repression. However, only 3 of these Q lines gave more than 95% dysgenic ovaries in cross 1, all others gave less than 80% suggesting that some of the repression in these strains is transmitted through both sexes.

Considering the phenotypic and molecular analysis together it is apparent that three of the four Q lines that do not contain *KP* elements transmit their repression maternally, while all 12 Q lines with *KP* elements transmit some, or all, of their repression through both sexes. One Q line however (Hunter Valley) does not

FIGURE 4.—*DdeI* digests of the A and C competition lines from Tables 1 and 2. A, *DdeI* digests of the A lines, probed with the 0.9 kb *PvuII* fragment of the *P* factor. Approximately 4 μ g of DNA was loaded per lane. Each A and B pair of lanes are the F₂ and F₁₄ DNAs (in that order) from one A line. The order of lines from 1 to 12 is as follows: AV1, AV2, A1.4, A1.1, A1.2, A1.3, A2, A3, A4.1, A4.2, A5 and A9. B, *DdeI* digests of the C lines, probed with the 0.9 kb *PvuII* fragment of the *P* factor. Approximately 4 μ g of DNA was loaded per lane. Each A and B pair of lanes are the F₂ and F₁₄ DNAs (in that order) from one C line. The order of lines from 1 to 12 is as follows: CV1, CV2, C1.4, C1.1, C1.2, C1.3, C2, C3, C4.1, C4.2, C5 and C9. C, Longer exposure of section A line filter (in A). D, Longer exposure of section of C line filter (in B). E, A line filter (in A) washed and re-probed with the 1.8-kb and 1.6-kb *HindIII* fragments of pA2-1.8H3, a plasmid containing a *D. melanogaster* actin gene fragment (FRYBERG *et al.* 1981). F, C line filter (in B) washed and re-probed as in E.

TABLE 4

Inheritance of the repression present in Q and M' lines

No.	Strain	Origin	A	A*	C	Cross 1	Cross 2
1	Hacateppe	Turkey	0	0	Q	100	0***
2	M. Isra	Israel	0	0	Q	100	38***
3	Merv	U.S.A.	0	0	Q	96	32***
4	Mt. Carmel	U.S.A.	0	0	Q	71	23***
5	Saint Cath.9	Canada	0	0	Q	70	31***
6	Ivory	Ivory Coast	0	0	Q	40	15***
7	Kravi-Hora	Czechoslovakia	3	8	Q/M'	40	8***
8	Quiryat-Anavin	Israel	0	0	Q	49	43
9	Sancerrre	France	0	0	Q	48	0***
10	Kingsbury	England	0	8	Q/M'	59	51
11	Melbourne	Australia	0	5	Q	36	41
12	Townsville	Australia	4.5	0	Q	45	10***
13	Iquitos	Peru	2	0	Q	51	24***
14	Wilkins	England	0	7	Q/M'	61	55
15	Hills	England	0	0	Q	52	34*
16	Hunter Valley	Australia	0	0	Q	25	4***
17	Cambridge (A1)	England	0	18	M'	62	63 ^a
18	Suchumi	Japan	0	25	M'	55	59
19	Sexi	Spain	0	60	M'	78	80 ^b
20	M-5-B	3	0	63	M'	100	100 ^c

The origin of each strain, its performance in the A and A* crosses, and its classification in the P-M system is given. The mating schemes for crosses 1 and 2 are shown in Table 3, and all crosses were performed as in MATERIALS AND METHODS except that 50 females were scored from every cross. A χ^2 analysis, using 2×2 contingency tables, was performed to look for heterogeneity between the cross 1 and 2 results for each line. (* = $P < 0.05$, *** = $P < 0.001$, with all other comparisons being nonsignificant.) C = classification.

^a Data from BLACK *et al.* (1987).

^b Data from KIDWELL (1985).

^c Origin of the P elements in this line is unknown.

contain KP elements but does exhibit strong repression in both cross 1 and 2.

DISCUSSION

Our current knowledge suggests that there are at least two types of repression of P element-induced hybrid dysgenesis in *D. melanogaster*. The first to be characterized, and the most extensively studied, is the P cytotype. This repression is inherited maternally, but ultimately depends on chromosomal factors (ENGELS 1979). In addition P cytotype has been shown to be strongly temperature dependent (RONSERRAY, ANXOLABEHRE and PERIQUET 1984; DANIELS *et al.* 1987). It has been suggested that the P cytotype may be determined by a P factor-encoded repressor (O'HARE and RUBIN 1983) or alternatively by extra-chromosomal copies of P elements persisting in the female cytoplasm that may encode a repressor (ENGELS 1979) or which may titrate out a limited amount of transposase (SIMMONS and BUCHOLZ 1985). More recently it has been suggested that a subset of chromosomal deletion derivatives may encode for a maternally inherited repressor (DANIELS *et al.* 1987; NITA-

SAKA, MUKAI and TSUNYUKI 1987). However, the precise mechanism underlying P cytotype repression, and the reason for its maternal inheritance still remain uncertain.

The second type of repression, initially documented in the Sexi strain by KIDWELL (1985), is transmitted through both sexes and, hence, has a chromosomally based component only. Until now it has been described only in M' strains. Two models have been proposed to account for the repression exhibited by M' strains. The first, the transposase titration model (SIMMONS and BUCHOLZ, 1985), proposes that the termini of P element deletion derivatives act as "dead ends" titrating out a limited amount of P transposase so reducing transposition. The second (BLACK *et al.* 1987) proposes that the KP element is responsible for the repression, through a KP-encoded protein.

Here we have shown that the addition of one or more KP elements only, derived from the Cambridge M' strain, to lines containing complete P elements from the Harwich strong P strain results in the evolution of M' strains with intermediate levels of bisexually transmitted P repression. These lines consistently show lower levels of P activity and intrastain sterility than control lines without KP elements. In general, the differences in sterility levels were marked, and not of a subtle nature, and the need for statistical analysis does not arise, except where this has been carried out. Molecular analysis revealed large increases in the copy number of the KP element in these lines during their evolution. Of the 20 lines studied, 16 were M' by F₁₃, a further 3 retained low levels of P activity but possessed the characteristic chromosomal inheritance of M' repression. Only one evolved to be phenotypically strong P: this one P line was shown to possess fewer KP elements than the other lines, and to transmit its repression maternally. It appears to be phenotypically and molecularly similar to the P strains found in N.E. Australia (I. A. BOUSSY, personal communication) and to the Senegal 1980 and Kerbinou lines examined in BLACK *et al.* (1987). Changes in the copy number of P element deletion-derivatives inherited on the Harwich chromosomes did occur. However, these changes were small in comparison to changes in the copy number of the KP element and did not correlate with the phenotypic variation of the lines. It was also clear from the molecular analysis that the competition lines which evolved to be phenotypically M' retained potentially complete P elements at F₁₄. However, these lines do not exhibit P activity as assayed by the A cross.

The KP element model of repression is consistent with the results reported here. It is clear that the introduction of a single KP element to lines containing complete P elements can fundamentally alter the evolution of these lines, preventing the build up of P

FIGURE 5.—*Dde*I digests of the Q and M' strains in Table 4. Approximately 4 μ g of total genomic DNA was used per lane and the 0.9-kb *Pvu*II fragment was used as the probe. The number of each lane refers to the strains in Table 4.

activity and P cytotype, and resulting in the evolution of M' strains. The observation that the deletion-derivative present in lines A9 and C9, which gives a 0.7-kb *Dde*I fragment of hybridization increases in copy number in these lines, in addition to the KP element (Figure 5), could suggest that it also possesses some repression ability. However, it is likely that selection for homozygosity of the entire chromosome (which harbors at least six KP elements) occurred in these lines, which by the A and A* tests were phenotypically M' by generation five. The possibility that the observed increase in the copy number of this element is due to its linkage to KP elements cannot be ruled out.

As suggested by BLACK *et al.* (1987), the most plausible cause of the spread of the KP element over all other deletion-derivatives through worldwide natural populations is an interaction between transposition and natural selection. A discussion of other interpretations of KP element accumulation can be found in BLACK *et al.* (1987). Our experimental lines all showed measurable levels of intrastain sterility and there would clearly be a great selective advantage for anything which reduces this sterility. We proposed earlier that natural variation in the copy number of the KP element, caused by variation in transposition, will produce variation in the ability to reduce levels of intrastain sterility on which natural selection can act. Our results consistently show that the introduction of KP elements to lines harbouring Harwich chromosomes greatly reduce levels of intraline sterility when compared to lines lacking KP elements. The molecular mechanism of KP element repression is unknown but it has been suggested that the 207 amino acid polypeptide capable of being produced by the 0.8-kb KP element transcript may interfere with P transposase function (BLACK *et al.* 1987). KP element constructs

have been made in this laboratory suitable for transformation of M embryos in order to investigate the exact way in which KP elements repress hybrid dysgenesis. In general, it is clear that the increase in KP element copy number in the experimental lines which began with one or two KP elements cannot be due to selective differences between chromosomes that are not related to KP elements.

We have shown that Q strains which have accumulated the KP element pass on some, or all, of their P element repression through both sexes, while three of the four Q strains we have studied which have not accumulated KP elements transmit all their repression maternally. The elements in the Australian Q strain Hunter Valley that transmits its repression through both sexes but does not contain KP elements are being studied further. This could hint at other types of repression which are not P cytotype or KP-induced. As the molecular mechanism of KP element action is unknown, it is possible that other deletion-derivatives with similar properties have been formed and spread in some natural populations. It is possible, however, that the classification of a strain as Q solely by the A and A* crosses does not reveal fundamental differences in the type of repression these strains contain. This variation could explain the finding by RONSERAY, ANXOLABEHERE and PERIQUET (1984) that the repression found in some, but not all, Q strains is sensitive to the age of females and the temperature at which they were raised. It seems, for the moment, that the three phenotypic classes of strains P, Q and M can be divided into a two fold classification of P cytotype and KP-induced types of repression.

Naturally occurring M' strains harboring similar numbers of KP elements vary greatly in their ability to repress hybrid dysgenesis (BLACK *et al.* 1987). Fur-

thermore, some single *KP* elements repress *P* element transposase whereas others may have little or no effect (D. BLACK, unpublished result). This suggests that the genomic locations in which these elements reside may be important in determining their repression ability.

Although our observations further complicate the analysis of *P* elements in natural populations, a full analysis of both types of repression is required to understand the current distribution and the population dynamics of this family of transposable elements, and possibly other families of eukaryotic transposable elements.

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