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Repression of *P* Element-Mediated Hybrid Dysgenesis in *Drosophila melanogaster*

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ABSTRACT

Inbred lines derived from a strain called Sexi were analyzed for their abilities to repress *P* element-mediated gonadal dysgenesis. One line had high repression ability, four had intermediate ability and two had very low ability. The four intermediate lines also exhibited considerable within-line variation for this trait; furthermore, in at least two cases, this variation could not be attributed to recurring *P* element movement. Repression of gonadal dysgenesis in the hybrid offspring of all seven lines was due primarily to a maternal effect; there was no evidence for repression arising *de novo* in the hybrids themselves. In one of the lines, repression ability was inherited maternally, indicating the involvement of cytoplasmic factors. In three other lines, repression ability appeared to be determined by partially dominant or additive chromosomal factors; however, there was also evidence for a maternal effect that reduced the expression of these factors in at least two of the lines. In another line, repression ability seemed to be due to recessive chromosomal factors. All seven lines possessed numerous copies of a particular *P* element, called *KP*, which has been hypothesized to produce a polypeptide repressor of gonadal dysgenesis. This hypothesis, however, does not explain why the inbred Sexi lines varied so much in their repression abilities. It is suggested that some of this variation may be due to differences in the chromosomal position of the *KP* elements, or that other nonautonomous *P* elements are involved in the repression of hybrid dysgenesis in these lines.

THE many families of transposable elements that have been identified in *Drosophila melanogaster* exhibit considerable variety in size, structure and behavior. One of these, the *P* family, consists of elements less than 3 kb long, and is responsible for a condition known as P-M hybrid dysgenesis (Kidwell, Kidwell and Sved 1977; Bingham, Kidwell and Rubin 1982). This condition is normally confined to the germ line, where it is manifested by elevated mutation rates, chromosome breakage, segregation distortion and sterility (Engels 1989).

As its name implies, hybrid dysgenesis occurs in the progeny of crosses between strains. However, not all inter-strain crosses produce dysgenic offspring. Genetic and molecular analyses have demonstrated that dysgenesis requires a specific protein, the P transposase, as well as a cellular environment that allows *P* element movement. The transposase is encoded by the four open reading frames of structurally complete (2.9 kb) *P* elements (Karess and Rubin 1984; Engels 1984; Laski, Rio and Rubin 1986; Rio, Laski and Rubin 1986). Incomplete elements, which are deletion derivatives of these 2.9-kb elements, cannot make the transposase, but, as long as their terminal and subterminal sequences are intact, they can be acted upon by it (Engels 1984, 1989; Spradling and Rubin 1982). Such elements are functionally nonautonomous since their movement requires the presence of complete, or autonomous, *P* elements.

Several laboratories are currently investigating the cellular conditions that control *P* element activity. Early studies had identified two alternative states, or cytotypes, that permit or repress movement (Engels 1979a, b, 1981b). The M cytotype is permissive, while the P cytotype is repressive. These two states are characteristic of certain types of Drosophila strains, which are designated M and P, respectively. M cytotype strains completely lack *P* elements, whereas P cytotype strains possess many different kinds.

Detailed studies have shown that cytotype is determined jointly by a combination of chromosomal and cytoplasmic factors, and that the influence of the latter can persist for at least two generations (Engels 1979a). These cytoplasmic factors account for the different levels of dysgenesis that are seen in genetically identical hybrids from reciprocal crosses between P and M strains (Engels 1979a, b; Simmons *et al.* 1980). In such crosses, the cytotype of a hybrid is usually the same as that of its mother. Thus, hybrids from the cross P female × M male inherit the P cytotype, which represses *P* element activity, whereas

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hybrids from the reciprocal cross inherit the M cytotype, which allows this activity. The latter type of hybrid is therefore much more likely to manifest dysgenic traits. The offspring of first generation backcrosses to the male parent also show the influence of these cytoplasmic factors, since they tend to have the same cytotype as their grandmothers; the persistence of this influence through two generations establishes that it is not simply a maternal effect, but rather, a *bona fide* case of maternal inheritance. In subsequent backcrosses, the cytotype is apt to switch to that of the backcrossing strain, indicating that it is ultimately determined by factors on the chromosomes. Such changes appear to be sudden and complete, for intermediate or transitional states are rarely observed (ENGELS 1979a).

More recent studies have revealed considerable variation in the inheritance of these regulatory states (KIDWELL 1983, 1985; SIMMONS *et al.* 1987; BLACK *et al.* 1987). This variation is seen in many strains that carry *P* elements, especially those derived from populations in Europe, Africa and Asia (ANXOLABÉHÈRE *et al.* 1984, 1985). Such strains are referred to as M′ or pseudo M because they permit *P* element movement in some of their hybrid progeny. Detailed studies with one of these strains, called Sexi, have suggested that it possesses a system for regulating *P* elements that is different from cytotype (KIDWELL 1985).

We have investigated *P* element regulation in seven inbred lines derived from the Sexi strain. Such lines should be fixed for many of the *P* elements they carry, especially if no autonomous *P* elements are present. The regulatory properties of these inbred Sexi lines have been investigated by studying the repression of gonadal dysgenesis, an abnormality that occurs when the embryonic germ line fails to develop. In the adult, this condition is manifested by rudimentary gonads that are incapable of producing gametes (ENGELS and PRESTON 1979; KIDWELL and NOVY 1979). The resulting sterility is often referred to as dysgenic or GD sterility. The precise etiology of this condition is not known, but it probably stems from *P*-element mediated chromosome breakage and dominant lethal mutations (ENGELS *et al.* 1987; SIMMONS *et al.* 1987; RASMUSSON *et al.* 1990).

Gonadal dysgenesis occurs in the hybrid offspring of certain crosses. Typically, one of the parents in these crosses induces *P* element activity in the offspring by contributing transposase-producing *P* elements. The other parent may contribute factors that regulate the level of this activity, thereby influencing the likelihood that gonadal dysgenesis will occur. If, in a series of crosses, the inducer contribution is held more or less constant, it is possible to estimate the relative effects of the regulatory factors that are contributed by the other parent. This is done simply by noting the proportion of dysgenic offspring that are obtained from each mating. A high proportion would indicate that the parent contributed weakly to the regulation of *P* element activity, whereas a low proportion would indicate that it contributed strongly. We shall refer to this measure of regulatory ability as the "repression potential" of the parent. If the parent is a male, this potential can only involve chromosomally transmitted factors, whereas, if it is a female, both chromosomal and cytoplasmic factors can be involved.

In this study we have sought to determine the strength and variability of the repression potential of flies from each of the inbred Sexi lines. We have investigated whether this potential varies within and among the lines, and whether it can exist stably as a state in between the M and P cytotypes. In addition, we have investigated whether or not the repression of gonadal dysgenesis involves a maternal effect; previous analyses of M′ strains have not considered this issue (KIDWELL 1985; BLACK *et al.* 1987). We have also studied the inheritance of repression potential, utilizing hybrids from reciprocal crosses between the Sexi lines and a true M strain. Experimental tests with these hybrids have allowed us to separate the genetic and cytoplasmic factors that contribute to repression potential. Finally, we have analyzed DNA from each of the Sexi lines, focusing on one type of *P* element that has been proposed to account for the repression of gonadal dysgenesis. This putative repressor, called the *KP* element, has been found in many M′ strains, including Sexi, where it is especially abundant (BLACK *et al.* 1987). Our combined genetic and molecular data have been used to evaluate the *KP* repressor hypothesis.

MATERIALS AND METHODS

Stocks: The stocks used in these experiments are listed below. Information about the genetic markers and rearranged chromosomes can be found in LINDSLEY and GRELL (1968) and in SIMMONS *et al.* (1987).

True M stocks (with the M cytotype and devoid of *P* elements):

1. *bw; st*, a stock homozygous for recessive autosomal markers.
2. *M5; bw; st*, a stock homozygous for the *X* chromosome balancer *Muller-5*, as well as the autosomal markers *bw* and *st*.
3. *C(1)DX, y f/Y/y cin w* f^{5} *su(f)*^{ts67g}, a stock 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 aids in the collection of virgin females.
4. *C(1)DX, y f/Y/y* shi^{ts}*; bw; st*, another attached-*X* stock in which the males have an *X* chromosome with a different temperature-sensitive lethal mutation (shi^{ts}). This stock is also homozygous for *bw* and *st*.
5. *y* sn^{3} *v car*, a stock homozygous for four recessive *X*-linked markers, including an extreme allele of the *singed* (*sn*) locus.

Stocks containing *P* elements:

6. π_2, an inbred, wild type strain with P cytotype that is capable of inducing a high frequency of GD sterility in dysgenic crosses (ENGELS and PRESTON 1979).

7. *C(1)DX, y f/y/sn*^{w}; π_2, an attached-*X* strain with the genetic background of the π_2 stock. The males in this strain carry the *P* element-insertion mutation *singed-weak* (*sn*^{w}) (ENGELS 1979b, 1984; ROIHA, RUBIN and O'HARE 1988), which becomes unstable in the presence of the P transposase. However, in this stock the *sn*^{w} mutation is stabilized by the P cytotype.

8. *y sn*^{w}/*y*$^+$ *Y; bw; st*, an M cytotype stock homozygous for *sn*^{w}. The only *P* elements in this stock are nonautonomous elements located in the vicinity of the *singed* locus.

9. M5-B#1, an inbred M′ strain derived from a Muller-5 balancer stock from the University of Birmingham, England (BINGHAM, KIDWELL and RUBIN 1982). All the *P* elements in this strain are nonautonomous (SIMMONS *et al.* 1987).

10. ν_6 an inbred P cytotype strain capable of repressing, but incapable of inducing, GD sterility (ENGELS and PRESTON 1981; SIMMONS *et al.* 1984).

11. *ry*^{506} *P*[*ry*$^+$ *Sal*I] (89D), a stock homozygous for a single *P* element in which a frameshift mutation has been created in the *Sal*I restriction site (KARESS and RUBIN 1984).

12. *C(1)DX, y f/Y/T-5 (p.3′), y sn*^{w}*; bw; st*, a subline (*p.3′*) of the *T-5* stock described by SIMMONS *et al.* (1987). The *T-5 X* chromosome in the males carries autonomous *P* elements capable of destabilizing *sn*^{w}. To maintain this stock, *T-5, y sn*^{w}*; bw; st* males that are phenotypically weak singed are crossed to *C(1)DX, y f; bw; st* females (from stock 4 above) at 21°. In previous experiments, the *T-5 X* chromosome was unable to induce a significant frequency of GD sterility in a pure M genetic background (SIMMONS *et al.* 1987); however, by the time of the present experiments, this chromosome had acquired a marked ability to do so.

13. Sexi, a wild type stock derived from a single inseminated female caught in Spain in the mid-1970s (KIDWELL 1985). Genetic analysis has indicated that this stock possesses some autonomous *P* elements (JONGEWARD, SIMMONS and HEATH 1987). Inbred lines were produced from this stock by 21 generations of full-sib mating at 25°. Thereafter, the lines were maintained by small mass matings at 21°. The inbreeding commenced with G_0 in May, 1985 and was completed by G_{24} in March 1986. In G_{11}, G_{12} and G_{18}, the inbreeding scheme was relaxed in order to obtain enough flies for genetic tests.

Experimental methods: Stocks and experimental cultures were raised in vials and half-pint milk bottles on a standard cornmeal-molasses medium. Typically, the flies that were used to initiate experiments came from cultures that had been reared at 25°. The basic methods for testing the germ line instability of *sn*^{w} and for determining the frequency of gonadal dysgenesis (GD sterility) in hybrid flies were given by KOCUR, DRIER and SIMMONS (1986) and by SIMMONS *et al.* (1987), respectively. Slight differences are noted in the text.

DNA for Southern analysis was extracted from adult females. Samples (3–6 μg) of DNA were digested with restriction enzymes according to the supplier's instructions (Bethesda Research Laboratories). The DNA fragments were separated in agarose gels by electrophoresis and then transferred to GeneScreen*Plus* nylon membranes (Dupont) by capillary blotting. The membranes were hybridized with radiolabeled DNA probes that had been synthesized with [^{32}P]dCTP (3000 Ci/mM, Amersham) from fragments of the plasmid pπ25.1 (O'HARE and RUBIN 1983) using a random primer DNA labeling system (Bethesda Research Laboratories). Plasmid fragments were isolated from restriction enzyme digestions that were electrophoresed in low melting point agarose gels. After hybridization, the membranes were washed at 65° in 0.1 × SSC, 0.1% SDS, sealed in plastic bags and exposed to X-ray film with intensifying screens (Dupont) at −80°. Membranes that were used for rehybridization were stripped of previously bound probe by shaking at 42° in 0.4 N NaOH and then in 0.1 × SSC, 0.5% SDS, 0.2 M Tris (pH 7.5).

RESULTS

Potential for inducing hybrid dysgenesis: To provide background information for the study of repression potential, each of the inbred lines derived from the M′ strain called Sexi was tested for its ability to induce hybrid dysgenesis. Two traits, *sn*^{w} mutability (ENGELS 1979b, 1984) and GD sterility (ENGELS and PRESTON 1979; KIDWELL and NOVY 1979), were studied. In the *sn*^{w} tests, males from each Sexi line were mass-mated to *y sn*^{w}; bw; *st* females at 25°. The resulting hybrids were then crossed to allow the detection of mutations of *sn*^{w} occurring in their germ lines. Both *sn*^{e} and *sn*$^{(+)}$ mutations could be detected in the crosses with the hybrid males, but only *sn*^{e} mutations could be detected in the crosses with the hybrid females; the reason is that these females already carried a wild type *singed* allele (KOCUR, DRIER and SIMMONS 1986). In both sets of crosses, some of the apparent mutations of *sn*^{w} were subsequently tested for complementation with *sn*^{3} to determine if they were genuine. Because the mutability of *sn*^{w} requires the *P* transposase, the occurrence of any *bona fide* mutations would indicate that an inbred line carried at least one transposase-producing *P* element on its chromosomes.

The *sn*^{w} mutability tests were initiated four times–in generations 11, 18, 38 and 45–during the propagation of the inbred lines (Table 1). In all these tests, only one of the inbred lines, Sexi.3, exhibited a marked ability to destabilize *sn*^{w} in hybrid flies. The persistence of this ability indicated that Sexi.3 maintained at least one autonomous *P* element in its genome. Autonomous elements were also present in Sexi.1, Sexi.2 and Sexi.4, since each of these induced a few *bona fide* mutations of *sn*^{w}; however, Sexi.4 seems to have lost this ability (and, presumably, its autonomous *P* elements) sometime during the experiments. The other inbred lines showed little or no ability to destabilize *sn*^{w}. No mutations were induced by Sexi.5 at anytime, and only a few apparent mutations (which were not tested for authenticity) were induced by Sexi.6 and Sexi.7–all in the experiment initiated in G_{11}. The absence of any *bona fide* mutations from these last three lines suggests that they did not have autonomous *P* elements, or if they did, that these elements were lost early in the propagation of the lines.

For the GD sterility tests, males from each of the inbred lines were individually mated to *bw*; *st* females

TABLE 1

Mutability of sn^w among the F_1 progeny of crosses between *y* sn^w; *bw*; *st* females and inbred Sexi males monitored over 44 generations

Stock	Sex of F_1	G_{11}				G_{18}				G_{38}				G_{45}			
		N[a]	w[b]	+[c]	e[d]	*N*	w	+	e	*N*	w	+	e	*N*	w	+	e
Sexi.1	Male	48	1320	0	0	101	2335	0	0	57	1303	0	0	79	1609	0	0
	Female	42	1376		0	89	2492		0	50	1458		0	67	1358		3
Sexi.2	Male	49	1084	0	0	49	980	0	0	58	1382	0	0	78	1521	1	0
	Female	44	1233		(2)[e]	46	1204		1	54	1586		2	76	1679		0
Sexi.3	Male	48	1002	(54)	(53)	60	1362	(45)	(62)	58	1376	(52)	(69)				
	Female	45	1270		(22)	49	1345		(14)	56	1614		1+ (15)				
Sexi.4	Male	45	941	3+ (3)	(1)	58	1424	0	0	57	1404	0	0	78	1529	0	0
	Female	47	1463		(1)	51	1657		(1)	57	1742		0	77	1704		0
Sexi.5	Male	48	1175	0	0	53[f]	977	0	0	59	1378	0	0	76	1618	0	0
	Female	47	1423		0	51	1522		0	52	1612		0	69	1493		0
Sexi.6	Male	49	1122	(8)	0	56	1070	0	0	58	1301	0	0	72	1385	0	0
	Female	38	1100		0	42	1087		0	49	1205		0	76	2139		0
Sexi.7	Male	48	1113	0	0	56	1309	0	0	58	1539	0	0	76	1558	0	0
	Female	47	1636		(1)	55	1730		0	50	1439		0	74	1994		0

F_1 flies were mated at 25° and the F_2 progeny were scored until the 15th day after mating.
[a] Number of F_1 cultures.
[b] Number of weak singed F_2 progeny.
[c] Number of wild type F_2 progeny.
[d] Number of extreme singed F_2 progeny.
[e] Numbers in parentheses indicate F_2 progeny that were not tested to confirm the apparent mutation of sn^w.
[f] One culture in which a suppressor of sn^w was segregating was not tallied in these data.

TABLE 2

GD sterility among the F_1 daughters of crosses between *bw*; *st* females and inbred Sexi males

Stock	No. males tested[a]	No. F_1 females examined	Percent F_1 females sterile
Sexi.1	6	62	0
Sexi.2	5	54	0
Sexi.3	5	55	0
Sexi.4	6	76	0
Sexi.5	4	52	1.9
Sexi.6	7	72	1.4
Sexi.7	4	46	0
bw; *st*[b]	5	56	0

[a] The inbred Sexi males came from G_{38}.
[b] Control.

at 29° to produce hybrid daughters. These were then examined for the presence or absence of eggs as described in SIMMONS *et al.* (1987). As Table 2 shows, not even Sexi.3 induced a significant percentage of sterility in hybrid females. Additional data from experiments discussed below corroborate these findings.

Potential for repressing gonadal dysgenesis: Females from the seven inbred Sexi lines exhibited different abilities to repress GD sterility in their hybrid offspring. Table 3 presents the results of experiments that monitored this ability over 29 generations. In each experiment, a sample of females from an inbred line were crossed individually to males from the P strain π_2 at 29°. As many as 12 of the daughters from each cross were then examined for GD sterility. Throughout the course of these experiments, Sexi.4 and Sexi.7 behaved like the control strain, *bw*; *st*, since nearly all their hybrid offspring were sterile. In contrast, Sexi.3 behaved like a P cytotype strain; its hybrids showed almost no sterility in any of the experiments. The other inbred lines, Sexi.1, Sexi.2, Sexi.5 and Sexi.6, produced a mixture of fertile and sterile offspring, indicating that they possessed intermediate repression potential.

The distributions of sterility among the hybrids from each of the intermediate lines are shown in Figure 1. Each distribution was constructed by plotting the frequency of sterility among the daughters of individually tested females. It is clear from these plots that the factors affecting hybrid sterility varied continuously within the lines. It is also clear that some of the lines changed gradually during the course of the experiments. For instance, the distribution of Sexi.1 was initially concentrated at the upper end of the scale; however, by G_{47} it was concentrated at the lower end, having passed through a highly dispersed state. The distributions of Sexi.2 and Sexi.6 were also initially concentrated at the upper end of the scale, but by G_{47}, both were dispersed over essentially the entire range. In all three cases, a leftward shift had occurred, indicating the emergence of greater repression potential. In contrast to these, the distribution of Sexi.5 remained more or less unchanged throughout the period of study.

Repression of sterility involves a maternal effect: The above experiments have shown that GD sterility is repressed in the hybrid offspring of females from some of the inbred Sexi lines. However, they have not shown whether this repression arises in the hybrids

TABLE 3

GD sterility among the F_1 daughters of crosses between π_2 males and inbred Sexi females monitored over 29 generations

	G_{18}			G_{24}			G_{32}			G_{40}			G_{47}		
Stock	N[a]	n[b]	GD[c] + SE	N	n	GD + SE	N	n	GD + SE	N	n	GD + SE	N	n	GD + SE
Sexi.1	32	383	96.8 ± 1.8	25	300	99.3 ± 0.5	25	300	70.7 ± 6.7	28	330	32.7 ± 3.9	19	207	11.4 ± 3.0
Sexi.2	35	420	86.7 ± 2.3	25	294	87.3 ± 2.7	25	300	64.0 ± 5.0	41	477	71.7 ± 3.5	34	351	48.0 ± 4.9
Sexi.3	34	408	5.9 ± 1.6	25	299	0	29	345	1.4 ± 0.6	25	295	0	27	323	0.3 ± 0.3
Sexi.4	27	318	99.4 ± 0.4	25	299	99.7 ± 0.3	25	292	94.3 ± 1.7	29	308	95.9 ± 3.2	19	223	86.4 ± 5.7
Sexi.5	26	309	60.5 ± 4.1	23	273	46.5 ± 4.5	29	334	59.7 ± 4.0	25	267	57.2 ± 4.4	36	406	41.3 ± 3.1
Sexi.6	17	160	86.3 ± 3.2	15	160	52.1 ± 6.0	23	194	43.7 ± 5.9	25	271	62.3 ± 3.5	22	212	58.6 ± 3.9
Sexi.7	35	416	97.5 ± 0.9	25	300	98.7 ± 0.9	25	293	99.2 ± 0.6	25	294	99.7 ± 0.3	38	433	97.0 ± 1.4
bw; st	11	132	100	14	131	99.1 ± 0.9	25	273	100	22	230	98.8 ± 0.6	33	296	98.5 ± 0.7

[a] Number of females crossed individually to π_2 males.
[b] Total number of F_1 daughters examined.
[c] Unweighted average percentage of daughters with GD sterility ± standard error.

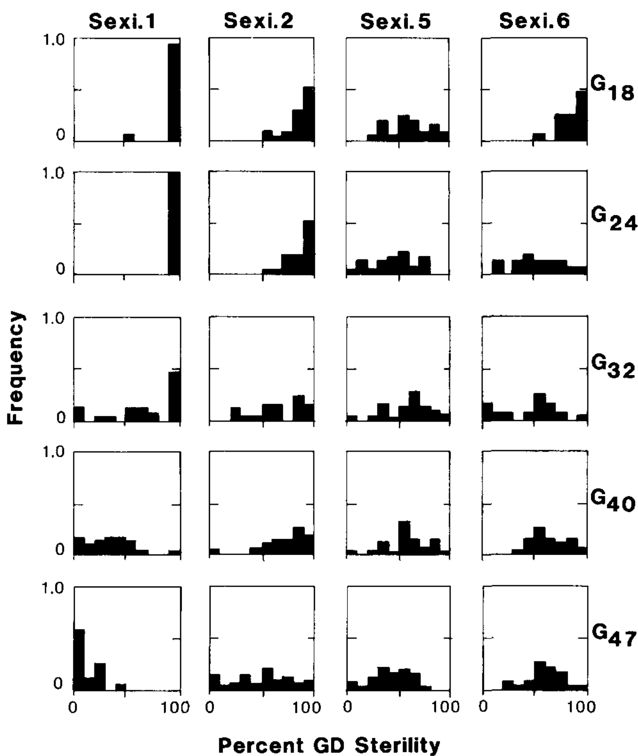

FIGURE 1.—Distributions of GD sterility among the daughters of inbred lines that had intermediate repression potential. The frequency indicates the proportion of daughters from a given female that were sterile. Results from five different generations are shown.

themselves, or is due to a maternal effect. To investigate this issue, each inbred line was crossed reciprocally to flies that carried a *T-5 X* chromosome. This chromosome has sn^w plus at least one transposase-producing *P* element and is able to induce high levels of GD sterility. By comparing the frequencies of sterility in the hybrid females from these crosses, it was possible to determine if a maternal effect was involved.

The *T-5*-bearing flies came from crosses between *T-5, y* sn^w; *bw*; *st* males and *C(1)DX, y f*; *bw*; *st* and *M5*; *bw*; *st* females; each *T-5* male was mated at 21° to both types of females. Then, from each pair of crosses, *T-5* sons from the first mating and *M5/T-5* daughters from the second were crossed, respectively, to females and males from each of the inbred Sexi lines; the same crosses were also carried out with two control stocks, *bw*; *st* and π_2, instead of the Sexi lines. In all these crosses, single pair matings were used and the cultures were incubated at 29°. In the next generation, samples of *T-5/+* (and, where appropriate, *M5/+*) females from each culture were examined for GD sterility. As a check on the frequency of sterility induced by the Sexi and control stocks themselves, a parallel experiment was performed in which the *T-5 X* chromosome was replaced by the *y* sn^w *X* chromosome from stock 8.

Before examining the results, it should be noted that the sn^w allele on the *T-5 X* chromosome was highly mutable. The average mutation rate, 0.423 ± 0.019, was estimated by scoring the sons of the matings between the *T-5* males and the *C(1)DX, y f*; *bw*; *st* females at the beginning of the experiment (26 matings, each transferred twice, for a total of 1174 progeny scored). This rate is comparable to the rates induced by the strongest P strains and indicates that the autonomous *P* elements on the *T-5 X* chromosome were able to generate much transposase activity. In contrast, the *y* sn^w *X* chromosome that was used in the parallel experiment was unable to generate any transposase activity.

Table 4 presents the main results. First, consider the data from crosses 1 and 2, which tested the ability of the Sexi and control strains to induce GD sterility without the *T-5 X* chromosome. In these crosses, *y* sn^w/*M5* females (cross 1) and *y* sn^w males (cross 2) were mated to flies from each of the Sexi and control stocks. Since these males and females did not carry any transposase-producing *P* elements, any sterility that occurred in their offspring must have been induced by elements contributed by their mates. It is clear from the data that none of the Sexi lines was able to induce much sterility in either cross 1 or cross

TABLE 4

Frequency of GD sterility induced by the *T-5 X* chromosome

Stock	Cross 1[a]: *M5/+* ♀♀			Cross 1: *sn*[w]/+ ♀♀			Cross 2[b]: *sn*[w]/+ ♀♀			Cross 3[c]: *M5/+* ♀♀			Cross 3: *T-5/+* ♀♀			Cross 4[d]: *T-5/+* ♀♀		
	N[e]	*n*[f]	GD ± SE[g]	*N*	*n*	GD ± SE	*N*	*n*	GD ± SE	*N*	*n*	GD ± SE	*N*	*n*	GD ± SE	*N*	*n*	GD ± SE
Sexi.1	26	262	1.7 ± 1.7	26	227	2.7 ± 2.7	36	416	11.1 ± 2.3	25	247	14.3 ± 3.5	25	247	86.8 ± 3.5	28	316	11.4 ± 2.4
Sexi.2	25	291	0	25	229	0	17	181	1.7 ± 1.7	24	241	25.7 ± 4.7	24	275	87.4 ± 3.8	15	149	8.3 ± 3.7
Sexi.3	28	321	0	28	312	0	41	473	1.2 ± 0.5	25	281	15.9 ± 2.9	25	293	79.5 ± 4.0	44	486	4.8 ± 1.4
Sexi.4	23	260	0	23	262	0	12	111	0	23	264	14.4 ± 4.7	23	264	88.4 ± 5.4	30	326	52.4 ± 4.8
Sexi.5	27	285	0	27	277	0	12	125	1.4 ± 1.4	24	234	10.1 ± 2.9	24	235	89.7 ± 4.0	12	143	2.1 ± 1.5
Sexi.6	26	274	0.8 ± 0.8	26	271	0.3 ± 0.3	6	67	0	22	228	11.0 ± 4.0	22	230	64.3 ± 6.8	9	74	26.8 ± 9.5
Sexi.7	29	332	0	29	338	0	53	598	0.6 ± 0.4	26	273	22.1 ± 5.9	26	279	92.9 ± 3.4	52	577	72.2 ± 3.3
bw; st	20	187	1.1 ± 0.8	21	228	2.2 ± 1.3	23	204	1.1 ± 0.8	24	218	7.8 ± 2.1	24	252	79.7 ± 3.7	23	105	73.1 ± 6.8
π_2	29	296	94.9 ± 2.5	29	301	95.6 ± 0.1	28	262	0.9 ± 0.7	25	168	98.9 ± 0.9	25	181	99.3 ± 0.7	43	423	2.8 ± 1.3

These data came from experiments initiated in G_{59}.

[a] *M5/sn*[w] ♀ × stock ♂.

[b] *sn*[w] ♂ × stock ♀.

[c] *M5/T-5* ♀ × stock ♂.

[d] *T-5* ♂ × stock ♀.

[e] Number of crosses.

[f] Number of daughters examined.

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

2, confirming earlier results (Table 2). In each case, the frequency of sterility was comparable to that observed in the crosses with the *bw*; *st* controls. In contrast, the other control strain, π_2, induced a high level of sterility in the offspring of cross 1, but almost none in the offspring of cross 2. These opposite results were expected from the known properties of the π_2 strain (ENGELS 1979a; ENGELS and PRESTON 1979).

Now consider the data from cross 3, which demonstrate that the *T-5 X* chromosome could induce a high frequency of GD sterility, both by itself and in combination with chromosomes from the inbred Sexi lines. In this cross, *T-5/M5* females were mated to males from each of the Sexi and control stocks. Both *T-5/+* and *M5/+* females emerged in the progeny. In all cases, the *T-5/+* females suffered appreciable sterility, whereas the *M5/+* females were usually fertile. (One exception is the cross with π_2, where a high frequency of sterility was observed–and expected–in both classes of progeny.) The large and consistent differences between these two classes of females indicate that the sterility-inducing factors were linked mainly to the *T-5 X* chromosome. This is most plainly seen in the results with the *bw*; *st* controls; 79.7% of the *T-5/+* females were sterile, compared to only 7.8% of the *M5/+* females. The low frequency of sterility in the *M5/+* class probably reflects the action of *P* elements that had transposed from the *T-5 X* chromosome to other chromosomes in the genome in a previous generation. This might also explain the low frequency of sterility among the *M5/+* females from the crosses involving the Sexi lines.

In cross 3 it is interesting to note that the *T-5/+* offspring of the Sexi lines tended to show more sterility than the *T-5/+* offspring of the *bw*; *st* controls. This suggests that the paternally derived Sexi chromosomes actually enhanced the sterility that was induced by the *T-5 X* chromosome (see also the data of RASMUSSON *et al.* 1990). In only one case (Sexi.6) was there noticeably less sterility than in the *bw*; *st* controls, but this was not significant ($0.05 < P < 0.10$ by a one-tailed Mann-Whitney rank sum test). The increased sterility that was observed with the Sexi lines is similar to that seen with another M′ strain, Muller-5 Birmingham (SIMMONS *et al.* 1987), and might be due to interactions between the *T-5*-generated transposase and the large number of *P* elements that were inherited from these lines (see RASMUSSON *et al.* 1990).

To see that the repression of GD sterility in the offspring of the Sexi lines involves a maternal effect, consider the data from cross 4. In this cross, *T-5* males were mated to females from each of the Sexi and control stocks. Although the daughters from this cross were genetically equivalent to the *T-5/+* daughters from cross 3, they showed much less GD sterility. The observed differences were significant ($P < 0.01$ by

one-tailed Mann-Whitney rank sum tests) in every case except one (the *bw*; *st* controls). Evidently, at least some of the females from each of the Sexi lines were able to provide their offspring with factors that prevented the onset of gonadal dysgenesis. Previous experiments, in which strong P strains had been used to induce GD sterility, failed to turn up any evidence for repression in the offspring of Sexi.4 and Sexi.7 females (Table 3). However, this experiment, which utilized a weaker inducer of sterility, revealed that females from both of these lines had a modicum of repression potential. The fact that Sexi.7 yielded as much sterility in cross 4 as the *bw*; *st* controls is probably explained by the large number of *P* elements present in the Sexi.7 hybrids–each a potential target for transposase attack–and by the extreme weakness of Sexi.7's maternal effect.

Note that the results of this experiment do not exclude the possibility of repression arising from the zygotic genotypes of the Sexi hybrids. However, they do establish that if repression arises in this way, it is not nearly so important as the repression that comes from the maternal effect. Thus, in the Sexi lines, repression potential appears to depend primarily on the ability of a female to establish a condition in her eggs that can prevent the onset of gonadal dysgenesis after fertilization.

Inheritance of repression potential: The inheritance of repression potential was studied by performing reciprocal crosses between each of the inbred Sexi lines and the *bw*; *st* strain. The hybrid females from these crosses were then mated to males capable of inducing GD sterility, and samples of their daughters were examined for gonadal dysgenesis. The results from these tests allowed us to determine if repression potential was due to genetic factors carried on the chromosomes (KIDWELL 1985), or to cytoplasmic factors transmitted through the egg (ENGELS 1979a, 1981b).

Experiments using sn^w; π_2 *males to induce GD sterility:* In one set of experiments, the reciprocal hybrids were mated individually to sn^w; π_2 males, which are strong inducers of GD sterility. The progeny from these crosses were reared at 29° and as many as 12 of the daughters from each were examined for gonadal dysgenesis. The daughters of crosses between individual inbred Sexi females and sn^w; π_2 males were also examined in order to assess the repression potential of each inbred stock. As controls, *bw*; *st* females were tested to show the sterility-inducing power of the sn^w; π_2 genome and the π_2 and ν_6 strains were included to demonstrate the maternal inheritance of cytotype (ENGELS 1979a; ENGELS and PRESTON 1981).

Table 5 and Figure 2 present the results of these experiments. The hybrid and stock females that were used in the test matings were raised at either 21° or 25°, so the data are reported according to temperature; however, this factor seems to have had little effect on the expression or inheritance of repression potential.

The test matings with the stock females confirmed that the inbred Sexi lines differed in repression potential. As before, Sexi.4 and Sexi.7 had very little ability to repress GD sterility in their offspring, Sexi.1, Sexi.2, Sexi.5 and Sexi.6 had a moderate ability to do so, and Sexi.3 repressed this sterility almost completely. The two P cytotype strains, π_2 and ν_6, also had very high repression potential.

The test matings with the reciprocal hybrid females revealed considerable variation in the inheritance of repression potential. To distinguish between the two types of hybrid females, we use the letters A and B, where A denotes the females that had *bw*; *st* mothers. With Sexi.1, neither type of hybrid female showed any ability to repress gonadal dysgenesis in its offspring; this suggests a system in which repression potential is controlled by purely recessive chromosomal factors. With Sexi.2 and Sexi.6, some repression was observed in the daughters of both types of hybrid females, but not nearly as much as in the daughters of the females from each of these inbred lines. Evidently in Sexi.2 and Sexi.6, partially dominant chromosomal factors were involved in the determination of repression potential. With Sexi.5, both types of hybrid females showed an ability to repress gonadal dysgenesis in their offspring. The strength of this ability, compared to that of the Sexi.5 females themselves, indicates control by approximately additive chromosomal factors. Curiously, in three separate experiments, the A hybrids from this line showed slightly more repression potential than the B hybrids. This difference between genetically equivalent females suggests a maternal effect; however, unlike the maternal effect discussed in the previous section, this one apparently reduces repression potential. There is also a weak indication of such an effect in the data from the Sexi.2 and Sexi.6 hybrids. With Sexi.3, repression was observed only in the daughters of the B hybrids; moreover, these hybrids varied quantitatively in the strength of their repression potential. These results clearly indicate that in this line cytoplasmic factors were involved. With π_2 and ν_6, again only the B hybrids showed any ability to repress GD sterility in their offspring, but in both cases almost no quantitative variation was observed. Instead, the B hybrids from both strains could be classified into two distinct groups; in one group, nearly all of the offspring were fertile, while in the other, nearly all were sterile. This dichotomy was especially pronounced with ν_6 and suggests that the high and low repression states (or cytotypes) were determined by the segregation of a

TABLE 5

Reciprocal cross analysis of the ability to repress GD sterility induced by sn^w; π_2 males

	25°									21°								
	Stock females			F_1-A females			F_1-B females			Stock females			F_1-A females			F_1-B females		
Stock	N^a	n^b	GD[c] ± SE	N	n	GD ± SE	N	n	GD ± SE	N	n	GD ± SE	N	n	GD ± SE	N	n	GD ± SE
Sexi.1	37	382	55.7 ± 6.6	47	559	99.8 ± 0.2	49	588	100	28	323	61.7 ± 5.6	30	360	99.7 ± 0.3	28	336	100
Sexi.2	44	528	50.6 ± 4.0	50	600	95.2 ± 0.9	40	480	98.3 ± 0.7	27	309	42.8 ± 4.7	30	360	97.2 ± 0.8	27	324	99.7 ± 0.3
Sexi.3	47	549	0.9 ± 0.5	49	574	99.8 ± 0.2	48	575	85.5 ± 2.6	30	359	5.3 ± 1.2	30	356	99.4 ± 0.4	28	336	82.4 ± 2.1
Sexi.4	43	487	99.6 ± 0.4	46	552	100	46	552	100	22	246	98.1 ± 0.9	30	360	99.7 ± 0.3	28	336	99.7 ± 0.3
Sexi.5	22	231	76.6 ± 3.8	49	555	89.3 ± 1.2	44	528	94.5 ± 1.1	38	328	45.5 ± 4.6	30	359	83.6 ± 2.4	30	360	92.8 ± 1.8
										45[d]	513	59.9 ± 3.5	44	524	87.1 ± 2.4	48	576	89.4 ± 1.5
Sexi.6	31	229	61.6 ± 4.5	50	600	92.5 ± 1.3	46	543	96.9 ± 1.0	25	233	66.8 ± 4.5	30	354	96.1 ± 1.2	27	324	96.6 ± 1.2
Sexi.7	35	339	97.6 ± 1.9	48	576	99.7 ± 0.2	47	563	99.8 ± 0.2	26	268	99.4 ± 0.4	29	348	99.7 ± 0.3	30	359	99.7 ± 0.3
π_2										41	486	0	48	569	98.6 ± 0.5	48	576	15.3 ± 4.6
ν_6	44	503	10.0 ± 4.3	45	540	99.3 ± 0.4	47	564	53.4 ± 7.1	30	353	8.9 ± 4.6	29	345	98.9 ± 0.9	29	348	48.3 ± 9.0
bw; *st*[e]	102	953	98.1 ± 1.1							75	550	100						

The tested flies were raised at the temperatures indicated. Altogether there were five experiments; the rearing temperatures for these are given in parentheses: experiment 1 (25°), initiated in G_{43} with Sexi.2, Sexi.4, Sexi.6 and Sexi.7; experiment 2 (25°C), initiated in G_{53} with Sexi.1, Sexi.3 and Sexi.5; experiment 3 (25°), with ν_6; experiment 4 (21°), initiated in G_{55} with Sexi.5 and π_2; experiment 5 (21°), initiated in G_{56} with all the inbred Sexi lines and ν_6. Females from the *bw*; *st* stock were tested in all of the expeirments.

[a] Number of females crossed individually to sn^w; π_2 males.

[b] Number of daughters examined.

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

[d] From experiment 4.

[e] Pooled results of all five experiments.

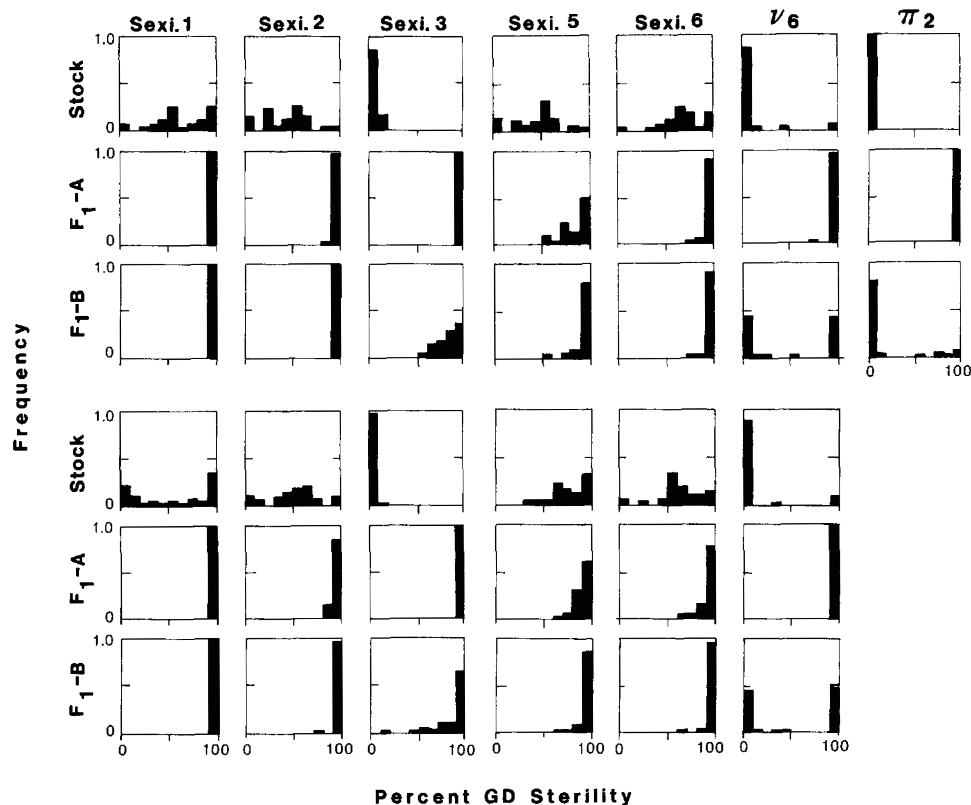

FIGURE 2.—Distributions of GD sterility among the daughters of females that were mated to sn^w; π_2 males. Reciprocal hybrid females (F_1-A and F_1-B) and stock females that had been reared at either 21° (upper panel) or 25° (lower panel) were used in these matings. Two other inbred lines (Sexi.4 and Sexi.7) were also tested, but nearly all of their daughters were sterile. As a result, the frequency distributions from these lines are not shown. See the text and Table 5 for details.

factor that was needed for the maintenance of the high repression state (ENGELS and PRESTON 1981).

Experiments using T-5 males to induce GD sterility: The inheritance of repression potential was studied in a second set of experiments in which GD sterility was induced by the *T-5 X* chromosome. Because this chromosome, by itself, induces less sterility than the sn^w; π_2 genome, it seemed that it might allow the detection of lower levels of repression potential. Reciprocal hybrid females from crosses between *bw*; *st* and each of the inbred Sexi lines were reared at 21° and then mated individually to two *T-5* males. These matings, also at 21°, were arranged so that both an A and a B female could be crossed to the same pair of males. The mated females were then transferred to fresh cultures that were kept at 29°, and samples of their daughters were examined for gonadal dysgenesis. Test matings between *T-5* males and individual inbred Sexi females were also carried out to assess the repression potential of each of the inbred stocks. In addition, these same males were mated to individual *bw*; *st* females to measure the sterility-inducing power of *T-5* in a pure M background. The π_2 and ν_6 strains were also included in these experiments to show the cytoplasmic inheritance of cytotype.

The results of these experiments are summarized in Table 6. First, it should be noted that by itself, the *T-5 X* chromosome induced a moderate level of GD sterility. This is evident from the crosses with the *bw*; *st* females, where 53.4% of the daughters were sterile. Second, it is clear that females from several of the tested stocks had the potential to repress this sterility; only Sexi.4 and Sexi.7 females failed to show this effect. Third, when compared to the *bw*; *st* females, hybrid females from six of the tested stocks (Sexi.2, Sexi.3, Sexi.5, Sexi.6, π_2 and ν_6) appeared to possess at least some repression potential. For Sexi.2, Sexi.5 and Sexi.6, this potential was greater in the A hybrids, while for Sexi.3 and ν_6, it was greater in the B hybrids; for π_2, repression potential was found in the B hybrids only.

These results amplify and refine the results of the previous experiments. As before, the repression potential of Sexi.1 appeared to be controlled by recessive chromosomal factors. Neither the A nor the B hybrids derived from this line showed any ability to repress sterility in their offspring; however, inbred females from the Sexi.1 stock clearly had a high potential for repression. In Sexi.2, Sexi.5 and Sexi.6, the control of repression potential seemed to be due to partially dominant or additive genetic factors. With these lines, sterility was repressed in the offspring of both types of hybrid females. Curiously, the A hybrids appeared to possess more repression potential than the B hybrids in all three cases; however, this superiority was statistically significant only for Sexi.2 and Sexi.5 ($P < 0.05$ by a one-tailed Mann-Whitney rank sum test). In these two cases, an "antirepression" maternal effect similar to that seen for Sexi.5 in the previous experiments is indicated. For Sexi.3 and ν_6, repression po-

TABLE 6

Reciprocal cross analysis of the ability to repress GD sterility induced by *T-5* males

	Stock females			F$_1$-A females			F$_1$-B females		
Stock	N^a	n^b	GD^c ± SE	N	n	GD ± SE	N	n	GD ± SE
Sexi.1	27	314	1.5 ± 0.6	45	532	59.1 ± 4.4	60	714	62.8 ± 2.9
Sexi.2	12	123	2.1 ± 1.1	36	440	18.5 ± 2.5	34	408	30.4 ± 3.5
Sexi.3	26	312	1.1 ± 0.8	43	516	30.8 ± 2.6	44	524	9.8 ± 1.7
Sexi.4	16	190	53.6 ± 6.7	47	564	63.3 ± 3.4	46	547	65.5 ± 4.2
Sexi.5	16	191	3.1 ± 1.0	42	501	9.5 ± 1.5	43	513	17.3 ± 2.3
Sexi.6	9	100	9.6 ± 4.5	31	372	9.9 ± 1.7	33	396	11.1 ± 1.7
Sexi.7	28	318	81.4 ± 2.9	50	600	77.3 ± 2.5	49	584	82.4 ± 2.1
π_2	25	279	0.7 ± 0.5	42	494	89.6 ± 1.9	41	490	2.2 ± 1.5
ν_6	23	263	2.7 ± 1.2	44	528	37.3 ± 3.4	47	563	18.2 ± 3.7
bw; st	254	1742	53.4 ± 1.8						

The tested flies were reared at 21° and came from G_{63}.
[a] Number of females crossed individually to *T-5* males.
[b] Number of daughters examined.
[c] Unweighted average percentage of daughters with GD sterility ± standard error.

tential appeared to be determined jointly by cytoplasmic and genetic factors. In the previous experiments, only the cytoplasmic factors were evident; here, a genetic component was revealed by the repression that occurred in the offspring of the A hybrids. This repression was demonstrated by comparing the data from the Sexi.3 and ν_6 A hybrid females with the data from the *bw*; *st* females. In both cases, the data were significantly different ($P < 0.05$ by a one-tailed Mann-Whitney rank sum test), suggesting the action of partially dominant or additive genetic factors. In addition, the influence of cytoplasmic factors was demonstrated by comparing the data from the A and B hybrids; for both strains, the B hybrids exhibited greater repression potential ($P < 0.05$ by a one-tailed Mann-Whitney rank sum test). In the case of ν_6, these hybrids appeared to fall into two distinct groups, one with high and one with low repression potential (data not shown); this finding therefore confirms the results of previous experiments (Figure 2). The π_2 strain also showed the influence of cytoplasmic factors on repression potential. With this strain, there was very high sterility in the daughters of the A hybrids and very low sterility in the daughters of the B hybrids. However, the latter did not exhibit any categorical differences, as happened with ν_6, possibly because the cytoplasmic factors governing repression potential were so strong.

Molecular analysis of the inbred Sexi lines: The repression of hybrid dysgenesis has been attributed to various types of *P* elements (DANIELS *et al.* 1987; ENGELS 1989; NITASAKA, MUKAI and YAMAZAKI 1987; SIMMONS *et al.* 1987), including a nonautonomous element called *KP* (BLACK *et al.* 1987; JACKSON, BLACK and DOVER 1988). Since this element has been reported to be abundant in the Sexi strain, Southern blots were performed to determine if it was also present in the inbred Sexi lines.

Genomic DNA was prepared from each of the inbred lines in generation 59; at the same time, DNA was extracted from the *bw*; *st*, π_2, M5-B#1, ν_6 and ry^{506} *P*[ry^+ *Sal*I] (89D) stocks, which served as controls. The DNA was digested with the restriction enzyme *Dde*I, electrophoresed, transferred to a membrane and then hybridized with a ^{32}P-labeled *Dde*I fragment of the plasmid pπ25.1 that contained bases 586-2762 of the complete *P* element sequence (Figure 3A). The autoradiograms that were obtained from this experiment are shown in Figure 3B.

Digestion of a complete *P* element with *Dde*I should produce an internal 2.18 kb fragment. As Figure 3B shows, such a fragment was detected in Sexi.1, Sexi.2 and Sexi.3, and among the controls, in π_2, ν_6 and ry^{506} *P*[ry^+ *Sal*I] (89D) (designated as *Sal*I). From these results and the genetic data reported in Table 1, we conclude that Sexi.1, Sexi.2 and Sexi.3 each had at least one complete *P* element in their genomes. By contrast, none of the other inbred Sexi lines yielded the 2.18-kb fragment; nor did M5-B#1. We therefore conclude that these stocks did not carry any complete *P* elements. The genetic data in Table 1 and in SIMMONS *et al.* 1987 also support this conclusion.

Digestion of a *KP* element with *Dde*I should produce a 0.42-kb fragment. As the map in Figure 3A indicates, this fragment should hybridize with the *Dde*I probe, but not with the smaller *Hin*dIII/*Sal*I probe. The results in Figure 3B show that the *Dde*I probe hybridized strongly with a 0.42-kb fragment in the DNA from all of the inbred Sexi lines, but not in the DNA from any of the other stocks. The same membranes that were hybridized with the *Dde*I probe were stripped and rehybridized with the *Hin*dIII/*Sal*I probe. In each case, the 0.42-kb fragment failed to bind the probe, even though other fragments, such as the 2.18 kb fragment from the complete *P* elements,

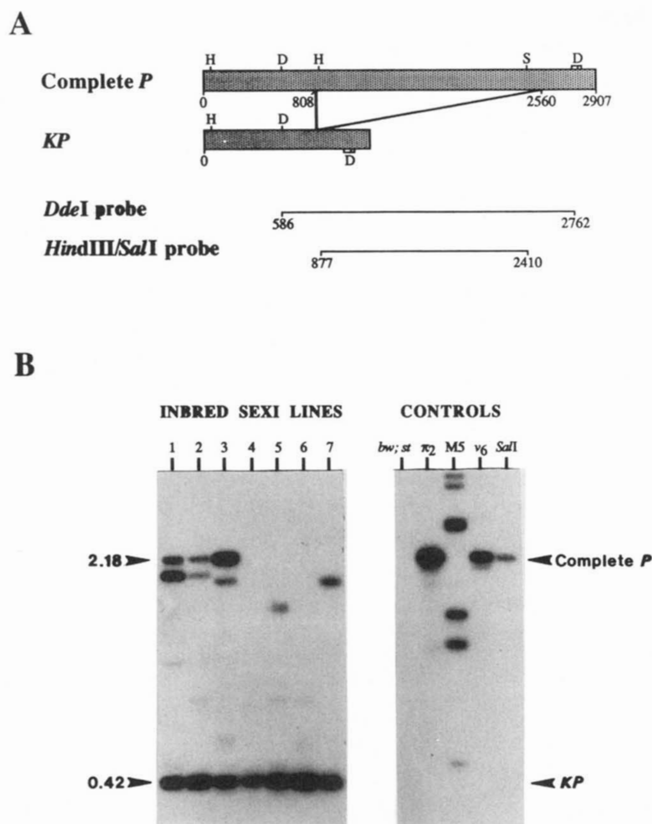

FIGURE 3.—Analysis of *P* elements in the inbred Sexi lines. A, Structures of complete *P* and *KP* elements and molecular probes used in this analysis. B, Autoradiograms from Southern blots of genomic DNA that had been digested with *Dde*I and hybridized with the *Dde*I *P* element probe shown in A. The DNA for the blots came from each of the inbred Sexi lines and five different control strains. Molecular sizes in kilobases are shown at the left. See the text for details.

did (data not shown). These results therefore suggest that many *KP* elements were present in each of the inbred Sexi lines, but not in any of the control stocks.

DISCUSSION

Assays for repression potential: The ability to detect the repression of *P* element activity obviously depends on the assay used and clearly, some assays are more sensitive than others. In this study, three strains were used to induce gonadal dysgenesis. Two of these, π_2 and sn^w; π_2, induced 100% hybrid sterility in crosses with pure M females. The other strain, *T-5*, induced only 50–80% sterility, making it more useful for detecting low levels of repression potential. Other studies have utilized sn^w hypermutability as an assay for the repression of *P* element activity (ENGELS 1979b, 1981b; SIMMONS *et al.* 1987). Although this assay is expected to be sensitive to rather low levels of repression potential, its use is complicated by a "titration effect" (SIMMONS and BUCHOLZ 1985; SIMMONS *et al.* 1987; RASMUSSON *et al.* 1990), in which the mutability of sn^w is reduced by competition among *P* elements for the transposase. Only in certain circumstances can this effect be separated from the genuine effects of repression (SIMMONS *et al.* 1987).

Variation in repression potential: Despite their origin from the same basic stock, the inbred Sexi lines displayed considerable variation in repression potential. One of these lines repressed GD sterility very effectively, four had intermediate repression potential, and two hardly repressed GD sterility at all. Furthermore, the four intermediate lines showed appreciable within-line variability, apparently much like the variability observed in the basic Sexi stock (KIDWELL 1985). Because of the high degree of inbreeding, genetic segregation is not likely to explain this within-line variation. *P* element movement might explain some of it, but certainly not in the lines that lacked autonomous *P* elements (*e.g.*, Sexi.5 and Sexi.6). In these, the best explanation is repression by factors with variable effects.

Unlike the inbred Sexi lines, the P cytotype strains π_2 and ν_6 showed little, if any, quantitative variability. Nearly all of the females from these strains repressed sterility in all of their daughters; only in the ν_6 stock did a few of the females seem to lack any repression potential. Even the hybrids that were produced by crossing π_2 or ν_6 females to true M males showed almost no quantitative variability. For the most part, these hybrids had either high or low repression potential–the P and M cytotypes, respectively; only a few cases of intermediate repression potential were observed. Previous studies with π_2 (ENGELS 1979a) and ν_6 (ENGELS and PRESTON 1981) have reported similar results. At present, the relationship between the discrete variation seen with π_2 and ν_6 and the quantitative variation seen with the inbred Sexi lines is not clear. However, it is possible that these two groups of strains reflect different systems of *P* element regulation (KIDWELL 1985).

It is worth noting that among the strains tested here, those with the highest repression potential (Sexi.3, π_2 and ν_6), all had autonomous *P* elements. It is not clear whether this association has any biological significance. However, HAGIWARA *et al.* (1987) have found a strain (IG489-6) that apparently lacks any autonomous elements even though it has high repression potential. If confirmed, this would demonstrate that high repression potential does not depend absolutely on the presence of autonomous *P* elements. However, these elements might be necessary for the evolution of high repression potential even if they are dispensable after it has evolved. Several investigators (DANIELS *et al.* 1987; KIDWELL, NOVY and FEELEY 1981; KIYASU and KIDWELL 1984; HEATH 1988; PRESTON and ENGELS 1989) have observed the emergence of high repression potential in strains that have autonomous *P* elements.

Repression of GD sterility: maternal *vs.* zygotic effects: Our experiments have demonstrated that repression of gonadal dysgenesis among the hybrid offspring of the inbred Sexi lines involves a maternal effect. Only the hybrids that had Sexi mothers repressed GD sterility, while those that had Sexi fathers were actually more susceptible to it. These experiments provided no evidence for repression arising from the zygotic genotypes of the hybrids themselves. However, HEATH (1988) has shown that repression in hybrids from other strains involves a zygotic component. This establishes that gonadal dysgenesis may be repressed by factors produced in the zygote as well as by factors synthesized in the mother and transmitted through the egg. The nature and origin of these factors is currently unknown. However, it has been proposed that they are the products of certain kinds of *P* elements (BLACK *et al.* 1987; ENGELS 1989; NITASAKA, MUKAI and YAMAZAKI 1987).

In general, it is not possible to distinguish between maternal and zygotic effects by studying the repression of GD sterility among the daughters of reciprocal hybrids between M′ and M strains (KIDWELL 1985; BLACK *et al.* 1987). However, as shown here, such experiments can reveal whether the establishment of a maternal effect is itself maternally influenced. For example, both types of hybrid females from reciprocal crosses between Sexi.5 and *bw; st* had the ability to repress gonadal dysgenesis in their offspring; however, on average, the females that had Sexi.5 mothers had slightly less of this ability. Since repression of gonadal dysgenesis is mediated principally by a maternal effect, these results suggest that the two types of hybrids differed in the efficiencies with which they established their respective effects. This difference cannot be due to genetic factors, for the two types of hybrids were genetically equivalent; rather, a maternal effect must be involved. It therefore appears that the ability to repress gonadal dysgenesis through a maternal effect is itself influenced by a maternal effect from the previous generation; however, this latter effect is inhibitory rather than facilitating. The simplest explanation of this paradoxical finding is that the maternally transmitted factors that repress gonadal dysgenesis also temporarily repress their own synthesis.

Determination of repression potential: As discussed above, repression potential may vary continuously in a strain, or it may exist only in high or low states. In the latter case, ENGELS (1979a) showed that these states, or cytotypes, are determined by a combination of genetic and cytoplasmic factors. In the short term, the cytoplasmic component appears to be stronger, but eventually this gives out and the chromosomal factors take over. ENGELS suggested that these chromosomal factors are closely associated, if not identical, to the factors that cause hybrid dysgenesis, namely, to the *P* elements themselves (BINGHAM, RUBIN and KIDWELL 1982; RUBIN, KIDWELL and BINGHAM 1982). Later, he proposed that the cytoplasmic component of cytotype was due to extrachromosomal factors–presumably *P* elements–with a limited ability for self-replication (ENGELS 1981a). Alternatively, he suggested that the two cytotypes might represent the "on" and "off" conditions of a set of genes which are regulated by a cytoplasmic particle. This second possibility was elaborated upon by O'HARE and RUBIN (1983), who proposed that the cytoplasmic component of cytotype is due to a *P* element-encoded repressor that is transmitted maternally and that positively feeds back to stimulate its own synthesis. Other investigators have presented hypotheses to explain repression by cytotype, but few have dealt explicitly with its cytoplasmic inheritance.

These efforts have been complicated by the discovery that M′ strains exhibit variation in repression potential (KIDWELL 1983, 1985; BLACK *et al.* 1987; SIMMONS *et al.* 1987) and that in some cases (*e.g.*, the basic Sexi stock), this potential seems to be determined solely by chromosomal factors (KIDWELL 1985; BLACK *et al.* 1987). Our data demonstrate that the phenomenology of the M′ strains is actually more complex.

First, in one of the inbred lines derived from the basic Sexi stock, repression potential was at least partially determined by cytoplasmic factors. In several other lines, only chromosomal factors seemed to be involved. This variability suggests that the basic Sexi stock was itself quite variable for the determinants of repression potential. Second, among the lines that showed only the chromosomal component of inheritance, several different kinds of genetic factors appeared to be operating. In Sexi.1, for example, repression potential was apparently due to recessive factors, whereas in Sexi.2, Sexi.5 and Sexi.6, it seemed that additive or partially dominant factors were involved. In addition, the expression of these factors was negatively influenced by a maternal effect–exactly the opposite of the effect postulated by O'HARE and RUBIN (1983). Third, the repression potential of two of the inbred lines (Sexi.4 and Sexi.7) was so weak that its chromosomal and cytoplasmic basis could not be studied. All these facts suggest that M′ strains may vary considerably in the ways they regulate *P* element activity.

Repression by the products of specific *P* elements: Many investigators have proposed that the repression of hybrid dysgenesis is due to the products of certain kinds of *P* elements. Initially it was thought that these repressors were produced by autonomous elements (O'HARE and RUBIN 1983; RONSSERAY, ANXOLABÉHÈRE and PERIQUET 1984), but recent evidence indicates that nonautonomous elements are more likely

candidates (BLACK *et al.* 1987; DANIELS *et al.* 1987; ENGELS 1989; HAGIWARA *et al.* 1987; NITASAKA, MUKAI and YAMAZAKI 1987; SIMMONS *et al.* 1987; ROBERTSON and ENGELS 1989). Data from two of the Sexi lines (Sexi.5 and Sexi.6) support this view; both of these lines had moderate repression potential even though they apparently lacked any autonomous *P* elements. Naturally, this raises the question of which nonautonomous elements are responsible for the ability to repress hybrid dysgenesis.

BLACK *et al.* (1987) have claimed that the repression potential of M′ strains is due to the polypeptide product of a specific nonautonomous *P* element called *KP* (Figure 3). This element is prevalent in the genomes of many M′ and Q strains, including Sexi, and could produce a polypeptide homologous to a portion of the *P* transposase. On the basis of indirect evidence, BLACK *et al.* (1987) hypothesize that this polypeptide represses *P* element activity by interfering with transposase function. These authors point out that the *KP* element is prevalent in many strains from diverse origins and that it seems to be conserved structurally. They note that it appears to be transcribed *in vivo*, and they also find that it is abundant in experimental lines that have developed an ability to repress gonadal dysgenesis; furthermore, these lines did not exhibit any cytoplasmic inheritance of repression potential. Although these facts are all consistent with the *KP* repressor hypothesis, they do not prove it. Moreover, other details presented by BLACK *et al.* (1987), but not discussed, reduce the force of their evidence. For instance, two M′ strains (Gomel and Kibris) clearly possessed many *KP* elements but apparently did not have any repression potential [see Figure 1 and Table 1 in BLACK *et al.* (1987)]. Also, several of the experimental lines that developed repression potential carried other types of *P* elements, in addition to *KP*, making it difficult to attribute repression to any one type.

In the present study, each of the inbred Sexi lines was shown to possess many *KP* elements. If these elements truly did produce repressor polypeptides, all of the lines might be expected to show repression potential. However, one of the lines had much repression potential, four had moderate potential, and two lines had little, if any; these last two lines are apparently similar to the Gomel and Kibris strains of BLACK *et al.* (1987). In addition, the rules governing the inheritance of repression potential varied among the inbred Sexi lines. Since these findings are not consistent with the *KP* repressor hypothesis ennunciated by BLACK *et al.* (1987), it is necessary to consider other explanations. One possibility is that the production of the *KP* repressor is affected by chromosomal position. On this view, the differences among the inbred Sexi lines could be explained by differences in the positions of their *KP* elements. Another possibility is that the repression potential of these lines is not due to *KP* elements at all, but rather to other nonautonomous *P* elements present in the genome. This latter possibility is strengthened by the finding that strains without *KP* elements show extensive variation in repression potential and that among these, the rules of inheritance are also quite variable (SIMMONS *et al.* 1987; HEATH 1988; J. RAYMOND, T. OJALA, J. WHITE and M. SIMMONS, personal communication). Given this diversity, it would not be surprising to find that *P* element regulation involves the products of many different kinds of nonautonomous *P* elements.

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