Repression of Hybrid Dysgenesis in *Drosophila melanogaster* by Individual Naturally Occurring *P* Elements

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ABSTRACT

Individual *P* elements that were genetically isolated from wild-type strains were tested for their abilities to repress two aspects of hybrid dysgenesis: gonadal dysgenesis and mutability of a double-*P* element-insertion allele of the *singed* locus (sn^w). These elements were also characterized by Southern blotting, polymerase chain reaction amplification and DNA sequencing. Three of the elements were 1.1-kb *KP* elements, one was a 1.2-kb element called *D50*, and one was a 0.5-kb element called *SP*. These three types of elements could encode polypeptides of 207, 204, and 14 amino acids, respectively. Gonadal dysgenesis was repressed by two of the *KP* elements (denoted *KP(1)* and *KP(6)*) and by *SP*, but not by the third *KP* element (*KP(D)*), nor by *D50*. Repression of gonadal dysgenesis was mediated by a maternal effect, or by a combination of zygotic and maternal effects generated by the *P* elements themselves. The mutability of sn^w was repressed by the *KP(1)* and *KP(6)* elements, by *D50* and by *SP*, but not by *KP(D)*; however, the *SP* element repressed sn^w mutability only when the transposase came from complete *P* elements and the *D50* element repressed it only when the transposase came from the modified *P* element known as Δ2-3. In all cases, repression of sn^w mutability appeared to be mediated by a zygotic effect of the isolated *P* element. Each of the isolated elements was also tested for its ability to suppress the phenotype of a *P*-insertion mutation of the *vestigial* locus ($vg^{21\text{-}3}$). *D50* was a moderate suppressor whereas *SP* and the three *KP* elements had little or no effect. These results indicate that each isolated *P* element had its own profile of repression and suppression abilities. It is suggested that these abilities may be mediated by *P*-encoded polypeptides or by antisense *P* RNAs initiated from external genomic promoters.

THERE are more than 40 families of transposable elements in the *Drosophila melanogaster* genome. Of these, the best understood is the *P* family, which consists of complete elements 2,907 base pairs (bp) long and a wide assortment of smaller elements derived from the complete ones by different internal deletions (O'HARE and RUBIN 1983; ENGELS 1989; O'HARE *et al.* 1992). The complete elements encode a *trans*-acting transposase, which is the catalytic agent for mobilizing the members of the *P* family (SPRADLING and RUBIN 1982; RUBIN and SPRADLING 1982; ENGELS 1984; KARESS and RUBIN 1984). Detailed molecular and biochemical studies have elucidated the primary structure of this polypeptide as well as the DNA sequences with which it interacts (O'HARE and RUBIN 1983; RIO, LASKI and RUBIN 1986; LASKI, RIO and RUBIN 1986; KAUFMAN, DOLL and RIO 1989; MULLINS, RIO and RUBIN 1989). The transposase is encoded by four exons, denoted 0–3, spanning the length of the *P* sequence. It contains 751 amino acids, has a mass of 87 kilodaltons (kD), and binds to regions near the 31-bp inverted terminal repeats of *P* elements. The synthesis of the transposase is restricted to the germ line because proteins in the somatic tissues prevent the removal of the last (2-3) intron in the pre-mRNA (RIO, LASKI and RUBIN 1986; LASKI, RIO and RUBIN 1986; LASKI and RUBIN 1989; SIEBEL and RIO 1990). Translation of this incompletely processed RNA results in a 66-kD protein that is identical with the transposase for much of its length; a stop codon in the 2-3 intron accounts for the smaller size. When the 2-3 intron is artificially removed from a complete *P* element, the 87-kD transposase is made in the somatic cells. Such modified elements, called Δ2-3 elements, have been introduced into Drosophila stocks, where they engender somatic *P* transposition (LASKI, RIO and RUBIN 1986; ROBERTSON *et al.* 1988).

The induction of *P* activity in the germ line leads to a syndrome of genetic abnormalities called hybrid dysgenesis (KIDWELL, KIDWELL and SVED 1977). These abnormalities include mutations, chromosome breakage, male recombination, chromosome nondisjunction, chromosome segregation distortion and sterility. Typically they occur in the progeny of a cross between a male that carries *P* elements in its genome (from a P strain) and a female that does not (from an M strain). The progeny of the reciprocal cross, P female × M male, are usually not dysgenic, indicating that *P* activity is repressed by factors contributed

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maternally from a P strain. Genetic analyses have suggested that these factors are products of the *P* elements themselves (ENGELS 1979a; KIDWELL 1981, 1985).

Early studies established a nomenclature to describe *P* element regulation. Strains with a strong ability to repress *P* activity were said to have the P cytotype and strains without this ability were said to have the M cytotype (ENGELS 1979a). Subsequent studies have shown that regulatory ability actually follows a continuous distribution in Drosophila populations, with the M and P cytotypes lying at the extremes (KIDWELL 1983, 1985; SIMMONS *et al.* 1987, 1990; HEATH and SIMMONS 1991). Strains with intermediate regulatory ability have been called M′ or pseudo-M, and molecular analyses have shown that, like P cytotype strains, they carry *P* elements in their genomes; true M cytotype strains, however, do not (BINGHAM, KIDWELL and RUBIN 1982). This variability suggests that *P* element regulation is determined by a complex array of genetic factors, presumably the *P* elements themselves.

Several investigators have proposed that regulation of the *P* family is mediated by *P*-encoded polypeptides that repress transposase activity [reviewed in RIO (1990)]. However, because the coding capacity of *P* elements is limited, these repressor polypeptides would have to share some of the same amino acid sequences as the transposase. These shared sequences might specify features such as DNA binding domains or oligomerization motifs that are critical for repressor function.

The most vigorously championed candidate for a repressor is the 66-kD polypeptide made from the incompletely processed transcripts of complete *P* elements (ROBERTSON and ENGELS 1989; MISRA and RIO 1990). Biochemical data suggest that this polypeptide is produced in the germ line and that it may act to repress the transcription of complete *P* elements (MISRA and RIO 1990; KAUFMAN and RIO 1991). Studies with modified *P* elements designed to produce this polypeptide have shown that it confers repression potential. However, the 66-kD polypeptide cannot be the only *P* element repressor since strains without it have been found to repress transposition (SIMMONS *et al.* 1987, 1990). Consequently, researchers have proposed that polypeptides from various incomplete *P* elements also function as repressors.

One candidate is the 207-amino acid polypeptide that could be made by a 1.15-kb element called *KP*. *KP* elements are present in Drosophila populations throughout the world, but they are especially abundant in the M′ populations of Eurasia (BLACK *et al.* 1987; C. PRESTON and W. ENGELS, personal communication). On the basis of indirect genetic analyses, it has been suggested that *KP* elements are responsible for intermediate repression potential (BLACK *et al.* 1987; JACKSON, BLACK and DOVER 1988). However, the existence of the *KP* polypeptide has not yet been demonstrated, nor has there been a published report of an individual *KP* element repressing hybrid dysgenesis. Other less well characterized incomplete *P* elements have also been proposed as possible sources of repressor polypeptides [*e.g.*, NITASAKA, MUKAI and YAMAZAKI (1987)]. Further research is needed to identify their potential polypeptide products and to test their repressor functions.

In this paper we analyze the repression of hybrid dysgenesis by three distinct types of incomplete *P* elements isolated from a strain called Sexi, which was derived from a natural population in Spain (KIDWELL 1985). Our results unambiguously demonstrate that individual, naturally occurring *P* elements possess repression potential and suggest that in some cases, repression may be mediated by an antisense *P* RNA rather than by a *P*-encoded polypeptide.

MATERIALS AND METHODS

Stocks: All stocks and experimental cultures were raised in vials or half-pint milk bottles on a standard cornmeal-molasses-agar medium. Unless otherwise stated, the culturing temperature was 25°. Additional information about the genetic markers and chromosome rearrangements can be found in LINDSLEY and ZIMM (1992) and SIMMONS *et al.* (1990).

Strains without P elements:

Canton S (CS), a wild-type strain.

bw; st, homozygous for the recessive eye color markers *brown* (*bw*) and *scarlet* (*st*), which are located on the second and third chromosomes, respectively.

C(1)DX, y f/Y/y cin w f^{s} su(f)^{ts67g}. The females have attached-*X* chromosomes and the males have an *X*-linked temperature-sensitive lethal mutation (*su(f)^{ts67g}*) which aids in the collection of virgin females.

C(1)DX, y f/Y/y shi^{ts}; bw; st, another attached-*X* stock in which the males have a different *X*-linked temperature-sensitive lethal mutation (*shi^{ts}*). Both males and females are homozygous for the autosomal markers *bw* and *st*.

C(1)DX, y f/Y/FM6, an attached-*X* stock in which the *X* chromosome in the male is the *FM6* balancer.

y sn^{3} v car, a stock homozygous for four recessive *X*-linked markers, *yellow* (*y*) body, extreme *singed* (*sn^{3}*) bristles, *vermilion* (*v*) eyes and *carnation* (*car*) eyes.

M5-CS, homozygous for the *M5* (*Muller-5*) balancer *X* chromosome. The genetic background is from a Canton S (CS) wild-type stock.

M5; bw; st, homozygous for the *M5* balancer *X* chromosome and the autosomal markers *bw* and *st*.

TM3, Sb Ser e/TM6, Tb e, abbreviated *TM3/TM6*, heterozygous for two third chromosome balancers, one carrying the dominant mutations *Stubble* (*Sb*) bristles and *Serrate* (*Ser*) wings and the other carrying the dominant mutation *Tubby* (*Tb*) body. Both chromosomes are homozygous lethal.

FM7, y 31^{d} sc^{8} sn^{x2} B/Ins(1) sc^{7} + AM, sc^{7} w^{a} ptg^{4} bx^{78h} l(1)^{78h}, abbreviated *FM7/sc^{7} l*, heterozygous for the *X* chromosome balancer *FM7*, which carries the dominant mutation *Bar* (*B*) eyes and the female sterilizing mutation *sn^{x2}*, and a lethal *X* chromosome.

Strains with P elements:

cn $vg^{21\text{-}3}$ *bw*, homozygous for the *P* element-insertion mutation $vg^{21\text{-}3}$ of the *vestigial* wing locus on the second chromosome (WILLIAMS, PAPPU and BELL 1988). There are no other *P* elements present. This stock is also homozygous for the recessive eye color markers *cinnabar* (*cn*) and *bw* which flank the *vg* locus.

C(1)DX, y f/Y; cn $vg^{21\text{-}3}$ *bw*, an attached-*X* stock homozygous for the *cn* $vg^{21\text{-}3}$ *bw* second chromosome.

ry^{506} *P*[ry^{+} *SalI*](89D), a transformed stock homozygous for a single, modified *P* element at cytological position 89D on chromosome *III* (ROBERTSON and ENGELS 1989). This *P* element, abbreviated *P*[*SalI*], has a frameshift mutation in the *Sal*I restriction site of exon 3 and therefore does not produce any active transposase (KARESS and RUBIN 1984). It is also marked with a wild-type allele of the *rosy* (*ry*) gene.

C(1)DX, y f/Y; ry^{506} *P*[ry^{+} *SalI*](89D), an attached-*X* stock homozygous for the *P*[*SalI*] third chromosome.

ry^{506} *P*[ry^{+} Δ2-3](99B), a transformed stock homozygous for a double *P* element insertion at 99B on chromosome *III* (H. ROBERTSON, personal communication). This insertion, abbreviated Δ2-3, consists of two modified *P* elements that lack the intron between exons 2 and 3 (LASKI, RIO and RUBIN 1986). It is marked with ry^{+} and produces a high level of transposase activity in the soma as well as in the germ line. However, the insertion itself is essentially immobile (ROBERTSON *et al.* 1988). There are no other *P* elements present in the Δ2-3 stock.

y sn^{w}/y^{+} *Y; bw; st*, homozygous for the double *P* element-insertion mutation sn^{w} [*weak-singed* bristles; ENGELS (1979b)]. In the presence of the *P* transposase, this *X*-linked mutation becomes unstable, mutating to a more extreme allele (sn^{e}) or to an essentially wild-type allele ($sn^{(+)}$). These changes are due to the excision of one or the other of the inserted *P* elements (ROIHA, RUBIN and O'HARE 1988) and therefore can be used as indicators of transposase action. The only other *P* element present in this stock is an approximately 0.6-kb element tightly linked to the *singed* locus. The stock is also homozygous for *bw* and *st*.

C(1)DX, y f/Y/y sn^{w}; ry^{506} *P*[ry^{+} *SalI*](89D), an attached-*X* stock in which the *X* chromosome in the males carries sn^{w} and the third chromosome is homozygous for the *P*[*SalI*] element.

C(1)DX, y f/Y/T-5, y sn^{w}; *bw; st*, an attached-*X* stock consisting of several sublines in which the male's *X* chromosome, called *T-5*, carries sn^{w} and at least one transposase-producing *P* element (SIMMONS *et al.* 1987). Due to the activity of the transposase in these sublines, the sn^{w} allele is unstable and mutates at a high rate. In order to maintain this stock, phenotypically weak-singed males from each subline are selected and mated individually at 21° to *C(1)DX, y f/Y; bw; st* females from a true M strain. *P* elements are therefore restricted mainly to the *T-5 X* chromosome since the *Y* chromosome and half the autosomes are replaced by M chromosomes every generation. The *T-5* sublines *K5*, *p3″* and *p3‴* were used to induce hybrid dysgenesis in these experiments.

C(1)DX, y f/Y/sn^{w}; π_2, an attached-*X* stock with autosomes derived from the wild-type P strain π_2 (ENGELS and PRESTON 1979). Because this stock has the P cytotype, the sn^{w} mutation is essentially stable.

D2.2, a wild-type stock containing a single *P* element in the genome. The stock was established from flies that were being used in germ line transformation experiments but its single *P* element was not derived from a transformation event. Rather, it was apparently acquired from one of the parental strains (*TM3/TM6* and ry^{506} *P*[ry^{+} Δ2-3]), which must have been segregating this element at low frequency.

Inbred Sexi lines: These lines were derived from a wild-type strain called Sexi by many generations of full-sib mating (RASMUSSON *et al.* 1990). The lines are denoted Sexi.1, Sexi.2, etc.

Genetic isolation of individual *P* elements: Individual *X*-linked *P* elements were isolated from three of the inbred Sexi lines (Sexi.1, Sexi.5 and Sexi.6) by recombination with multiply marked *X* chromosomes that were devoid of *P* elements. The autosomes in these recombination experiments were all from an M strain. Southern blotting and *in situ* hybridization were used to monitor the number and position of the *X*-linked *P* elements during the isolation process. The final recombinant chromosomes were made homozygous using the *M5-CS* balancer or kept in stock with attached-*X* females. Full details of the isolation process are found in RASMUSSON (1991).

Tests for the suppression of $vg^{21\text{-}3}$: Males with an isolated *P* element on the *X* chromosome were crossed to *C(1)DX, y f/Y; cn* $vg^{21\text{-}3}$ *bw* females. Their sons were then backcrossed to produce males hemizygous for the isolated *P* element and homozygous for $vg^{21\text{-}3}$. Suppression of the $vg^{21\text{-}3}$ phenotype was assessed in these males.

Tests for the repression of gonadal dysgenesis (GD sterility): Our experimental design utilized "reciprocal" crosses that permitted the identification of separate maternal and zygotic contributions to the repression of gonadal dysgenesis (Figure 1). In the G_1, flies bearing a sterility-inducing *T-5 X* chromosome were crossed to males and females carrying the chomosome to be tested for repression potential. Because in some cases this chromosome carried an isolated *P* element, it is denoted as *(P)* in the Figure. Only a single pair of flies was used in each G_1 cross; all these crosses were initiated at 21°, but after 2 days, the mated flies were transferred to fresh cultures, which were incubated at 29°. The progeny that emerged by day 11 were transferred to fresh vials and aged at 21° for 2–3 days. Then from each vial, as many as 12 females of particular genotypes were examined for egg production by squashing them in colored water between two glass plates. Those females without any eggs were considered to have gonadal dysgenesis. A low frequency of dysgenesis indicated repression, presumably by factors associated with the isolated *P* elements. Repression by zygotic factors was ascertained in the *T-5/(P)* daughters of cross 1, repression by maternal factors was ascertained in the *FM7/T-5* daughters of cross 2, and repression by a combination of zygotic and maternal factors was ascertained in the *(P)/T-5* daughters of cross 2. As a positive control in these experiments, the *P*[*SalI*] element was also tested for its effect on *T-5*-induced GD sterility. In the G_1, *T-5/M5* females were crossed to males homozygous for the *P*[*SalI*] element (cross 1) and *T-5* males were crossed to *P*[*SalI*]/*TM3* females (cross 2). Daughters from these crosses were then examined for gonadal dysgenesis.

Tests for the repression of sn^{w} mutability: *X* chromosomes carrying an isolated *P* element and the *P* element-insertion mutation sn^{w} were constructed by recombination. The presence of the isolated *P* element was ascertained by the polymerase chain reaction (PCR) with *P*-specific primers. Recombinant sn^{w} chromosomes that lacked the isolated *P* element were also identified so they could be used as controls in the repression tests. Two types of tests were conducted. In the first, sn^{w} mutability was induced in the germ line by a *T-5 X* chromosome that carried a complete *P* element and a sn^{e} allele. The sn^{w} allele that was destabilized was located on the homologous *X* chromosome. In the second test, sn^{w} mutability was induced in the soma and in the germ line by the Δ2-3 *P* element.

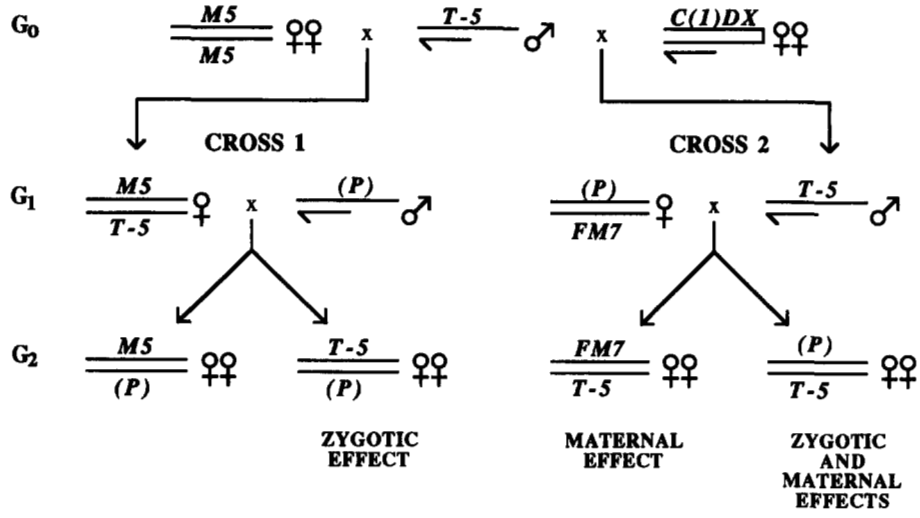

FIGURE 1.—Mating scheme to test for the repression of gonadal dysgenesis by individual *P* elements. The recessive autosomal markers *bw* and *st*, which were present in the G_0 flies, are not shown. Many replicate cultures of the G_0 double matings were established at 21° to produce the flies used in the G_1 matings.

The *T-5* experiments for repression of sn^w mutability (Figure 2) commenced with males from a *T-5* subline in which sn^w had already mutated to sn^e. The sn^w chromosome to be tested for repression potential is denoted sn^w *(P)*. In the G_2, phenotypically weak-singed *T-5*, sn^e/sn^w *(P)* females from each of thet two types of G_1 crosses were individually mated to *y* sn^3 *v car* males at 25° and the progeny were scored for bristle phenotype through day 17 (sn^w is dominant to sn^e and sn^3, and sn^+ is dominant to sn^w, sn^e and sn^3). The germ-line mutability of sn^w was estimated by calculating the proportion of wild-type flies among the wild-type and weak-singed progeny. This calculation ignores mutations to sn^e because they could not be distinguished from the sn^e allele that was already present on the *T-5* chromosome; it therefore yields a partial mutation rate. As a positive control, the *P*[*SalI*] element was also tested for its effect on *T-5*-induced sn^w mutability; sn^w; *P*[*SalI*] males were mated at 21° to *T-5*, $sn^e/M5$ females and their *T-5*, sn^e/sn^w; *P*[*SalI*]/+ daughters were crossed individually to *y* sn^3 *v car* males. The progeny of these crosses were then scored for bristle phenotype.

The Δ2-3 experiments for repression of sn^w mutability were performed in two ways, one testing for repression in males (Figure 3A) and the other testing for repression in females (Figure 3B). In the tests for repression in males, G_2 sn^w *(P)* males that were heterozygous for Δ2-3 were examined for bristle mosaicism to determine if the isolated *P* element had any effect on the somatic mutability of sn^w. These males were then mated individually at 25° to *C(1)DX*, *y f/Y*; π_2 females, which had the P cytotype, and their sons were scored for bristle phenotype to determine if the isolated *P* element had any effect on the germ-line mutability of sn^w. The extreme-singed, weak-singed and wild-type phenotypes in these sons could be distinguished because the P cytotype, which they had inherited maternally, suppressed the somatic mosaicism that ordinarily occurs when Δ2-3 is combined with sn^w (ROBERTSON *et al.* 1988). The proportion of extreme-singed and wild-type sons was used to estimate the germ-line mutability of sn^w. As a positive control, the *P*[*SalI*] element was also tested for its effect on Δ2-3-induced sn^w mutability. These tests began by mating sn^w; *P*[*SalI*] males separately to *C(1)DX, y f/Y*; *P*[*SalI*] and *P*[*SalI*] females at 25°. sn^w; *P*[*SalI*] sons from the first mating were crossed to *C(1)DX, y f/Y*; Δ2-3 females (cross A) and $sn^w/+$; *P*[*SalI*] daughters from the second mating were crossed to Δ2-3 males (cross B); the cultures from both sets of crosses were reared at 21°. In both cases, the sn^w; *P*[*SalI*]/Δ2-3 males

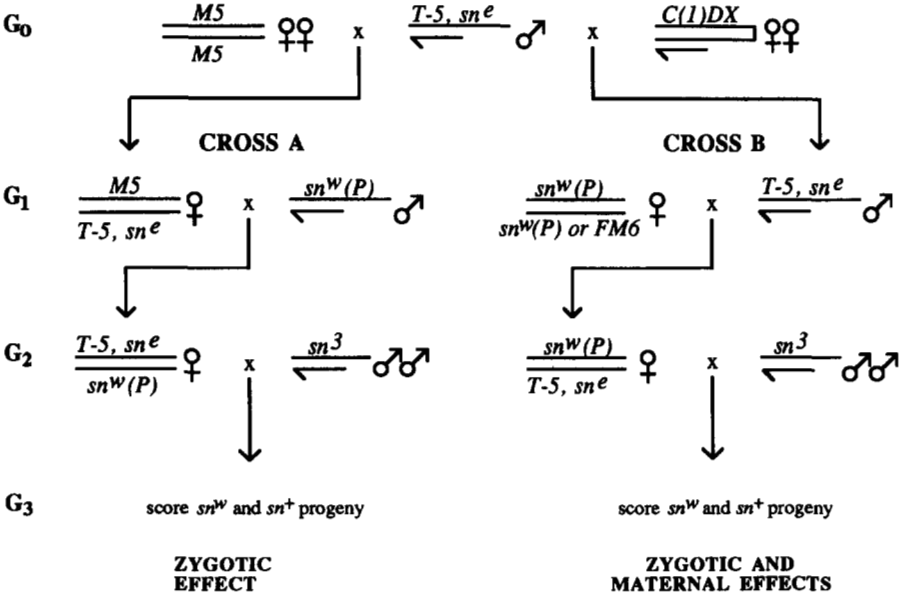

FIGURE 2.—Mating scheme to test for the repression of *T-5*-induced sn^w mutability by individual *P* elements. The recessive autosomal markers *bw* and *st*, which were present in the G_0 flies, are not shown. The G_0 and G_1 matings were replicated many times and the cultures were incubated at 21°.

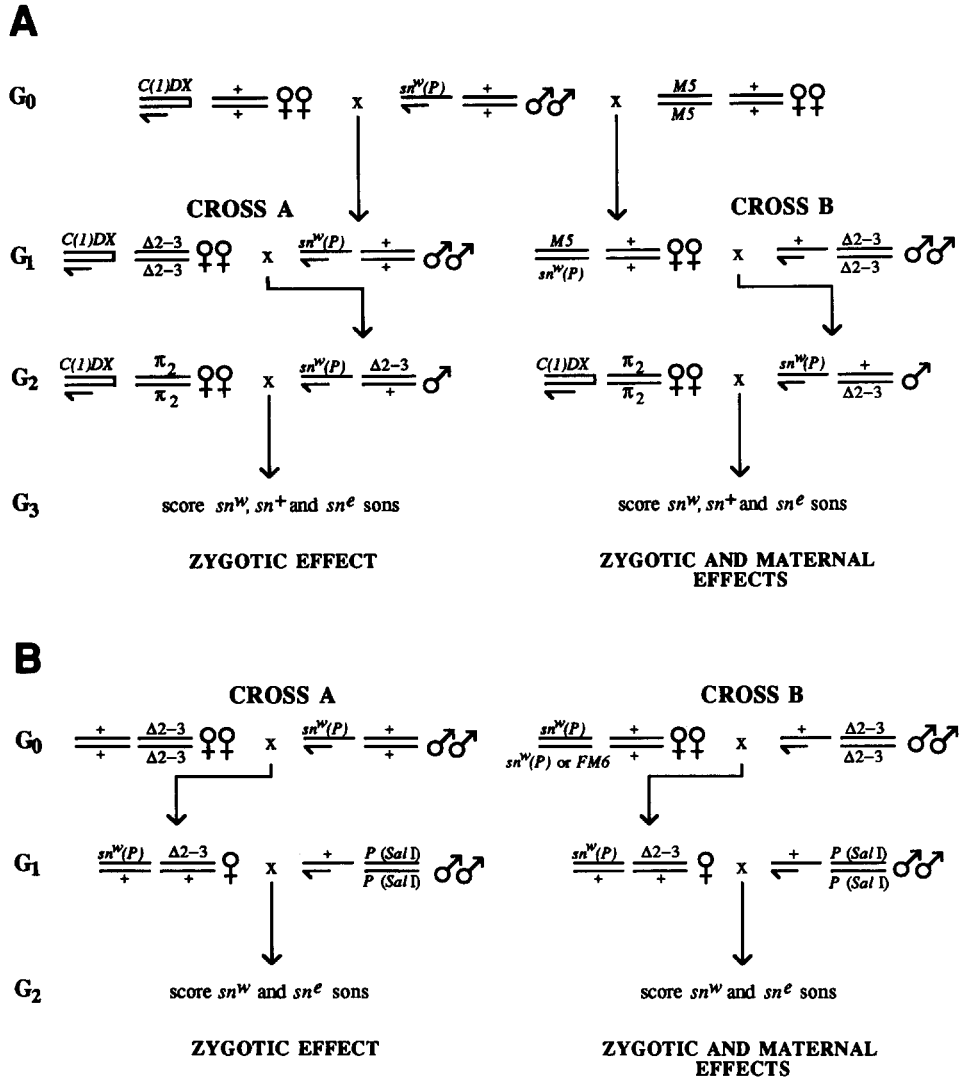

FIGURE 3.—Mating schemes to test for the repression of Δ2-3-induced sn^w mutability by individual *P* elements in males (panel A) and in females (panel B). In panel A, the autosomal recessive markers *bw* and *st* were present in the G_0 females; also, the G_1 cultures were incubated at 21°. In panel B, the G_0 cultures were incubated at 21°.

that emerged were all phenotypically weak-singed, indicating that *P*[*Sal*I] completely repressed somatic sn^w mutability, as expected (ROBERTSON and ENGELS 1989). These males were then mated individually to *C(1)DX, y f/Y*; π_2 females at 25° and their sons were scored for bristle phenotype to estimate the germ-line mutability of sn^w.

In the tests for repression of Δ2-3-induced sn^w mutability in females, G_1 sn^w *(P)*/+; Δ2-3/+ females were mated to *P*[*Sal*I] males at 25° and their sons were scored by bristle phenotype. The different bristle phenotypes could be distinguished because the *P*[*Sal*I] element represses the somatic mosaicism caused by Δ2-3. However, only the weak and extreme singed sons were tallied in these tests because the wild-type class confounded germ-line mutations of sn^w with the sn^+ allele that was already present in the maternal genotype. The mutation rate was therefore estimated by the proportion of the tallied sons that were extreme singed.

In both the *T-5* and Δ2-3 experiments, a reduction in sn^w mutability in the flies of cross A would indicate repression by zygotic factors, whereas a reduction in mutability in the flies of cross B would indicate repression by either zygotic or maternal factors, or by a combination of these.

Southern blotting: Genomic DNA for Southern analysis was extracted from 1 to 20 adults, precipitated with ethanol and resuspended in sterile double-distilled water. DNA samples (3–12 μg) were digested with restriction enzymes according to the supplier's instructions (Bethesda Research Laboratories) and the resulting fragments were separated in 0.8% or 1.5% agarose gels by electrophoresis. The gels were blotted by capillary action to GeneScreen*Plus* (Du Pont) or Hybond (Amersham) nylon membranes using 0.4 M NaOH, 0.6 M NaCl as a transfer solution and then hybridized with radioactively labeled probes according to standard procedures (RASMUSSON 1991).

***P* element probes:** Probes for Southern blots were made from the plasmid pπ25.7BWC by random primer labeling with [^{32}P]dCTP (3000 Ci/mM, Amersham). This plasmid contains a *P* element that is complete except for a deletion of 39 bp from the 5′ end and 23 bp from the 3′ end; it does not contain any other Drosophila DNA sequences (O'HARE *et al.* 1992).

***In situ* hybridization:** *In situ* hybridizations were carried out according to published methods (JOHNSON-SCHLITZ and LIM 1987). Probes were prepared from the plasmid pπ25.1 (O'HARE and RUBIN 1983) by random primer labeling with [^{3}H]thymidine. This plasmid contains a complete *P* element flanked by genomic sequences from cytological position 17C on the *X* chromosome.

PCR amplification of genomic DNA: Genomic DNA templates for the polymerase chain reaction were obtained by homogenizing single flies in 100 μl grinding buffer (10 mM Tris-Cl, pH 8.2; 1 mM EDTA; 25 mM NaCl; 200 ng/μl

Proteinase K) and incubating at 37° for 20 min. (GLOOR *et al.* 1991). The proteinase was then inactivated by treatment at 95° for 2 min. The resulting solution contained approximately 30 ng/μl single-stranded DNA (G. GLOOR, personal communication) and 1–2 μl were used for each amplification reaction. Amplifications were carried out in 20–50-μl volumes using Taq DNA polymerase (Promega) and a Coy Company TempCycler. Each reaction contained 1× Promega reaction buffer (10 mM Tris, pH 9.0; 50 mM KCl; 1.5 mM $MgCl_2$; 0.01% gelatin (w/v); 0.1% Triton X-100), 0.2 mM each dATP, dCTP, dGTP, dTTP, 1.5 ng/μl each of two oligonucleotide primers (or 3.0 ng/μl inverted repeat primer, see below), approximately 1.5 ng/μl genomic DNA and 0.025 units/μl Taq DNA polymerase. The reaction mixtures were covered with a minimal volume of mineral or paraffin oil to prevent evaporation. The temperature profile for each reaction cycle was 0.5–1.0 min at 94° to denature the DNA, 1 min at 60° to anneal the primers and 1.5 min at 72° to synthesize new DNA strands; 25–30 cycles were performed. Tracking dye was added to the PCR products and samples were fractionated in 1.0% agarose gels.

DNA sequencing: DNA for sequencing was prepared using asymmetric PCR (GYLLENSTEN and EHRLICH 1988). Each reaction was carried out in 50 μl with 0.04 unit of Taq DNA polymerase and 1.5 ng/μl of one primer and 0.015 ng/μl of the other. Buffer, deoxyribonucleotides and genomic DNA were included as in standard PCR. The temperature profile of each reaction was 45 sec at 92°, 2 min at 55° and 2 min at 72°. There were 40 cycles. The amplified DNA was extracted with 2–4 volumes of chloroform-isoamylalcohol (24:1), precipitated with ethanol and resuspended in 7 μl double-distilled water. DNA sequencing was performed using the Sequenase Version 2.0 kit (U.S. Biochemical Corp.) with slight modifications of the supplier's instructions. Sequencing reaction products were fractionated in 0.4 mm gels of 6% polyacrylamide, 7 M urea prepared in 100 mM Tris-borate electrophoresis buffer.

DNA primers: Oligonucleotides for PCR and sequencing of *P* element DNA were provided by WILLIAM ENGELS (University of Wisconsin, Madison) or were purchased from Oligos, Etc. All oligonucleotide sequences are written 5′ to 3′ and their positions in the sequence of a complete *P* element are shown in parentheses.

Inverted repeat (IR) primer, (12/2896) AACATAAGGTGGTCCCGTCG (31/2877). This primer is derived from a sequence within the 31-bp inverted terminal repeats of *P* and primes DNA synthesis inward. It was used to amplify *P* elements with intact termini.

5′-subterminal primer, (35) GCCGAAGCTTACCGAA GTAT (54). This primer is derived from a sequence just inside the 5′ inverted terminal repeat of *P* and primes DNA synthesis toward the 3′ end.

KP-specific primer, (2577) ATCAACATCGACGTTTC ;cdCAC (805). This primer, designed by G. GLOOR and W. ENGELS, spans the deletion breakpoint (Δ) in *KP* elements and primes DNA synthesis toward the 5′ end. It was used with the 5′-subterminal primer in PCR amplifications to identify *KP* elements. Extensive tests have established that it amplifies only *KP* elements (C. PRESTON and W. ENGELS, personal communication).

Oligonucleotides that were used strictly for DNA sequencing are listed in RASMUSSON (1991).

RESULTS

Molecular characterization of individual naturally occurring *P* elements: Naturally occurring *P* elements were isolated by meiotic recombination from the *X* chromosomes of the inbred lines Sexi.1, Sexi.5 and Sexi.6 and from the *X* chromosome of the wild-type stock D2.2. Each of the strains carrying these isolated elements is listed in Table 1, along with its origin and *X* chromosome genotype. Southern blotting was used to show that only a single *P* element was present in the genome and *in situ* hybridization and PCR were used to determine the cytological location and size of this element.

The results of the Southern analysis are shown in Figure 4. Genomic DNA from each strain was digested with *Bam*HI, a restriction enzyme which does not cleave within the *P* sequence. Except for the M strain control, each lane of the autoradiogram shows a single band of hybridization, indicating that only one *P*-containing fragment was present in the digested DNA. For strains 1-F and 5-F, the hybridizing bands were apparently the same size, suggesting that these two strains might carry the same isolated *P* element.

The cytological locations of the isolated *P* elements were established by *in situ* hybridization with a *P* element probe made from the plasmid pπ25.1. Because this plasmid contains DNA from cytological location 17C on the *X* chromosome, all the hybridizations showed label at this site. Five of the six strains that were examined also showed label at one other *X* chromosome site (Table 1), revealing the location of the isolated *P* element. The sixth strain, 6-N, did not show this additional label. Subsequent PCR amplification of the *P* element in strain 6-N demonstrated that it was too small to be detected by our *in situ* hybridization procedure (see below). Two of the strains (1-F and 5-F) showed label in cytological region 4C. These were the same strains that had given apparently identical results in the Southern analysis, reinforcing the suggestion that they carried the same isolated *P* element.

The sizes of the isolated *P* elements were determined by PCR amplification using a primer complementary to a sequence in the inverted terminal repeats. Figure 5A shows the results of amplifications with this IR primer. As expected, the apparently identical *P* elements in strains 1-F and 5-F were the same size, about 1.2 kb long. The *P* elements in strains 1–5, 6-Z and D2.2 were also the same size (1.1 kb) even though they were at different cytological positions. Strain 6-N carried the smallest of the isolated *P* elements, about 500 bp long, and was therefore too small to be detected by our *in situ* hybridization technique.

Because of their size and origin, it seemed possible that the 1.1 kb *P* elements in strains 1-5, 6-Z and D2.2 might be *KP* elements (BLACK *et al.* 1987). This possibility was investigated by PCR amplification of genomic DNA using a primer near the 5′ end of the *P*

TABLE 1

A summary of genetic and cytological data on strains containing individual *P* elements

Strain	Origin	*X* chromosome genotype[a]	Cytological location[b]	*P* element	Phenotype of vg^{21-3} homozygotes[c]
1-F	Sexi.1	*y w fa * cv v f car*	4C	*D50(1)*	Intermediate
5-F	Sexi.5	*y w fa * cv v f car*	4C	*D50(5)*	Intermediate
1-5	Sexi.1	** cv v f car*	2F	*KP(1)*	Very severe
6-Z	Sexi.6	g^2 ** f (fs)*	13F	*KP(6)*	Severe
D2.2	D2.2	+	14F	*KP(D)*	Very severe
6-N	Sexi.6	g^2 ** (fs)*		*SP*	Very severe
*Sal*I	Transformed stock	+	89D[d]	*P*[*Sal*I]	Wild type

[a] An asterisk shows the location of the *P* element relative to the markers present. Two strains (6-Z and 6-N) had unmapped recessive female-sterilizing mutations (*fs*) on the X chromosome.

[b] Determined by *in situ* hybridization of a *P* element probe to polytene chromosomes. Because of its small size, the element in strain 6-N could not be localized by this method. However, the genetic derivation of the *X* chromosome in this strain implies that the element is proximal to *g*, which is located in 12B.

[c] The intermediate phenotype means wings about half normal size with notches in the distal and posterior margins. The severe phenotype means wings about one-fourth normal size with scalloped posterior margins. The very severe phenotype means club-shaped wings about one-fifth normal size.

[d] ROBERTSON and ENGELS (1989).

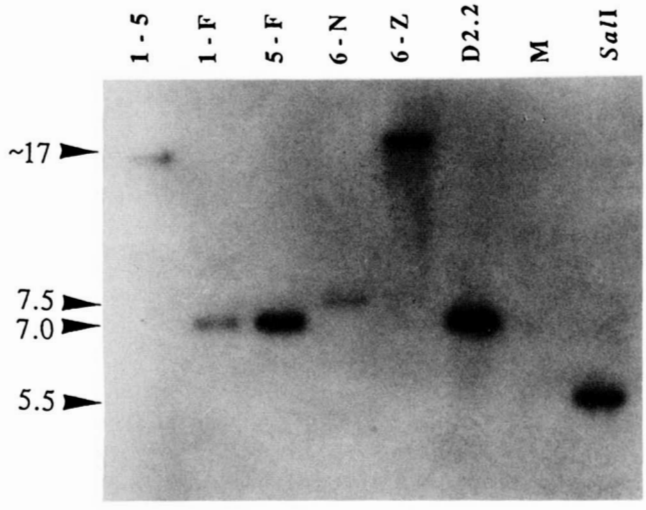

FIGURE 4.—Southern analysis of genomic DNA from strains with isolated *P* elements. The lanes are labeled with the strain names. See text and Table 1 for details.

sequence and another primer spanning the deletion in *KP* elements (see MATERIALS AND METHODS); these primers will amplify *P* element DNA only if the *KP* deletion is present (G. GLOOR, C. PRESTON and W. ENGELS, personal communication). For strains 1-5, 6-Z and D2.2, a PCR product of the expected size was obtained (Figure 5B), indicating that each carried a *KP* element. For the other strains, no PCR product was obtained indicating that some other type of *P* element was present.

The nature of each isolated *P* element was definitively established by DNA sequencing. Asymmetric PCR was used to generate material for the sequencing experiments. Primers for these experiments were chosen on the basis of preliminary studies using genomic Southern blotting and restriction enzyme digestion of standard PCR products. Only regions containing the *P* element deletion were sequenced.

Analysis of the sequencing data indicated that, as expected, the isolated *P* elements in strains 1-F and 5-F were identical. Each had a deletion from bp 819 to bp 2527 plus a 21-bp insertion of non-*P* DNA at the deletion breakpoints. A search of GeneBank DNA sequences revealed that an element of this type had previously been identified in a mutant allele (*D50*) of the *RpII* locus (SEARLES *et al.* 1986; W. ENGELS, personal communication). Henceforth, we shall refer to this type of *P* element as "*D50*," with the isolate from strain 1-F denoted as *D50(1)* and the isolate from strain 5-F denoted as *D50(5)* (Table 1).

Sequencing of the *P* elements in strains 1-5 and 6-Z confirmed that they were indeed *KP* elements. Both had a deletion from bp 809 to bp 2563, with a 2-bp ambiguity due to duplicate sequences at the deletion breakpoints. The presumptive *KP* element in strain D2.2 was not sequenced. We shall distinguish among the three isolated *KP* elements by denoting them as *KP(1)*, *KP(6)* and *KP(D)*, with the symbol in parentheses referring to the carrier strain (Table 1).

The *P* element in strain 6-N was only 517 bp long. Its deletion spanned bp 186–2577 and apparently arose within a 3-bp direct repeat. To date, no *P* element with this particular deletion has been described. We therefore denote this type of *P* element as "*SP*" in recognition of the fact that it was isolated from a derivative of the Sexi strain (Table 1).

The structures of the three types of isolated *P* elements are shown in Figure 6. This figure also shows the sizes of the polypeptides these elements could encode. The *KP* and *D50* elements are expected to produce similar polypeptides slightly more than 200 amino acids long, with most of the sequence identical with the N-terminal part of the P transposase (203

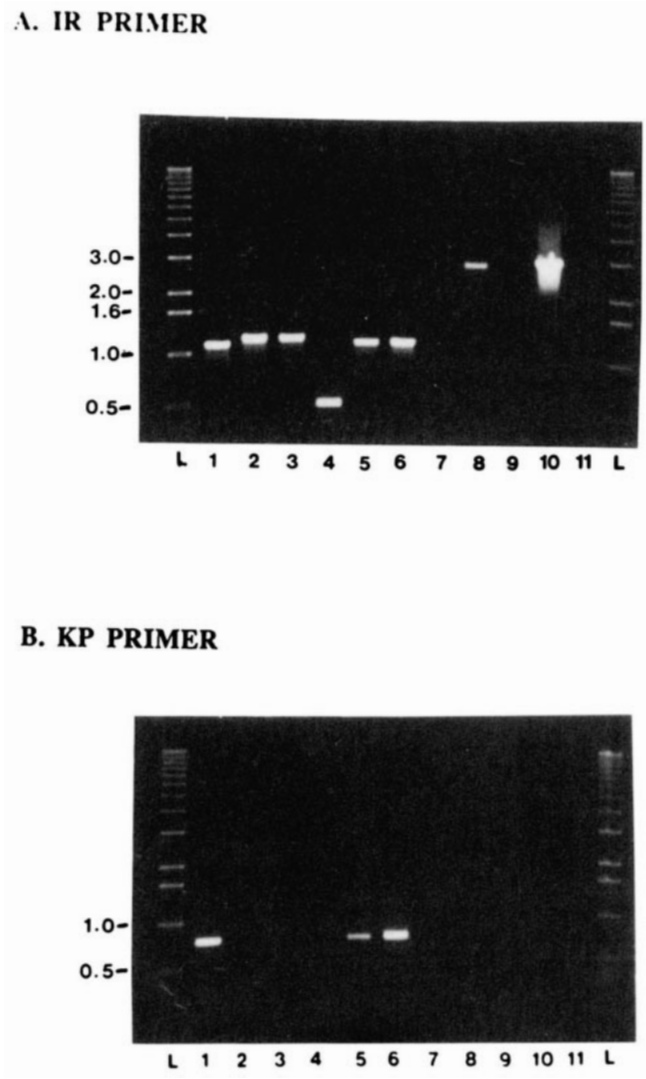

FIGURE 5.—PCR amplification of *P* sequences in strains with isolated *P* elements. (A) Amplifications with the inverted repeat (IR) primer. (B) Amplifications with the *KP*-specific and 5′-subterminal primers. In both panels, L denotes the lanes with DNA size markers. The other lanes contain PCR products from the tested strains or from controls: 1 (strain 1-5), 2 (1-F), 3 (5-F), 4 (6-N), 5 (6-Z), 6 (D2.2), 7 (Canton S), 8 (*cn* $vg^{21\text{-}3}$ *bw*), 9 (*P*[*SalI*]), 10 (plasmid pπ25.1) and 11 (no template DNA).

amino acids for *KP* and 199 for *D50*). There has been considerable speculation that the putative polypeptide product of a *KP* element could function to repress hybrid dysgenesis, possibly by combining with authentic transposase polypeptides and poisoning the holoenzyme (BLACK *et al.* 1987; JACKSON, BLACK and DOVER 1988; RIO 1990, 1991). To date, however, there has been no rigorous proof that this polypeptide is produced, nor has there been a demonstration that a single *KP* element can repress transposase action. This latter issue is addressed in the experiments discussed below. We also investigate the repression potential of a single *D50* element.

The putative polypeptide of the *SP* element is only 14 amino acids long, 11 of which would be identical with the N-terminal sequence of the P transposase. Because it is doubtful that such a polypeptide would influence transposase activity, we originally included the *SP* element as a negative control in our tests for repression of hybrid dysgenesis. However, to our surprise, this element proved to be an effective repressor of hybrid dysgenesis (see below). As a positive control for the repression tests, we included the *P*[*SalI*] element, which has previously been shown to repress some dysgenic traits (ROBERTSON and ENGELS 1989).

Effect on a "cytotype-dependent" allele: Some *P*-insertion alleles appear to be sensitive to the presence of *P* element repressors. This is manifested by a change in the phenotype, which becomes either more or less mutant depending on the particular *P*-insertion allele (ROBERTSON and ENGELS 1989). The $vg^{21\text{-}3}$ allele of the *vestigial* locus is especially useful in these kinds of studies (WILLIAMS, PAPPU and BELL 1988). In the absence of repressors, *i.e.*, in a pure M genetic background, $vg^{21\text{-}3}$ homozygotes exhibit a severe reduction in wing size. In contrast, $vg^{21\text{-}3}$ homozygotes with a P genetic background have wild-type wings. Preliminary studies by BENZ (1989) have indicated that intermediate phenotypes can be obtained in $vg^{21\text{-}3}$ homozygotes that carry different types of *P* elements. Each of the *P* elements that was isolated here was therefore tested for its ability to suppress $vg^{21\text{-}3}$ (see MATERIALS AND METHODS). The results (Table 1) indicate that only the *D50* element in strains 1-F and 5-F suppressed the $vg^{21\text{-}3}$ phenotype significantly. The three *KP* elements and the *SP* element had little or no effect. However, as expected, the known repressor *P*[*SalI*] suppressed $vg^{21\text{-}3}$ completely (BENZ 1989). From these results, it might therefore be predicted that among the naturally occurring *P* elements, *D50* would have the greatest ability to repress hybrid dysgenesis.

Effect on gonadal dysgenesis (GD sterility): Each of the isolated *P* elements was tested for its ability to repress gonadal dysgenesis. In these tests we used a scheme designed to reveal the separate contributions of maternal and zygotic repressors (see Figure 1). This involved "reciprocal" crosses between flies carrying an isolated *P* element and flies carrying a sterility-inducing *X* chromosome called *T-5*. Segregation of these in the progeny allowed an assessment of the sterility-inducing potential of *T-5* in the absence of the isolated *P* element, and more importantly, an assessment of the effects of sterility-repressing factors that might be associated with the maternal or zygotic expression of this element.

The results of the initial tests for repression of GD sterility are shown in Table 2 and Figure 7. There were two experiments, each using sterility-inducing *X* chromosomes from the *p3‴* subline of the *T-5* stock. The ability of these chromosomes to induce GD sterility is evident from the tests with the nonrepressing

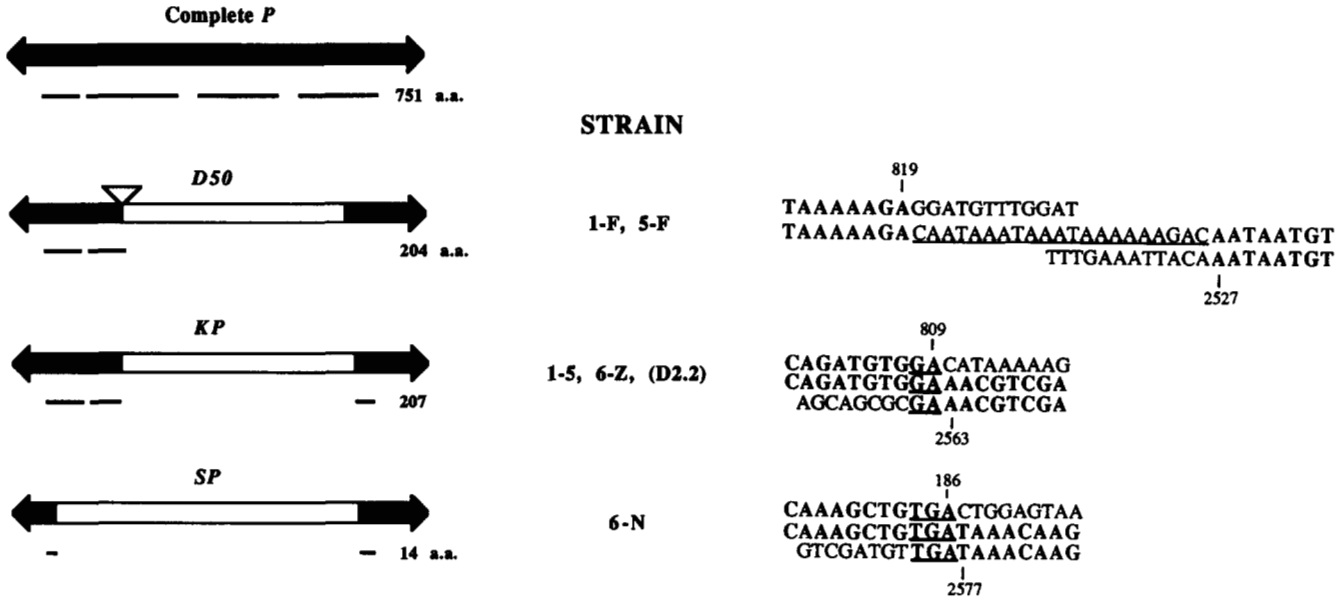

FIGURE 6.—The *D50*, *KP* and *SP* elements compared to the complete *P* element. The open regions indicate deletions and the triangle indicates an insertion of non-*P* DNA. The coding regions are shown beneath each element, with the number of amino acids (a.a.) in the expected polypeptide indicated at the right. At the far right, the DNA sequences spanning the deletion breakpoints are shown in between sequences from the complete *P* element; the upper and lower sequences demarcate the left (5′) and right (3′) breakpoints, respectively, with boldface denoting the portions shared by the complete and incomplete elements. The non-*P* DNA in the *D50* element is underlined. Regions spanning the deletions were sequenced in all the elements except the *KP* element in strain D2.2, which is listed in parentheses.

TABLE 2

Repression of GD sterility by chromosomes with individual *P* elements

		Cross 1: *M5/T-5*[a] female × *(P)*[b] male						Cross 2: *(P)/FM7*[c] female × *T-5* male						
		M5/(P)			*T-5/(P)*			*FM7/T-5*				*(P)/T-5*		
Experiment	*P* element	*N*[d]	*n*[e]	GD[f] ± SE	*n*	GD ± SE	GD′[g]	*N*	*n*	GD ± SE	GD′	*n*	GD ± SE	GD′
I	*bw; st* control	60	641	7.0 ± 1.5	626	74.2 ± 3.7	1.00	59	497	65.3 ± 3.7	1.00	589	53.6 ± 3.9	1.00
	D50[h]	45	451	8.9 ± 2.3	435	76.1 ± 4.1	1.02	60	588	54.5 ± 3.6*	0.83	663	58.1 ± 3.6	1.08
	KP(1)	30	309	4.7 ± 1.4	324	66.7 ± 5.3	0.90	30	300	41.4 ± 4.5***	0.63	326	22.4 ± 4.2***	0.41
	KP(6)	30	309	14.5 ± 2.8	307	75.5 ± 5.1	1.01	30	228	21.6 ± 4.2***	0.33	270	20.7 ± 4.2***	0.39
	KP(D)	30	319	4.8 ± 1.8	314	83.4 ± 3.7	1.12	30	320	44.7 ± 6.0***	0.68	339	50.3 ± 5.8	0.93
	SP	30	301	3.9 ± 1.8	308	68.1 ± 4.0*	0.91	30	270	15.5 ± 2.7***	0.24	315	4.8 ± 1.3***	0.09
	P[SalI]	30	327	1.8 ± 0.8	321	39.4 ± 5.7***	0.53	30	177	34.2 ± 4.1***	0.52	283	0.6 ± 0.6***	0.01
II	*bw; st* control	108	1097	8.3 ± 1.3	1121	44.1 ± 3.6	1.00	100	952	50.5 ± 4.0	1.00	1090	45.1 ± 3.7	1.00
	D50[h]	64	595	12.2 ± 1.9	583	54.0 ± 4.8	1.22	60	576	56.4 ± 4.8	1.12	692	55.2 ± 4.6	1.22
	KP(1)	30	299	9.0 ± 2.1	293	50.5 ± 6.8	1.15	30	358	45.7 ± 6.3	0.90	360	20.0 ± 3.7***	0.44
	KP(6)	30	331	5.7 ± 1.6	336	50.8 ± 6.9	1.15	30	307	13.7 ± 3.1***	0.27	350	8.7 ± 2.6***	0.19
	KP(D)	30	321	11.6 ± 2.9	344	52.9 ± 6.8	1.20	30	266	40.1 ± 6.4	0.79	344	36.1 ± 5.9	0.80
	SP	30	285	16.5 ± 4.7	261	62.8 ± 6.8	1.42	29	300	27.0 ± 4.9***	0.53	348	14.4 ± 3.3***	0.31
	P[SalI]	30	342	4.1 ± 1.1	332	25.8 ± 5.3**	0.58	33	297	16.2 ± 4.1***	0.32	381	3.9 ± 1.3***	0.09

The Mann-Whitney rank sum test was used to determine if the percentage of GD sterility was significantly less than that of the *bw; st* control, with each culture treated as an independent unit. Asterisks indicate conventional levels of significance.

[a] The *T-5* subline *p3′′′* was used in these experiments.

[b] *(P)* denotes the *X* chromosome under test. A third chromosome carrying the *P[SalI]* element was also tested. The genotypes of the daughters in these tests were *M5/+*; *P[SalI]/+* (left column) and *T-5/+*; *P[SalI]/+* (right column).

[c] In the tests involving the *P[SalI]* element, *P[SalI]/TM3* females were used in this cross. Their daughters had the genotypes *T-5/+*; *TM3/+* (left column) and *T-5/+*; *P[SalI]/+* (right column).

[d] Number of cultures.

[e] Number of females examined.

[f] Unweighted average percentage of females with GD sterility ± standard error, which was calculated empirically.

[g] Average percentage of females with GD sterility normalized to the *bw; st* control.

[h] Although the *D50(1)* and *D50(5)* elements were tested separately, the data have been pooled in this analysis.

M strain, *bw; st*. In cross 1 of these tests, moderate to high frequencies of sterility (74.2% in experiment I and 44.1% in experiment II) were observed in the daughters that carried a *T-5 X* chromosome, but little

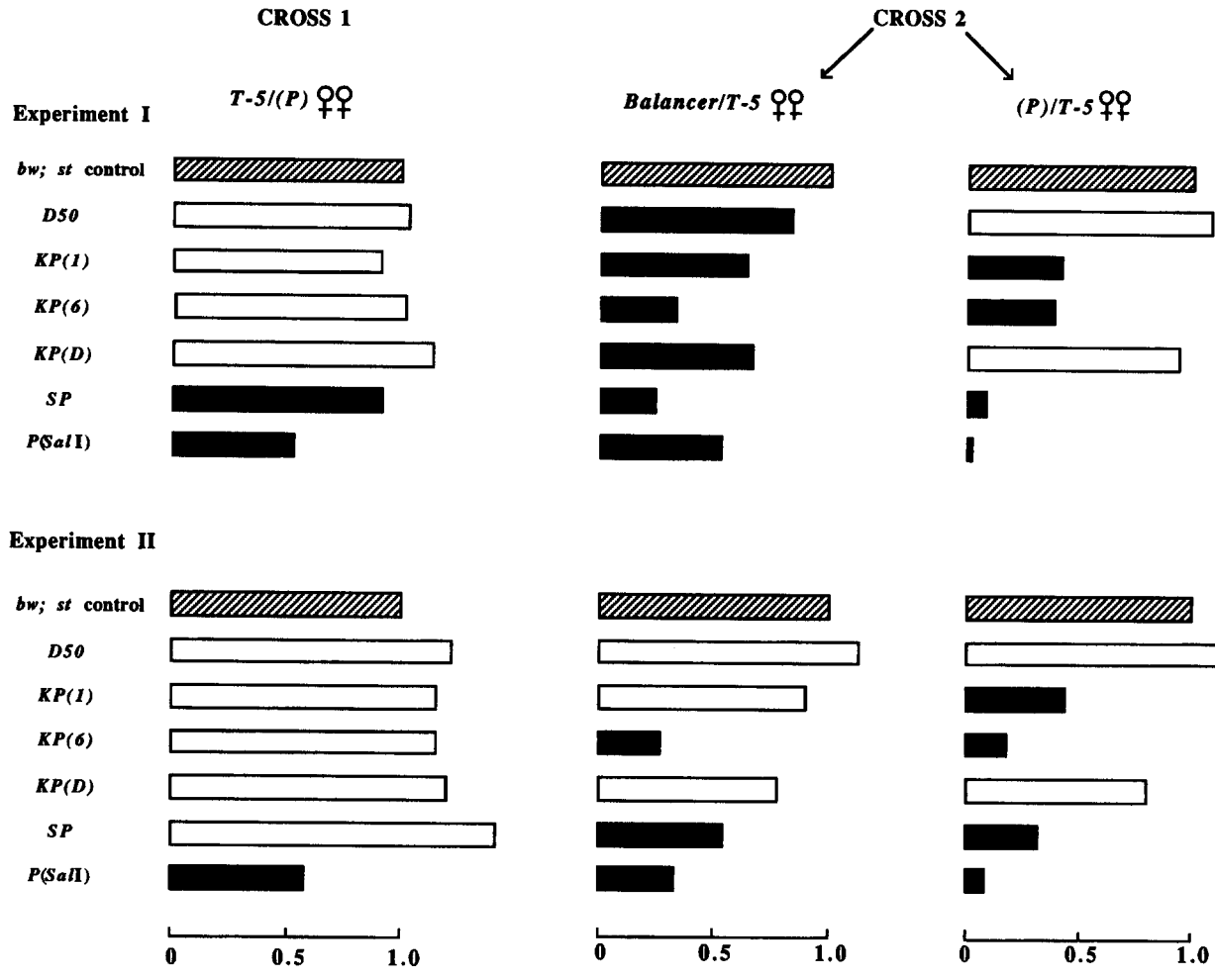

FIGURE 7.—Repression of gonadal dysgenesis by individual *P* elements in experiments I and II. Cross-hatching is used for the *bw; st* controls; the results that were significantly less than these are indicated by a solid bar. See text and Table 2 for details.

sterility (7.0% in experiment I and 8.3% in experiment II) was seen in the daughters that lacked this chromosome.

In these experiments, repression of *T-5*-induced sterility was ascertained by comparing the results of the tests with the isolated *P* elements to those with the *bw; st* controls. Accordingly, all the data were normalized to these controls. The *P*[*SalI*] element was included as a positive control and, as expected, it significantly reduced the frequency of sterility in both experiments. Furthermore, the repressing effect of *P*[*SalI*] seemed to be due to a combination of maternally and zygotically expressed factors. The zygotic factors were evident in the data from cross 1, in which *P*[*SalI*] was transmitted paternally. Among the *T-5/+; P*[*SalI*] daughters from this cross, the frequency of sterility was only about half that seen among the daughters from the *bw; st* controls–a highly significant difference. The *P*[*SalI*] element also repressed sterility through a purely maternal effect. This was evident in the *T-5/+; TM3/+* daughters from cross 2 which inherited cytoplasm from their *P*[*SalI*]*/TM3* mothers even though they did not inherit *P*[*SalI*] itself. These daughters showed 50–70% less sterility than the daughters from the *bw; st* controls–a highly significant difference, implying that factors transmitted maternally but independently of the *P*[*SalI*] element were influential in repressing gonadal dysgenesis in the offspring. When these maternally transmitted factors were combined with the *P*[*SalI*] element itself (see the *T-5/+; P*[*SalI*]*/+* daughters of cross 2), gonadal dysgenesis was almost completely repressed.

None of the naturally occurring *P* elements showed much ability to repress dysgenesis through a purely zygotic effect. For each of the elements, the frequency of sterility among the *T-5/(P)* daughters of cross 1 was

approximately equal to or greater than the corresponding frequency for the *bw; st* controls. In contrast, two of the elements consistently showed an ability to repress dysgenesis through a purely maternal effect. This was seen in the *FM7/T-5* daughters of cross 2, where *KP(6)* and, surprisingly, *SP* caused significant reductions (46–76%) in the frequency of sterility. The *D50*, *KP(1)* and *KP(D)* elements also appeared to reduce this sterility somewhat (17–37%), but their effects were significant only in experiment I. The combined maternal and zygotic effects of the isolated *P* elements were assessed in the *(P)/T-5* daughters of cross 2. Among these, the *KP(1)*, *KP(6)* and *SP* elements caused large and highly significant reductions in sterility (56–91%) in both experiments but the *D50* and *KP(D)* elements did not.

Additional experiments using more rigorous controls were performed to determine if the repression of sterility by *KP(1)*, *KP(6)* and *SP* was due specifically to these elements or to some extraneous factor. *X* chromosomes that were completely isogenic with the chromosomes that carried these isolated elements were made by excising the elements through transposase action. This was accomplished by passing the *X* chromosomes that carried the elements through females that were heterozygous for the Δ2-3 transposase source. In these females, the *X* chromosomes were balanced by *FM7*. Non-*FM7*, non-Δ2-3 sons from these females were mated to *C(1)DX, y f/Y; bw; st* females to establish lines and then screened by PCR with the IR *P* element primer to determine which had lost the isolated *P* element. Lines so identified were then backcrossed to *C(1)DX, y f/Y; bw; st* females to fix the recessive autosomal markers. *X* chromosomes carrying the isolated *P* elements were also put into this genetic background. All the *X* chromosomes were then tested for their abilities to repress *T-5*-induced gonadal dysgenesis using the scheme in Figure 1. For each *P*-containing chromosome, there were two independently generated isogenic controls, each having been double-checked for the loss of its *P* element. A *bw; st* control was also included in these experiments.

The results of two experiments that utilized different *T-5* sublines are shown in Table 3 and Figure 8. The effect of each *P* element was ascertained by comparing it with its two isogenic controls, which were pooled. This pooling was justified since only one significant difference at the 5% level was detected in 18 independent comparisons between the isogenic controls. Only *SP* repressed dysgenesis through a purely zygotic effect (see the *T-5/(P)* daughters from cross 1); although small, this effect was significant in both experiments. *SP* also repressed dysgenesis through a purely maternal effect, as did *KP(6)* (see the *FM7/T-5* daughters from cross 2); however, for *SP* this effect was significant only in experiment III and for *KP(6)* it was significant only in experiment IV. Both *SP* and *KP(6)* repressed dysgenesis very significantly when their zygotic and maternal effects were combined (see the *(P)/T-5* daughters from cross 2) and they did so in both experiments. *KP(1)*, by contrast, had no significant repression potential in either experiment.

These results demonstrated that the *KP(6)* and *SP* elements were directly responsible for the repression of gonadal dysgenesis, but they did not confirm the repression that was previously found to be associated with *KP(1)*. This may mean that *KP(1)* is not a *bona fide* repressor of gonadal dysgenesis, or that its ability to repress was obscured by other factors. In this context, it should be noted that neither *KP(6)* nor *SP* was as effective a repressor in experiments III and IV as it was in experiments I and II. One possible explanation is the genetic background, which may have weakened the repression ability of all three *P* elements, especially *KP(1)*, in Experiments III and IV. Another is the *T-5* chromosomes, which were clearly stronger inducers of sterility in experiments III and IV than they were in experiments I and II (compare the results from the *bw; st* controls). By presenting a greater challenge, these chromosomes may have made it more difficult to detect repression by the isolated *P* elements.

Effect on sn^w mutability: Each of the isolated *P* elements was tested for its ability to repress the mutability of the double-*P* element-insertion mutation sn^w. In experiments I and II, the sn^w allele was destabilized by different *T-5 X* chromosomes (Table 4 and Figure 9) and in experiments III and IV it was destabilized by Δ2-3 (Table 5 and Figure 10). "Reciprocal" crosses (A and B) were used in all four experiments (see the schemes in Figures 2 and 3) to identify separate zygotic and maternal components of repression. The *P*[*Sal*I] element was included as a positive control, and there were also several independent negative controls (see MATERIALS AND METHODS).

As expected, *P*[*Sal*I] was a strong repressor of sn^w mutability (ROBERTSON and ENGELS 1989). In Experiments I and II, it reduced the germ-line mutation rate to a small fraction of that seen with the *bw; st* controls. This happened even though the element was inherited paternally, indicating that repression was caused by a purely zygotic effect. (There was no separate test for a maternal effect of *P*[*Sal*I] in experiments I and II.) In experiment III, *P*[*Sal*I] reduced the germ-line mutability of sn^w to about 20% of the *bw; st* controls, regardless of whether it was inherited paternally or maternally. This indicated that repression by *P*[*Sal*I] did not involve a maternal effect, confirming a finding of ROBERTSON and ENGELS (1989). In experiment IV, the reduction in sn^w mutability was even greater, suggesting that *P*[*Sal*I] was a stronger repressor in the female germ line than it was

TABLE 3

Repression of GD sterility by chromosomes with individual *P* elements compared to isogenic chromosomes lacking these elements

		Cross 1: *M5/T-5*[a] female × *(P)*[b] male						Cross 2: *(P)/FM7* female × *T-5* male						
		M5/(P)			*T-5/(P)*			*FM7/T-5*				*(P)/T-5*		
Experiment	*P* element	*N*	*n*	GD ± SE	*n*	GD ± SE	GD′	*N*	*n*	GD ± SE	GD′	*n*	GD ± SE	GD′
III	*bw; st* control	34	268	5.3 ± 1.7	225	82.4 ± 3.5	1.00	32	172	86.5 ± 4.4	1.00	244	78.5 ± 4.9	1.00
	KP(1)⁻ 6.2 control	29	143	10.2 ± 3.0	135	71.3 ± 5.8	0.87	32	175	82.0 ± 5.3	0.95	226	77.3 ± 4.9	0.98
	KP(1)⁻ 16.1 control	30	198	7.2 ± 2.2	192	78.1 ± 4.5	0.95	28	157	80.4 ± 5.5	0.93	207	72.4 ± 6.0	0.92
	KP(1)	32	178	11.5 ± 3.3	164	76.4 ± 5.4	0.93	32	176	77.2 ± 6.0	0.89	217	66.3 ± 5.8	0.84
	KP(6)⁻ 14.2 control	31	183	24.9 ± 4.5	190	74.4 ± 6.4	0.90	35	219	71.0 ± 5.9	0.82	295	61.8 ± 5.4	0.79
	KP(6)⁻ 9.2 control	32	177	13.8 ± 3.2	161	77.1 ± 4.9	0.93	32	153	85.3 ± 4.1	0.99	212	78.0 ± 4.4	0.99
	KP(6)	32	194	13.2 ± 3.0	157	70.6 ± 5.2	0.85	29	164	63.5 ± 6.8*	0.73	212	33.5 ± 4.4***	0.43
	SP⁻ 13.2 control	32	192	8.1 ± 2.6	170	84.1 ± 5.0	1.02	29	180	72.7 ± 6.0	0.84	207	67.9 ± 5.9	0.86
	SP⁻ 11.2 control	36	237	8.1 ± 1.7	202	77.4 ± 4.8	0.94	32	163	80.3 ± 5.2	0.92	245	72.7 ± 4.7	0.93
	SP	35	202	5.5 ± 1.9	212	70.3 ± 4.9	0.85*	34	201	77.4 ± 5.5	0.89	277	53.8 ± 5.4**	0.68
IV	*bw; st* control	40	326	13.1 ± 3.1	304	69.8 ± 5.0	1.00	36	237	67.9 ± 4.9	1.00	311	64.3 ± 5.1	1.00
	KP(1)⁻ 6.2 control	40	278	21.4 ± 4.9	294	80.0 ± 4.1	1.15	39	304	82.5 ± 5.0	1.21	372	74.8 ± 5.4	1.16
	KP(1)⁻ 16.1 control	34	263	30.8 ± 4.7	236	77.0 ± 4.9	1.10	37	328	77.5 ± 5.1	1.14	397	78.3 ± 4.5	1.22
	KP(1)	34	238	18.7 ± 4.3	247	81.5 ± 3.8	1.17	36	273	86.6 ± 3.9	1.28	340	73.6 ± 4.4	1.14
	KP(6)⁻ 14.2 control	44	303	13.7 ± 2.3	319	79.1 ± 3.9	1.13	38	229	78.2 ± 4.2	1.15	371	69.7 ± 3.7	1.08
	KP(6)⁻ 9.2 control	40	272	25.7 ± 4.5	280	74.4 ± 4.7	1.07	36	211	73.5 ± 6.0	1.08	283	64.9 ± 5.3	1.01
	KP(6)	40	289	13.0 ± 2.3	280	73.6 ± 4.4	1.05	39	240	65.0 ± 5.2	0.96	368	41.5 ± 4.7***	0.65
	SP⁻ 13.2 control	42	265	30.3 ± 4.5	282	76.4 ± 4.6	1.09	31	177	75.5 ± 5.9	1.11	219	70.1 ± 5.8	1.09
	SP⁻ 11.2 control	36	281	29.6 ± 5.7	262	72.9 ± 5.7	1.04	42	227	76.9 ± 5.0	1.13	357	60.3 ± 5.7	0.93
	SP	43	333	17.0 ± 3.7	323	66.4 ± 3.7	0.95**	41	277	58.2 ± 5.5**	0.86	371	40.8 ± 4.3***	0.63

Isogenic control *X* chromosomes that had independently lost the isolated *P* elements are denoted with a minus sign and an identification number. Each isolated *P* element was compared to its pooled isogenic controls by the Mann-Whitney rank sum test. Asterisks indicate conventional significance levels and statistical symbols are defined in Table 2.

[a] The *T-5* sublines *p3′′′* and *p3′′* were used in experiments III and IV, respectively.

[b] *(P)* denotes the *X* chromosome under test.

in the male germ line. In the soma, *P*[*Sal*I] completely repressed Δ2-3-induced sn^w mutability, as expected (ROBERTSON and ENGELS 1989).

Among the naturally occurring *P* elements, none repressed sn^w mutability in the soma but four clearly repressed it in the germ line. *KP(1)* was a moderate to strong repressor, reducing sn^w mutability by 20–40% in experiments I and II and by 50–75% in experiments III and IV (see Tables 4 and 5 for significance levels). Clearly, this element was a more effective repressor with the Δ2-3 transposase source than it was with the *T-5* transposase source. *KP(6)* was also a stronger repressor with the Δ2-3 transposase source. Its effects were not significant in experiments I and II, but they were highly significant in experiments III and IV (27–62% reduction in mutability). The *D50* element also had no effect in experiments I and II, but it was a weak repressor in experiments III and IV, indicating that it was somewhat effective against the Δ2-3 transposase source. The *SP* element gave the opposite results: strong repression in experiments I and II (40–70% reduction in mutability), but no repression in experiments III and IV. This element was therefore effective only against the *T-5* transposase source. All four elements seemed to repress sn^w mutability through a zygotic effect; there was no substantial evidence for an additional maternal effect. The other naturally occurring *P* element, *KP(D)*, had no significant effect on sn^w mutability in any of the experiments.

DISCUSSION

Repression by naturally occurring *P* elements: We have established that individual naturally occurring *P* elements are capable of repressing different aspects of hybrid dysgenesis, GD sterility and sn^w mutability. Each of the isolated elements exhibited a distinct profile of repression abilities (Table 6).

Two of the three *KP* elements repressed both dysgenic traits, but not in a parallel fashion. *KP(1)* was the stronger repressor of sn^w mutability and *KP(6)* was the stronger repressor of GD sterility. The third *KP* element, *KP(D)*, had no significant effect on either trait. These functional differences suggest that genomic position may affect the repression ability of a particular type of *P* element.

The *D50* element, which is structurally very similar to *KP*, was, at best, a weak repressor of hybrid dysgenesis. It had no discernable effect on sterility and only a marginal effect on sn^w mutability, and this was seen only in the experiments using Δ2-3 as the transposase source. Experiments are currently underway to test *D50* elements in other genomic positions for

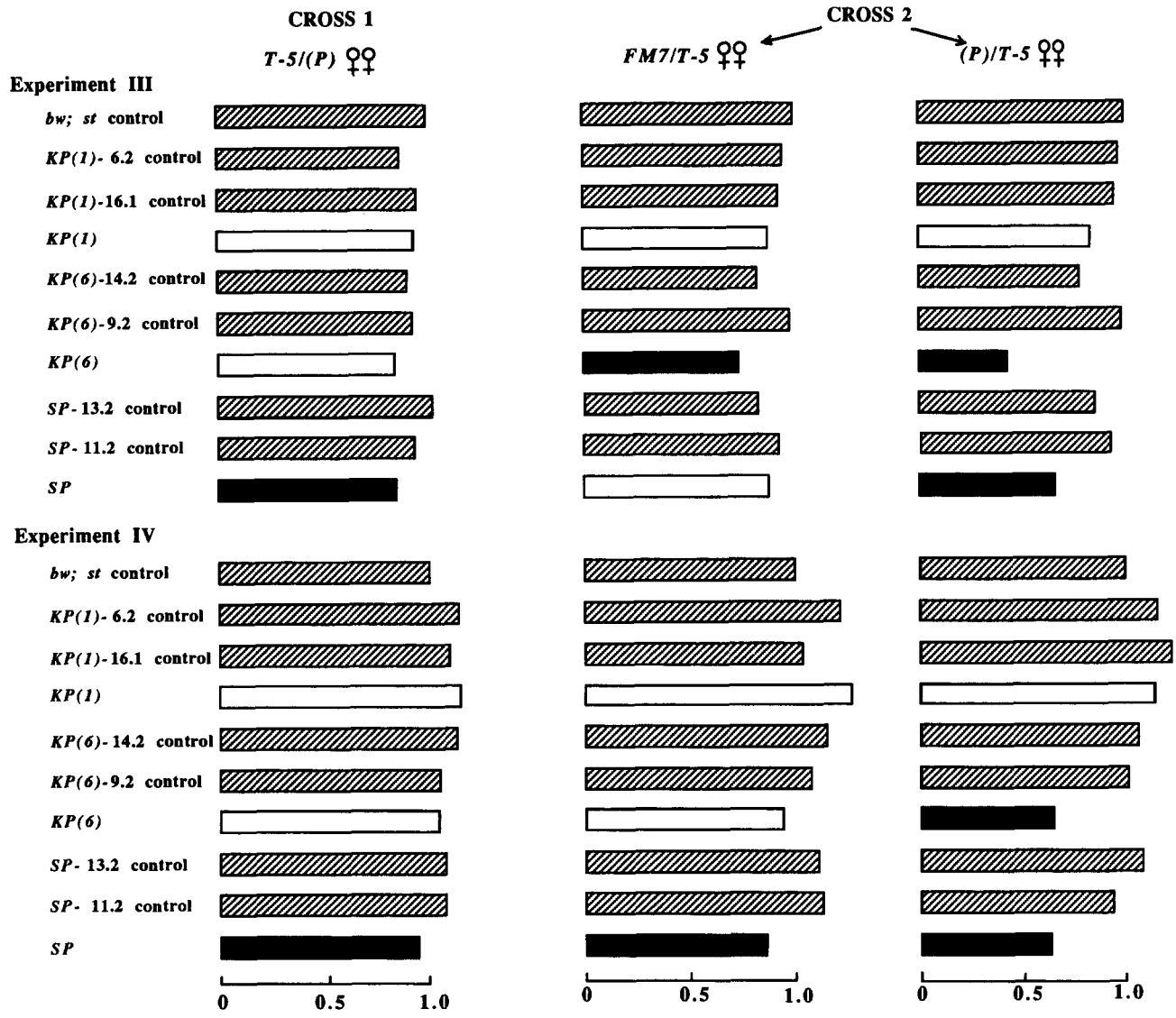

FIGURE 8.—Repression of gonadal dysgenesis by individual *P* elements in exeriments III and IV. Cross-hatching denotes the *bw; st* and various isogenic controls and solid color denotes the cases in which the frequency of sterility was significantly less than the controls. See text and Table 3 for details.

repression of dysgenic traits (C. PRESTON and W. ENGELS, personal communication).

The most surprising finding was that the *SP* element, only 517 bp long, was a strong repressor of GD sterility and *T-5*-induced sn^w mutability. This element was originally included in the experiments because we thought it was too small to encode a polypeptide repressor and could therefore serve as a negative control. Instead, it turned out to be the strongest repressor among the naturally occurring elements that were tested. The positive control element *P*[*Sal*I] also repressed GD sterility and sn^w mutability, as expected (ROBERTSON and ENGELS 1989).

Zygotic and maternal effects, results with different transposase sources and phenotypic suppression of vg^{21-3}: In all cases, the naturally occurring *P* elements seemed to repress GD sterility through a maternal effect, or through a combination of maternal and zygotic effects. In contrast, they seemed to repress sn^w mutability through a zygotic effect alone. One possible explanation is that a reduction in sn^w mutability can result from zygotically produced repressors acting in the developed, functioning germ line, whereas a reduction in GD sterility requires repressor action early during germ line development when the events leading to sterility occur (ENGELS and PRESTON 1979). These early acting repressors would most likely be provided by the maternal cytoplasm, explaining the maternal effect. However, there was also clear evidence for a zygotic effect working in concert with the maternal effect. This suggests a threshold phenomenon in which maternally produced repressors

TABLE 4

Repression of *T-5*-induced sn^w mutability by individual *P* elements

Experiment[a]	*P* Element	Cross A (*P* element paternally derived)				Cross B (*P* element maternally derived)			
		N[b]	*n*[c]	*u*[d] ± SE	*u'*[e]	*N*	*n*	*u* ± SE	*u'*
I	*bw; st* control	74	549	45.2 ± 2.8	1.00	55	706	39.6 ± 2.5	1.00
	D50$^-$ control	40	573	38.2 ± 2.1	0.84	40	798	34.4 ± 2.7	0.87
	D50[f]	40	431	43.6 ± 3.2	0.96	36	482	29.0 ± 2.8	0.73
	KP(1)	35	324	27.7 ± 2.5***	0.61	36	543	30.4 ± 3.0**	0.76
	KP(6)$^-$ control	23	146	44.6 ± 3.5	0.99	39	275	33.8 ± 3.9	0.85
	KP(6)	33	152	34.2 ± 5.3	0.76	38	415	25.2 ± 3.2	0.64
	KP(D)$^-$ control	39	704	42.2 ± 1.8	0.93	40	641	34.0 ± 2.1	0.86
	KP(D)	40	446	42.1 ± 2.8	0.93	39	577	33.1 ± 2.4	0.84
	SP$^-$ control	39	424	39.6 ± 2.7	0.88	40	551	33.8 ± 3.2	0.85
	SP	36	256	12.6 ± 2.2***	0.28	39	760	13.6 ± 1.4***	0.34
	P[*Sal*I]	40	299	2.8 ± 0.8***	0.06				
II	*bw; st* control	62	1394	45.3 ± 2.0	1.00	63	1819	40.3 ± 1.5	1.00
	D50$^-$ control	39	853	41.2 ± 2.1	0.91	40	848	40.6 ± 2.2	1.01
	D50[f]	33	780	43.6 ± 1.8	0.96	39	1096	42.0 ± 1.5	1.04
	KP(1)	33	587	36.5 ± 3.1	0.81	34	505	32.5 ± 2.7**	0.81
	KP(6)$^-$ control	34	622	41.6 ± 2.6	0.92	38	880	37.6 ± 2.2	0.93
	KP(6)	34	535	45.2 ± 3.5	1.00	40	1016	36.1 ± 1.7	0.90
	KP(D)$^-$ control	28	824	44.0 ± 1.8	0.97	39	1220	41.2 ± 1.2	1.02
	KP(D)	32	505	40.4 ± 3.4	0.89	40	994	42.5 ± 1.1	1.05
	SP$^-$ control	38	786	41.8 ± 2.2	0.92	38	1394	40.1 ± 1.5	0.99
	SP	40	825	26.4 ± 1.8***	0.58	39	1461	19.2 ± 1.7***	0.49
	P[*Sal*I]	36	349	5.2 ± 1.4***	0.11				

The Mann-Whitney rank sum test was used to determine if an isolated *P* element gave a significantly lower mutation rate than its control, which is denoted by a minus sign. The derivation of the control chromosomes is explained in the MATERIALS AND METHODS. The *KP(1)* and *P*[*Sal*I] elements were compared to the *bw; st* control. In the significance tests, each culture was treated as an independent unit. Asterisks indicate conventional significance levels.

[a] The *T-5*, sn^t chromosomes from sublines *K5* and *p3'''* were used in experiments I and II, respectively.

[b] Number of cultures.

[c] Number of progeny scored.

[d] Unweighted average partial mutation rate as a percentage ± standard error, which was calculated empirically.

[e] Average mutation rate normalized to the *bw; st* control.

[f] Although the *D50*(1) and *D50*(5) elements were tested separately, the data have been pooled in this analysis. Likewise the data for the two *D50* control chromosomes have been pooled.

bring dysgenic activity down to a level where nascent zygotically produced repressors can have an impact. A response threshold is also consistent with evidence that weak or moderate repressors are ineffective against strong inducers of sterility (see also SIMMONS *et al.* 1990). Curiously, the *in vitro* modified *P* element, *P*[*Sal*I], repressed sterility through a separate zygotic effect, implying that its repressor is synthesized early during development.

The different tests for repression of sn^w mutability revealed that the ability to repress can depend on the transposase source (*T-5* or Δ2-3). The *SP* element repressed sn^w mutability only when it was induced by the complete *P* elements carried by the *T-5 X* chromosomes, whereas the *KP(6)* and *D50* elements repressed sn^w mutability only when it was induced by the intron-deficient Δ2-3 *P* element. These observations suggest that repressors may be sensitive to the structure of transposase-producing *P* elements or to the structure of the primary transcripts of these *P* elements.

The repression abilities of the naturally occurring *P* elements were not correlated with their effects on the phenotype of the $vg^{21\text{-}3}$ *P*-insertion mutation. This mutation is one of several known "cytotype-dependent" alleles whose expression is thought to be influenced by repressors of hybrid dysgenesis (ROBERTSON and ENGELS 1989; BENZ 1989). Our results, although based on a limited sample of elements, indicate that if there is a relationship between the repression of hybrid dysgenesis and the phenotypic modification of a cytotype-dependent allele, it is not a simple one.

Mechanisms of repression: The prevalent view is that the *P* family is regulated by *P*-encoded polypeptides [reviewed in RIO (1990)]. These polypeptides have been postulated to repress dysgenesis by binding to *P* elements and either barring transposase access or interfering with transposase synthesis, or by binding to transposase polypeptides and destroying their catalytic function. Another model (SIMMONS and BUCHOLZ 1985), that extrachromosomal *P* element DNA titrates the transposase away from chromosomal *P*

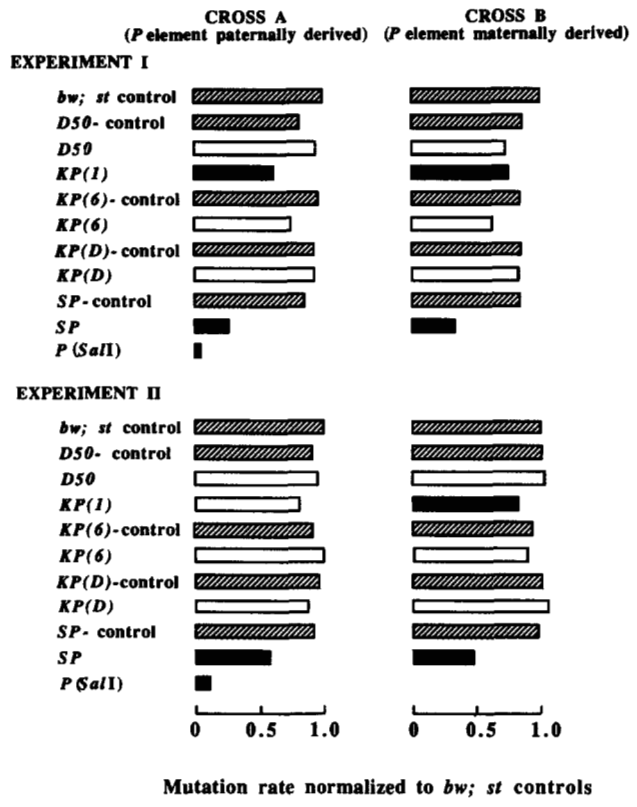

FIGURE 9.—Repression of *T-5*-induced sn^w mutability by individual *P* elements. Cross-hatching denotes the various controls and solid color denotes the cases in which sn^w mutability was significantly less than that of the relevant control. See text and Table 4 for details.

elements, thereby making them less active, has received little support.

The polypeptide repressor hypothesis is supported by evidence from tests with experimentally modified *P* elements that have been introduced into Drosophila strains by germ-line transformation. ROBERTSON and ENGELS (1989) showed that *P*[*SalI*] was an effective repressor of Δ2-3-induced GD sterility, sn^w mutability and pupal lethality. Our results have confirmed and extended their findings. This modified *P* element has a frameshift mutation in the last exon of the transposase gene, making it incapable of encoding a catalytically active product. It could, however, produce a polypeptide that would be isosequential with the transposase for much of its length. Because such a polypeptide could possess some of the binding and oligomerization properties of the transposase, it might be able to function in a competitive or antagonistic way as a repressor of transposase activity. Other modified *P* elements with frameshift mutations in exons 0, 1 or 2 did not repress transposase activity, suggesting that these exons are critical for repressor function (ROBERTSON and ENGELS 1989). Taken together, these results are consistent with the idea that the 66-kD polypeptide made from the first three exons of incompletely processed complete *P* element transcripts is a natural repressor of transposase activity (LASKI, RIO and RUBIN 1986; ROBERTSON and ENGELS 1989; MISRA and RIO 1990). Molecular constructs designed to produce this polypeptide under the control of a heat shock-inducible promoter have provided some supporting evidence (MISRA and RIO 1990).

The polypeptide repressor hypothesis could also be used to explain repression by small *P* elements, such as *KP*, as long as their polypeptide products retain some of the binding or oligomerization properties of the transposase. The predicted KP polypeptide would contain 207 amino acids, including an oligomerization motif that might allow it to bind to transposase polypeptides and disrupt their catalytic function (RIO 1990). However, there is currently no evidence for this polypeptide, much less for a disrupting interaction with the transposase. Consequently, although repression by individual *KP* elements has been established in fact, its underlying mechanism is still a matter of speculation. However, assuming the polypeptide repressor hypothesis to be correct, the inability of some *KP* elements such as *KP(D)* to repress dysgenesis could be attributed to transcriptional inactivity caused by a nearby silencer sequence (O'KANE and GEHRING 1987) or to a nonsense or missense mutation in the coding sequence of the element itself.

Our results with the *SP* element are not very easily explained by the polypeptide repressor hypothesis. The predicted *SP* polypeptide would contain only 14 amino acids–probably too small to be of any significance. However, *SP* was a potent repressor of both GD sterility and *T-5*-induced sn^w mutability. One possible explanation is that repression by *SP* is mediated by an antisense RNA that interferes with the processing or translation of complete *P* element transcripts. This antisense RNA could be produced by transcription backward through the *SP* element from an external promoter. Once formed, the antisense RNA molecules could bind to sense RNA molecules produced by complete *P* elements, blocking their processing or translation, or even causing their degradation by RNases. Any of these events would impair the synthesis of transposase polypeptides and ultimately reduce the incidence and severity of hybrid dysgenesis. Of course any kind of *P* element could insert backwards and downstream of a genomic promoter, leading to the synthesis of antisense *P* RNA. *P* elements apparently do have a tendency to insert in promoter regions (KELLEY *et al.* 1987). This mechanism of repression could therefore be quite general, and could even apply to complete *P* elements. In addition, the requirement for an inverted orientation downstream of a genomic promoter would explain why a particular type of *P* element, such as *KP*, might repress dysgenesis in one genomic location but not in another. Furthermore, because some promoters would be expected to initiate

TABLE 5

Repression of Δ2-3-induced sn^w mutability by individual *P* elements

Experiment[a]	*P* element	Cross A (*P* element paternally derived)				Cross B (*P* element maternally derived)			
		N	*n*	*u* ± SE	*u'*	*N*	*n*	*u* ± SE	*u'*
III	*bw; st* control	94	1422	88.1 ± 0.8	1.00	96	1696	88.9 ± 1.0	1.00
	D50$^-$ control	81	1350	92.1 ± 0.8	1.04	81	1134	93.5 ± 0.7	1.05
	D50	63	1012	78.5 ± 1.5***	0.89	43	608	77.6 ± 2.2***	0.87
	KP(1)	50	942	47.1 ± 2.1***	0.53	48	800	49.2 ± 2.7***	0.55
	KP(6)$^-$ control	45	964	87.1 ± 1.3	0.99	49	898	90.7 ± 1.2	1.02
	KP(6)	49	923	64.2 ± 1.6***	0.73	49	977	64.5 ± 1.6***	0.73
	KP(D)$^-$ control	47	664	86.9 ± 1.7	0.99	48	821	88.3 ± 1.3	0.99
	KP(D)	39	537	86.4 ± 1.6	0.98	41	633	86.8 ± 1.7	0.98
	SP$^-$ control	48	957	90.3 ± 1.3	1.02	47	914	88.7 ± 1.2	1.00
	SP	50	985	89.6 ± 0.8	1.01	50	1142	90.0 ± 0.9	1.01
	P[*SalI*]	50	1036	21.9 ± 1.7***	0.24	50	1071	15.8 ± 1.5***	0.18
IV	*bw; st* control	29	381	62.4 ± 3.9	1.00	30	354	55.9 ± 3.2	1.00
	D50$^-$ control	57	688	53.8 ± 3.2	0.86	55	593	54.9 ± 2.8	0.98
	D50	46	440	46.5 ± 3.7	0.75	45	531	42.0 ± 4.1*	0.75
	KP(1)	29	576	17.1 ± 2.8***	0.27	29	482	12.7 ± 2.8***	0.23
	KP(6)$^-$ control	30	445	57.4 ± 3.4	0.92	30	450	54.7 ± 3.5	0.98
	KP(6)	29	467	27.8 ± 3.9***	0.45	30	640	21.3 ± 2.8***	0.38
	KP(D)$^-$ control	30	377	59.4 ± 4.5	0.95	29	458	50.9 ± 3.6	0.91
	KP(D)	21	319	52.9 ± 5.2	0.85	19	287	55.3 ± 5.4	0.99
	SP$^-$ control	29	399	56.2 ± 2.9	0.90	30	366	61.5 ± 4.8	1.10
	SP	30	355	51.8 ± 3.4	0.83	29	371	56.4 ± 4.0	1.01
	P[*SalI*]	30	537	5.8 ± 1.0***	0.09				

Statistical tests were performed as in Table 4. Symbols are as in Table 4 except for *u*; in experiment III this was calculated as the percentage of all the sons that were extreme-singed or wild-type whereas in experiment IV it was calculated as the percentage of the sons that were extreme-singed among those that were either extreme-singed or weak-singed. See text for details.

[a] In experiment III, the *sn* mutations occurred in sn^w/Y; Δ2-3/+ males whereas in experiment IV, they occurred in $sn^w/+$; Δ2-3/+ females.

transcription strongly in the maternal germ line, this hypothesis can explain repression through a maternal effect.

The antisense repressor hypothesis can also explain why different kinds of *P* elements seem to be able to function as repressors of hybrid dysgenesis, albeit with different efficiencies and repression profiles [see also SIMMONS *et al.* (1987, 1990) and HEATH and SIMMONS (1991)]. In this study, for example, *KP(1)* repressed *T-5*-induced GD sterility and sn^w mutability as well as Δ2-3-induced sn^w mutability whereas *SP* repressed *T-5*-induced GD sterility and sn^w mutability but not Δ2-3-induced sn^w mutability. These differences can be explained by postulating that the antisense transcripts of these elements and their flanking DNAs have different abilities to bind with the sense transcripts of complete and Δ2-3 *P* elements.

The antisense repressor hypothesis is also consistent with the way repression potential evolves in experimental populations. Typically, this occurs many generations after *P* elements have been introduced into an M population, but once repression ability appears, it spreads rapidly and usually rises to a very high level (DANIELS *et al.* 1987; KIDWELL, KIMURA and BLACK 1988; KIYASU and KIDWELL 1984; PRESTON and ENGELS 1989; HEATH and SIMMONS 1991). Molecular analyses have suggested that subtle changes in the array of genomic *P* elements are responsible for this dramatic transition. Under the antisense repression hypothesis, the kind of change that is needed is the backwards insertion of a *P* element downstream of a fairly strong genomic promoter. Such events may be relatively rare, explaining the long lag time in the evolutionary process. However, once they occur, they might confer high repression potential, *i.e.*, switch the cytotype from M to P, and would then be expected to spread rapidly through a population under natural selection.

NANCY KLECKNER and colleagues have demonstrated that a bacterial transponson, IS*10*, is regulated by antisense RNA initiated from a promoter located a short distance from the 5′ end of the transposase gene within this transposon (SIMONS and KLECKNER 1988; CASE, SIMONS and SIMONS 1990; MA and SIMONS 1990). This promoter, called pOUT, generates an RNA that is complementary to the ribosome binding site in the transposase sense RNA. Pairing between sense and antisense RNA blocks ribosome binding, thereby preventing translation of the transposase message. Although this system indicates that transposon activity can be regulated by antisense RNA, it differs from the one that we have proposed in an important way: the IS*10* antisense promoter, pOUT, is located inside the IS*10* element, whereas the one we have

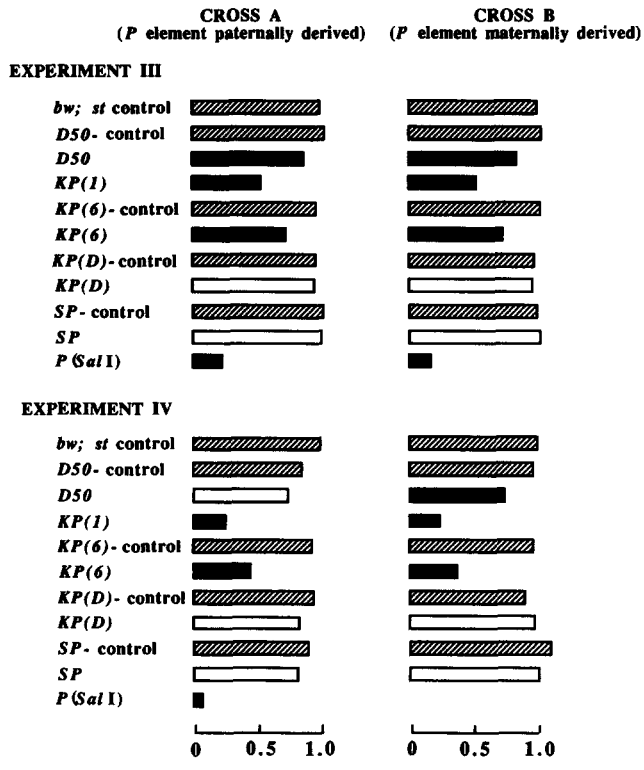

FIGURE 10.—Repression of Δ2-3-induced sn^w mutability by individual *P* elements. Cross-hatching denotes the various controls and solid color denotes the cases in which sn^w mutability was significantly less than that of the relevant control. See text and Table 5 for details.

TABLE 6

Summary of the properties of the isolated *P* elements

Element	Repression of				Suppression of
	GD[a,d]	*snw* [a,d]	*snw* [b,c]	*snw* [b,d]	*vg21-3*
KP(1)	++	++	+++	+++	−
KP(6)	+++	−	++	++	±
KP(D)	−	−	−	−	−
D50	−	−	+	+	++
SP	+++	+++	−	−	−
P[*Sal*I]	+++	+++	+++	+++	+++

The strength of the effect is indicated by the number of plus signs; a minus sign means no significant effect.
[a] Induced by the *T-5 X* chromosomes.
[b] Induced by the Δ2-3 *P* element.
[c] Assessed in males.
[d] Assessed in females.

proposed to initiate the synthesis of antisense *P* RNA is located outside the *P* element.

The hypothesis enunciated here may apply to other families of transposable elements. In *Drosophila melanogaster*, the copy number of each transposon family appears to be regulated, at least approximately (CHARLESWORTH and LANGLEY 1989). We suggest that in many cases antisense RNA may be the regulatory agent. This hypothesis obviates the need for regulatory proteins that are specific for each transposon family and capitalizes on the most outstanding property of transposons, namely, that they transpose. Occasionally, a transposon should insert backwards and downstream of a strong genomic promoter, leading to the production of antisense transposon RNA. This RNA could then interfere with the expression of sense RNA from other copies of the transposon, thereby blocking the synthesis of the proteins needed for transposition. Under this hypothesis, the evolution of the regulatory state depends on the ability of genomic promoters to initiate the synthesis of antisense transposon RNA. Such promoters would therefore provide the genome with general and effective instruments to combat the accumulation of a multitude of parasitic transposable elements.

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