INHERITANCE OF MUTATOR ACTIVITY IN *ZEA MAYS* AS ASSAYED BY SOMATIC INSTABILITY OF THE *bz2-mu1* ALLELE

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ABSTRACT

Mutator lines of maize were originally defined by their high forward mutation rate, now known to be caused by the transposition of numerous *Mu* elements. A high frequency of somatic instability, seen as a fine purple spotting pattern on the aleurone tissue, is characteristic of *Mu*-induced mutable alleles of genes of the anthocyanin pathway. Loss of such somatic instability has been correlated with the *de novo*, specific modification of *Mu* element DNA. In this report the presence or loss of somatic instability at the *bz2-mu1* allele has been monitored to investigate the inheritance of the Mutator phenomenon. The active state is labile and may become weakly active (low fraction of spotted kernel progeny) or totally inactive (no spotted kernel progeny) during either outcrossing to non-Mutator lines or on self-pollination. In contrast, the inactive state is relatively permanent with rare reactivation in subsequent crosses to non-Mutator lines. Cryptic *bz2-mu1* alleles in weakly active lines can be efficiently reactivated to somatic instability when crossed with an active line. However, in reciprocal crosses of active and totally inactive individuals, strong maternal effects were observed on the inactivation of a somatically unstable *bz2-mu1* allele and on the reactivation of cryptic *bz2-mu1* alleles. In general, the activity state of the female parent determines the mutability of the progeny.

ABOUT a decade ago, Robertson described the Mutator stock of maize. It was characterized by a high frequency of recessive mutations in progeny of self-pollinated plants, non-Mendelian transmission of the Mutator phenotype to nearly all of the progeny, and recovery of up to 35% somatically unstable alleles among new mutants (ROBERTSON 1978). These characteristics suggested the presence of a family of transposable elements; however, ROBERTSON and MASCIA (1981) demonstrated that the Mutator activity could not substitute for the autonomous member of the known *Ac/Ds*, *Dt*, *I/En(Spm)*, or *r-cu/Fcu* controlling element families. From characterization of an unstable allele of *alcohol dehydrogenase-1* (*Adh1*) selected from a Mutator background, a new transposable element family has been identified in Mutator stocks. The *Adh1-S3034* allele contains an insertion of about 1.4 kilobase pairs (kbp), called *Mu1* (BENNETZEN *et al.* 1984). By DNA sequence analysis, *Mu1* was shown to be 1367 base pairs (bp) long, to contain approximately 200-bp terminal inverted

repeats, and to create a 9-bp host sequence duplication in *Adh1* at the site of its insertion (BARKER *et al.* 1984).

By DNA hybridization to genomic DNA from Mutator plants, *Mu1* defines a family of transposable elements, the members of which share homology to the repetitive termini and middle portion of this element; these related sequences are called *Mu* elements and are typically found in 10–50 copies in Mutator stocks (BENNETZEN 1984). That *Mu* elements are the major cause of the high forward mutation frequency in Mutator stocks is strongly supported by detection of *Mu* elements in additional unstable mutants selected from Mutator backgrounds. Thus far, four alleles of *Adh1* (FREELING and BENNETT 1985) and several unstable loci of the anthocyanin pigment biosynthesis pathway (*a1-Mum2* [O'REILLY *et al.* 1985], *bz1-mu1* and *bz2-mu2* [TAYLOR, CHANDLER and WALBOT 1986], and *bz2-mu1* [M. MCLAUGHLIN and V. WALBOT, unpublished data]) have been shown to contain *Mu* element insertions. This correlation between *Mu* elements and the high mutation rate of Mutator lines is strengthened by the observation that non-Mutator lines of maize contain few *Mu*-homologous sequences (BENNETZEN 1984; CHANDLER, RIVIN and WALBOT 1986).

The inheritance of Mutator activity is atypical of transposable element functions in maize. For example, the autonomous elements *Ac* and *En(Spm)* show Mendelian inheritance; usually only one or two autonomous elements exist in a stock, and they segregate with exceptional cases of an increase or decrease in copy number (reviewed in FEDOROFF 1983; FREELING 1984). In fact, the dependence of somatic instability at mutable loci containing *Ds* elements on the simple segregation of *Ac* was the key genetic proof of *Ac* autonomy. With the Mutator phenomenon, ROBERTSON (1978) found that most progeny of crosses between Mutator and non-Mutator plants inherit Mutator activity, as assayed by the mutation frequency in seedlings from self-pollinated ears of the subsequent generation. This assay for new recessive seedling mutations scores the ability of *Mu* elements to insert at new locations. From the results of this assay, it seemed plausible that the small fraction of progeny which failed to exhibit Mutator behavior would be missing an autonomous *Mu* element required for transposition. To explain the lack of segregation of autonomous elements, ROBERTSON suggested that at least three copies of such autonomous elements would have to exist and that these elements would have to increase in copy number before meiosis to be represented in most progeny.

An alternative and more immediate assay for Mutator activity is observation of somatic reversion to wild-type activity at alleles containing *Mu* elements. This assay can be a very sensitive indicator if mutable alleles of the anthocyanin pigment biosynthethic pathway are utilized, because the presence of purple color is readily observed on the aleurone tissue (COE 1978). In this assay it is assumed that revertant sectors result from the excision of the *Mu* element from the affected allele; thus, loss of somatic instability is an indication that at least one *Mu* element no longer excises.

By monitoring somatic reversion at *bz2-mu1* we discovered numerous cases of loss of the characteristic fine spotting somatic reversion phenotype (WALBOT

1984; CHANDLER and WALBOT 1986). Such lines lacking somatic instability at an indicator allele are called inactive Mutator. These cases were not readily explained by simple segregation of autonomous elements, because in many instances transmission of the spotted kernel phenotype was as expected in either self-pollination or outcross progeny of a single plant, but not in both. The loss of somatic instability behaved like a change of phase (WALBOT *et al.* 1986), a process defined by MCCLINTOCK (1965) as a potentially reversible alteration in the ability of autonomous elements to program the activities of their transposable element system.

Thus far, all of the inactive lines we have examined at the molecular level contain one or more *Mu* elements with an increased level of DNA modification; this modification masks *Hin*fI sites in the terminal inverted repeats of *Mu* and other restriction endonuclease recognition sites in the internal portion of the element (WALBOT, CHANDLER and TAYLOR 1985; CHANDLER and WALBOT 1986). Consistent modification of one internal site in Mutator lines that have lost their characteristic high forward mutation rate has also been reported (BENNETZEN 1985). Interestingly, modification may play a role in regulating the activities of the *Ac/Ds* family as well. In stocks carrying an active *Ac/Ds* transposable element family, multiple copies of sequences cross-hybridizing to *Ac*-specific sequences do exist, but most are highly modified and do not restrict with several enzymes; only the genetically active *Ac* is unmodified at these sites (FEDOROFF, WESSLER and SHURE 1983). Thus, DNA modification may be a general mechanism for repressing the activity of transposable element functions in maize.

To measure the frequency of loss of the somatic instability phenotype and to begin to elucidate the genetic control of the *Mu*-specific modification process, I have investigated the inheritance of the active and inactive Mutator states by monitoring somatic mutability at *bz2-mu1* over several generations. The active state is much more labile in our Mutator stocks than in the original report of ROBERTSON (1978), and the inactive state is relatively permanent. Analysis of the outcome of crossing active and inactive Mutator lines demonstrates that there are maternal effects on the state of the Mutator system, the first cases of maternal effects unrelated to element dosage in a transposable element system of maize.

MATERIALS AND METHODS

Stocks: The original Mutator stock used in these studies was a gift from D. S. ROBERTSON; the line contained all of the genes required for anthocyanin pigmentation of the aleurone tissue and is referred to by ROBERTSON as a purple Mutator line (ROBERTSON *et al.* 1985). The *bz2* tester in hybrid W23/K55 background was a gift of E. H. COE; the *anther ear bz2* deletion stock was obtained from the Maize Genetics Cooperative; this deletion encompasses approximately 2 map units on the long arm of chromosome *1* and eliminates both *an* and *bz2* gene functions. As shown in Figure 1 the *bz2-mu1* allele was selected in 1982 at Stanford University by crossing the purple Mutator stock to *bz2* tester (WALBOT, BRIGGS and CHANDLER 1985). The original mutable kernel was planted in the greenhouse during the winter of 1983 and, subsequently, was propagated by outcrossing to the *bz2* testers and by self-pollination. Molecular classification of *Mu* element modification has been previously reported for some second

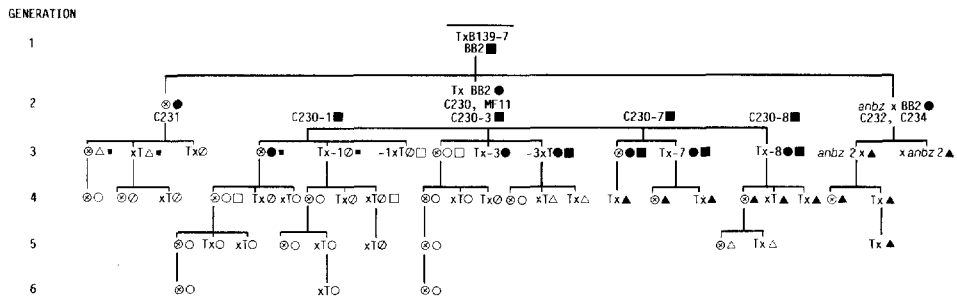

FIGURE 1.—Lineage relationship among the stocks used in these experiments. In all cases the female parent is listed first. (X) indicates self-pollination, × T indicates crosses of Mutator by *bz2* tester, and T × represents *bz2* tester × Mutator. Selected progeny of these crosses are listed on the line below. The transmission of the spotted kernel phenotype characteristics of *bz2-mu1* is indicated to the right of crosses as follows: ●, expected segregation of spotted kernels on all ears examined; ▲, expected segregation of spotted kernels on more than 50% of ears; △, expected segregation of spotted kernels on fewer than 50% of ears; Ø, no ears with the expected ratio of spotted:colorless kernels, but some spotted kernels present; and O, no spotted kernels on any ears from this cross. The original mutable kernel was selected from the cross *bz2* tester × B139-7, a purple Mutator plant. This kernel was grown as plant BB2. From this plant and some plants of the C230 lineage of the next generation, DNA was extracted from a leaf and restricted with *Hin*fI to assay *Mu* element modification; modification level is indicated to the right of plant number designations using the following code: ■, no modification; ■, mixture of modified and unmodified elements; □, all modified elements. In other cases the presence of modified *Mu* elements was assayed in DNA prepared from seedlings grown from the indicated cross, and sibling kernels were grown to maturity for crossing; in these cases the state of *Mu* element modification is indicated to the right of the cross using the same code.

generation plants and their progeny (CHANDLER and WALBOT 1986); this information is included in Figure 1.

Analysis of somatic instability: In most cases, somatic instability was scored on 100 kernels of each ear, counting from the base of the ear toward the tip sampling about 5–8 rows. In the case of outcrosses in which 1:1 segregation of the spotted:bronze kernel phenotype was expected, ears ranging from 40:60 to 60:40 ($P < 0.05$ using the χ^2 statistical test) were considered to show expected segregation, and precise kernel counts are not reported. For all ears showing skewed segregation, the precise spotted:bronze segregation ratio of each ear is reported in the tables, as well as the significance calculated from the χ^2 statistic. A similar criterion was applied to the analysis of crosses with different expectations of spotted:bronze kernel segregation. In a few instances all of the kernels on an ear were counted, or all of the rare, spotted kernels on an ear were counted and the number of bronze kernels estimated by multiplying the number of rows × number of kernels in one row and rounding off to the nearest, lower number that was a multiple of 50; this latter case is reported as, for example, 3:>300 to indicate that three spotted kernels were found on an ear containing greater than 300 but fewer than 350 kernels.

RESULTS

Somatic instability at *bz2-mu1*: As shown in Figure 2, the mutability at *bz2-mu1* results in kernels with a high frequency of somatic reversions late in development. This somatic instability was inherited in expected Mendelian ratios in the immediate progeny of the original *bz2-mu1/bz2* individual in both self-pollination and outcrossing to tester stocks. However, in the next genera-

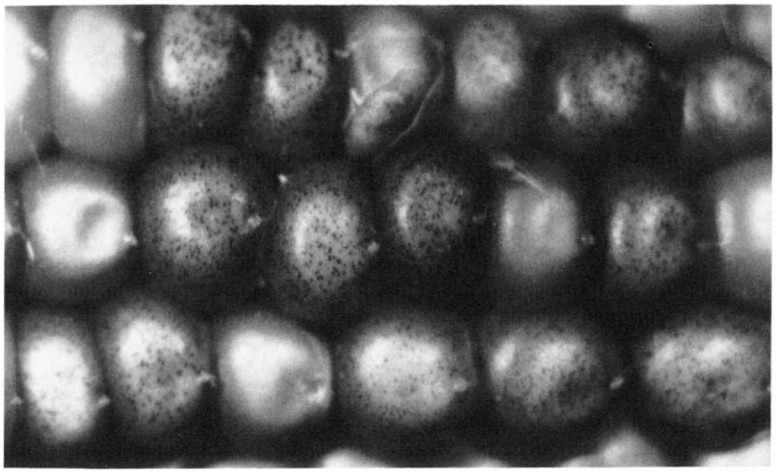

FIGURE 2.—The pattern of somatic reversion at *bz2-mu1* in an active Mutator line: *bz2* × C230-7.

tion, deviations from the expected inheritance of the spotted kernel phenotype were detected in multiple derivatives of both the self-pollination and outcross progeny (Figure 1). Materials showing complete somatic stability are termed inactive Mutator lines, and those with a deficiency of spotted kernels are called weakly active lines.

Molecular classification of Mutator lines: At the molecular level the presence of modified *Mu* elements has been demonstrated in several inactive and weakly active Mutator lines by digesting genomic DNA with the restriction endonuclease *Hin*fI; this enzyme has a recognition site in the inverted repeats of *Mu* elements. In an active line, the *Hin*fI sites in all or nearly all *Mu* elements are recognized, whereas in inactive and weakly active lines there is a lack of restriction of one or both *Hin*fI sites in most or all elements (CHANDLER and WALBOT 1986). The molecular status of the *Mu* elements in selected lineages is summarized in Figure 1. Importantly, leaf DNA prepared from the original mutable plant and eight outcross progeny in the second generation showed complete *Hin*fI digestion suggesting that modified *Mu* elements were not present in the lineage at the beginning of the experiment.

Frequency of loss of the spotted kernel phenotype: Previously, ROBERTSON (1983) reported an initial increase in forward mutation rate in Mutator lineages following self-pollinated or Mutator × Mutator crosses compared to outcrosses of Mutator to non-Mutator lines. Consequently, the frequency of Mutator inactivation as detected by loss of somatic instability at *bz2-mu1* has been measured separately for self-pollinated and outcross progeny. Spotted kernels (one-third *bz2-mu1* and two-thirds *bz2/bz2-mu1*) among self-pollinated progeny of the original mutator plant (BB2 [X]) were planted as family C231 and subsequently self-pollinated and outcrossed to tester (Table 1). In these crosses, about three-fourths of the plants failed to transmit the expected ratio of spotted:bronze kernels. Five of ten ears showing a skewed ratio were completely inactive, and among the weakly active derivatives the average frequency of

TABLE 1

Genetic analysis of the inheritance of somatic instability in the progeny (C231) of the selfed ear of the original mutable plant

Type of cross (expected sp:bz segregation)	No. of ears	Ears with abnormal segregation ($P < 0.0005$)	sp:bz[a] K ratios on abnormal ears	Average % sp K on abnormal ears
C231(X) (3:1 or 1:0)	4	3	0:258, 51:310, 0:78	5
C231 × *bz2* tester (1:1 or 1:0)	6	4	1:127, 0:178, 19:57, 0:19	9
bz2 tester × C231 (1:1 or 1:0)	3	3	49:207, 0:168, 4:231	7

Spotted kernels were picked from the selfed ear BB2(X), of which one-third were *bz2-mu1* and two-thirds were *bz2/bz2mu-1*, and were grown as family C231 during the summer of 1983. The plants were self-pollinated or testcrossed to, or by, *bz2* tester as indicated. The female parent is listed on the left in all crosses.

[a] Abbreviations: sp = spotted, bz = bronze, K = kernels.

FIGURE 3.—Reduction in frequency of somatic reversion at *bz2-mu1* in a lineage in which some individuals are switching to an inactive Mutator state in the fourth generation of outcrossing of the C230-8 lineage.

spotted kernels was less than 10%, rather than the 50, 75 or 100% expected. Furthermore, the spotted kernels present had a lower frequency of revertant sectors than do typical kernels in fully active lines (Figure 3).

Among the outcross progeny of the original mutable plant (Table 2) fewer switches from active to a weakly active line occurred. Loss of the spotted kernel phenotype was found in the third generation in about one-third of the progeny (15 of 43 ears). The affected ears averaged ~30% spotted kernels, of 50% expected, a level considerably higher than in the self-pollination lineage. However, in both the self-pollination and outcross lineages, the active Mutator state is very labile. Even though a family possesses the expected phenotypic segregation of mutable kernels and has no evidence of *Mu* element modification, activity can be lost in a substantial fraction of its progeny (Figure 2).

Most individuals in a weakly active lineage such as *bz2* × C230-1 are likely

TABLE 2

Inheritance of somatic mutability in outcross progeny of the original mutable plant

Type of cross (expected sp:bz segregation)	No. of ears	Ears with abnormal segregation ($P <$ 0.0005)	sp:bz K ratios on abnormal ears	Average % sp K on abnormal ears
C230(X) (3:1)	12	4	0:168, 252:118, 188:85, 45:55	46
C230 or MF11 × *bz2* (1:1)	7	2	1:275, 3:316	1
bz2 × C230 or MF11 (1:1)	11	7	63:2842,[a] 108:179, 22:78, 128:263, 73:167, 171:253, 0:100	22
C233 or 234 × *bz2* (1:1)	11	0		
bz2 × C233 or 234 (1:1)	1	1	30:70	30

Spotted kernels, all of genotype *bz2mu-1/bz2*, were selected from the outcross progeny of the original mutable plant and grown in 1983. Families C230 and MF9 were derived from the outcross to *bz2* tester, and families C233 and C234 from the outcross to *an bz2*. The female parent is listed on the left in each cross.

[a] Sum of kernels on 11 individual ears of *bz2* × C230-1.

to become less active in the next generation (Table 3). When transmission was tested in plants grown from the rare spotted kernels (63:2842) in this lineage, all self-pollinated progeny showed altered transmission, with an average of 24% spotted kernels among the progeny (of 75% or 100% expected). Among the outcross progeny, 96% of the progeny ears showed a deficit in spotted kernels, with an average of <10% spotted kernels compared to 50% expected. About one-half of the self-pollination and outcross progeny had completely bronze ears characteristic of a fully inactivated line.

Stability of the inactive Mutator state: From Mendelian expectations of *bz2* transmission, a predictable number of inactive individuals should contain a cryptic *bz2-mu1* allele. Thus, reappearance of the spotted kernel phenotype might be expected if inactive lines were readily reactivated. The stability of the bronze kernel phenotype among inactive progeny containing the *bz2-mu1* allele was tested by outcrossing such plants to *bz2* tester and scoring the spontaneous reappearance of the spotted kernel phenotype. Bronze kernels of the weakly active stock, *bz2* tester × C230-1, were selected from a population of 63:2842 spotted:bronze kernels; one-half should be *bz2/bz2mu-1*, but loss of somatic instability masks the mutable phenotype. In subsequent outcrosses with *bz2* tester (Table 3) only one plant of 56 examined showed spontaneous reactivation of somatic instability at *bz2-mu1*. Because half of the individuals tested should be *bz2* and will lack the mutable allele, reactivation should be considered as one in 27, or about 4%. This result suggests that the weakly active Mutator lines are primarily composed of inactive individuals and that this inactivity cannot be readily reversed by crosses to non-Mutator lines.

The progeny of inactive line C230-3(X) were all bronze, and the *Hin*fI sites of all *Mu* elements were modified in the 12 individuals tested (CHANDLER and WALBOT 1986). There was no evidence for reactivation of the spotted kernel

TABLE 3

Transmission of the spotted kernel phenotype in the C230-1 lineage

Parent (kernel phenotype)	Type of cross (segregation if *bz2-mu1* present)	No. of Ears	Ears with abnormal segregation ($P <$ 0.0005)	sp:bz K ratios on abnormal ears	Average % sp K on abnormal ears
		A. Third generation			
bz2 × C230-1[a] (bronze)	Self (3:1 or 1:0)	12	12	All 0:>200	0
	bz2 tester × stock (1:1)	10	10	All 0:>200	0
	Stock × *bz2* tester (1:1)	46	46	45 0:>200, 27:107	<1
bz2 × C230-1[b] (spotted)	Self	6	6	0:100, 20:39, 4:75, 3:76, 26:31, 44:43	24
	bz2 tester × stock (1:1)	11	10	28:81, 28:55, 18:90, 5:300, 38:168, 36:138, 4 0:>300	12
	Stock × *bz2* tester (1:1)	15	15	4:>300, 2:202, 1:>250, 1:>300, 1:>300, 10 ears, 0:>300	<1
C230-1(X) (spotted)	Self (1:0 or 3:1)	10	10	All 0:>200	0
	bz2 tester × stock (1:0 or 1:1)	13	13	3:303, 12 0:>200	<1
	Stock × *bz2* tester (1:0 or 1:1)	12	12	All 0:>200	0
		B. Fourth generation			
CC CH[c] (bronze)	Self	47	47	All 0:>200	0
	bz2 tester × stock	39	39	All 0:>200	0
	Stock × *bz2* tester	22	22	All 0:>150	0
		C. Fifth generation			
D91 E321[d] (bronze)	Self	18	18	All 0:>200	0
	bz2 tester × stock	18	18	All 0:>150	0

[a] Bronze kernels were removed from *bz2* tester × C230-1 and grown as families D84 in 1984 and E302 in 1985. The parent C230-1 was genotypically *bz2-mu1/bz2*, but failed to transmit the spotted kernel phenotype to most outcross progeny; the spotted:bronze kernel ratio was 63:2482. At the molecular level, modified *Mu* elements were detected in all outcross progeny tested. Thus, of the bronze kernels planted, about one-half should be heterozygous for the *bz2-mu1* allele, which, if reactivated, would exhibit the spotted kernel phenotype. Consequently, of the 56 outcross progeny examined, only 28 would be predicted to contain the mutable allele.

[b] Fifteen of the rare spotted kernels (63:2482) of *bz2* × C230-1 were planted as D85 in 1984 and E303 in 1985. The genotype of these kernels should be *bz2/bz2-mu1*.

[c] In the fourth generation, five bronze kernels were taken from each of the ten (X) ears propagated from the C230-1(X) and were planted as CC2 and 3 or CH47-51 in the winter 1984 crop and crossed as indicated.

[d] A bronze kernel was selected from each selfed ear of the fourth generation and was grown as D91 in 1984 or E321 in 1985 and crossed as indicated.

TABLE 4

Transmission of the spotted kernel phenotype in the C230-3 lineage

Culture (kernel phenotype)	Type of cross (segregation if *bz2-mu1* present)	No. of ears	Ears with abnormal segregation ($P < 0.0005$)	sp:bz K ratios on abnormal ears	Average % sp K on abnormal ears
C230-3(X)[a] (bronze)	Self (1:0 or 3:1)	4	4	All 0:>200	0
	bz2 × D80 E309 (1:0 or 1:1)	8	8	6:94, 7 0:>200	<1
	D80 E309 × *bz2*	8	8	All 0:>200	0
C230-3 × *bz2*[b] (spotted)	Self (3:1 or 1:0)	22	22	All 0:>200	0
	bz2 × E308 (1:1)	20	15	33:67, 23:77, 14:86, 37:63, 18:82, 1:99, 9 all 0:>200	8
	E308 × *bz2*	18	13	7:93, 29:71, 1:99, 21:79, 3:97, 30:70, 16:84, 1:99, 27:73, 19:80, 30:>200	12

[a] Only bronze kernels were observed on the C230-3(X) ear; three-fourths of these should contain the *bz2-mu1* allele. Kernels were grown as family D80 in 1984 and E309 in 1985. At the molecular level, 12 of 12 seedlings tested contained completely modified *Mu* elements.

[b] Bronze kernels were removed from the C230-3 × *bz2* tester ear and grown as family D82 in 1984 and E308 in 1985. One-half of these kernels should contain the *bz2-mu1* allele. At the molecular level none of the four seedlings tested contained modified *Mu* elements; in the reciprocal cross *bz2* × C230-3, six of 24 seedlings tested contained modified *Mu* elements.

phenotype among four self-pollinated progeny ears of this lineage, even though three-fourths of the individuals should contain the *bz2-mu1* allele (Table 4). Among the 16 outcross ears analyzed, there was one Mutator plant that produced an ear sector with six spotted kernels, but otherwise all ears were bronze. Thus, reactivation can occur spontaneously, albeit very infrequently, even in an inactive line.

In contrast to the inactive C230-3(X) ear and lineage, the progeny of C230-3 × *bz2*, the second ear on plant C230-3, showed normal segregation and no evidence of modified *Mu* elements. However, plants derived from spotted kernels of C230-3 × *bz2* usually failed to transmit this somatic instability in the subsequent generation (Table 4). All (22 of 22 ears) of the self-pollinated progeny became completely inactivated, and about two-thirds of the outcross progeny became weakly active (28 of 38 ears). This lineage showed a much higher propensity for loss of somatic instability at *bz2-mu1* than other active lineages (see Table 6), suggesting that the Mutator system in the C230-3 plant was altered in some way that resulted in the immediate inactivation of one ear (C230-3[X]) and the near complete, but delayed inactivation of progeny from the second, outcross ear.

Similarly, the two outcross and self-pollination progeny of C230-1 were phenotypically quite distinct in the second generation, but all were producing mainly inactive progeny in the third generation (Figure 1, Table 3). The C230-1(X) ear showed a near-normal spotted:bronze kernel ratio (124:59, $P < 0.04$), whereas the outcross progeny were weakly active or inactive (63:2842 with *bz2*

TABLE 5

Test for restoration of somatic mutability by crossing individual inactive Mutator plants to each other or by crossing descendants of the inactive C230-1 and C230-3 lineages together

Type of cross (lineage)	No. of ears	Ears with abnormal segregation ($P < 0.005$)	Spotted:bronze kernels on abnormal ears
Reciprocal in lineage (*bz2* × C230-1)[a]	34	34	33 0:>200 11:179[b]
Reciprocal in lineage (C230-3(X)[c]	28	28	28 0:>200
Between lineages ([bz2 × C230-1] × C230-3(X)[d]	21	21	All 0:>200

[a] Bronze kernels from *bz2* × C230-1 were picked and grown in 1984 and 1985; as discussed in Table 3, about half of these kernels should be *bz2/bz2-mu1*. In pairwise crosses, plants were reciprocally pollinated, *i.e.*, plant −1 × −2 and −2 × −1.

[b] The sectored ear had 11:179 sp:bz kernel ratio; at the base there was a 10:49 sp:bz sector and one additional spotted kernel elsewhere.

[c] Bronze kernels were picked from C230-3(X); three-fourths of these kernels should contain at least one *bz2-mu1* allele, and one-fourth should be *bz2*. Exact reciprocal crosses were performed.

[d] Individuals of the *bz2* × C230-1 and C230-3(X) lineages as described above were crossed to each other, although exact reciprocal crosses were not always completed.

tester as female and 1:168 with *bz2* as male). Despite the high fraction of spotted kernels on the C230-1(X) ear, at the molecular level DNA from most seedlings sampled contained modified *Mu* elements (CHANDLER and WALBOT 1986). As shown in Table 3, 34 of 35 third generation progeny and all fourth generation progeny of this lineage were completely inactive. Thus, the outcross progeny of C230-1 showed immediate inactivation, whereas there was a one-generation delay in the inactivation of the C230-1(X) lineage.

Tests for segregation of factors required for reactivation: Inactive individuals transmitting a stable bronze phenotype in crosses to *bz2* tester were crossed together to determine if factors required for somatic instability at *bz2-mu1* are segregating in inactive stocks (Table 5). If combined, such factors might restore somatic instability. Because the copy number of such factors might be critical—as is the case with *Ac*, in which increasing copy number suppresses somatic instability (FEDOROFF 1983)—insofar as possible exact pairwise reciprocal crosses were carried out within a lineage resulting in triploid endosperm tissue of different genetic constitution in the two products. In 33 of 34 ears analyzed, all kernels were stably bronze. This result suggests that individual inactive progeny, half of which should contain a cryptic *bz2-mu1* allele do not contain other segregating factors required for somatic instability at this locus. Using a similar test, no evidence for reactivation was found upon crossing derivatives of the C230-3(X) lineage with each other, a cross in which three-fourths of the parent bronze kernels should contain *bz2-mu1* (Table 5).

The one exceptional case was an ear sector in a reciprocal cross of two C230-1 lineage plants; this sector demonstrates that reactivation can occur. However, because the spotted kernel phenotype was restored in just part of an ear and was not transmitted through the pollen of this individual, the ear sector is taken as evidence that spontaneous reactivation occurred during the

somatic development of the maternal plant and was not affected by the cross with another inactive Mutator plant. That is, the *bz2* × C230-1 derivative already contained all of the factors required for somatic instability at *bz2-mu1*.

Another test for spontaneous reactivation was carried out by crossing derivatives of the C230-3(X) and *bz2* × C230-1 lineage together (Table 5). However, as was found with most within-lineage crosses, there is no restoration of somatic instability at *bz2-mu1*. More limited tests with progeny from other inactive individuals of the C230 and C231 lineages confirm that no restitution of Mutator activity occurs in crosses between inactive lines (data not shown). Furthermore, such tests have been pursued for two additional generations in the C230-1 and C230-3 lineages, each time sampling a number of individuals, some fraction of which should contain the *bz2-mu1* allele, without finding another instance of restoration of somatic instability (data not shown). Taken together, these data strongly suggest that factors required for somatic instability at *bz2-mu1* are not segregating in different inactive lineages.

Stability of the active Mutator state: To further test the stability of the active state of the Mutator system, the progeny of *bz2* × C230-8 have been examined in some detail. When C230-8 was crossed to 22 tester plants, each ear showed the expected 1:1 spotted:bronze kernel ratio. At the molecular level both the 18 spotted and 16 bronze progeny tested showed complete *Hin*fI restriction of all *Mu* element DNA. Thus, this appears to be a completely active lineage at the phenotypic and genotypic level. However, in subsequent generations, weakly active and inactive individuals were found (Figure 1, Table 6).

Because the *bz2* × C230-8 ears yielded the expected 1:1 spotted:bronze phenotypic ratio, the bronze kernels should be simply *bz2*. As expected, such bronze kernels show no somatic instability when crossed to *bz2* tester. On the other hand, plants grown from spotted kernels of *bz2* × C230-8 did transmit the spotted kernel phenotype in a near Mendelian fashion in self-pollination (95% normal transmission) and outcross progeny (79% as male, 95% as female). Only nine of 86 ears showed a deviation from the expected fraction of spotted kernels, and only one ear was completely bronze. Thus, the switch from active to weakly active (eight of 86) or inactive (one of 86) was much lower in the *bz2* × C230-8 lineage than among the progeny of the C230 family as a whole in the previous generation (nine of 30 weakly active and two of 30 inactive progeny, Table 2). The average percent spotted kernels among new weakly active derivatives in C230-8 was 25% (Table 6), and several individuals showed precisely 1:3 sp:bz segregation; this would be expected for the independent segregation of *bz2-mu1* and an autonomous controlling element. However, most new derivatives of *Mu* inactivation do not conform to this expectation.

The *bz2* × C230-8 lineage also remained more active than other lineages which were normal in the second generation of propagation. Progeny of other C230 lineages that showed normal segregation and no evidence of *Mu* element modification were tested for the transmission of somatic stability in the third generation (Table 7). Self-pollination of such progeny yielded only 16 of 43 ears with normal segregation: 63% of the progeny became weakly active or

TABLE 6

Transmission of the spotted kernel phenotype in progeny of *bz2* tester × C230-8

Kernel phenotype (genotype)	Type of cross (expected segregation)	No. of ears	Ears with abnormal segregation ($P < 0.0005$)	sp:bz K ratios on abnormal ears	Average % sp K on abnormal ears
	A. Third generation crosses[a]				
Bronze (*bz2*)	*bz2* tester × stock (0:1)	43	0		
Spotted (*bz2/bz2-mu1*)	Self (3:1)	22	1	165:85	66
	bz2 tester × stock	34	7	35:110, 72:128, 38:190, 59:102, 80:184, 22:78, 0:100	24
	Stock × *bz2* tester	25	1	26:74	26

B. Fourth and Fifth Generation Crosses

	Self-pollination			Outcross progeny[b]		
	No. of ears		Abnormal % sp K	No. of ears		Abnormal % sp K
Generation	Normal	Abnormal		Normal	Abnormal	
Fourth[c]	17	8	32	8	4	28
Fifth[d]	16	15	26	17	14	18

[a] Kernels were taken from several of 22 ears of *bz2* × C230-8 and were planted as families CF49 in winter 1984, D88 in 1984 and E301 and E306 in 1985. At the molecular level, 18 of 18 spotted and 16 of 16 bronze kernels of this genotype contained *Mu* elements completely restricted by *Hin*fI.

[b] In all cases the Mutator plant was used as the pollen parent and was crossed to tester.

[c] A single spotted kernel was planted from each selfed ear showing normal segregation in the self-pollinations of spotted kernels from *bz2* × C230-8 (panel A), and the plants were self-pollinated and crossed onto tester. The kernels should be one-third homozygous *bz2-mu1* and two-thirds heterozygous for the mutable allele.

[d] Two spotted kernels were planted from each selfed ear showing normal segregation in the fourth generation, and the plants were self-pollinated and crossed onto tester.

inactive. Upon outcrossing about one-third (54 of 147 ears) showed evidence for loss of somatic instability. Thus, lines picked because of their normal segregation pattern and absence of modified *Mu* elements are likely to remain active to some extent. However, the presence of somatic instability and *Mu* elements capable of complete digestion by *Hin*fI in siblings or parents does not guarantee that a particular individual will remain active.

Further evidence that active lines are labile comes from tests in subsequent generations of the C230-8 lineage. The third generation self-pollination progeny (Table 6) showed 95% expected segregation, but a progressive loss of Mutator activity occurred in the next two generations. When spotted kernels were planted from normally segregating ears and outcrossed to tester, only 68% of the fourth generation ears showed expected ratios, and the remainder were weakly active lines. In the fifth generation, phenotypically normal Mutator parents gave rise to ears with the expected phenotype only 50% of the time. The ears with a deficiency in spotted kernel progeny still retained a

TABLE 7

Transmission of the spotted kernel phenotype in other lineages lacking modified *Mu* elements

	Self-pollination			Outcross progeny			
	No. of ears			No. of Ears			
				By *bz2*	To *bz2*		
Culture[a]	Normal	Abnormal	Abnormal % spK	Normal	Normal	Abnormal	Abnormal % sp K
C230-3 × *bz2*	0	22	0	ND[b]	10	18	10
C230-4 × *bz2*	10	3	32	2	18	5	30
bz2 × C230-6	0	1	22	ND	4	16	10
C230-6 (X)	ND	ND		ND	8	5	44[c]
C230-7 (X)	ND	ND		ND	18	4	41[c]
bz2 × C230-7	6	1	50	8	25	6	33
Total ears	16	27		10	83	54	

[a] In all cases, spotted kernels were removed from ears showing a normal segregation ratio in the previous generation. Outcrosses were performed by *bz2* tester pollen and by using the Mutator plant as pollen donor to *bz2* tester as indicated.

[b] ND, Not determined; no ears of this type were produced.

[c] For these tests, one-third of the kernels should have been homozygous *bz2-mu1* and two-thirds heterozygous for the mutable allele. Consequently, the percentage of spotted kernels sums results from ears that deviated from 3:1 and 1:1 segregation.

substantial fraction of somatically unstable kernels characteristic of a weakly active line.

Considering all of the data on the transmission of the active Mutator state as monitored by somatic instability at *bz2-mu1*, it is clear that lines can become weakly active or inactive at any time, and once inactive, they tend to remain so. Inactivation is progressive, noticed first as a deviation in the percentage of ears showing expected spotted:bronze kernel ratios; the abnormal ratio ears often have a substantial percentage of spotted kernels when loss of activity is first noted. In the next and subsequent generations both the percentage of normal ears and the percentage of spotted kernels per ear usually decrease.

Maternal influences on maintenance of Mutator activity: In the first instance of *Mu* inactivation in the outcross lineage (Table 2) there is a significant bias in the transmission of the Mutator phenotype: 16 of 18 progeny ears showed expected segregation when the Mutator plant was the female parent, whereas only four of 12 ears showed expected segregation when the Mutator plant was the male parent. In subsequent generations, combining data for all active C230 lineages (Tables 6 and 7) nearly all (35 of 36) of the outcross ears in which the Mutator plant was the female parent showed normal segregation; the one abnormal ear retained 26% spotted kernels. However, when these active individuals were used as the pollen parent in crosses to *bz2* tester, normal segregation was found in approximately two-thirds of the progeny ears (110 of 171).

A maternal effect was also noted in the maintenance of the spotted kernel phenotype in the plants grown from the rare spotted kernels (63 spotted:2482 bronze) from *bz2* tester × C230-1. At the molecular level, all of the bronze

TABLE 8

Reactivation of somatic instability of a cryptic *bz2-mu1* allele in a weakly active Mutator background in crosses with an active Mutator line

	Type of cross	
	E301 × E302[a] (active × inactive)	E302 × E301 (inactive × active)
Total no. of ears scored	36	37
No. of ears with spotted kernels	20	18
No. of exact reciprocal crosses with spotted kernels	12	10
Average % spotted kernels on reactivated ears	33	23

[a] Bronze kernels from *bz2* × C230-8 were used as the active line; when testcrossed to *bz2* tester, none of these plants produced spotted kernels (Table 6). At the molecular level, DNA was prepared from seedlings of bronze siblings, and 16 of 16 contained *Mu* elements totally restricted by *Hin*fI. The weakly active line was *bz2* × C230-1; one-half of the bronze kernels should contain *bz2-mu1*. At the molecular level, DNA samples from 10 of 10 sibling seedlings contained modified *Mu* elements. This weakly active line shows a low level of spontaneous reactivation on crossing to tester (Table 3).

kernels tested from this cross had modified *Mu* elements; of the five spotted kernels examined at the molecular level, one contained modified elements, but four did not [CHANDLER and WALBOT 1986). As shown in Table 3, in the next generation these spotted kernels differentially transmitted the somatic instability trait, depending on the direction of the cross. Although ten of 11 ears showed abnormal transmission when the Mutator plant was used as male, the percentage of spotted kernels was still reasonably high at 12%, and only four ears were completely bronze. In the reciprocal crosses, 100% of the progeny showed abnormal segregation ratios; two-thirds (ten of 15) of the progeny were completely inactive, and the weakly active progeny averaged only 1% spotted kernels. Thus, there is a maternal effect on the maintenance of Mutator in a weakly active line, but it is opposite to that seen in the more active C230 lineages.

Reactivation of cryptic *bz2-mu1* alleles in weakly active or inactive Mutator lines on crossing with an active line: To determine whether an active Mutator line can supply functions required to restore instability at *bz2-mu1* in an inactive line, such stocks were reciprocally crossed (Table 8). Bronze kernels of *bz2* tester × C230-8 which should be simply *bz2* in an active Mutator stock (Table 6) were crossed as male and female by plants grown from bronze kernels of *bz2* tester × C230-1 carrying a cryptic *bz2-mu1* allele. Although the spontaneous reactivation of the cryptic allele in this family is only 4% when crossed onto non-Mutator plants [Table 3, section *bz2* × (*bz2* × C230-1), contains the outcross ears of the individuals reported in Table 8], in crosses as male with an active line, 20 of 36 ears analyzed showed at least a few spotted kernels; this is close to the expected fraction (18 of 36) of these plants that should be *bz2/bz2mu-1*. Reactivation was substantial because the average percentage of spotted kernels was 27% (50% spotted would be full reactivation). Thus, an active Mutator line can provide activities required for the restoration

TABLE 9

Reactivation of somatic instability of a cryptic *bz2-mu1* allele in an inactive Mutator background in crosses with an active Mutator line

	Type of cross	
	Active[a] × inactive[b]	Inactive × active
Total no. of ears scored	21	28
No. of ears with spotted kernels	12	0
No. of exact reciprocal crosses with spotted kernels	11	0
Average % spotted kernels on reactivated ears	16	0

[a] For the active lineage, bronze kernels from *bz2* × C230-8 were used. That these individuals did not contain a *bz2-mu1* allele was confirmed by crossing to *bz2* tester. At the molecular level all sibling seedling DNA samples of this type contained *Mu* elements completely restricted by *Hin*fI.

[b] Bronze kernels from C230-3(X) were used as the inactive lineage. Three-fourths of the kernels should contain at least one *bz2-mu1* allele. At the molecular level all sibling kernels of this type contained a population of modified *Mu* elements.

of somatic instability at *bz2-mu1*, even when this allele has been in a lineage with modified *Mu* elements.

In this test, 12 pairs of exact reciprocal crosses showed evidence of reactivation. Of these, two instances were found in which somatic instability was restored only when the active line was the female parent. The spotted:bronze kernel ratios on these ears were 22:278 and 7:282, compared to 0:>300 for the reciprocal crosses. Additional support for a positive maternal effect by active plants on reactivation of the cryptic *bz2-mu1* allele comes from a comparison of the average percent spotted kernels: with the active line as female, the average was 33%; with the inactive line as female, the average was 23%.

Although reactivation was reasonably successful when an active and weakly active line were crossed together, reactivation of inactive lines occurred less frequently (Table 9). Again, bronze kernels of the *bz2* × C230-8 lineage were used as the source of an active Mutator system. On crossing with the C230-3(X) lineage, in which three-fourths of the progeny should contain a cryptic *bz2-mu1* allele, only one-fourth of the ears examined (12 of 49) showed restoration of the spotted kernel phenotype. All of the reactivation events involved an active *bz2* female parent crossed by the inactive line carrying a cryptic mutable allele. By the criterion of percentage of spotted kernels, the extent of reactivation was low, only 16% compared to the 50% and 100% expected from heterozygous and homozygous donors, respectively.

Maternal effects on inactivation: The final evidence for a maternal effect comes from an inactivation experiment in which spotted kernel progeny of *bz2* tester × C230-8 were crossed by progeny of the completely inactive C230-3 (X) lineage. As in the reactivation experiment, each individual was crossed as male to tester to demonstrate that the classification as active or inactive was appropriate, and exact reciprocal crosses were completed between active and inactive individuals (Table 10). All ears on an active plant crossed by an inactive line showed the expected 1:1 segregation for spotted:bronze kernels. In

TABLE 10

Inactivation of the *bz2-mu1* allele in an active Mutator background when crossed as male to an inactive line

	Type of cross	
	E306 × E307[a] (Active × inactive)	E307 × E306 (Inactive × active)
No. of ears with normal segregation	13	3
No. of ears with abnormal segregation	0	10
% normal segregation	100	23
Phenotypic segregation on abnormal ears		16:24, 5:36, 38:62, 12:25, 8:47, 12:29, 17:23, 22:43, 4:66, 19:34

[a] Family E306 is derived from active Mutator line *bz2* × C230-8. Family E307 is derived from the inactive C230-3(X) lineage, selfed again as D80-1(X); bronze kernels were taken from this ear.

the reciprocal crosses, however, only three of 13 ears showed normal segregation. Those showing abnormal segregation were still weakly active lines with an average of 28% spotted kernels. In other experiments in which these and other pairs of active and inactive lines were crossed together, 48 of 49 ears on active female parents transmitted the expected ratio of spotted kernels; when used as the male parent, 14 of 51 showed normal transmission of somatic instability.

DISCUSSION

Using somatic instability at *bz2-mu1* as an indicator of Mutator activity over several generations, it is clear that inactivation of the Mutator system is a common feature of our Mutator stocks. Upon selfing, such inactive lines rarely show new recessive mutants (data not shown), confirming a previous report that modification of *Mu* elements correlates with a loss of the forward mutation rate as well (BENNETZEN 1985). Inactivation of somatic instability is usually progressive; active lines typically give rise to weakly active lines, which subsequently become fully inactive. Among the inactive lineages, there are few instances of spontaneous reactivation on crossing to non-Mutator lines.

Somatic instability in the endosperm is a reasonably accurate indicator of an active state in the embryo. However, the one-generation delay in the recognition of inactivation of sister ears in two C230 plants may indicate special cases in which the somatic reversion in the endosperm did not accurately reflect the inactive state of the *Mu* elements in the diploid embryo. This loss of somatic instability appears to involve more than just the segregation of a factor required for somatic instability, because crosses of different inactive individuals show only rare reactivation of the cryptic *bz2-mu1* alleles. All of these features of inactivation are presumed to result from DNA modification of *Mu* elements. In a few instances, weakly active ears contained kernel ratios consistent with independent segregation of the *bz2-mu1* allele and an autonomous element; further work will be required to clarify whether such an autonomous element is now segregating in some stocks.

It is intriguing that two kinds of maternal effects were detected on the maintenance of the active and inactive states of the Mutator system. Two kinds of enzymatic functions are hypothesized to specifically recognize *Mu* elements: specific *de novo* masking of *Hin*fI sites presumably requires a modification enzyme, whereas excision requires a transposase. In active lines such as C230-8, loss of somatic instability occurred preferentially when individuals were crossed as male onto tester. This maternal effect suggests that a positive factor(s) required to maintain Mutator activity is being diluted in the sperm. In contrast, lines such as C230-1, in which the switch to inactivity is occurring at high frequency, are more likely to retain activity when crossed as male onto tester, perhaps because they are escaping a modification system. Two kinds of maternal effect were also found in the reactivation and inactivation experiments. An active *bz2* line can more efficiently reactivate cryptic *bz2-mu1* alleles when used as the female parent (Tables 8 and 9): In the reciprocal crosses of an active line carrying *bz2-mu1* with an inactive line (Table 9), the active line shows completely normal spotted:bronze kernel segregation when used as the female parent, but not as the male.

All of the experiments monitoring somatic instability (*Mu* excision) at *bz2-mu1* as an indicator of activity suggest that the state of the female parent is critical in determining the extent to which the active and inactive states are maintained. These maternal effects are the first reported for maintenance of a maize transposable element system. ROBERTSON (1985) did recently report that the frequency of *Mu*-mediated mutation in one lineage differed in male and female germ cells in one generation but that the bias in mutation frequency disappeared in subsequent generations.

It is possible that Mutator activities are preserved when active plants are used as female because transposase or other factors required to promote the excision of the *Mu* element at *bz2-mu1* are found in a higher concentration in the maternal nucleoplasm or cytoplasm than in the pollen. Nonchromosomal factors are likely to show maternal effects in maize because the egg and primary endosperm nucleus of the maternal parent are considerably larger than the sperm nuclei (KIESSELBACH 1949). In addition, because somatic instability is scored in the triploid endosperm, maternal *Mu* dosage will be twice that of the pollen parent. There is some evidence that the absolute number of *Mu* elements in a stock is positively correlated with the frequency of loss of Mutator activities (ROBERTSON *et al.* 1985), but more data will be required to evaluate how different dosages of modified and unmodified *Mu* elements might interact. For example, in studies of *P* element-mediated somatic instability in *Drosophila melanogaster* SIMMONS and BUCHOLZ (1985) have recently demonstrated that increasing the dosage of defective *P* elements depresses instability at the *singed bristle* locus; they interpret their results as a titration of transposase by the defective elements.

Both the inactivation and reactivation of *Mu* activities suggest that changes of phase occur in the Mutator system. Because DNA modification is known to be reversible (JONES 1985), the genetic phenomenon of change of phase could be readily explained by DNA modification. However, until the activities en-

coded by *Mu* elements have been discovered, many questions about the nature of active and inactive Mutator states will remain unanswered. For example, does element modification suppress production of transposase functions resulting in an inactive phase?

Because approximately 25% of the cytosine residues in the maize genome are modified to 5-methylcytosine (HAKE and WALBOT 1980), specific modification of the *Mu* elements could utilize existing modification enzymes combined with some additional feature that confers sequence specificity. Potential specific features of *Mu* elements would include the possibility of palindrome formation and of the transposase binding. Once established, a specific modification pattern is thought to be maintained with reasonable fidelity through subsequent DNA replication (GRAFSTROM, HAMILTON and YUAN 1984). Consequently, even if *Mu*-encoded functions are part of the initial signal for modification of *Mu* elements, their presence might not be required for the maintenance of modification in inactive lines. In this regard it is significant that, in the non-Mutator lines we have studied, there are a few *Mu*-homologous sequences, but they are in an extensively modified state, presumably maintained without any Mutator activities present (CHANDLER, RIVIN and WALBOT 1986).

Several questions raised by the observations reported here are amenable to investigation. Now that the *bz2-mu1* allele has been cloned (M. MCLAUGHLIN and V. WALBOT, unpublished data), active and inactive lines can be screened to determine if DNA modification of the *Mu* element present in this locus is necessary or sufficient to suppress its somatic instability; conversely, does reactivation of the *bz2-mu1* phenotype depend on the modification state at this locus only? Also in progress are studies to determine whether the inactive lines show a decrease in *Mu* element copy number in outcrosses to *bz2* tester stock, as has been reported for Mutator stocks that have lost their characteristic high forward mutation rate (ALLEMAN and FREELING 1986). If element copy number does decrease as a result of the simple segregation of now immobile *Mu* elements, this may further suppress the Mutator system by diluting the number of *Mu* elements capable of encoding transposase functions. Although simple segregation of active elements is not the proximate cause of inactivation, it may eventually result in fixation of the inactive state.

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