# Genetic and molecular analysis of repression in the P–M system of hybrid dysgenesis in *Drosophila melanogaster*

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**Summary**

Twelve inbred lines derived from an M′ strain of *Drosophila melanogaster* were used to study the repression of P-element-mediated hybrid dysgenesis. Initial assessments indicated that the lines differed in the ability to repress gonadal dysgenesis, and that this ability was highly correlated with the ability to repress $sn^{w}$ hypermutability. Later assessments indicated that most of the lines with low or intermediate repression potential evolved to a state of higher repression potential; however, Southern analyses failed to reveal significant changes in the array of genomic P elements that could account for this evolution. In addition, none of the lines possessed the incomplete P element known as KP, which has been proposed to explain repression in some *D. melanogaster* strains. One of the lines maintained intermediate repression potential throughout the period of study (52 generations), indicating that the intermediate condition was not intrinsically unstable. Genetic analyses demonstrated that in some of the lines, repression potential was influenced by factors that were inherited maternally through at least two generations; however, these factors were not as influential as those in a classic P cytotype strain. Additional tests with a dysgenesis-inducing X chromosome called *T-5* indicated that repression itself was mediated by a combination of maternal effects and paternally inherited factors that were expressed after fertilization. These tests also suggested that in some circumstances, the P transposase, or its message, might be transmitted through the maternal cytoplasm.

## 1. Introduction

In *Drosophila melanogaster*, transposable P elements are responsible for an array of genetic abnormalities known as hybrid dysgenesis (Kidwell *et al.* 1977; Bingham *et al.* 1982). These abnormalities are restricted to the germ line and include traits such as chromosome breakage (Berg *et al.* 1980; Simmons & Lim, 1980; Engels & Preston, 1981), male recombination (Kidwell & Kidwell, 1976), mutation (Simmons *et al.* 1980), transmission ratio distortion (Engels, 1979*a*), and sterility (Engels & Preston, 1979; Kidwell & Novy, 1979). Molecular analyses have shown that the elements that are responsible for these traits are structurally and functionally heterogeneous (Rubin *et al.* 1982; O'Hare & Rubin, 1983; Karess & Rubin, 1984). Complete, or autonomous, P elements are 2907 bp long and encode an 87 kDa polypeptide, called the P transposase, which is needed for transposition (Laski *et al.* 1986; Rio *et al.* 1986). Incomplete, or nonautonomous, P elements differ from complete elements by deletions of internal sequences; consequently, these elements cannot synthesize the transposase. However, since the transposase is trans-acting, incomplete P elements can be mobilized whenever a complete element is present in the genome (Spradling & Rubin, 1982; Engels, 1984).

P elements are present in some *Drosophila* strains but are absent from others (Bingham *et al.* 1982). The latter are called true M strains, while the former are classified either as P, Q or M′ (Kidwell, 1983). P strains typically possess many P elements, including complete ones, and are proficient in causing hybrid dysgenesis in the offspring of certain crosses. Specifically, crosses between P males and true M females produce dysgenic offspring, but crosses between P females and true M males usually do not. This difference between the reciprocal crosses indicates that P females are able to repress hybrid dysgenesis through a maternally transmitted condition, which is called the P cytotype (Engels, 1979*b*, *c*). M females lack this ability, and are said to possess the M cytotype. Q strains resemble P strains because they carry many P elements, including complete ones, and

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because they possess the P cytotype; however, compared to P strains, Q strains are less proficient in causing hybrid dysgenesis (Engels & Preston, 1981; Kidwell, 1981; Simmons *et al.* 1985). M′ strains constitute a heterogeneous class and are characterized mainly by a partial ability to repress hybrid dysgenesis in crosses to P males (Kidwell, 1983, 1985). These strains sometimes have many P elements (Black *et al.* 1987; Simmons *et al.* 1987), including complete ones (Jongeward *et al.* 1987), but they do not possess the very strong repression ability that is associated with the P cytotype. Statements that M′ strains carry only incomplete P elements have appeared in the literature, and have actually been used to define this class of strains; however, such statements overlook the molecular and phenotypic complexity of this class, and are, therefore, misleading.

In general, the ability of P, Q and M′ females to repress hybrid dysgenesis in their offspring can be called 'repression potential'. Genetic analyses of representative strains have indicated that repression itself involves a maternal effect, and that repression potential is determined by a combination of chromosomal and cytoplasmic factors (Engels, 1979*b*, *c*; Engels & Preston, 1981). Based on these findings, Engels (1979*c*, 1981) proposed that the factors that are responsible for repression potential are the P elements themselves.

Recently several investigators have attempted to identify P elements with repressor functions. Initially, attention was focused on complete P elements (O'Hare & Rubin, 1983), but the finding that some strains with moderate to high repression potential did not have complete elements caused attention to shift to the incomplete elements (Sakoyama *et al.* 1985; Simmons *et al.* 1987). Accordingly, some researchers have proposed that particular incomplete or mutant P elements are responsible for the P cytotype (Nitasaka *et al.* 1987; Robertson & Engels, 1989), or for the partial repression abilities of M′ strains (Black *et al.* 1987; Jackson *et al.* 1988); however, more work is needed to elucidate these and other possibilities.

In the current study, lines derived by inbreeding an M′ strain called B13 were used to investigate the repression of hybrid dysgenesis. These lines were used because intense inbreeding would be expected to fix much of the variation that was segregating in the basic stock. The objectives of the study were to ascertain if there were differences in repression potential among and within the lines, to monitor temporal changes in repression potential and to correlate these with changes in the array of genomic P elements, and to determine the manner in which repression potential was inherited.

## 2. Materials and methods

### (i) *Drosophila strains*

B13 lines: 12 lines were created by inbreeding an M′ stock called B13 that was homozygous for the autosomal markers *cn* (cinnabar eyes) and *bw* (brown eyes). The B13 stock was constructed by introducing an X chromosome from a Brownsville, Texas population into a *cn bw* laboratory stock (Curtsinger, 1984). After 7 years of laboratory culture, inbred lines were derived from this stock by 20 generations of full-sib mating. At the end of inbreeding, each line was named for its ability to repress dysgenic sterility (H3, H8, H15 and H23 had high ability, L1, L2, L3 and L4 had low ability and I5, I6, I9 and I10 had intermediate ability). Thereafter, the lines were maintained by mass transfer in half-pint milk bottles.

*bw*; *st*: a true M strain with the recessive eye colour mutations *bw* (brown) and *st* (scarlet), located on chromosomes II and III, respectively.

$y\ sn^{w}/Y\ y^{+}$; *bw*; *st*: a strain homozygous for a double-P-element-insertion mutation ($sn^{w}$) of the X-linked *singed* locus. In the presence of the P transposase, this phenotypically weak-singed allele becomes unstable, mutating in the germ line to a more extreme ($sn^{e}$) or an essentially wild-type ($sn^{(+)}$) allele (Engels, 1979*b*, 1984). These changes involve the excision of one or the other of the inserted P elements (Roiha *et al.* 1988) and can be detected in the next generation by appropriate matings. The only other P element present in this stock is a nonautonomous element tightly linked to the *singed* locus.

$y\ sn^{3}\ v\ car$: a true M strain with an extreme allele ($sn^{3}$) of the *singed* locus that is recessive to $sn^{+}$ and $sn^{w}$.

$C(1)DX, y\ f/Y/y\ cin\ w\ f^{s}\ su(f)^{ts67g}$: a true M stain in which the females have attached-X chromosomes and the males have an X chromosome with a temperature-sensitive lethal mutation ($su(f)^{ts67g}$) that facilitates the collection of virgin females.

*M5*; *bw*; *st*: a true M strain homozygous for the Muller-5 (*M5*) balancer X chromosome and the autosomal markers *bw* and *st*.

$C(1)DX, y\ f/Y/y\ shi^{ts}$; *bw*; *st*: a true M strain with attached-X chromosomes in the females and an X-linked, temperature-sensitive lethal mutation ($shi^{ts}$) in the males. This stock is also homozygous for the autosomal markers *bw* and *st*.

$C(1)DX, y\ f/Y/T\text{-}5, sn^{w}$; *bw*; *st*: a strain with attached-X chromosomes in the females and an X chromosome with autonomous P elements and a P insertion mutation of the singed locus ($sn^{w}$) in the males (Simmons *et al.* 1987). The $sn^{w}$ mutation in this stock is unstable in the germ line as a result of P-element excisions from the *singed* locus. Consequently, the stock is maintained by mating phenotypically weak singed males (genotypically *T-5*, $y\ sn^{w}$; *bw st*) to $C(1)DX, y\ f/Y/y$; *bw*; *st* females from the true M strain described above. Since this is a dysgenic mating,

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the cultures are kept at 21 °C to minimize sterility in the offspring. In previous experiments (Simmons *et al.* 1987), *T-5* X chromosomes were not able to induce dysgenic sterility when they were introduced into a pure M background; however, by the time of the experiments reported here, *T-5* chromosomes from a subline called p3′ had acquired a marked ability to do so.

$\pi_2$: a standard P strain that was derived from a natural population in Madison, Wisconsin (Engels & Preston, 1979). This strain has the P cytotype and also has a strong ability to induce hybrid dysgenesis.

*C(1)DX, y f/Y/sn*$^w$; $\pi_2$, referred to as *sn*$^w(\pi_2)$ in the text: a P strain with attached-X chromosomes in the females and an X-chromosome with the *sn*$^w$ mutation in males. The genetic background of this strain was derived from $\pi_2$. Because it has the P cytotype, the *sn*$^w$ mutation is stable; however, males from this strain have a strong ability to induce hybrid dysgenesis in crosses to M strain females.

Sexi.7: a wild type M′ strain known to contain numerous copies of the incomplete P element known as KP (Black *et al.* 1987; Simmons *et al.* 1990).

*ry*$^{506}$ *P*[*ry*$^+$*Sal* I](89D), referred to as *Sal* I in the text: a stock homozygous for a single P element in which a frameshift mutation has been created in the *Sal* I restriction site (Karess & Rubin, 1984).

Further information about many of the stocks listed above can be found in Lindsley & Grell (1968).

### (ii) *Tests of the ability to repress dysgenic sterility*

The ability of females from the B13 lines to repress dysgenic (or GD) sterility was determined by sampling 9–36 females from each line and mating them individually to 2 or 3 $\pi_2$ males. Each culture was incubated at 29 °C for 11–13 days; then the progeny that had emerged were transferred to fresh vials to age for 2–6 days at 22 °C. After aging, 8–12 of the females in each culture were examined for egg production by squashing them between glass slides. A solution of distilled water and food colouring helped to visualize the eggs. Only those females that failed to extrude any eggs were considered to be sterile. These tests were also performed with a true M strain (*bw*; *st*), which served as a negative control.

### (iii) *Tests of the ability to repress* sn$^w$ *instability*

The ability to repress *sn*$^w$ mutability was studied in flies obtained by mass-mating B13 (or control) females to *sn*$^w(\pi_2)$ males at 21 °C. A sample of the *sn*$^w$/+ $F_1$ daughters from these matings were then crossed individually to *y sn*$^3$ *v car* males at 25 °C to determine the germ line instability of *sn*$^w$. Progeny from these crosses were scored for the *sn*$^w$ and *sn*$^e$ phenotypes 13 and 16 days after mating; the *sn*$^+$ progeny were not counted because *sn*$^{(+)}$ derivatives of *sn*$^w$ could not be distinguished from the *sn*$^+$ allele that was present on the B13 X chromosome. In each culture, the mutation rate was calculated as the proportion of *sn*$^e$ flies among those scored; the mean mutation rate of a group of cultures was estimated by computing the unweighted average (Engels, 1979*d*).

### (iv) *Tests for transposase activity*

Transposase activity was detected by observing the germ-line mutability of *sn*$^w$ in hybrids produced by crossing B13 (or control) males with *y sn*$^w$; *bw*; *st* females at 21 °C. The male hybrids were mated individually to *C(1)DX, y f* (M cytotype) females at 25 °C and their sons were scored for the *sn*$^w$, *sn*$^{(+)}$ and *sn*$^e$ phenotypes. The female hybrids were mated individually to 3 *y sn*$^3$ *v car* males at 25 °C and their progeny (both sexes) were scored for the *sn*$^w$ and *sn*$^e$ phenotypes [the *sn*$^+$ flies were not scored for the same reason given in section 2(iii)]. In these tests, the offspring of the hybrid flies were counted on the 13th and 16th days after mating.

Transposase activity was also investigated in crosses to induce GD sterility. In these, B13 males were mated individually to females from the true M strain *bw*; *st* at 29 °C; 8–12 of the daughters from each culture were then examined for egg production as described in section 2(ii) above.

### (v) *Crosses to study the inheritance of repression potential*

In G68, reciprocal mass matings were performed between each of the B13 lines and a true M strain (*bw*; *st*). The matings that utilized *bw*; *st* females were denoted as cross A, while those that utilized B13 females were denoted as cross B. The genetically equivalent hybrid females that were obtained from these crosses were tested for repression potential by mating them individually to 2 or 3 *sn*$^w(\pi_2)$ males at 29 °C. From each mating, a sample of daughters was aged 2–4 days at 22 °C and then examined for gonadal dysgenesis as described above.

### (iv) *Crosses to identify maternal and zygotic contributions to the repression of GD sterility*

In G74, flies carrying a *T-5* X chromosome were crossed to males and females from each of the B13 lines. To obtain the *T-5*-bearing flies for these matings, individual *T-5*, *y sn*$^w$; *bw*; *st* males were double-mated, first to *M5*; *bw*; *st* females, and then to *C(1)DX, y f/Y*; *bw*; *st* females. The progeny of both matings were reared at 21 °C to minimize sterility. Then *T-5/M5* females from the first mating were crossed to B13 males (denoted cross 3), and *T-5* males from the second mating were crossed to B13 females (denoted cross 4). Only a single pair of flies were used in each culture. All these crosses were initiated at 21 °C, but after 3 days, the mated flies were transferred

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to fresh cultures, which were incubated at 29 °C; then, as many as 12 of the $F_1$ daughters from each culture were examined for GD sterility. As controls, M (*bw*; *st*) and P ($\pi_2$) cytotype flies were substituted for the B13 flies in the above matings. In addition, a parallel series of crosses was carried out in which the *T-5* X chromosome was replaced by the *y sn*$^w$ X chromosome from an M-cytotype stock. Since this latter chromosome lacked any autonomous P elements, these crosses provided information on the ability of the B13 and control stocks to induce sterility by themselves. The crosses between *y sn*$^w$/*M5*; *bw*; *st* females and B13 or control males were denoted as cross 1 and those between *y sn*$^w$; *bw*; *st* males and B13 or control females were denoted as cross 2. The *y sn*$^w$/*M5*; *bw*; *st* females and *y sn*$^w$; *bw*; *st* males that were used in these crosses were obtained by mass-mating females from the *M5*; *bw*; *st* and *C(1)DX*, *y f*/*Y*; *bw*; *st* stocks to *y sn*$^w$/$y^+$*Y*; *bw*; *st* males.

### (vii) Drosophila *culturing conditions*

All *Drosophila* cultures were reared on a standard cornmeal–molasses–yeast–agar medium in vials or half-pint milk bottles. Unless specified, the culturing temperature was 25 °C.

### (viii) *Southern blotting*

DNA was extracted from adult flies and restriction enzyme digestions were performed according to the manufacturer's recommendations (Bethesda Research Laboratories). Digested DNA was separated using agarose gel electrophoresis and then transferred by capillary blotting to a GeneScreen Plus nylon membrane (Dupont) using 0·4 M NaOH/0·6 M-NaCl. Each membrane was prehybridized and hybridized at 42 °C in a solution consisting of 5 × SSCP (0·75 M-NaCl, 0·075 M sodium citrate, 0·05 M potassium phosphate; pH 6·8), 50% deionized formamide, 50 mM Tris pH 7·5, 1 × Denhardt's solution (0·02% Ficoll-400, 0·02% polyvinylpyrrolidone, 0·02% nuclease-free bovine serum albumen), 1% SDS, 5% dextran sulfate and 100 $\mu$g/ml heat-denatured salmon sperm DNA. DNA restriction fragments used for probes (Fig. 1) were gel-purified from the vector p$\pi$25.1 which contained a complete P element (O'Hare & Rubin, 1983). Probe DNA was prepared using random primer labelling (Amersham or Bethesda Research Laboratories labelling kits) by incorporating $^{32}$P-labeled dCTP at 3000 Ci/mM (Amersham) to a specific activity of 1–2 × $10^8$ dpm/$\mu$g. The final posthybridization washes were at 65 °C using 0·1 × SSC/0·1% SDS. Bound probe was removed according to the protocol described in the GeneScreen Plus technical booklet; each membrane was agitated at 42 °C with 0·4 N-NaOH for 30–60 min and then with stripping buffer (0·1 × SSC, 0·5% SDS, 0·2 M Tris, pH 7·5) for 30–60 min.

## 3. Results

### (i) *Repression of dysgenic sterility*

The ability to repress P-element-mediated dysgenic sterility was ascertained by determining the frequency of sterile daughters from matings between individual B13 females and $\pi_2$ males. Those females that produced low frequencies of sterile daughters were considered to have high repression potential. Each of the B13 lines was tested with this assay in G22, following the period of inbreeding. On average, 13·5 females from each line were tested and 11·2 daughters from each of the tested females were examined for dysgenic sterility.

Figure 2 gives the frequency distributions of sterility among the daughters of the tested females. Since all of the daughters of the *bw*; *st* control females were sterile, it is clear that the $\pi_2$ males that were used in these tests were powerful inducers of dysgenic sterility. Nevertheless, some of the females from each of the inbred lines were able to repress dysgenesis in at least some of their daughters. In four of the lines (H3, H8, H15 and H23), the potential for repression was clearly very high, in four (I5, I6, I9 and I10) it was intermediate, and in the remaining four (L1, L2, L3 and L4) it was rather low. Figure 2 also presents the results of repression tests that were performed with females taken from the B13 base population at G0 ($n = 60$) and, again, at G22 ($n = 61$). In both cases, the frequency distributions indicate considerable variation in the ability to repress dysgenic sterility, thereby suggesting a basis for the differences that were observed among the inbred lines.

### (ii) *Repression of* sn$^w$ *hypermutability*

The inbred B13 lines were also tested for their abilities to repress *sn*$^w$ hypermutability. In these tests, the germ

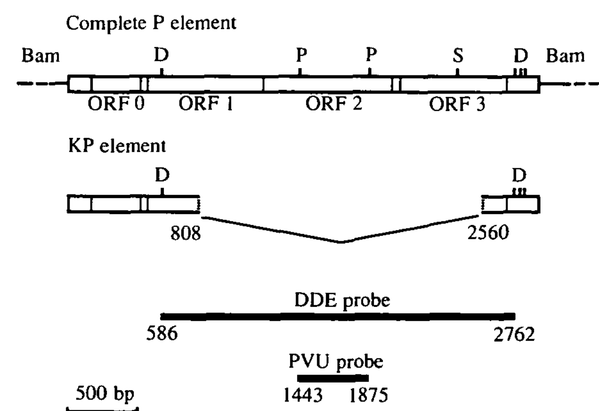


Fig. 1. Maps of complete P and KP elements and probes. Abbreviations and recognition sites (bp) for the restriction enzymes are: Bam, *Bam*H I (none); D, *Dde* I (586, 2762, 2796, 2814); P, *Pvu* I (1443, 1875); S, *Sal* I (2410). The locations of the four open reading frames (ORFs) are also shown.

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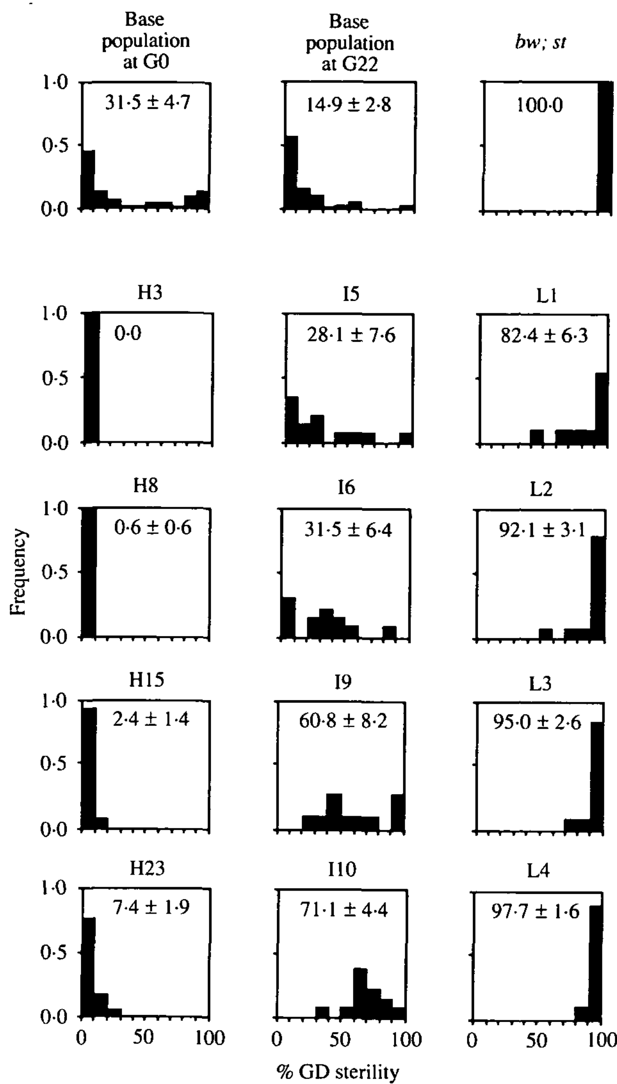


Fig. 2. Distributions of GD sterility in the daughters of females from the B13 base population and from the inbred lines. The data from the base population were obtained from tests performed at the beginning (G0) and at the end of inbreeding (G22). The data for the inbred lines were obtained at G22, when the lines were designated as having high (H), intermediate (I) or low (L) repression potential. The data for the *bw*; *st* stock were also obtained at G22. For each distribution, the mean and standard error are given.

line mutability of $sn^w$ was estimated for hybrid females derived from matings between B13 females and $sn^w(\pi_2)$ males. These tests were carried out for different groups of lines in G23, G25 and G27, and in each group, the *bw*; *st* strain was included as a negative control. Repression of $sn^w$ mutability was detected by a reduction in the mutation rate, which was calculated as the percentage of $sn^e$ flies among those scored. Altogether, 22827 flies from 602 test cultures were examined to obtain the data. In addition, parallel tests for the repression of dysgenic sterility were carried out in conjunction with the tests for the repression of $sn^w$ hypermutability. In these, individual B13 females were mated to $\pi_2$ males and the frequency of dysgenic sterility among their daughters was determined. This

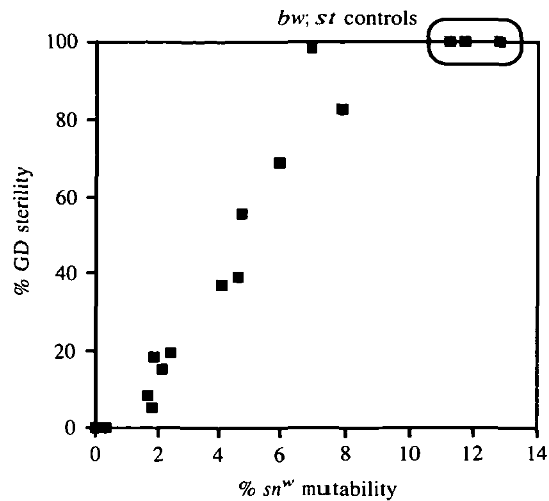


Fig. 3. The relationship between repression of $sn^w$ mutability and repression of GD sterility. GD sterility and $sn^w$ mutability were determined in hybrid females from each of the 12 inbred lines. The results with hybrids from the *bw*; *st* strain are also shown.

experimental design therefore permitted the relationship between the repression of $sn^w$ mutability and the repression of GD sterility to be investigated.

The combined results of the mutability and sterility tests are given in Figure 3. From a comparison to the *bw*; *st* controls, it appears that nearly all of the B13 lines were able to repress both dysgenic traits; the only exception was L4, which did not repress GD sterility in G23 but did in G27 (This is the only line that was tested twice). Figure 3 also shows that the incidence of the two traits was highly correlated (Kendall's $\tau = 0{\cdot}92$ using only the data from the inbred lines, $P < 0{\cdot}01$), suggesting that the repression of each was due to a common factor. However, since the sterility and mutability data were obtained from groups of lines tested at different times, it is possible that the correlation was due to differences among the groups. This possibility was investigated by performing a Kruskal–Wallis test, but no evidence for group heterogeneity was found ($P > 0{\cdot}25$).

In Fig. 3, notice that the B13 data form a line that intersects the top of the figure about 4 percentage points to the left of the data from the *bw*; *st* controls. This difference, amounting to about one third of the total distance on the horizontal scale, suggests that $sn^w$ mutability was repressed in some of the B13 stocks even though GD sterility was not. One possible explanation is that part of the repression of $sn^w$ mutability was due to titration of the transposase by P elements on the B13 chromosomes (Simmons & Bucholz, 1985).

### (iii) *Temporal changes in repression potential*

The sterility assay was used to determine the repression potential of females from each of the B13 lines at various times between G22 and G68. During this

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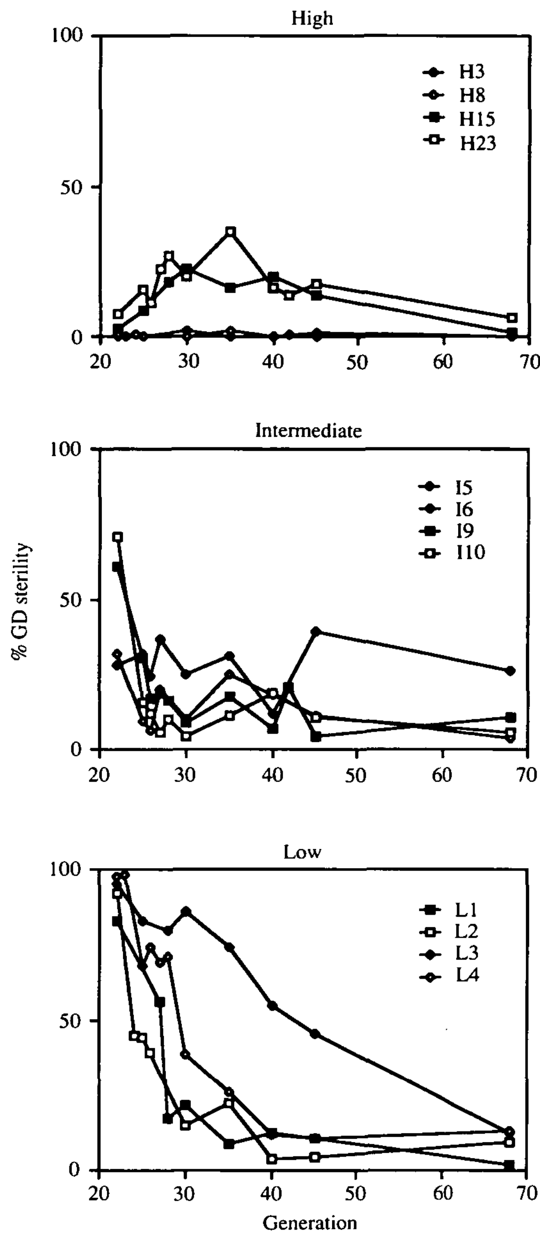


Fig. 4. Temporal changes in repression potential of the inbred lines following inbreeding. Lines with high, intermediate or low repression potential at G22 were monitored for repression of GD sterility until G68.

period, the lines were maintained by mass culturing in half-pint bottles. For each test, 8–36 females from a line were mated individually to $\pi_2$ males and 8–12 of the daughters from each mating were examined for GD sterility. On average, 19·9 females were mated in each test, and 11·6 daughters from each female were examined for sterility. Altogether, there were 113 tests, or 9·4 per line. As controls, *bw*; *st* females were included in every group of tests, and they consistently yielded > 99 % sterile daughters.

Figure 4 summarizes the results from the B13 lines for the whole period between G22 and G68. Two of the lines (H3 and H8) maintained high repression potential throughout this period; one line (I5) maintained intermediate repression potential and two lines (H15 and H23) changed from high to intermediate, and then back to high. All the other lines changed from low to high repression potential (L1, L2, L3, L4), or from intermediate to high repression potential (I6, I9 and I10) during this period. The observed changes therefore suggest that high repression potential was systematically favored in the lines, and that once it was acquired, it was a fairly stable state. Other studies with laboratory populations have obtained similar results (Kiyasu & Kidwell, 1984; Hirara *et al.* 1985; Anxolabéhère *et al.* 1986; Kidwell *et al.* 1988). However, one of the lines (I5) maintained intermediate repression potential during the entire period of study. Figure 5 shows the frequency distributions that were obtained from different tests with this line. Except for G40, all of these distributions were similar in shape and had similar means. This suggests that intermediate repression potential could be maintained as a stable state, even in a highly inbred population.

### (iv) *Transposase activity in the B13 lines*

Some of the changes documented above might have been due to the accumulation of repressor-producing

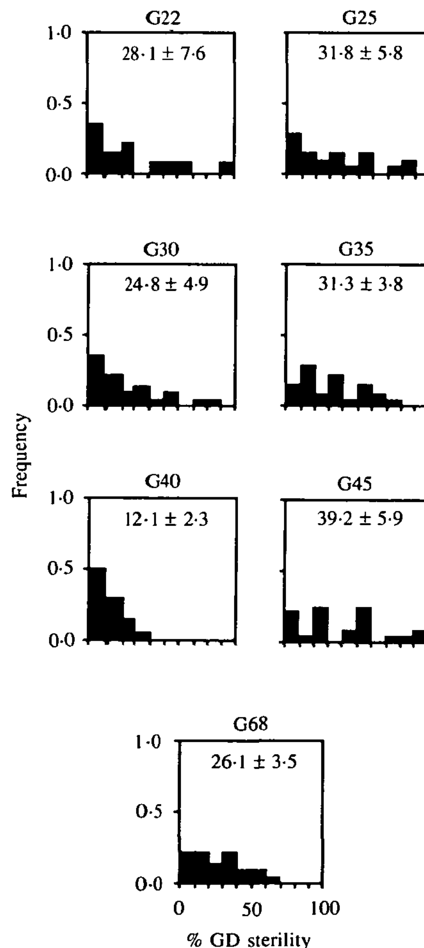


Fig. 5. Distributions of GD sterility in daughters of a line with intermediate repression potential from G22 until G68. The means and standard errors of the distributions are shown.

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Table 1. *Detection of transposase activity in hybrids from the inbred lines*

| | | Tests of female hybrids | | | | | Tests of male hybrids | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Line | Generation | Cultures scored | $sn^w$ | $sn^e$ | Mutation rate (%) | S.E. | Cultures scored | $sn^w$ | $sn^{(+)}$ | $sn^e$ | Mutation rate (%) | S.E. |
| H3 | 22 | 94 | 2662 | 8 | 0·30 | 0·13 | 99 | 2159 | 0 | 0 | 0·00 | |
| H8 | 24 | 100 | 3774 | 144 | 3·81 | 0·44 | 95 | 2136 | 244 | 192 | 17·59 | 1·25 |
| H15 | 25 | 61 | 2911 | 14 | 0·61 | 0·27 | 44 | 1023 | 0 | 0 | 0·00 | |
| H23 | 26 | 62 | 3064 | 16 | 0·63 | 0·24 | 62 | 1501 | 0 | 0 | 0·00 | |
| I5 | 26 | 61 | 2541 | 31 | 1·13 | 0·22 | 63 | 1428 | 3 | 0 | 0·30 | 0·21 |
| I6 | 26 | 46 | 1578 | 7 | 0·48 | 0·17 | 52 | 1198 | 0 | 0 | 0·00 | |
| I9 | 26 | 62 | 3124 | 18 | 0·64 | 0·20 | 55 | 1238 | 2 | 1 | 0·22 | 0·17 |
| I10 | 26 | 63 | 3373 | 36 | 1·09 | 0·29 | 59 | 1343 | 0 | 0 | 0·00 | |
| L1 | 27 | 58 | 2819 | 34 | 1·27 | 0·33 | 64 | 1441 | 0 | 0 | 0·00 | |
| L2 | 24 | 96 | 3755 | 27 | 0·72 | 0·20 | 85 | 1953 | 0 | 0 | 0·00 | |
| L3 | 25 | 64 | 2594 | 15 | 0·61 | 0·18 | 62 | 1588 | 4 | 5 | 0·73 | 0·53 |
| L4 | 22 | 99 | 2996 | 16 | 0·59 | 0·19 | 100 | 1944 | 12 | 0 | 0·88 | 0·77 |
| L4 | 26 | 63 | 3267 | 44 | 1·22 | 0·35 | 57 | 1246 | 0 | 0 | 0·00 | |
| $\pi_2$ | 26 | 61 | 1724 | 135 | 7·69 | 0·84 | 58 | 795 | 182 | 131 | 28·54 | 2·04 |

P elements, possibly by transposition. To determine whether the inbred lines were capable of synthesizing the transposase needed for this, hybrid males and females from crosses between B13 males and *y* $sn^w$; *bw*; *st* females were screened for germ line mutations of $sn^w$. The occurrence of any mutations would indicate that at least one transposase-producing P element was present on the B13 chromosomes.

These tests for transposase activity were carried out between G22 and G27; the $\pi_2$ strain, which is known to carry many transposase-producing P elements, was included as a positive control. As the results in Table 1 show, each of the inbred lines was able to induce at least some germ line mutations of $sn^w$; thus, each of the inbred lines carried at least one transposase-producing P element. These tests for transposase activity also show that hybrid females were generally more mutable than hybrid males. This observation might be explained by a concentration of P elements on the B13 X chromosomes, which were present in the hybrid females but not in the hybrid males. This explanation is consistent with the origin of the B13 strain, which was constructed by introducing an X chromosome from a natural population into a true M genetic background.

Tests were also conducted between G22 and G28 to determine if B13 males could induce GD sterility in the offspring of crosses with true M females. From each line 11–21 males were mated singly to *bw*; *st* females and 8–12 daughters of each were examined for GD sterility (altogether, 1966 hybrid females were scored). The highest frequency of sterility that was observed was 6% (line L3), indicating that B13 males had little ability to induce GD sterility.

### (v) *Inheritance of repression potential*

Previous studies have shown that maternally inherited, i.e. cytoplasmic, factors can contribute to the determination of repression potential (Engels, 1979*c*; Kidwell, 1981; Jackson *et al.* 1988; Simmons *et al.* 1990). To see if such factors were present in the B13 lines, reciprocal hybrid females produced by crossing B13 flies to flies from the *bw*; *st* M strain were mated individually to $sn^w(\pi_2)$ males and samples of their daughters were examined for GD sterility. These reciprocal hybrids, denoted $F_1$-A and $F_1$-B according to whether the B13 parent was male (A) or female (B), were chromosomally equivalent; therefore, any differences in the frequency of sterility among their daughters would indicate that cytoplasmic factors were involved in the determination of repression potential. Hybrids produced by intercrossing the *bw*; *st* and $\pi_2$ strains were also tested in this way to provide comparative data.

The results of these experiments are summarized in Table 2. This Table also presents the results of tests with females taken directly from the B13 stocks. At this time (G68), four of the stocks (H3, H8, H15 and L1) exhibited repression potential as great as that seen in the $\pi_2$ control, seven (H23, I6, I9, I10, L2, L3 and L4) showed slightly less potential and one (I5) showed only moderate potential.

The data from the tests with the reciprocal hybrids indicated that cytoplasmic factors inherited from the B13 females contributed to repression potential in at least six lines (H3, H8, I9, L1, L2 and L3; see Fig. 6). For each of these, the daughters from the $F_1$-B hybrids showed significantly less sterility than those from the $F_1$-A hybrids ($P < 0.05$ by a one-tailed Mann–Whitney rank sum test). In no case, however, was the influence of the cytoplasmic factors as great as that seen with the $\pi_2$ strain. In five other lines (H15,

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Table 2. *Reciprocal cross analysis of the inheritance of repression potential*

| | Stock (G68) | | | $F_1$-A hybrids | | | $F_1$-B hybrids | | |
|---|---|---|---|---|---|---|---|---|---|
| Line | $N^a$ | $n^b$ | GD±S.E.[c] | $N$ | $n$ | GD±S.E. | $N$ | $n$ | GD±S.E. |
| H3 | 25 | 289 | 0·3±0·3 | 23 | 276 | 96·0±1·7 | 24 | 268 | 38·8±5·6* |
| H8 | 21 | 242 | 0 | 24 | 280 | 98·6±0·8 | 25 | 300 | 82·7±3·7* |
| H15 | 13 | 134 | 1·3±0·9 | 24 | 288 | 98·6±0·7 | 25 | 300 | 98·7±0·6 |
| H23 | 20 | 233 | 6·2±1·6 | 24 | 288 | 97·6±0·9 | 24 | 288 | 96·5±1·2 |
| I5 | 23 | 276 | 26·1±3·5 | 24 | 288 | 97·2±1·0 | 25 | 300 | 96·7±1·1 |
| I6 | 12 | 137 | 3·5±2·2 | 24 | 288 | 94·5±1·4 | 24 | 288 | 98·6±0·7 |
| I9 | 21 | 241 | 10·5±2·8 | 25 | 300 | 97·7±0·9 | 25 | 300 | 91·0±2·3* |
| I10 | 16 | 176 | 5·5±3·0 | 25 | 300 | 97·3±0·8 | 25 | 300 | 96·3±1·4 |
| L1 | 15 | 171 | 1·7±0·9 | 25 | 300 | 97·3±1·3 | 24 | 288 | 94·5±1·5* |
| L2 | 21 | 246 | 9·2±3·2 | 25 | 300 | 97·7±0·8 | 25 | 300 | 92·7±2·0* |
| L3 | 24 | 255 | 12·7±3·3 | 25 | 300 | 99·7±0·3 | 22 | 264 | 97·0±1·4* |
| L4 | 15 | 176 | 13·0±3·0 | 25 | 300 | 98·3±0·7 | 25 | 299 | 97·0±1·1 |
| $\pi_2$ | 19 | 220 | 1·4±0·7 | 24 | 287 | 99·3±0·7 | 25 | 300 | 17·0±6·0* |
| *bw*; *st* | 12 | 127 | 100·0 | | | | | | |

[a] Number of cultures.
[b] Number of daughters examined for GD sterility.
[c] Unweighted average percentage of daughters with GD sterility±standard error.
* Significantly less than $F_1$-A

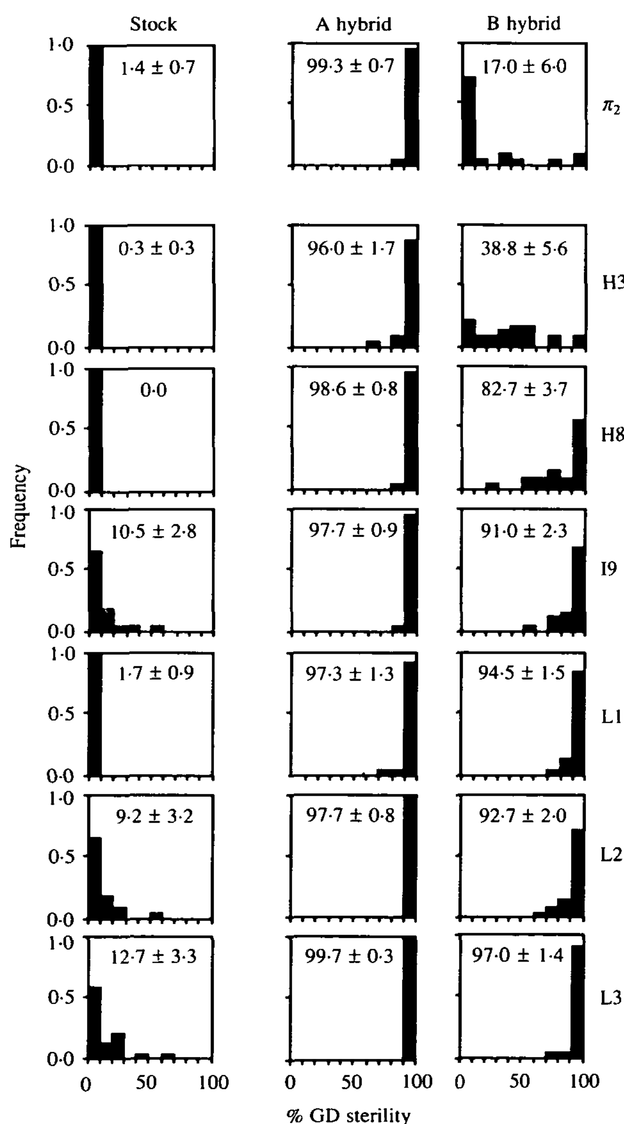


Fig. 6. Inheritance of repression potential in reciprocal hybrid females. The hybrid females were derived from reciprocal matings between the inbred lines and the *bw*; *st* strain. Only the distributions from lines with a significant difference between the reciprocal hybrids are shown. The means and standard errors of these distributions are also given.

H23, I5, I10, and L4) no evidence for cytoplasmic factors was obtained. However, one of the lines (I6) showed a significantly greater frequency of sterility among the daughters of the $F_1$-B hybrids ($P < 0·05$ by a one-tailed Mann–Whitney rank sum test), suggesting that in some cases, antagonists of repression potential could be transmitted through the maternal cytoplasm. Similar results have been obtained in studies with other, unrelated strains (Simmons *et al.* 1990). It should also be noted that since the $F_1$-A hybrids from each of the B13 lines showed little or no ability to repress GD sterility in their daughters, the repression potential of these lines did not appear to be influenced by partially dominant chromosomal factors. Studies using other strains have documented the influence of such factors (Kidwell, 1985; Black *et al.* 1987; Simmons *et al.* 1990).

### (vi) *Maternal and zygotic contributions to the repression of GD sterility*

Although the preceding experiments have provided information about the chromosomal and cytoplasmic basis of repression potential, they have not considered the nature of repression itself. Previous studies have indicated that the repression of GD sterility is due primarily, if not exclusively, to a maternal effect (Engels, 1979*c*; Simmons *et al.* 1987, 1990); however,

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Table 3. *Genetic analysis of maternal and zygotic contributions to the repression of GD sterility*

| Line | Cross 1: *M5*/+ | | | Cross 1: $sn^w$/+ | | | Cross 2: $sn^w$/+ | | | Cross 3: *M5*/+ | | | Cross 3: *T-5*/+ | | | Cross 4: *T-5*/+ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $N^a$ | $n^b$ | GD ± S.E.[c] | *N* | *n* | GD ± S.E. | *N* | *n* | GD ± S.E. | *N* | *n* | GD ± S.E. | *N* | *n* | GD ± S.E. | *N* | *n* | GD ± S.E. |
| H3 | 28 | 288 | 0·3 ± 0·3 | 28 | 312 | 0 | 28 | 309 | 0 | 25 | 276 | 6·5 ± 1·9 | 25 | 274 | 41·0 ± 5·3 | 23 | 273 | 0 |
| H8 | 28 | 288 | 0 | 28 | 313 | 0 | 22 | 207 | 0·4 ± 0·4 | 24 | 284 | 12·7 ± 2·8 | 24 | 281 | 50·7 ± 6·8 | 17 | 186 | 0·5 ± 0·5 |
| H15 | 24 | 267 | 0 | 24 | 280 | 0 | 17 | 184 | 0 | 29 | 316 | 11·4 ± 2·5 | 29 | 309 | 54·1 ± 4·8 | 14 | 164 | 0 |
| H23 | 26 | 198 | 0 | 26 | 213 | 0 | 21 | 148 | 0 | 25 | 240 | 15·0 ± 2·8 | 23 | 237 | 45·9 ± 5·3 | 27 | 316 | 1·2 ± 1·2 |
| I5 | 29 | 340 | 0·3 ± 0·3 | 29 | 337 | 0 | 24 | 241 | 0·4 ± 0·4 | 28 | 270 | 14·4 ± 3·3 | 28 | 278 | 51·9 ± 5·1 | 24 | 249 | 1·4 ± 0·8 |
| I6 | 27 | 309 | 0 | 27 | 314 | 0 | 27 | 294 | 0 | 27 | 307 | 10·3 ± 1·9 | 27 | 308 | 40·4 ± 5·5 | 25 | 267 | 0 |
| I9 | 27 | 309 | 0 | 27 | 306 | 0·3 ± 0·3 | 26 | 290 | 1·0 ± 1·0 | 28 | 289 | 5·3 ± 1·2 | 28 | 294 | 49·9 ± 4·5 | 27 | 300 | 0·6 ± 0·4 |
| I10 | 29 | 334 | 0·3 ± 0·3 | 29 | 342 | 0·3 ± 0·3 | 10 | 94 | 0·8 ± 0·8 | 27 | 281 | 6·7 ± 2·0 | 27 | 278 | 55·3 ± 4·7 | 15 | 166 | 1·1 ± 0·8 |
| L1 | 30 | 360 | 0·3 ± 0·3 | 30 | 360 | 0·3 ± 0·3 | 24 | 247 | 0 | 25 | 270 | 10·0 ± 2·9 | 25 | 269 | 25·4 ± 4·5 | 26 | 212 | 0·4 ± 0·4 |
| L2 | 26 | 301 | 0 | 26 | 308 | 0 | 22 | 249 | 0·4 ± 0·4 | 24 | 264 | 5·7 ± 2·3 | 24 | 263 | 20·0 ± 4·1 | 26 | 256 | 0·3 ± 0·3 |
| L3 | 23 | 264 | 0 | 23 | 266 | 0 | 13 | 121 | 2·1 ± 2·1 | 25 | 266 | 16·9 ± 3·0 | 25 | 254 | 66·0 ± 4·7 | 16 | 178 | 0·5 ± 0·5 |
| L4 | 29 | 336 | 0·9 ± 0·5 | 29 | 329 | 0·9 ± 0·5 | 25 | 272 | 0 | 28 | 282 | 14·2 ± 3·4 | 28 | 284 | 52·5 ± 4·8 | 21 | 151 | 0·8 ± 0·8 |
| *bw*; *st* | 76 | 735 | 0·1 ± 0·1 | 76 | 790 | 0·2 ± 0·2 | 40 | 516 | 1·7 ± 0·6 | 54 | 510 | 21·4 ± 3·0 | 56 | 534 | 87·4 ± 1·9 | 44 | 312 | 63·6 ± 4·3 |
| $\pi_2$ | 29 | 312 | 100 | 29 | 312 | 99·7 ± 0·3 | 12 | 137 | 0 | 26 | 227 | 97·1 ± 2·6 | 26 | 241 | 100 | 21 | 244 | 0 |

Cross 1 (*M5*/$sn^w$ ♀ × line ♂); Cross 2 ($sn^w$ ♂ × line ♀); Cross 3 (*M5*/*T-5* ♀ × line ♂); Cross 4 (*T-5* ♂ × line ♀).

[a] Number of crosses.

[b] Number of daughters examined.

[c] Unweighted average percentage of daughters with GD sterility ± standard error.

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it is formally possible that repression might also involve chromosomal factors that are expressed after fertilization. To determine whether this was so, flies from each B13 line was crossed in G74 with flies carrying a dysgenesis-inducing *T-5* X chromosome. This chromosome was used because previous experiments had demonstrated that the B13 lines were largely incapable of inducing GD sterility by themselves. With each of the lines, two types of crosses were performed: B13 male × *T-5/M5* females (denoted cross 3) and B13 female × *T-5* (denoted cross 4). In each case, many replicate matings were established and from each mating, a samples of the *T-5/+* and, where appropriate, *M5/+* daughters were examined for GD sterility. In addition, a parallel series of crosses between B13 flies and flies bearing a *y* $sn^w$ X chromosome in place of the *T-5* X chromosome was performed to assess the frequency of GD sterility induced by the B13 lines themselves (B13 male × *y* $sn^w$/*M5* female = cross 1 and B13 female × *y* $sn^w$ male = cross 2). As controls, crosses 1–4 were also carried out using flies from the *bw*; *st* and $\pi_2$ strains in place of the B13 flies.

The results of these experiments are presented in Table 3. Notice that for crosses 1 and 3 there are two sets of data, corresponding to the two types of daughters, *M5/+* and either $sn^w$/+ or *T-5/+*, that were produced. In contrast, crosses 2 and 4 each produced only a single type of daughter.

From crosses 1 and 2 it is clear that, by themselves, the B13 lines could not induce more than a trivial level of GD sterility. This is in contrast to the $\pi_2$ strain, which induced a high frequency of sterility in the daughters from cross 1, but not in those from cross 2; these opposite results are consistent with the known properties of the $\pi_2$ strain (Engels, 1979*c*; Engels & Preston, 1979).

Cross 3 demonstrates that the *T-5* X chromosome could induce a high frequency of sterility, either by itself or in combination with the B13 chromosomes; 87·4% of the *T-5/+* daughters from the *bw*; *st* controls were sterile, compared to 21·4% of their *M5/+* sisters. Smaller, but still significant differences were also observed between the *T-5/+* and *M5/+* classes from each of the B13 lines. These data therefore indicate that the ability to induce GD sterility was strongly associated with the *T-5* X chromosome. The sterility that was seen among the *M5/+* females from these crosses probably reflects the action of P elements that had transposed from the *T-5* X chromosomes to other chromosomes in a previous generation.

Cross 3 also established that the paternally derived B13 chromosomes contributed substantially to the repression of GD sterility. With every line, the frequencies of sterility for both the *M5/+* and *T-5/+* females were significantly less than the corresponding frequencies for the *bw*; *st* controls. This finding is surprising since other studies have shown that P-element-containing chromosomes act synergistically with *T-5* X chromosomes to increase the frequency of GD sterility (Simmons *et al.* 1987; Rasmusson *et al.* 1990). The present observations therefore imply that the B13 chromosomes carried factors that were expressed after fertilization to repress the sterility-inducing effects of the *T-5* X chromosome. Perusal of the data that cross 3 shows that the efficacy of these factors varied among the B13 lines. The most effective line, L2, reduced sterility among the *T-5/+* females to 20%, while the least effective line, L3, reduced it to only 66%. The effects of these factors were not seen in the experiments that studied the inheritance of repression potential, possibly because a stronger inducer of gonadal dysgenesis [the $sn^w(\pi_2)$ strain] was employed.

From cross 4 it is clear that GD sterility was strongly repressed in the *T-5/+* daughters of the B13 females; few, if any of these were sterile, compared to 63·6% from the *bw*; *st* controls. Furthermore, since

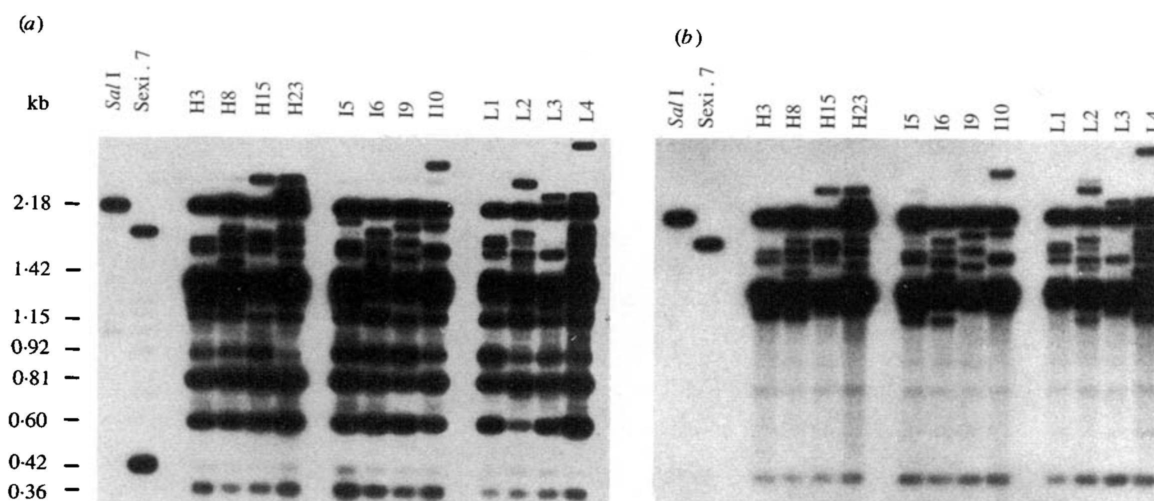


Fig. 7. Analysis of *Dde* I restriction fragments from lines with high, intermediate or low repression potential at G22. A Southern membrane containing *Dde* I-digested DNA from each line was hybridized with the DDE probe (*a*) and also with the PVU probe (*b*).

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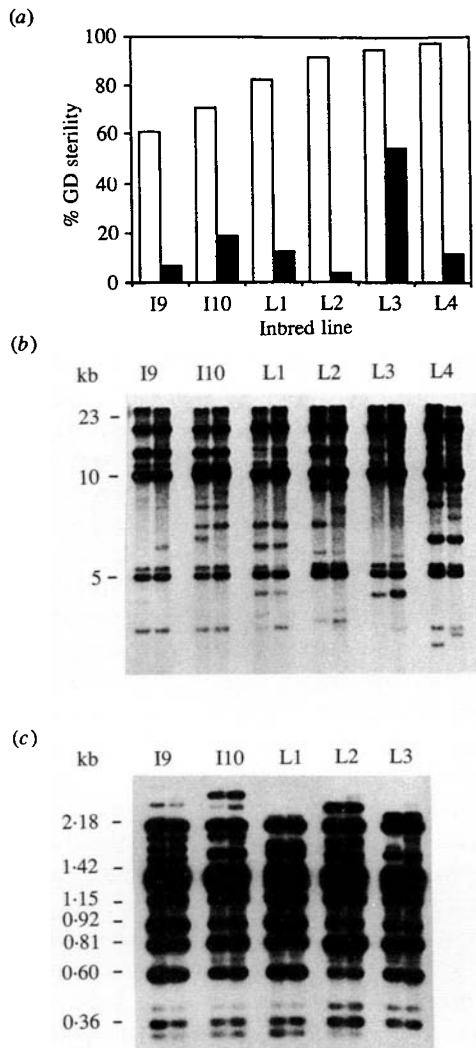


Fig. 8. Analysis of *Bam*H I and *Dde* I restriction fragments of several inbred lines that had acquired increased repression potential. Differences in the ability to repress GD sterility between generations 22 (□) and 40 (■) are shown for 6 inbred lines (*a*). A membrane containing *Bam*H I-digested DNA from the six lines at G22 (left lane) and G40 (right lane) was hybridized with the PVU probe (*b*). A membrane containing *Dde* I-digested DNA from 5 of the lines at G22 (left lane) and G40 (right lane) was hybridized with the DDE probe (*c*).

such strong repression was not seen among the chromosomally equivalent daughters from cross 3, a maternal effect was apparently involved. However, this inference must be qualified by noting that the *T-5*/+ daughters from the *bw*; *st* controls also showed significantly less sterility in cross 4 than they did in cross 3. This unexpected (Simmons *et al.* 1987, 1990) finding suggests that sterility was intrinsically more likely when the *T-5* X chromosome was maternally inherited, possibly because of maternal transmission of the transposase itself.

### (vii) *Molecular analysis of the B13 lines*

Several analyses were carried out in an attempt to explain the repression abilities of the B13 lines. One approach was to examine the kinds of P elements that were present in each of the lines. DNA was extracted from flies collected in G22 and digested with the enzyme *Dde* I, which cleaves complete P elements in two locations and releases an internal 2·18 kb fragment. The digested DNA was fractionated in an agarose gel, transferred to a membrane by Southern blotting, and then hybridized with radiolabelled P-element probes. DNA from two control strains, *Sal* I (with a single artificially mutated P element, see Karess & Rubin, 1984) and Sexi.7 (with many copies of a particular incomplete element called KP, see Simmons *et al.*, 1990), was also analysed.

Figure 7 shows the autoradiograms that were obtained by hybridizing the Southern membrane with two different P-element probes. The DDE probe (see Fig. 1) was expected to hybridize with no more than one size of *Dde* I restriction fragment from each kind of P element present. The many hybridizing fragments evident in the autoradiogram in Fig. 7*a* therefore demonstrate that each of the B13 lines had many different kinds of P elements. This molecular diversity was not seen in the control strains; *Sal* I produced only one hybridizing band, reflecting the fact that it carried only one kind of P element, and Sexi.7 produced only two major hybridizing bands, implying the presence of two principal kinds of elements, one of which was the KP element. Upon digestion with *Dde* I, this latter element produces an internal fragment that is 420 bp long. The B13 lines also appeared to produce such a fragment, but as the autoradiogram in Fig. 7*b* shows, this fragment also hybridized with the PVU probe, which is not homologous to the DNA sequences of KP elements (see Fig. 1). Consequently, the 0·42 kb fragment seen in the B13 lines could not have been derived from KP.

From Fig. 7*a* it is apparent that the majority of the bands that hybridized with the DDE probe were common to all of the B13 lines. This includes a 2·18 kb band, which could have come from complete P elements (see Fig. 1). Because of these similarities, it was not possible to identify particular P elements that were responsible for the variation in repression potential among the lines.

Another approach was to compare DNA samples from lines before and after the acquisition of high repression potential. As shown above, 6 of the 8 I and L lines acquired high repression potential between G22 and G40 (see Figs 4 and 8*a*). Consequently, an attempt was made to determine if these phenotypic changes were correlated with any changes in the array of genomic P elements.

Figure 8*b* shows an autoradiogram of *Bam*H I-digested DNA from the lines at G22 and at G40; the DNA was hybridized with the centrally located PVU



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probe, which is expected to detect P elements greater than 1·2 kb in length. Since *Bam*H I does not cleave within P elements, this autoradiogram provided data on the genomic distribution of such elements. Within each line, only a small number of bands appeared or disappeared between G22 and G40, implying that the evolution of high repression potential was associated with very little P-element movement. However, there was a net increase in the number of resolvable bands in all 6 lines.

To check on possible qualitative differences among the P elements in a line before and after the acquisition of high repression potential, *Dde* I-digested DNA from five of the lines was hybridized with the DDE probe. As Fig. 8*c* shows, there are few, if any, differences between the paired samples. Thus, using Southern analysis, it was not possible to discern any striking molecular changes associated with the acquisition of high repression potential. This is essentially the same conclusion that was reached by Daniels *et al.* (1987) and by Kidwell *et al.* (1988).

## 4. Discussion

The repression of P-element-induced hybrid dysgenesis has proven to be a complex phenomenon. Early studies (Engels, 1979*c*, 1981; Engels & Preston, 1981) had indicated that individual *D. melanogaster* females repressed dysgenesis in either all of their progeny, or in none of them; only a few cases of intermediate repression potential were found. This dichotomy led Engels (1979*c*) to define the M and P cytotypes as opposite regulatory states, with the former permitting dysgenesis and the latter repressing it. Through genetic analysis, Engels (1979*c*) showed that the P cytotype was jointly determined by chromosomal and cytoplasmic factors, and that the chromosomal factors were genetically correlated with the factors that induced hybrid dysgenesis. Subsequent analyses (Bingham *et al.* 1982; Rubin *et al.* 1982) demonstrated that these latter factors were transposable P elements. It is now widely held that certain types of P elements are responsible for the establishment of the P cytotype (Daniels *et al.* 1987; Nitasaka *et al.* 1987; Engels, 1989; Robertson & Engels, 1989).

Additional genetic studies have shown that the ability to repress hybrid dysgenesis can vary quantitatively. In some strains, this variation is essentially continuous (Kidwell, 1985; Black *et al.* 1987; Simmons *et al.* 1987, 1990; Jackson *et al.* 1988), suggesting that P-element activity might be regulated by a mechanism that is different from the P cytotype. Black *et al.* (1987) have attributed this regulation to a non-autonomous P element, called KP, which is prevalent in the genomes of many Q and M′ strains. Genetic analysis of some of these strains has indicated that the ability to repress dysgenesis is determined primarily, if not exclusively, by additive chromosomal factors (Kidwell, 1985; Jackson *et al.* 1988). However, another study with KP-containing strains has revealed considerable variation in the way in which repression potential is determined, including determination by cytoplasmic factors (Simmons *et al.* 1990). In addition, this study has identified two strains that have numerous KP elements, but little or no repression potential. It is therefore unclear in what way KP elements contribute to the repression of hybrid dysgenesis.

In the present study, inbred lines lacking KP elements were examined for the ability to repress hybrid dysgenesis. Two traits, GD sterility and $sn^w$ hypermutability, were used to assess the repression potential of the lines. Although the results with these traits were highly correlated, each of the lines seemed to be slightly more effective in repressing $sn^w$ mutability. This greater effectiveness might be due to a titration effect (Simmons & Bucholz, 1985; Simmons *et al.* 1987; Rasmusson *et al.* 1990), in which P elements from the inbred lines titrate the transposase away from the $sn^w$ allele, thereby reducing its mutability. Such an effect would be expected even if the P elements from the inbred lines were unable to synthesize repressors of P-element activity.

In this study, repression potential was monitored during an extensive period in which the inbred lines were maintained by random mating. Initially, there were striking differences among and within lines, but over time, these tended to disappear as the less potent lines evolved to a state of high repression potential. This evolution occurred rather rapidly (< 8 generations), suggesting that changes in only one or a few P elements might have been responsible. In support of this view, Southern blot analyses demonstrated that none of the lines underwent dramatic changes in the number or types of genomic P elements.

Genetic analyses revealed that cytoplasmic factors contributed to the determination of repression potential in some of the inbred lines; however, these factors were not as strong as those that were found in a classic P-cytotype strain. Overall, the diverse modes of inheritance of repression potential (Engels, 1979*c*; Engels & Preston, 1981; Kidwell, 1981, 1985; Black *et al.* 1987; Jackson *et al.* 1988; Simmons *et al.* 1990) suggest that many different kinds of genetic and cytoplasmic factors may be involved.

Previous studies have indicated that the repression of gonadal dysgenesis is mediated primarily by a maternal effect (Engels, 1979*c*; Simmons *et al.* 1987, 1990); however, in the present study it was found that repression could also be caused by paternally contributed chromosomal factors that are expressed after fertilization. This zygotic effect is not likely to come from transposase titration since there is an intrinsic tendency for paternally contributed chromosomes to increase the incidence of gonadal dysgenesis, possibly by providing more targets for the transposase to attack (Simmons *et al.* 1987; Rasmusson *et al.* 1990).

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Other investigators (Kidwell, 1981; Robertson & Engels, 1989) have also reported repression by zygotic effects.

While it is generally agreed that repressors of hybrid dysgenesis can be maternally transmitted, there has apparently been no consideration of the possibility that the P transposase (or its message) might also pass through the maternal cytoplasm. This prospect has been raised by the results of the experiments with the transposase-producing *T-5* X chromosomes. In particular, it was found that the offspring of crosses between *T-5/M-5* females and M cytotype males were more likely to be dysgenic than the chromosomally equivalent offspring of crosses between *T-5* males and M cytotype females. This observation may be explained in one of two ways; either the P elements on a maternally inherited *T-5* X chromosome are more susceptible to mobilization than the P elements on a paternally transmitted *T-5* X, or the P transposase (or its message) is transmitted through the maternal cytoplasm, thereby enhancing the mobilization of all P elements in the next generation. In *D. simulans*, Bryan *et al.* (1987) have found a case of maternal enhancement of transposon instability that formally resembles the situation reported here.

The results of this and other studies have provided a complex picture of P-element regulation. This regulation appears to vary quantitatively and qualitatively, and is determined by a combination of chromosomal and cytoplasmic factors; in some cases, the cytoplasmic factors have been shown to persist for more than two generations. In addition, although P-element regulation often involves a maternal effect, it may sometimes arise from factors that are expressed post-zygotically. Population studies have shown that the evolution of regulatory ability need not be associated with dramatic changes in the array of genomic P elements; nonetheless, there is strong evidence that certain types of P elements influence P activity, possibly synthesizing repressors of transposition. Further work will be needed to elucidate the nature of these repressors, as well as the factors that modulate their production.

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