The Influence of Nonautonomous *P* Elements on Hybrid Dysgenesis in *Drosophila melanogaster*

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ABSTRACT

An inbred line of the M′ strain Muller-5 Birmingham was studied for its abilities to affect P-M hybrid dysgenesis. This strain possesses 57 *P* elements, all of which are apparently defective in the production of the P transposase. In combination with transposase-producing elements, these nonautonomous elements can enhance or diminish the incidence of hybrid dysgenesis, depending on the trait that is studied. Dysgenic flies that have one or more paternally-derived chromosomes with these elements partially repress the instability of the *P* element insertion mutation, sn^w; however, such flies have elevated frequencies of another dysgenic trait, GD sterility, and also show distorted segregation ratios. An explanation is presented in which all of these phenomena are unified as manifestations of the kinetics of *P* element activation in the germ line. The progeny of Muller-5 Birmingham females exhibit partial repression of both sn^w instability and GD sterility. This repression appears to involve a factor that can be transmitted maternally through at least two generations. This mode of repression therefore conforms to the pattern of inheritance of the P cytotype, the condition that brings about nearly total repression of *P* element activity in some strains. Models in which this repression could arise from the nonautonomous *P* elements of Muller-5 Birmingham are discussed.

P-M hybrid dysgenesis in *Drosophila melanogaster* arises from the movement of transposable *P* elements in a cellular condition called the M cytotype (ENGELS 1983). This movement is restricted to the germ line, where it causes sterility due to gonadal dysgenesis (GD sterility), chromosome breakage, mutation and segregation distortion (KIDWELL, KIDWELL and SVED 1977). All of these traits depend on the action of a transposase encoded by structurally intact *P* elements. Detailed molecular studies have shown that the 2.9-kb DNA sequence of these elements consists of four exons and three introns (O'HARE and RUBIN 1983; LASKI, RIO and RUBIN 1986; RIO, LASKI and RUBIN 1986) and that all four exons are needed for the production of the transposase (KARESS and RUBIN 1984). The germ line specificity of transposition is due to an inability of somatic cells to splice out the third intron in the RNA transcript of the transposase gene (LASKI, RIO and RUBIN 1986; RIO, LASKI and RUBIN 1986).

P elements that produce their own transposase can move autonomously, probably by means of an interaction between the transposase and the short inverted repeats at their ends. This property is missing in *P* elements with deletions in the transposase gene; however, since the transposase is *trans*-acting, these nonautonomous elements can be mobilized so long as their ends are intact and at least one autonomous *P* element is present (SPRADLING and RUBIN 1982; ENGELS 1984).

Some strains of Drosophila lack *P* elements completely. These are called pure M strains because they possess the M cytotype. Other strains possess both kinds of *P* elements, but their movement is repressed by a condition called the P cytotype. Although the P cytotype appears to originate from the *P* elements themselves (ENGELS 1979a), it is not clear which type of element produces it.

Still other strains possess only nonautonomous *P* elements (SIMMONS and BUCHOLZ 1985). These do not suffer from hybrid dysgenesis because the transposase is lacking. However, when females from such strains are crossed to males carrying autonomous *P* elements, dysgenesis does occur in the progeny. Typically, this is less extreme than that seen in the progeny of crosses involving pure M females, suggesting that these strains have some ability to repress hybrid dysgenesis (KIDWELL 1983, 1985). Because these strains appear to have an attenuated form of the M cytotype, they are called pseudo-M or M′.

This paper describes experiments with an M′ strain called Muller-5 Birmingham. All the *P* elements of this strain appear to be nonautonomous (BINGHAM, KIDWELL and RUBIN 1982; SIMMONS and BUCHOLZ 1985). The purpose of these experiments was to determine the effects of these nonautonomous elements on various manifestations of hybrid dysgenesis, including GD sterility, segregation distortion and the mutability of a *P* element insertion mutation of the *X*-linked *singed* bristle locus.

MATERIALS AND METHODS

Stocks: Brief descriptions of the stocks used in these experiments are given below. More detailed information

about the markers and chromosomes can be found in LINDSLEY and GRELL (1968).

The following stocks were pure M:

1. *bw; st*, a stock with the recessive eye color mutants *bw* (*brown*, on chromosome *II*) and *st* (*scarlet*, on chromosome *III*); the combination of these two mutants gives white eyes.

2. *M5; bw; st*, a stock with the same autosomal markers as above but also with the Muller-5 (*M5*) balancer *X* chromosome. This balancer contains recombination-suppressing inversions, two recessive markers (*sc* = *scute* bristles and w^a = *white-apricot* eyes), and the semi-dominant marker *B* = *Bar* eyes.

3. *C(1)DX, y f/Y/y cin w* f^s *su(f)*^{ts67g}, an attached-*X* chromosome stock in which the males have a temperature-sensitive lethal mutation that permits the collection of virgin females easily.

4. *y* sn^3 *v car*, a stock with four recessive *X*-linked markers. Of these, the most important from the standpoint of these experiments is sn^3, an extreme allele of the *singed* bristle locus.

The following two stocks carried numerous *P* elements, had the P cytotype, and were strong inducers of P-M hybrid dysgenesis:

5. π_2, an inbred strain derived from a natural population in Madison, Wisconsin (ENGELS and PRESTON 1979).

6. *C(1)DX, y f/Y/*sn^w; π_2, a strain with the genetic background of π_2. The females in this strain have attached-*X* chromosomes, while the males have a double *P* element insertion mutation of the *singed* locus. This mutation, called singed-weak, arose with the insertion of two nonautonomous *P* elements into the *singed* locus and causes a slight malformation of the bristles (ENGELS 1979b, 1984; H. ROIHA, K. O'HARE and G. RUBIN, personal communication). In the M cytotype, this mutation responds to the presence of the P transposase and becomes unstable, mutating to sn^e (a more extreme allele) and to sn^+ (an apparently wild-type allele). These mutations occur in the germ line and can be detected in the next generation by appropriate matings. The dominance relations of these *singed* alleles is $sn^+ > sn^w > sn^e = sn^3$, permitting the wild type, weak and extreme alleles to be distinguished in heterozygotes with sn^3. Because this strain had the P cytotype, the sn^w allele in it was stable.

The following strains were M′:

7. *y* sn^w; *bw; st/* y^+ *Y*, a stock with the sn^w mutation and one other nonautonomous *P* element close to *singed*, but otherwise free of *P* elements.

8. M5-B#1, an inbred strain derived from a Muller-5 balancer stock from Birmingham, England. The balancer was the standard Muller-5 *X* chromosome, with inversions and markers as discussed above. However, unlike the *M5* strain listed above, this one carried many *P* elements (BINGHAM, KIDWELL and RUBIN 1982). M5-B#1 was derived from Muller-5 Birmingham by ten generations of sib-mating; thereafter it was maintained with mass matings.

Using *in situ* hybridization, JOHNG LIM (personal communication) has determined the number and positions of the *P* elements in this inbred strain. There are 19 elements in the *X* chromosome, 17 in chromosome *II*, 21 in chromosome *III* and zero in chromosome *IV*. No elements were detected in the chromocenter.

Three other strains, designated as *T-5*, *T-7* and *T-11*, were used in these experiments. In each, *P* elements were present on the *X* chromosome. These strains were maintained by mating individual males that had the *P* element-bearing *X* chromosome to females with the *C(1)DX, y f* attached-*X* chromosomes; these females were free of *P* elements and were also homozygous for *bw* and *st*. Thus, *Y* chromosomes and autosomes potentially contaminated by *P* elements in the male could be replaced by maternally-contributed chromosomes each generation. In these three stocks, the male's *X* chromosome carried sn^w and at least one autonomous *P* element derived from a wild type strain called ν_6 (SIMMONS and BUCHOLZ 1985). This latter fact is known because sn^w was unstable in the crosses used to maintain these stocks and such instability requires an autonomous element. The purpose of these stocks was to test the response of sn^w to the transposase made by the autonomous *P* elements linked to it. We therefore refer to the *X* chromosomes in each of these stocks as tester *X* chromosomes.

Experimental methods: Stocks and experimental cultures were raised in vials and half-pint milk bottles on a standard cornmeal-molasses medium.

The germ line instability of sn^w was ascertained by scoring the progeny of sn^w carriers. sn^w males were mated individually to attached-*X* females and their sons were scored for bristle phenotype. *M5*(sn^+)/sn^w and sn^+/sn^w females were mated individually to *y* sn^3 *v car* males. In the case of the *M5* heterozygotes, all the progeny not carrying the *M5* chromosome were scored. For the other heterozygotes, only the weak and extreme singed progeny were scored; in these cultures the wild type flies were not counted because mutations of sn^w to sn^+ could not be distinguished from the sn^+ allele already present in the heterozygous mothers. All cultures in which sn^w mutability was measured were reared at 25° and were scored on the 13th and 15th days after they were established. The mutation rate was estimated by the proportion of mutants among the flies counted.

To estimate the frequency of gonadal dysgenesis (GD sterility) among hybrid females, the cultures that produced these females were raised at 29°. The flies that hatched by day 11 were transferred to fresh culture vials and aged for 2 days. These were then etherized and the females were squashed in water between two glass plates. Each female was examined for the presence of extruded eggs; females not showing these were classified as sterile. Food coloring was added to the water between the glass plates to make scoring easier.

Chromosome segregation was studied by scoring the progeny of individual heterozygous males crossed to tester homozygous females at 25°. The recessive markers *bw* and *st* were used to follow the segregation of chromosomes *II* and *III*, respectively. The progeny of these crosses were counted on the 13th and 15th days after starting the cultures.

RESULTS

Do *P* elements of M5-B#1 produce transposase?

To determine whether the *P* elements of M5-B#1 produce transposase, we tested the ability of M5-B#1 males to induce hybrid dysgenesis in crosses to M cytotype females.

In the first test, M5-B#1 males were crossed individually at 29° to M cytotype females from the *bw; st* stock. The hybrid daughters of this cross were then examined for GD sterility. In a total of 25 crosses, 291 hybrid daughters were scored but only 11 (3.8%) were sterile. Since the background sterility in this test is typically 1–5%, M5-B#1 does not appear to have the ability to induce GD sterility.

In the second test, M5-B#1 males were crossed en masse at 25° to M cytotype *y* sn^w; *bw; st* females. Individual sons and daughters of these matings were then tested for sn^w instability. A total of 197 sons were tested and all of their 5600 male progeny were sn^w;

thus, from these crosses there was no evidence for transposase action. Among the 197 $sn^w/M5\text{-}B\#1$ daughters that were tested, one produced a cluster of 14 sn^+ males along with 27 sn^w females. Another daughter produced a single sn^+ female along with 37 sn^w flies. However, the 6293 progeny from the other 195 females that were tested were all sn^w.

Further testing of the exceptional sn^+ female revealed that its sn^+ *X* chromosome carried an inversion with breaks in polytene bands 6A1-2 and 10F-11A, which are the breakpoints of *In(1)S*, one of the inversions present in the Muller-5 balancer chromosome (J. K. LIM, personal communication). This exceptional sn^+ chromosome probably arose through a rare double exchange between the sn^w and Muller-5 *X* chromosomes, giving the appearance of a mutation from sn^w to sn^+.

The nature of the exceptional sn^+ males was also investigated. The procedure was to mate each male to attached-*X* females and to *y* sn^3 *v car* females. All the sons of the crosses to the attached-*X* females were phenotypically wild type, as expected, but all the daughters from the other crosses were weak singed, as if the sn^+ allele were actually a cryptic sn^w allele. Subsequent analysis has shown that the wild phenotype of the exceptional males was due to an *X*-linked modifier of the sn^w mutation. When this modifier was separated from the *singed* locus by recombination, the weak singed phenotype was restored (M. J. SIMMONS and J. D. RAYMOND, unpublished observations).

Based on these tests for sn^w destabilization, we are reasonably confident that none of the *P* elements of M5-B#1 makes the P transposase. W. R. ENGELS and C. R. PRESTON (personal communication) have reached a similar conclusion in their more extensive studies with another subline of the Muller-5 Birmingham stock.

The cytotype of M5-B#1

The cytotype of M5-B#1 was assessed in two tests. In the first, M5-B#1 females were crossed individually at 29° to males from the strong P stock π_2 and their F_1 daughters were examined for GD sterility. If M5-B#1 had the P cytotype, we would expect very little GD sterility, whereas if it had the M cytotype, much sterility would be expected. Control crosses of π_2 males to M cytotype females from the *bw*; *st* and *M5*; *bw*; *st* stocks were performed simultaneously.

The results of this assay for cytotype are shown in Figure 1. The F_1 females from the control crosses were almost all sterile, as expected, but the F_1 females from the crosses involving M5-B#1 showed great variation in the frequency of sterility. These data indicate, therefore, that M5-B#1 has a cytotype that is intermediate between M and P.

The second test made use of sn^w hypermutability. M5-B#1 females were mated individually at 21° to single sn^w; π_2 males and three F_1 daughters from each

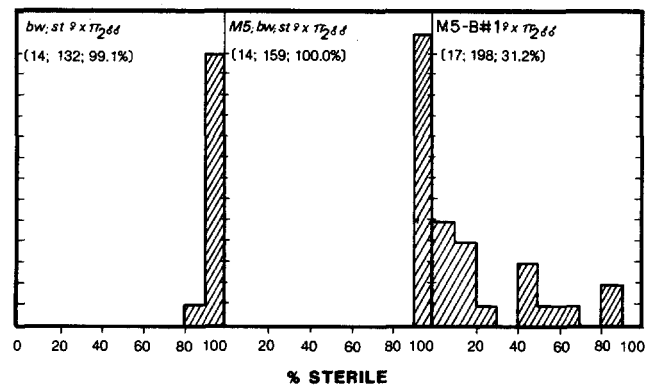

FIGURE 1.—Assessment of cytotype using the GD sterility assay. The distributions of sterility for the hybrid females of three crosses are shown. Each unit in a distribution represents one hybrid female. As many as 12 daughters of each of these were examined for GD sterility. The total number of hybrids that were tested, the total number of daughters from them that were examined, and the unweighted average percent of these that were sterile are given in parentheses.

TABLE 1

Assessment of the cytotype of M5-B#1 using the sn^w assay

Female parent	No. families	No. cultures	F_2 progeny scored				$u \pm$ SE
			sn^w	sn^+	sn^s	Total	
M5-B#1	43	117	4275	74	130	4479	0.044 ± 0.004
M5;bw:st	42	122	3339	434	480	4253	0.223 ± 0.009

The mutation rate, *u*, was averaged over cultures using ENGELS' (1979c) unweighted procedure.

culture were tested for sn^w mutability. The lower temperature of these matings was to reduce GD sterility among the F_1. The same males that were mated to the M5-B#1 females were also mated to individual M cytotype *M5*; *bw*; *st* females. Then the F_1 daughters of these second matings were also tested for sn^w mutability.

The data from the sn^w cytotype test are shown in Table 1. The mean mutation rate of sn^w among the daughters of the control *M5*; *bw*; *st* mothers was 0.223; however, among the daughters of the M5-B#1 mothers it was only 0.044. This substantial difference indicates that M5-B#1 has considerable ability to repress sn^w instability. Owing to the experimental design, it was possible to compare the average mutation rates of daughters with the same sn^w; π_2 father but with different mothers. In the 42 half-sib families in which a comparison was possible, the females with M5-B#1 mothers always had lower average mutation rates than the females with *M5*; *bw*; *st* mothers. This clearly indicates the ability of M5-B#1 to repress hybrid dysgenesis.

Effects of paternally derived M5-B#1 chromosomes on manifestations of hybrid dysgenesis

We performed experiments to assess the effects of chromosomes derived paternally from the M5-B#1

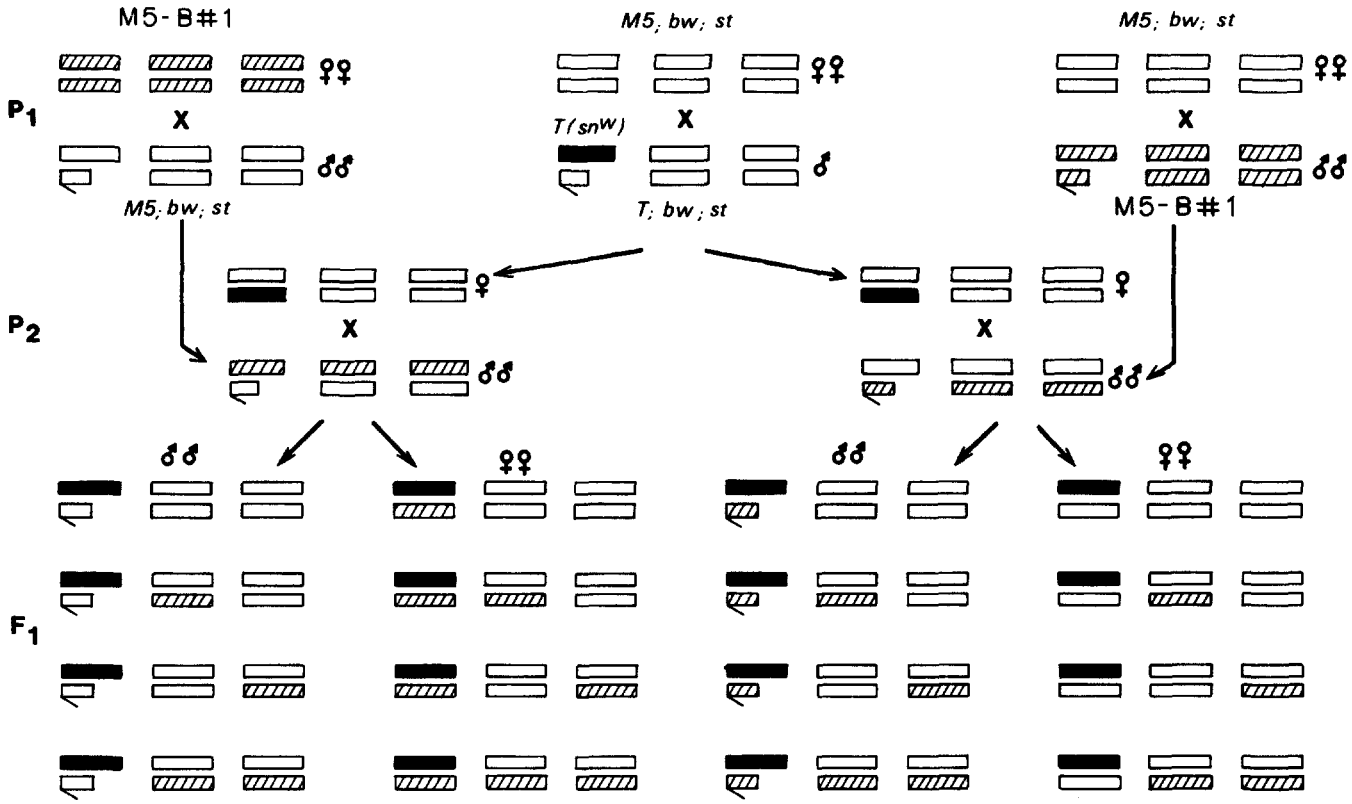

FIGURE 2.—Mating scheme for testing the effects of M5-B#1 chromosomes on hybrid dysgenesis. The hatched bars represent the major chromosomes of M5-B#1, the open bars represent the chromosomes of pure M strains, and the darkened bars represent the tester *X* chromosomes carrying sn^w. See text for details.

stock, individually and in combinations, on three manifestations of hybrid dysgenesis—sn^w mutability, GD sterility and segregation distortion. The purpose was to ascertain whether the nonautonomous *P* elements carried by these chromosomes had any influence on these traits. The experiments utilized the tester *X* chromosomes, symbolized *T*, as the source of the transposase needed to induce dysgenesis. The mating scheme for all the experiments is shown in Figure 2.

As shown in the center of Figure 2, single males of the genotype *T*; *bw*; *st* were crossed to homozygous *M5*; *bw*; *st* females, which were free of *P* elements, to produce *M5/T*; *bw/bw*; *st/st* P_2 daughters. Except for new transpositions to the autosomes, these should have *P* elements only on the tester *X* chromosome. Also in the P_1, reciprocal crosses between M5-B#1 and *M5*; *bw*; *st* flies were carried out (shown on the two sides of Figure 2). These produced *M5/Y*; +/*bw*; +/*st* P_2 sons, which carried a complement of autosomes derived from M5-B#1 (symbolized by the + signs in the text and the hatched symbols in Figure 2) and, depending on the cross, an *X* chromosome derived either from M5-B#1 or from *M5*; *bw*; *st*; in these males, the *Y* chromosome came from the opposite stock that contributed the *X* chromosome. The *M5/Y*; +/*bw*; +/*st* P_2 males were then mated to single *M5/T*; *bw/bw*; *st/st* females, whose derivation was given above, to produce an assortment of male and female progeny. Owing to the segregation of the autosomes in the P_2 males, the F_1 flies that inherited the tester *X* chromosome had different eye colors; some had red eyes (genotype +/*bw*; +/*st*), others brown (*bw/bw*; +/*st*), scarlet (+/*bw*; *st/st*) or white (*bw/bw*; *st/st*). In each case, the + chromosome was derived paternally from M5-B#1. Samples of flies in each of these phenotypic classes were then tested for sn^w mutability, GD sterility and segregation distortion using the procedures given in MATERIALS AND METHODS. The tiny fourth chromosome, which was not marked, could not be followed in these experiments; however, this chromosome did not carry any *P* elements visible by *in situ* hybridization.

sn^w mutability in males: Two experiments utilizing the design just described were carried out to assess the effect of the chromosomes from M5-B#1 on the mutability of sn^w in males. In each of these, F_1 males carrying a tester *X* chromosome with the sn^w allele were collected for mating to attached-*X* females. These males came from cultures that had been incubated at 25° and were themselves mated at 25°. In experiment I, we selected two males of each phenotype from each culture; these were split into two groups, each phenotype being represented once in a group. To spread out the effort, the groups were tested 4 days apart, so each group was treated as a separate run of the experiment. In the second experiment, only one male of each phenotype was collected from a culture and all of these were tested at the same

TABLE 2

Effects of chromosomes from M5-B#1 on sn^w mutability and progeny size in males carrying the *T-5* tester *X* chromosome

M5-B#1 chromosomes	Experiment I							Experiment II						
	No. males	Progeny scored				$N \pm$ SE	$u \pm$ SE	No. males	Progeny scored				$N \pm$ SE	$u \pm$ SE
		sn^w	sn^+	sn^e	Total				sn^w	sn^+	sn^e	Total		
Y, II, III	40	389	121	298	808	20.20 ± 2.37	0.507 ± 0.051	47	1199	215	194	1608	34.21 ± 2.07	0.268 ± 0.026
Y, III	39	428	115	164	707	18.13 ± 2.42	0.420 ± 0.044	43	1148	195	238	1581	36.77 ± 2.33	0.305 ± 0.025
Y, II	45	742	387	467	1596	35.46 ± 2.70	0.538 ± 0.034	44	1356	262	286	1904	43.27 ± 2.55	0.301 ± 0.024
Y	45	810	673	453	1936	43.02 ± 3.17	0.593 ± 0.033	44	1281	284	350	1915	43.52 ± 2.99	0.361 ± 0.030
II, III	43	620	245	262	1127	26.21 ± 2.10	0.461 ± 0.039	51	1177	177	210	1564	30.67 ± 2.00	0.256 ± 0.024
III	42	581	257	277	1115	26.55 ± 2.42	0.510 ± 0.041	50	1194	111	223	1528	30.56 ± 1.73	0.235 ± 0.021
II	40	896	324	382	1602	40.05 ± 2.49	0.455 ± 0.024	52	1236	191	274	1701	32.71 ± 1.59	0.268 ± 0.018
O	47	1031	834	592	2457	52.28 ± 2.39	0.587 ± 0.027	51	1425	330	357	2112	41.41 ± 2.00	0.324 ± 0.020

The average number of progeny, *N*, and the average mutation rate, *u*, were computed over all males tested using ENGELS' (1979c) unweighted procedure. Only male progeny were scored in these experiments.

time. For all the tests, the F_1 males were mated individually to three attached-*X* females; the mated flies were then transferred to fresh vials twice at 4–5-day intervals to obtain as many progeny as possible. All three tester chromosomes were used in experiment I, but only tester *T-5* was used in experiment II. Over 56,000 flies were scored to obtain the data.

The *T-5* tester chromosome induced very high sn^w mutation rates, 0.40–0.60 in experiment I and 0.24–0.36 in experiment II. The other two tester chromosomes induced much lower mutation rates, 0.04–0.08 for *T-7* and 0.01–0.06 for *T-11*. Apparently the *T-5* chromosome produced substantially higher levels of the P transposase. Another explanation, that *T-5* had an altered sn^w allele that was unusually sensitive to destabilization by the P transposase, was ruled out by experiments in which sn^+ and sn^e derivatives of the sn^w *T-5* chromosome were tested for their ability to destabilize in *trans* the sn^w allele from stock 7. The mutation rate of this sn^w allele was between 0.15 and 0.38, indicating that the *T-5* chromosome produced unusual amounts of transposase.

The data from the cultures with the *T-5* tester chromosome show that there were significant differences in the mutation rates of the different genotypes tested in these experiments (see Table 2). In experiment I, the flies lacking both of the M5-B#1 autosomes had the highest average mutation rate, about 0.59, whether or not the M5-B#1 *Y* chromosome was present. Compared to these, the flies with either or both of the M5-B#1 autosomes had average mutation rates that were proportionately reduced by 8–28%. In experiment II, the flies without either of the M5-B#1 autosomes again had the highest average; the other genotypes had averages that were proportionately reduced by 15–25%. These differences can also be seen by comparing the actual mutation rate distributions, which are shown in Figure 3. In experiment I, the distributions for the flies without either of the major M5-B#1 autosomes are shifted to the right on the scale, indicating generally higher mutability. In experiment II, this shift is less pronounced, but still perceptible.

To evaluate the significance of these differences, we used the sign test. The mutation rates of males with different genotypes but that came from the same culture and test group were compared systematically. The number of positive differences between two genotypes was then evaluated using the binomial distribution under the null hypothesis that the two genotypes had the same mutation rate. This is equivalent to the hypothesis that positive and negative differences occur with equal probability. Table 3 gives the results of this test applied to all possible comparisons using the pooled data of experiments I (both runs) and II.

From Table 2 it is clear that the *T-5* flies that had either of the major M5-B#1 autosomes were significantly less mutable than the flies with neither of these autosomes. This establishes that either of the major M5-B#1 autosomes can partially repress sn^w mutability. However, there was no evidence for a difference in the repression abilities of the two autosomes. In fact, both autosomes together did not bring about significantly more repression than either one of them alone.

These sn^w experiments also provided information on the fertility of the tested males. The average number of progeny produced by the different types of *T-7* and *T-11* males in experiment I ranged from 55.4 to 68.3 (data not shown). In contrast, in this experiment the averages for the different types of *T-5* males ranged from 18.1 to 52.3 (see Table 2). This large difference in fertility cannot be due to environmental factors, since all three types of males were reared and tested together under the same culture conditions. Rather, it appears that the *T-5* chromosome itself caused a significant reduction in fertility. However, the M5-B#1 chromosomes also played a role, since males carrying these in combination with *T-5* were significantly less fertile than males without them. The M5-B#1 chromosome *III* had an especially pro-

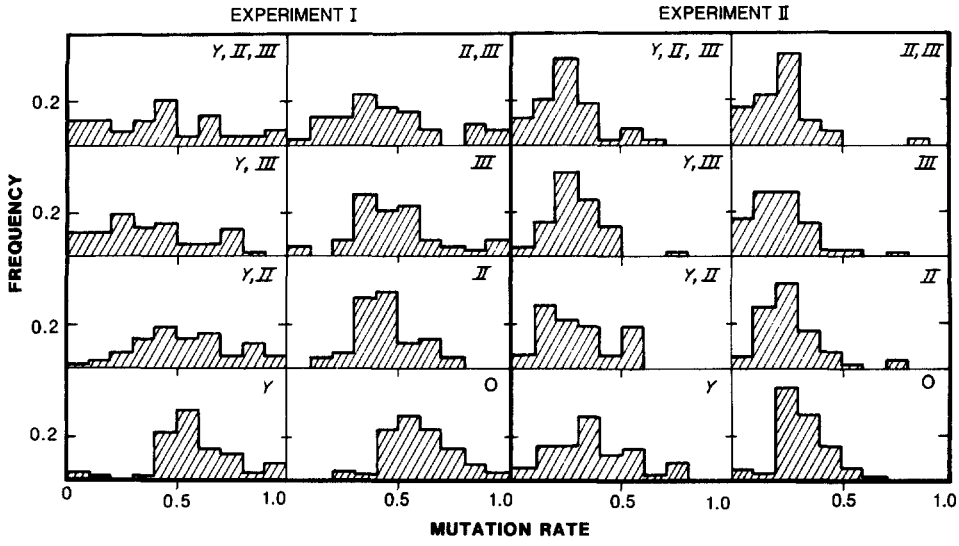

FIGURE 3.—Distributions of the mutation rate of sn^w in males with the *T-5* tester *X* chromosome. The data from experiments I and II are shown. Within an experiment, each distribution represents tests with a different genotype. The M5-B#1 chromosomes that were present are given above the distribution. All the distributions have been scaled so that the area under each of them sums to one.

TABLE 3

Comparisons of mutation rates and fertilities of males carrying the *T-5* tester *X* chromosome and different complements of M5-B#1 chromosomes

Chromosomes	Mutation rate			Fertility		
	III	*II*	*O*	*III*	*II*	*O*
M5-B#1 *Y* chromosome present:						
II, III	36/77	47/82	53/80**	39/76	62/82**	57/79**
III		40/75	52/77**		57/75**	61/76**
II			52/84*			50/83*
M5-B#1 *Y* chromosome absent:						
II, III	38/79	50/87	62/90**	39/78	58/89**	71/90**
III		41/79	55/82**		52/77**	80/95**
II			64/89**			64/87**

** $P < 0.01$; * $P < 0.05$.

The chromosomes in each genotype that were derived from M5-B#1 are indicated as column and row headings. Genotypes with and without the M5-B#1 *Y* chromosome were compared separately, as indicated. The entries, x/n, in the table give the number of times (x) the genotype at the left (row heading) had a lesser mutation rate or fertility than the genotype at the top (column heading) in a total of n comparisons; *e.g.*, genotype *II, III* with the M5-B#1 *Y* chromosome had a lesser mutation rate than genotype *III* in 36 out of 77 comparisons. Comparisons were tallied over experiments I (both runs) and II and all ties were excluded. Statistical significance was determined with a one-tailed sign test by summing the probabilities from x to n.

nounced fertility-depressing effect; in experiment I, for example, *T-5* males with this chromosome were only about 50% as fertile as *T-5* males lacking any of the M5-B#1 chromosomes (see Table 2). Application of the sign test to the pooled data of experiments I and II, summarized in Table 3, shows that 10 of the 12 comparisons between the fertilities of genotypes with the *T-5* chromosome were significant; the two nonsignificant comparisons involved the fertilities of males with the M5-B#1 third chromosome and those with both of the major M5-B#1 autosomes. None of the 24 comparisons between the genotypes with the other two tester *X* chromosomes was significant by this test (data not shown). These data imply that a relative abundance of the P transposase reduces male fertility, especially if chromosomes with nonautonomous *P* elements are present. This reduction might arise from the induction of dominant lethal mutations by transposing *P* elements.

***sn*^{w} mutability in females:** In a third experiment utilizing the mating scheme in Figure 2, we assessed the effects of each of the major M5-B#1 chromosomes on sn^w mutability in females. This experiment employed the *T-5* tester *X* chromosome, which, as in the previous experiments, was transmitted maternally to the tested F_1 flies. However, because the tested flies were females heterozygous for *T-5* and a sn^+ Muller-5 balancer *X* chromosome, the *T-5* chromosome might have carried a *singed* allele that had mutated in a previous generation. Such an allele would be hidden by the sn^+ allele on the Muller-5 *X* chromosome. To minimize this possibility, any F_1 *T-5/M5* heterozygote that did not have at least one sn^w brother was discarded. This eliminated cases in which the sn^w allele had mutated in the germ cells of the *T-5* grandfathers of the F_1 flies; however, it did not exclude those cases in which sn^w had mutated in the germ cells of the P_2 females. Such cases had to be identified by examining the progeny of each F_1 female. These females were mated individually to *y* sn^3 *v car* males so that weak, wild and extreme phenotypes could be distinguished in the offspring inheriting the *T-5* chromosome. Clearly, any F_1 female that carried either sn^+ or sn^e on the *T-5* chromosome would be expected to transmit primarily that allele to her offspring. The occasional appearance of another allele could be due to a secondary mutation of sn^+ or sn^e. Using this logic, it was possible to exclude potential cases of preexisting sn^+ and sn^e mutations by discarding data in which either

TABLE 4

Effects of chromosomes from M5-B#1 on sn^w mutability in females

M5-B#1 chromosomes	No. families	No. cultures analyzed	No. cultures excluded	sn^w	sn^+	sn^e	Total	$u \pm$ SE
II, III	38	56	47	2247	638	529	3414	0.342 ± 0.033**
III	28	39	38	1198	515	370	2083	0.431 ± 0.038
II	26	38	53	1269	565	361	2195	0.447 ± 0.044
O	33	55	37	1748	859	599	3206	0.466 ± 0.028**
X, II, III	36	62	32	2456	775	551	3782	0.362 ± 0.029
X, III	30	44	39	1456	421	288	2165	0.356 ± 0.030
X, II	34	53	39	1810	477	467	2754	0.357 ± 0.029
X	29	37	38	1065	344	250	1659	0.383 ± 0.040

The average mutation rate, *u*, was computed over all females analyzed using ENGELS' (1979c) unweighted procedure. Non-Bar male and female progeny were scored in this experiment. The number of families is the number of P_2 cultures whose offspring were represented in the analysis. Comparisons between different genotypic classes were based on the weighted averages of each class of females in a family. These were evaluated by means of the sign test. Only two comparisons, indicated by the ** next to the pertinent numbers, were significant ($P < 0.01$).

the wild type or extreme classes predominated in the progeny of the F_1 females (see below for the exact criteria for exclusion).

We attempted to test three F_1 females of each of the four eye color phenotypes segregating from each of the P_2 cultures. The test crosses between these F_1 females and *y* sn^3 *v car* males were incubated in vials at 25° and transferred to fresh vials after 5 days. When the first vials were scored, we determined which females produced exclusively sn^+ or sn^e progeny and discarded the corresponding transfer cultures without scoring them. The other transfer cultures were scored and the results were pooled with those from the original cultures; we decided that any female that produced an excess of wild type or extreme singed progeny (one or the other type greater than 80% of the total) should be excluded from the analysis. This criterion was chosen because there was a natural discontinuity between 0.8 and 0.9 in the distribution of the partial mutation rate, calculated either as the proportion of wild type or the proportion of extreme singed among the progeny scored.

The results of this experiment are given in Table 4. Over 21,000 progeny were scored. The flies that carried an *X* chromosome from M5-B#1 generally had lower average mutation rates than those with an *X* from *M5*; *bw*; *st*. In addition, among those flies that did not have the M5-B#1 *X* chromosome, those with both of the major M5-B#1 autosomes were significantly less mutable (by the sign test) than those lacking either of these autosomes. Both of these findings are also evident in the distributions of the mutation rate shown in Figure 4. It therefore appears that the M5-B#1 chromosomes, including the *X*, can repress the mutability of sn^w in females.

Segregation distortion: In experiment II described above, *T-5* and control F_1 males of each phenotype were tested for chromosome segregation. The control males came from a mating scheme identical to the one in Figure 2, except that the *T-5* tester chromosome was replaced with the *y* sn^w *X* chromosome from stock 7. All the F_1 males were mated individually to three *bw*; *st* females at 25° and their progeny were counted by eye color and sex until the 15th day after mating. Altogether, over 23,000 flies were scored in these experiments.

The frequency of recovery of each of the M5-B#1 autosomes was calculated for each culture and then tallied into a frequency distribution. Figure 5 shows these distributions for the control (hatched) and *T-5* (cross-hatched) cultures. Any overlap between the two distributions is indicated by the darkened area. The left panel in Figure 5 gives the distributions for the cultures in which the tested male was heterozygous for a single M5-B#1 autosome. The right panel gives the distributions for each autosome in the cases where the tested male was a double heterozygote. The analysis was performed separately on the cultures with and without the M5-B#1 *Y* chromosome.

As is clear from Figure 5, the control distributions are centered in the middle of the scale. Compared to these, the *T-5* distributions are shifted significantly to the left, indicating much reduced recovery of the M5-B#1 autosomes. In every case, there is very little overlap between the *T-5* and control distributions. This dramatic distortion induced by *T-5* against the M5-B#1 autosomes is further documented in Table 5, which gives the summary statistics for these segregation experiments.

T-5 males heterozygous for only the M5-B#1 third chromosome produced proportionately far fewer adult progeny with this chromosome (on average, 0.18 and 0.23 of the total) than did the corresponding controls (0.51 and 0.52 of the total). By the rank sum test, the differences between the *T-5* and control means are statistically significant ($P < 0.05$). Likewise, *T-5* males heterozygous for the M5-B#1 second chromosome exhibited significant segregation distortion (on average, 0.28 and 0.30 M5-B#1 second chromosomes among the progeny compared to 0.47 and 0.50

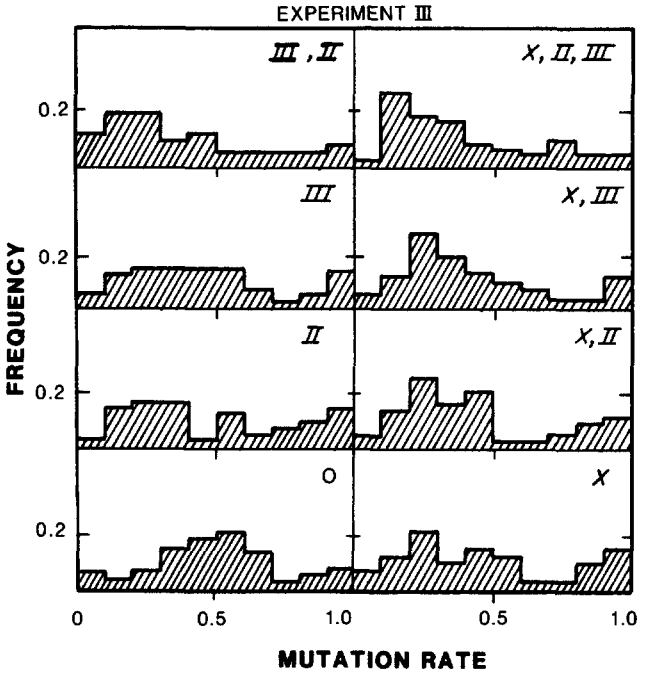

FIGURE 4.—Distributions of the mutation rate of sn^w in females with the *T-5* tester *X* chromosome. The data come from experiment III. Each distribution represents tests with a different genotype. The M5-B#1 chromosomes that were present are given above the distribution. Before scaling, these distributions were truncated by excluding cultures in which the frequency of sn^+ or sn^e exceeded 0.80. See text for details.

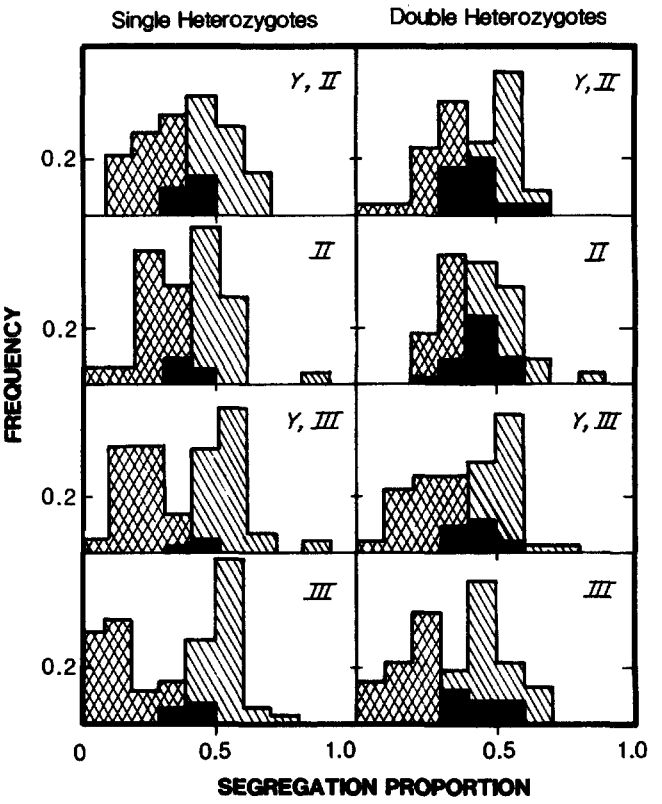

FIGURE 5.—Distributions of the autosomal segregation proportion in tests with males heterozygous for one or two M5-B#1 autosomes. In all cases, the proportion represents the fraction of progeny that carried one of the M5-B#1 autosomes. The autosome in question is indicated in each of the frames. In some tests, the M5-B#1 *Y* chromosome was present and this is also indicated. All distributions were scaled so that the area under each of them sums to one. The *hatched* distributions refer to tests with males that had the control $y\ sn^w\ X$ chromosome, the *cross-hatched* distributions refer to tests with males that had the *T-5* tester *X* chromosome, and the *darkened* areas show the overlap between these two distributions.

for the controls). From these data it appears that there was greater distortion for chromosome *III* than for chromosome *II*. This is reminiscent of the greater effect that chromosome *III* had on male fertility.

The crosses with the doubly heterozygous males reinforce these findings. Curiously, however, the degree of distortion for each of the autosomes in these double heterozygotes was less than it was in the single heterozygotes. For instance, the mean proportion of M5-B#1 third chromosomes recovered from the double heterozygotes with a M5-B#1 *Y* chromosome was 0.28, whereas from the corresponding single heterozygotes, it was 0.23 (not significantly different by the rank sum test). Another reduction in the degree of distortion was observed with the double heterozygotes lacking the M5-B#1 *Y* chromosome. For these the mean segregation proportion for chromosome *III* was 0.24 compared to 0.18 for the single heterozygotes (significantly different at $P < 0.05$ by the rank sum test). For chromosome *II*, the segregation proportions were 0.35 (double heterozygotes) and 0.30 (single heterozygotes) for the crosses with the M5-B#1 *Y* chromosome (not significantly different), and 0.38 (double heterozygotes) and 0.28 (single heterozygotes) for the crosses without this chromosome ($P < 0.01$ by the rank sum test). The consistency of these differences indicates that when both of the M5-B#1 autosomes are present, each is recovered more often in the progeny than it would be if it were the only M5-B#1 autosome in the genome. This attenuation of segregation distortion can also be seen in Figure 5, which shows that there is greater overlap between the *T-5* and control frequency distributions of the double heterozygotes.

The absence of any pronounced deviation from Mendelian expectations in the control crosses strongly suggests that the *T-5* chromosome interacts specifically with the autosomes from M5-B#1 to cause segregation distortion. A plausible model is one in which the P transposase generated by the *T-5* chromosome acts on the nonautonomous elements of the M5-B#1 autosomes, inducing breakage or dominant lethal mutations. Either of these outcomes could result in an under-representation of the M5-B#1 chromosomes among the progeny.

Although the *T-5 X* chromosome clearly influenced the recovery of the M5-B#1 autosomes, there was no evidence for abnormal recovery of the *T-5* chromosome itself. The proportion of females was reasonably close to the Mendelian expectation in all cases. Preferential loss of the *T-5 X* chromosome would produce *XO* males, which, unfortunately, are phenotypically indistinguishable from ordinary *XY* males; thus, in this

TABLE 5

Segregation of M5-B#1 autosomes in the presence and absence of the *T-5* tester *X* chromosome in males

Parental genotype	No. cultures	+/*bw*; +/*st*	*bw*/*bw*; +/*st*	+/*bw*; *st*/*st*	*bw*/*bw*; *st*/*st*	No. ♀	Total	Proportion ♀ ± SE	Mean segregation prop. ± SE *II*	Mean segregation prop. ± SE *III*
(with M5-B#1 *Y*)										
T-5/*Y*'; +/*bw*; +/*st*	25	152	187	272	533	661	1141	0.548 ± 0.026	0.348 ± 0.026	0.292 ± 0.023
sn^w/*Y*'; +/*bw*; +/*st*	36	450	469	478	456	960	1853	0.518 ± 0.015	0.497 ± 0.013	0.500 ± 0.014
T-5/*Y*'; *bw*/*bw*; +/*st*	21		267		878	59	1145	0.515 ± 0.020		0.266 ± 0.020
sn^w/*Y*'; *bw*/*bw*; +/*st*	51		1633		1530	1648	3163	0.526 ± 0.008		0.522 ± 0.012
T-5/*Y*'; +/*bw*; *st*/*st*	14			189	421	296	610	0.474 ± 0.029	0.302 ± 0.026	
sn^w/*Y*'; +/*bw*; *st*/*st*	31			858	829	857	1687	0.494 ± 0.013	0.505 ± 0.013	
T-5/*Y*'; *bw*/*bw*; *st*/*st*	17				898	429	898	0.476 ± 0.021		
sn^w/*Y*'; *bw*/*bw*; *st*/*st*	30				1642	854	1642	0.517 ± 0.017		
(without M5-B#1 *Y*)										
T-5/*Y*; +/*bw*; +/*st*	28	173	223	366	665	704	1427	0.485 ± 0.012	0.377 ± 0.021	0.245 ± 0.024
sn^w/*Y*; +/*bw*; +/*st*	34	403	454	487	453	908	1797	0.512 ± 0.014	0.493 ± 0.014	0.480 ± 0.015
T-5/*Y*; *bw*/*bw*; +/*st*	28		269		1109	668	1378	0.465 ± 0.018		0.180 ± 0.023
sn^w/*Y*; *bw*/*bw*; +/*st*	41		1321		1268	1370	2589	0.527 ± 0.009		0.511 ± 0.011
T-5/*Y*; +/*bw*; *st*/*st*	17			227	552	377	779	0.474 ± 0.018	0.280 ± 0.022	
sn^w/*Y*; +/*bw*; *st*/*st*	23			641	749	691	1390	0.494 ± 0.014	0.473 ± 0.019	
T-5/*Y*; *bw*/*bw*; *st*/*st*	21				1019	487	1019	0.493 ± 0.016		
sn^w/*Y*; *bw*/*bw*; *st*/*st*	30				1610	853	1610	0.551 ± 0.021		

In the genotypes at the left, the + chromosomes were derived from M5-B#1. Each genotype with the *T-5* tester *X* chromosome is compared to one with the ordinary sn^w *X* in place of *T-5*. The M5-B#1 *Y* chromosome is indicated by *Y*'. Segregation proportions refer to the proportion of progeny that received the M5-B#1 autosome indicated. These, as well as the proportion of females, were averaged over cultures using the unweighted procedure of ENGELS (1979c).

experiment, the only way of documenting the loss of *T-5* was through a shift in the sex ratio, which was not observed.

GD sterility: From experiment I it was clear that the tester *X* chromosome *T-5* was a potent inducer of sn^w destabilization. We therefore investigated the ability of this chromosome to induce GD sterility, by itself and in combination with chromosomes from M5-B#1.

In one experiment, the sterility-inducing potential of *T-5* was assessed simultaneously with its ability to destabilize sn^w. The procedure involved mating *T-5* males individually to attached-*X* females, on the one hand, and to *bw*; *st* females, on the other. The first mating, conducted at 25°, was to produce sons that could be tested for sn^w mutability. These were crossed individually at 25° to attached-*X* females and the male progeny were scored in the usual way. The second mating, conducted at 29°, was to obtain F_1 daughters that could be examined for GD sterility. In order to compare *T-5* to a strong inducer of GD sterility, we performed concurrent control experiments utilizing sn^w; π_2 males in place of the *T-5* males.

The average mutation rate of sn^w was 0.428 ± 0.024 for the *T-5* flies. This was based on 1625 progeny from 111 different cultures. In the control experiment, there were 1042 progeny from 81 cultures and the average mutation rate was 0.321 ± 0.036. Thus *T-5* was more effective than π_2 in destabilizing sn^w. However, as the other part of the experiment showed, *T-5* was considerably less effective than π_2 in causing GD sterility. Only 6.4% of the 298 F_1 females that were examined from the *T-5* cultures were sterile. In contrast, 99.5% of the 196 F_1 females from the π_2 cultures had GD sterility. Thus, the flies with the higher ability to destabilize sn^w were decidedly less able to induce GD sterility. Obviously, these results cannot be explained by any simple model in which the induction of GD sterility is proportional to the level of sn^w instability.

Important clues toward an understanding of these paradoxical results came from two experiments in which *T-5* was tested in combination with chromosomes from M5-B#1. These tests were actually parts of experiments II and III, already discussed. In each of these experiments, the P_2 crosses between *M5*/*T-5*; *bw*/*bw*; *st*/*st* females and the two kinds of *M5*/*Y*; +/*bw*; +/*st* males were transferred to fresh culture vials after five days and incubated at 29°. These transfer cultures produced the F_1 females that were examined for GD sterility.

Each of the F_1 females was heterozygous for *T-5* and a Muller-5 *X* chromosome, the latter coming either from M5-B#1 or from *M5*; *bw*; *st*. Segregation of the autosomes produced four genotypic classes: (1) +/*bw*; +/*st*, (2) *bw*/*bw*; +/*st*, (3) +/*bw*; *st*/*st* and (4) *bw*/*bw*; *st*/*st*, where the + chromosomes were derived from M5-B#1. We endeavored to examine 4–6 females of each genotype from each culture for GD sterility, but owing to the vicissitudes of chromosome segregation, this was not always possible. It is important to recognize, however, that the different classes of females that were examined came from the same set of cul-

TABLE 6

GD sterility among females with M5-B#1 chromosomes in the presence and absence of the *T-5* tester *X* chromosome

M5-B#1 chromosomes	Experiment II				Experiment III				Control			
	No. cultures	No. females	No. sterile	Percent sterile	No. cultures	No. females	No. sterile	Percent sterile	No. cultures	No. females	No. sterile	Percent sterile
II, III	45	193	11	5.3	55	191	96	50.3	57	187	0	0.0
III	45	208	8	3.8	56	198	103	52.0	57	164	0	0.0
II	44	176	13	7.4	54	173	79	45.7	55	133	0	0.0
0	44	157	1	0.6	55	148	7	4.7	53	120	0	0.0
X, II, III	53	208	35	16.8	66	236	136	57.6	56	184	0	0.0
X, III	54	200	24	12.0	66	228	148	64.9	56	164	0	0.0
X, II	53	166	25	15.1	66	209	120	57.4	53	147	1	0.7
X	51	163	29	17.8	62	180	99	55.0	53	119	0	0.0

The M5-B#1 chromosomes in the genome are listed at the left for two experiments in which the *T-5* tester *X* chromosome was present, as well as for control series in which *T-5* was absent.

tures. This minimized variation due to the environment and to maternally inherited factors. It is also important to note that if any M5-B#1 chromosome was present in an F_1 female, it was inherited from her father; in contrast, the *T-5* tester chromosome was always inherited from her mother.

The results of these experiments are presented in Table 6, along with those of a control experiment in which the *T-5* chromosome was replaced by the *y* sn^w *X* chromosome. By itself, *T-5* caused negligible sterility (0.6% in experiment II and 4.7% in experiment III). Likewise, the M5-B#1 chromosomes caused negligible sterility (only 0.1% overall in the control experiment), so long as they were not combined with the *T-5 X* chromosome. However, there was low to moderate sterility (3.8–64.9%) among the females that had the *T-5* tester *X* chromosome and at least one of the M5-B#1 chromosomes. These experiments therefore demonstrate that *T-5* interacts with the chromosomes from M5-B#1 to cause GD sterility.

Experiment III showed the strongest interactions between *T-5* and the M5-B#1 chromosomes. Among the F_1 females that had *T-5* and at least one M5-B#1 chromosome, the frequency of sterility ranged from 45.7 to 64.9%. The M5-B#1 *X* chromosome appeared to be the most potent cause of sterility, although each of the major autosomes was almost as powerful. There was no strong evidence for a cumulative effect of the M5-B#1 chromosomes, although the genotypes with the M5-B#1 *X* and either or both of the autosomes were slightly more susceptible to *T-5*-induced sterility.

The data from experiment II bear out these conclusions, although less dramatically. For some unknown reason, the potential for inducing GD sterility was less in this experiment than in experiment III. It is interesting to note that the mutation rate of sn^w was lower in this experiment than it was in experiment I or in the experiment that compared *T-5* and π_2. It is possible that the *T-5* chromosomes used in experiment II carried fewer autonomous *P* elements than the ones used in the other experiments.

These results demonstrate the ability of *T-5* to induce GD sterility by interacting specifically with chromosomes carrying nonautonomous *P* elements. One possible interpretation is that the transposase generated by *T-5* acts on these *P* elements to cause dominant lethal mutations in the developing germ line, thereby wiping out the cells that should form the adult gonad.

The ability of M5-B#1 to repress hybrid dysgenesis involves a maternally transmitted factor

These last results demonstrate that the nonautonomous *P* elements of M5-B#1 interact with *T-5* to cause hybrid dysgenesis. However, one of the first experiments that was discussed showed that in some crosses M5-B#1 partially represses hybrid dysgenesis. These apparently contradictory results can be reconciled by noting that the crosses that led to repression involved M5-B#1 females that might have transmitted a cytoplasmic repressor to their progeny. The other crosses involved paternal transmission of the M5-B#1 chromosomes and would therefore have excluded the action of a cytoplasmic repressor. To test this explanation, we bred two groups of flies that had *T-5* and a set of M5-B#1 chromosomes. One group received its M5-B#1 chromosomes maternally, the other group received them paternally. If a maternally transmitted factor were responsible for the repression of hybrid dysgenesis, then these two genetically equivalent groups should have different frequencies of GD sterility.

The actual experiment began by mating two *T-5* males, which were brothers, to *C(1)DX, y f/Y; bw; st* females at 21°. Each mating produced a subline of *T-5* flies, hereafter designated as H or L. The same males used in these matings were also mated at 21° to *M5; bw; st* females. These produced *M5/T-5* heterozygotes, which were then mated individually at 29° to M5-B#1 or *M5; bw; st* males. The daughters of these latter matings were examined for GD sterility. This allowed the combined effects of *T-5* and the

TABLE 7

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

Cross		Progeny tested	H (high) subline				L (low) subline			
			No. cultures	No. females	No. sterile	Percent sterile ± SE	No. cultures	No. females	No. sterile	Percent sterile ± SE
T-5 ♂ × M5-B#1 ♀	(1)	*M5-B#1/T-5*	26	266	16	7.47 ± 3.01	32	340	0	0
T-5 ♂ × *M5; bw; st* ♀	(2)	*M5/T-5*	23	234	10	5.15 ± 1.80	35	362	3	0.95 ± 0.56
M5/T-5 ♀ × M5-B#1 ♂	(3a)	*M5/M5-B#1*	36	296	148	48.31 ± 7.20	47	477	41	9.04 ± 1.45
	(3b)	*T-5/M5-B#1*	36	380	218	56.32 ± 6.73	47	541	113	20.64 ± 1.91
M5/T-5 ♀ × *M5; bw; st* ♂	(4a)	*M5/M5*	35	101	3	4.76 ± 3.17	39	127	2	0.69 ± 0.48
	(4b)	*T-5/M5*	38	419	40	9.30 ± 2.72	41	438	0	0

paternally inherited M5-B#1 chromosomes to be determined. At the same time, *T-5* males from each of the sublines were crossed at 29° to M5-B#1 or *M5; bw; st* females. The males used in these single-pair matings were the immediate progeny of the two males used to initiate the sublines. Their *M5/T-5* daughters were then examined for GD sterility, permitting the maternal effects of M5-B#1 to be determined. To complete the experiments, *T-5* brothers of the males used in these last matings were crossed individually at 25° to attached-*X* females so that the mutability of sn^w could be estimated.

The results of the sterility tests are summarized in Table 7. By itself, the *T-5* chromosome induced little or no GD sterility. This can be seen in the *M5/T-5* females that received their *M5* chromosome from the *M5; bw; st* stock. In the table, these females are represented by genotypes 2 and 4b, which are genetically equivalent even though they came from different crosses. For both sublines, the frequency of sterility for these two genotypes was low. In contrast, the females that had paternally derived chromosomes from M5-B#1 had much higher sterility frequencies. These are represented by genotypes 3a and 3b, the former lacking *T-5* and the latter possessing it. For the H subline, the frequencies of GD sterility for these genotypes were 48.31 and 56.32%, respectively. For the L subline, the corresponding frequencies were 9.04 and 20.64%. Obviously, the H subline induced more sterility than the L subline, but the main point is that the M5-B#1 chromosomes were involved in this sterility. This is most clearly seen by comparing genotypes 3b and 4b, which had the same kind of mothers but different fathers. As mentioned above, no M5-B#1 chromosomes were present in genotype 4b and the frequency of sterility was low. In contrast, for genotype 3b, which had a paternally derived set of M5-B#1 chromosomes, the sterility frequency was 20–56%. By a *t*-test, the difference between genotypes 3b and 4b was statistically significant for both sublines ($P < 0.05$).

One problem in these data concerns the high frequency of sterility for genotype 3a, which had the M5-B#1 chromosomes but lacked the *T-5* chromosome. This suggests that in the 3a females the M5-B#1 chromosomes caused GD sterility independently of the *T-5 X* chromosome. However, from previous work we know that without the *P* transposase, the M5-B#1 chromosomes are incapable of doing this. One possible explanation is that the sterile 3a females inherited one or more autonomous *P* elements that had transposed from the *T-5 X* chromosome to another chromosome in the genome. The transposase produced by these transposed elements would be able to activate nonautonomous *P* elements inherited from M5-B#1, leading to GD sterility.

This explanation is supported by a detailed examination of the data. For the H subline, where the data are more informative, the distribution of GD sterility among the daughters of the *M5/T-5* females that were mated to the M5-B#1 males was bimodal. This was true of both types of daughters (3a and 3b); moreover, the frequency of sterility in these two types was positively correlated (Kendall's tau = 0.54, $P < 0.01$). These facts suggest that some of the *M5/T-5* females possessed autonomous *P* elements able to activate the nonautonomous elements derived from M5-B#1. Other females lacked these elements and produced essentially 100% fertile daughters. This patchy distribution is consistent with the idea that transpositions occurred in the germ line of the single *T-5* male that sired each of these *M5/T-5* females. Such transpositions would lead to considerable genetic heterogeneity, including cases in which autonomous *P* elements had inserted into autosomes. These autosomal elements could then be passed on to both the 3a and 3b females in the next generation, where they could cause GD sterility in both groups. There would also be cases in which the *T-5 X* chromosome had lost autonomous *P* elements, leading to 3b females that were not at risk to become sterile. Thus, the presence of some sterility among the 3a females and the absence of total sterility among the 3b females is probably due to *P* element transpositions that occurred in an earlier generation.

In these data, the maternal effect of the M5-B#1 stock is evident by comparing the sterility frequencies of genotypes 1 and 3b. These females were genetically

equivalent except that those in class 1 had M5-B#1 mothers while those in class 3b had M5-B#1 fathers. Clearly, there was much greater sterility in class 3b and the difference between the two classes was statistically significant for both sublines. Since these two classes had the same chromosome complement, the difference between them must be due to a non-chromosomal factor. Evidently, the class 1 females inherited such a factor from their M5-B#1 mothers. The results in Table 7 therefore solve the paradox posed by the results of the previous experiments. The chromosomes of M5-B#1 do indeed contribute to the induction of GD sterility, so long as a source of the P transposase is present. However, this contribution is significantly reduced when M5-B#1 females are used in the crosses; the reason is that these females transmit a cytoplasmic repressor of hybrid dysgenesis to their progeny.

In these experiments, the H subline consistently induced more sterility than the L subline, suggesting that it generated higher levels of the P transposase. This was confirmed by measuring the mutability of sn^w in the two sublines. The H subline was tested in 11 cultures, and based on a total of 251 progeny, the average mutation rate was 0.493 ± 0.076. The L subline was tested in 32 cultures, and based on 980 progeny, the average mutation rate was 0.047 ± 0.009. This large difference persisted when males from the two sublines were tested in subsequent generations. It is important to note that because these two sublines were derived from *T-5* males that were brothers, the difference between them must have arisen in a single generation. This suggests that perhaps the male that produced the L subline had inherited an *X* chromosome that had lost one or more autonomous *P* elements.

The maternally transmitted factor of M5-B#1 persists for at least two generations

The experiments discussed above demonstrate that a maternally transmitted factor is responsible for the ability of M5-B#1 to repress GD sterility. ENGELS (1979a) showed that P cytotype strains have such a factor and that it persists in the maternal line for at least two generations. To investigate whether or not the repressing factor in M5-B#1 conforms to this pattern of inheritance, we tested the abilities of hybrids between M5-B#1 and the true M strain *bw*; *st* to repress GD sterility and sn^w mutability.

The M5-B#1 and *bw*; *st* strains were crossed at 25° in both ways to produce reciprocal hybrid females. We denote the females that had *bw*; *st* mothers as the A hybrids and those that had M5-B#1 mothers as the B hybrids. Both types were then used in matings with sn^w; π_2 males. These matings were arranged so that each male was mated to an A and a B female. Initially, these cultures were kept at 21° to minimize GD sterility among the progeny, but after 6 days the inseminated females were transferred to fresh culture vials that were incubated at 29°. This higher temperature was to induce GD sterility. From each primary culture, *M5-B#1*/sn^w and +/sn^w daughters were collected and mated individually to *y* sn^3 *v car* males at 25° so that the germ line mutability of sn^w could be assessed. We attempted to test two daughters of each genotype from each primary culture. Owing to the structure of the experiment, it was therefore possible to compare the average mutation rates of the daughters of each A female with the corresponding average of each B female. It must be remembered that these daughters were half-sisters because they had the same sn^w; π_2 father. From the transfer cultures we examined as many as 12 females of each of the two genotypes for GD sterility. In addition to these crosses, a set of control matings was carried out. In these, sn^w; π_2 males were crossed to M5-B#1 and *bw*; *st* females. Each male was mated at 21° to each kind of female and after six days, the mated females were transferred to fresh cultures, which were incubated at 29°. The mutability of sn^w was assessed using daughters from the primary cultures and the frequency of GD sterility was determined using daughters from the transfer cultures.

The results of these experiments are given in Table 8. The A and B hybrid females, as well as the control *bw*; *st* females, produced essentially 100% sterile daughters at 29°. None of these females, therefore, had any ability to repress GD sterility. In contrast, the control M5-B#1 females produced only a moderate frequency of GD sterility, indicating some repression of this dysgenic trait. The daughters of these females also had less sn^w mutability than the daughters of the control *bw*; *st* females; this difference was highly significant by the sign test, since 40 out of 41 half-sib family comparisons showed that the daughters of the M5-B#1 females were less mutable. As previous experiments have shown, this partial repression of dysgenesis is evidently due to a maternally transmitted factor. The inability of the A and B hybrids to repress GD sterility suggested that this factor could not be transmitted through an additional generation. However, the mutation data in the right half of Table 8 showed that this was not the case.

The mean mutation rates of the tested flies were less if they had the M5-B#1 maternal lineage. An examination of the mutation rates of females from individual half-sib families bears this out. For instance, the *M5-B#1*/sn^w flies with B mothers were less mutable than those with A mothers in 32 out of the 49 half-sib families for which comparisons were possible, a result that is highly significant by the sign test (P = 0.022). For the +/sn^w flies, a similar result was obtained. Those with B mothers were less mutable than those with A mothers in 34 out of 45 comparisons (P = 0.0004). It is clear, therefore, that for this trait, the flies with the M5-B#1 maternal lineage were less mutable than their counterparts with the *M5*; *bw*; *st* mater-

TABLE 8

Suppression of GD sterility and sn^w mutability by reciprocal hybrids between M5-B#1 and *bw; st*

Cross	Progeny tested	GD sterility tests				sn^w Hypermutability tests							
		No. cultures	No. females	No. sterile	Percent sterile (weighted)	No. families	No. cultures	sn^w	sn^+	sn'	Total	$u \pm$ SE	$v \pm$ SE
M5-B#1/+ ("A") ♀ × sn^w; π_2 ♂	*M5-B#1/*sn^w	62	679	679	100.0	59	110	2110	241	280	2631	0.202 ± 0.011	
	$+/sn^w$	62	704	704	100.0	57	103	2975		327	3302		0.095 ± 0.007
M5-B#1/+ ("B") ♀ × sn^w; π_2 ♂	*M5-B#1/*sn^w	61	700	700	100.0	59	99	2672	212	218	3102	0.147 ± 0.015	
	$+/sn^w$	61	716	715	99.9	54	94	2692		157	2849		0.057 ± 0.007
M5-B#1/M5-B#1 ♀ × sn^w; π_2 ♂	*M5-B#1/*sn^w	25	276	122	44.2	46	155	5403	93	159	5655	0.043 ± 0.004	0.028 ± 0.003
+/+ (bw;st) ♀ × sn^w; π_2 ♂	$+/sn^w$	44	506	506	100.0	52	147	3429		391	3820		0.104 ± 0.007

The mutation rates *u* and *v* were calculated by averaging the proportion of mutants among the flies scored; for *u*, both singed-extreme and wild type flies were counted, whereas for *v*, the wild-type flies were ignored.

nal lineage, even though the two groups were chromosomally identical. This means that the repressor that M5-B#1 possesses can be transmitted maternally for at least two generations. The pattern of transmission of this "partial P cytotype" therefore conforms to that already established for the complete P cytotype (ENGELS 1979a).

DISCUSSION

Muller-5 Birmingham was the first strain to be identified that had many *P* elements but did not have the full-fledged P cytotype (BINGHAM, KIDWELL and RUBIN 1982). Subsequent investigations have identified several other such strains (KIDWELL 1983, 1985 and unpublished observations from our laboratory). These M′ or pseudo-M strains have some ability to repress hybrid dysgenesis, suggesting that their cytotype is intermediate between the extremes of pure M, which has no repressing ability, and P, which brings about nearly complete repression.

This paper reports experiments with an inbred subline of Muller-5 Birmingham called M5-B#1. These experiments have established that although all the *P* elements in this subline are nonautonomous, they can still have important effects on hybrid dysgenesis.

Chromosomal repression of hybrid dysgenesis: We have found that individual chromosomes derived paternally from M5-B#1 reduce the mutation rate of sn^w. These results are consistent with the earlier results of SIMMONS and BUCHOLZ (1985), who found this effect when whole sets of chromosomes from the Muller-5 Birmingham parent strain were tested. The demonstration that individual chromosomes from M5-B#1 can reduce sn^w instability suggests that the *P* elements carried by each of these chromosomes is responsible for this effect. Since the effect is seen when the chromosomes are paternally derived, it cannot be ascribed to a cytoplasmic repressor. SIMMONS and BUCHOLZ (1985) suggested that this paternally based repression of sn^w mutability was the result of transposase titration. On this hypothesis, each *P* element in the genome competes for the P transposase. When many *P* elements are present, there is a lower probability for the transposase to interact with sn^w, leading to a reduction in the mutation rate.

Chromosomal enhancement of hybrid dysgenesis: The same experiments that showed that chromosomes derived paternally from M5-B#1 have the potential to repress sn^w mutability also demonstrated that these chromosomes increase the incidence of GD sterility. This paradoxical finding cannot be explained solely by the action of the *T-5 X* chromosome, which was the source of the transposase, nor can it be explained solely by the action of the M5-B#1 chromosomes. Rather, it is clear that an interaction between *T-5* and the M5-B#1 chromosomes was responsible. It is likely that the transposase provided by the *T-5* chromosome

activates the *P* elements on the M5-B#1 chromosomes, causing dominant lethal mutations in the developing germ line. Such mutations might be associated with chromosome breakage and might be frequent enough to wipe out the primordial germ tissue; this would lead to GD sterility in the adult. In cases where the germ tissue survives and the individual is fertile, the chromosomes damaged by *P* element activity could cause aneuploidy in the next generation. Aneuploid individuals have reduced viability, so the segregation proportion would be skewed against those progeny inheriting a chromosome that had potentially damaging *P* elements. Distorted segregation among the progeny could therefore reflect the same basic cellular events that cause GD sterility. On this model, the fact that the distortion for the M5-B#1 third chromosome was greater than that for the second chromosome could be explained by a greater number of nonautonomous target elements on the third chromosome. It is also possible to explain the fact that when both of these autosomes were present simultaneously, each was distorted less than when either was the only M5-B#1 autosome in the genome. This attenuation of distortion would indicate competition for the transposase that was generated by the autonomous *P* elements on the *T-5 X* chromosome. With both of the M5-B#1 autosomes present, the transposase would be spread over a greater number of targets, thereby reducing the likelihood that either autosome would be "killed." In this view, the levels of sn^w mutability, GD sterility and segregation distortion all reflect the kinetics of *P* element activation in the germ line.

Cytoplasmic repression of hybrid dysgenesis: Our experiments showed that when M5-B#1 females were used in dysgenic crosses, there were significant reductions in both sn^w mutability and GD sterility. This suggests that M5-B#1 females transmit a cytoplasmic repressor of hybrid dysgenesis to their offspring. Further experiments using a sn^w assay have shown that the putative repressor can be transmitted maternally for at least two generations. However a sterility assay was not sensitive enough to detect this.

The demonstration that M5-B#1 possesses a maternally transmitted repressor of hybrid dysgenesis has a double significance. First, it establishes that M′ strains with a partial ability to repress hybrid dysgenesis transmit this ability in the same way as strains with essentially complete repression potential. The pattern of inheritance of this "partial P cytotype" therefore conforms to the pattern of the full P cytotype. Second, since evidently none of the *P* elements in M5-B#1 is autonomous, the ability to repress *P* element activity appears to be independent of the P transposase. This casts doubt on the assumption of O'HARE and RUBIN (1983) and SIMMONS and BUCHOLZ (1985) that autonomous *P* elements play an essential role in the regulation of *P* element activity.

Models of *P* element regulation: To date, most thinking about the mechanism of *P* element regulation has focused on the role of autonomous elements. In one model, proposed by O'HARE and RUBIN (1983), these elements encode a protein repressor that inhibits transposase activity. This model now seems unlikely for several reasons. First, detailed analysis of an autonomous element has shown that all four of its open reading frames are needed for transposase synthesis (KARESS and RUBIN 1984). Without differential transcription or splicing, autonomous *P* elements do not seem to have the ability to produce both a repressor and a transposase. Second, some strains that possess autonomous *P* elements have failed to evolve the ability to regulate *P* element activity (DANIELS *et al.* 1987; W. R. ENGELS, personal communication). The existence of such strains suggests that autonomous elements do not produce a repressor, although it may be argued that these strains simply have not accumulated enough autonomous elements to generate the quantities of the repressor that are needed for *P* element control. Third, M′ strains such as Muller-5 Birmingham apparently lack autonomous *P* elements, but nonetheless have the potential to repress hybrid dysgenesis. The existence of these strains strongly implies that the key to understanding *P* element regulation lies with the nonautonomous *P* elements.

How might nonautonomous *P* elements bring about the control of *P* element activity and the repression of hybrid dysgenesis? One possibility is that some of these elements encode a mutant transposase that binds to autonomous *P* elements and inhibits their transcription. This binding might be mediated by amino acid sequences that serve this purpose in the normal enzyme, but that bind more tightly in the mutant because of a structural alteration.

Another possibility is for a mutant transposase to bind to all *P* elements and block their access to the normal transposase. In this model, transposase synthesis would not be inhibited, but its functions would be thwarted by tightly binding mutant molecules.

Both of these models assume that a mutant transposase acts as a repressor of *P* element activity by binding to at least some of the *P* elements in the genome. H. ROBERTSON and W. R. ENGELS (personal communication) have obtained indirect evidence for such binding by studying the fertility of flies heterozygous for sn^w and sn^{x2}. The latter is an allele that interferes with the function of the *singed* locus in the female germ line; sn^{x2} homozygotes produce defective eggs and are therefore sterile. sn^w/sn^{x2} heterozygotes also produce defective eggs, but only if they have the P cytotype; individuals with the M cytotype are reproductively normal. These facts suggest that the agent that produces the P cytotype also interferes with *singed* expression, possibly by binding to the *P* elements inserted in the sn^w allele. More refined studies have shown that individual defective *P* elements can sometimes cause reproductive failure in sn^w/sn^{x2} females.

This indicates that these elements might encode a protein able to bind to the *P* elements of sn^w and impair the expression of the *singed* locus.

Although the work of ROBERTSON and ENGELS supports a model of repressor binding, it is possible that *P* element regulation involves some other mechanism. For instance, proteins produced by defective *P* elements could aggregate with normal transposase molecules and poison the holoenzyme. However this and both of the repressor models suffer from an inability to explain the maternal inheritance of cytotype. This inadequacy was pointed out by ENGELS (1981), who postulated that extrachromosomal *P* elements might play a role in the control of *P* element activity. Such elements might have the ability to replicate themselves and could therefore be transmitted maternally for a few generations. SIMMONS and BUCHOLZ (1985) elaborated on this possibility by proposing that extrachromosomal *P* elements might titrate the transposase made by autonomous chromosomal elements and thereby repress chromosomal *P* element activity. They hypothesized that extrachromosomal elements might be generated through the excision of chromosomal *P* elements, presumably by the action of transposase. However, since Muller-5 Birmingham and other partially-repressing strains apparently lack the normal transposase, this hypothesis cannot stand without modification. One possibility is that mutant transposases might replicate chromosomal *P* elements and generate extrachromosomal copies. This, of course, is tenable only if the normal transposase has a replicative function, a possibility that is suggested by the proliferation of *P* elements in strains where single autonomous elements have been introduced by transformation (DANIELS *et al.* 1987; W. R. ENGELS, personal communication).

At present, all the models discussed above remain as formal possibilities; there is no decisive evidence to eliminate any of them. It may turn out as KIDWELL (1985) has conjectured that *P* element regulation involves more than one molecular mechanism.

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