The *Mutator*-Related *Cy* Transposable Element of *Zea mays* L. Behaves as a Near-Mendelian Factor

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ABSTRACT

The *bz-rcy* allele arose in a single gamete of the TEL (transposable-element laden) population, when the *rcy* receptor element inserted into the *Bronze1* locus. This newly arisen receptor allele conditions a stable bronze kernel phenotype in the absence of the independently segregating regulatory element, *Cy*. In the presence of *Cy*, *bz-rcy* conditions fully colored spots on a bronze background. The spots represent clonal sectors arising from mutations of *bz-rcy* to *Bz'*. Although *Cy* exhibits genetic interactions with the *Mutator* system it differs from *Mu*-homologous elements in its near-Mendelian behavior which is in contrast to the non-Mendelian inheritance of *Mutator* and *Mu*-homologous elements. Evidence is presented which suggests that the timing and mode of *Cy* transposition differ from those of *Mu1*.

TEN transposable-element systems that cause genetic instability in maize have been defined at the genetic level (for recent reviews see: FEDOROFF 1983; FREELING 1984; NEVERS, SHEPHERD and SAEDLER 1985; PETERSON 1987; LILLIS and FREELING 1986). Within a system two classes of maize transposable elements usually exist: regulatory and receptor elements. Regulatory elements are autonomously transposition-competent; they do not require a second factor in order to transpose. In contrast, receptors are not capable of autonomous transposition; they cannot transpose in the absence of a *trans*-acting factor supplied by regulatory elements.

The regulatory elements of nine of these systems exhibit Mendelian or near-Mendelian inheritance. The remaining system, *Mutator*, is inherited in a non-Mendelian fashion; 90% of the progeny from a *Mutator* plant exhibit *Mutator* activity (ROBERTSON 1978). A family of transposable elements having in common termini homologous to those of *Mu1* is responsible for the *Mutator* phenomenon. It appears likely that neither the 1.4-kb *Mu1* element nor the 1.7-kb element is autonomous (LILLIS, SPIELMANN and SIMPSON 1985; ALLEMAN and FREELING 1986). The "master element" that regulates the transposition of these *Mu* elements remains to be detected.

The *Cy* system exhibits certain genetic interactions with the *Mutator* system (SCHNABLE and PETERSON 1988). For example, *Mutator* activity (in *Mutator*-derived lines) and mutability at *a-mum2* (a *Mutator*-derived allele with a 1.4-kb *Mu1*-like insert) are correlated with *Cy* activity. Strongly active *Cy* elements are found only in the TEL population (in which *Cy* was first detected) and *Mutator*-derived lines (SCHNABLE and PETERSON 1986). In this report we describe the origin and near-Mendelian inheritance of the two components of this transposable element system with particular emphasis on the *Cy* element which may be the elusive "master element" of the *Mutator* system.

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MATERIALS AND METHODS

Origin of *bz-m805137*: In 1979 an isolation plot (PETERSON 1978) was established by planting fully colored kernels from the TEL (transposable-element laden) population (see Figure 3 in SCHNABLE and PETERSON 1986) as females. The TEL population was developed by crossing stocks carrying *En* by Line C and was so termed because it has been shown to contain five different regulatory elements: *Cy*, *En*, *Uq*, *Ac*, and *Dt* (SCHNABLE 1986). Because all individuals within the TEL population are homozygous for the wild-type alleles of the closely linked *Bz* and *Sh* loci, a *bz sh* male parent could be used to expose changes at these two loci (cross 2 in Figure 1). The *sh bz* stocks used in this cross and throughout this study do not carry *Cy* (SCHNABLE and PETERSON 1986).

From a total of 4.15×10^5 gametes, one new stable *sh* allele and one mutable *bz* allele were recovered. The latter was isolated as a individual spotted bronze kernel from ear 79 6203-67 (Figure 3 in SCHNABLE and PETERSON 1986) and has been designated *bz-m805137*. The following report addresses the inheritance of mutability at this allele.

Two tests to establish the basis of losses of mutability: In the first test receptor function was assayed by exposing the putative mutant receptor element to *Cy* (Figure 2). The appearance of mutability confirms that the receptor is still functional. If, however, *Cy* does not trigger mutability, the receptor, is said to have become nonresponsive, and is designated *bz-n(rcy)*. Of course, depending on the particular cross, some bronze round nonspotted selections selected from nonspotted ears and sectors will represent crossovers, *i.e.*, *Sh bz/sh bz*. Such selections would not be expected to exhibit mutability when crossed by *Cy*. Hence the appearance of an occasional ear lacking mutability and arising from

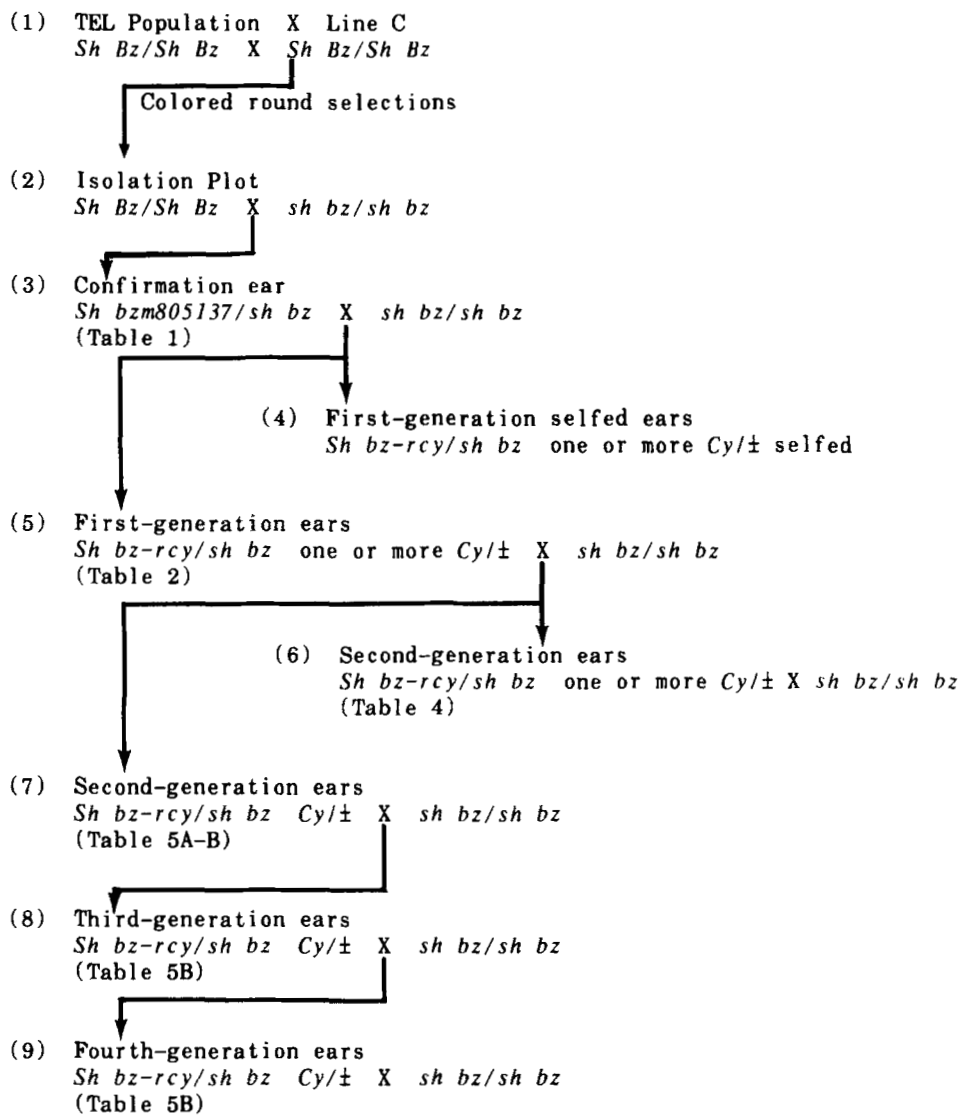

FIGURE 1.—Flow diagram illustrating the origin of *bz-rcy* and of the lines used to establish the inheritance of *Cy*. Arrows indicate lines of descent. Unless otherwise indicated bronze round spotted selections were taken each generation. Parenthetical values are cross numbers.

this cross is not evidence for loss of receptor function, assuming sib ears yield mutability in this test.

In the second test, regulatory element function was assayed using a variable number of bronze shrunken nonspotted selections (but always at least five) from a nonspotted ear or ear sector (Figure 2). These kernels, which would be expected to segregate for *Cy* if the regulatory element function were still present, were tested for *Cy* activity in crosses by *bz-rcy* (Figure 2). The appearance of mutability in some of the resulting ears demonstrates that an active *Cy* is segregating in the nonspotted tissue. If however, five or more such ears lack mutability, the chance of this result being due merely to segregation is 1/32 ($1/2^n$, n = number of ears tested, SEDCOLE 1977), or less (if more than one *Cy* were present). If at this point the receptor has been shown to be active, it can be concluded that the loss of mutability was due to the loss of *Cy* activity.

Estimation of the number of *Cy* segregating on an ear: Goodness of fit χ^2 tests were used to estimate the number of *Cy* segregating on an ear. The observed ratio of spotted to nonspotted kernels that were also round was tested against the 10 ratios expected if 1–10 *Cy* were segregating. The first three of these ratios can be calculated from Table 1 by multiplying the indicated percentages by two (to correct for the exclusion of shrunken kernels). The rare ears with ambiguous ratios were classified in such a way as to underestimate the rate of *Cy* increase.

RESULTS

Heritability of the mutable phenotype: To confirm the heritability of the exceptional spotted bronze kernel rescued from the isolation plot (MATERIALS AND METHODS and cross 2 in Figure 1), the plant grown from it was crossed by *sh bz* to generate the confirmation ear (cross 3 in Figure 1). The heritability of the mutable phenotype, and its association with the *Bronze1* locus was established by the appearance of bronze mutable kernels on this ear (80 5137/5621, Table 1). The ratio of spotted to nonspotted kernels on this ear is not significantly different from the expectation for a two-element system with two independently segregating regulatory elements, *i.e.*, *Sh bz-rcy/sh bz Cy/+ Cy/+*, where *bz-rcy* represents the receptor and *Cy* the regulatory element of the two-element system. However, this ratio could also be explained

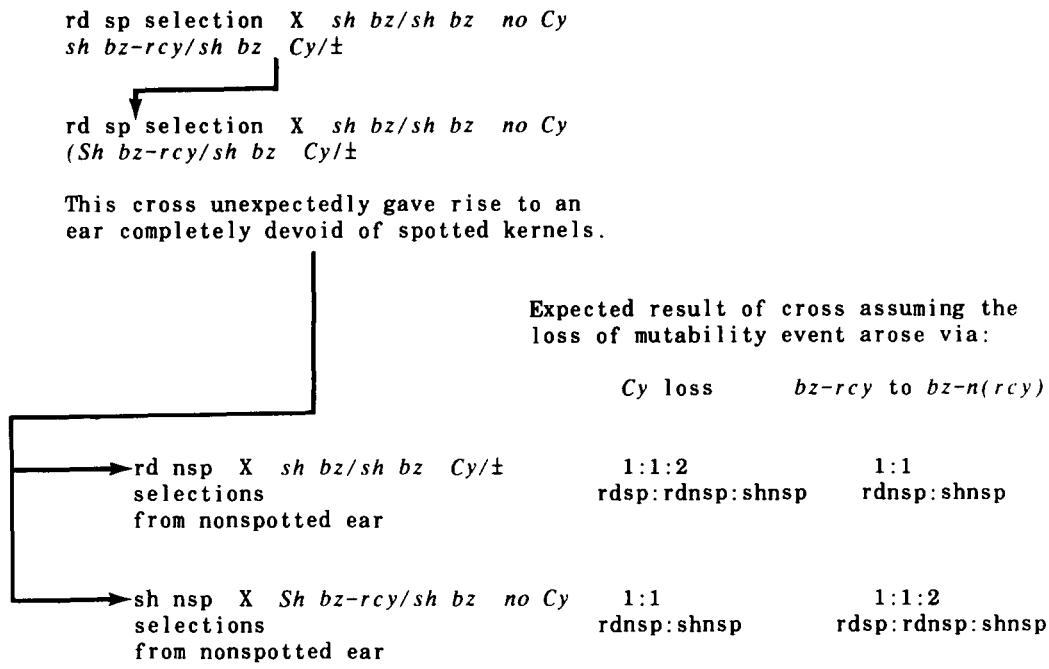

FIGURE 2.—Tests to determine the basis of loss-of-mutability events that arose via discordant plants (the phenotype of the selected kernel and the resulting ear unexpectedly did not agree). Similar tests were performed on ear and plant sectors exhibiting loss-of-mutability events by using bronze round nonspotted and bronze shrunken nonspotted selections from within the region of loss. Kernel phenotypes are indicated as follows: rd = round, sh = shrunken, sp = spotted, nsp = nonspotted. All kernels were bronze. Ratios assume no crossovers. Arrows indicate lines of descent.

TABLE 1

Phenotypic frequencies associated with the confirmation ear (cross 3, Figure 1) and expected segregation ratios under a nonautonomous model with one, two and three *Cy* segregating

	Kernel No. (%)			
	Bronze round		Bronze shrunken	
Confirmation ear	Spotted	Nonspotted	Spotted	Nonspotted
1. 80 5137/5621	86 (32.3)	26 (9.8)	2 (0.8)	152 (57.1)
2. One *Cy*	(24.5)	(25.5)	(0.5)	(49.5)
3. Two *Cy*	(36.75)	(13.25)	(0.75)	(49.25)
4. Three *Cy*	(42.875)	(7.125)	(0.875)	(49.125)

The ratio of spotted round:nonspotted round on the confirmation ear is not significantly different at the 0.01 level from the expectation for the segregation of two *Cy* (with a χ^2 value of 0.621 and 1 d.f.). However, this ratio is significantly different from the segregation of one and three *Cy* (with χ^2 values of 34.60 and 7.37, respectively, and with 1 d.f.). The spotting pattern on this ear was, in most cases, fine-high (SCHNABLE and PETERSON 1986), although three individual kernels, each of which displayed exceptional patterns (fine-low), were also present. The values presented in lines 2–4 are expected segregation ratios.

by an autonomously controlled allele that generates nonresponsive derivatives at a high rate. An autonomously controlled allele is one where a regulatory element resides at the controlled locus.

Demonstration that *bz-rcy* is the receptor of a two-element system: To test the inheritance of the mutability of *bz-rcy*, plants grown from spotted selections from the confirmation ear (cross 3 in Figure 1) were backcrossed by or on a *sh bz* line (cross 5 in Figure 1) which is completely devoid of *Cy* activity (SCHNABLE and PETERSON 1986) to generate first-generation ears. If, as the nonautonomous model predicts, the common parent of these ears (80 5137/5621, Table 1) did in fact carry two *Cy*, then this model would also predict (assuming Mendelian segregation) that these progeny ears should exhibit segregation ratios suggestive of one and two independent *Cy* (lines 2 and 3 in Table 1). Although most of the first-generation ears displayed ratios which were consistent with these expectations (Table 2) and thereby support the nonautonomous model, unexpected segregation patterns, suggestive of more than two *Cy*, were also present (discussed later). These results made it impossible to settle, without further tests, the question of whether *bz-rcy* is the receptor for a two-element system in which the regulatory elements are capable of replication or is an autonomously mutable allele that generates nonresponsive alleles at various rates a situation known to

TABLE 2

Phenotypic frequencies from crosses of plants with the genotype *Sh bz-m805137/sh bz* and grown from bronze spotted kernels selected from the confirmation ear, 80 5137/5621 (Table 1), by or on *sh bz* (cross 5, Figure 1)

Cross[a]	Kernel No. (%) Bronze round Spotted	Bronze round Nonspotted	Bronze shrunken Spotted	Bronze shrunken Nonspotted	No. *Cy*[b]	χ^{2c}
A. Cross = *Sh bz-m805137/sh bz* × *sh bz/sh bz*						
1. 80g37-1/44	52 (22.9)	51 (22.5)	2 (0.9)	122 (53.7)	1	0.09
2. 80g37-2/44[d]	118 (41.8)	20 (7.1)	7 (2.5)	137 (48.7)	3	0.01
3. 80g37-3/45	64 (21.3)	72 (24.0)	2 (0.7)	162 (54.4)	1	0.21
4. 80g37-4/44	16 (14.0)	29 (25.4)	1 (0.9)	68 (59.6)	1	3.25
5. 80g37-6/45[d]	148 (52.5)	29 (10.3)	4 (1.4)	101 (35.8)	3	0.66
6. 80g37-7/45	101 (41.6)	37 (15.2)	1 (0.4)	104 (42.8)	2	0.01
7. 80g38-3/44[d]	123 (45.2)	5 (1.8)	4 (1.5)	140 (51.5)	4	3.05
8. 80g38-5/45[d]	80 (31.5)	10 (3.9)	9 (3.5)	155 (61.0)	3	0.73
9. 80g38-6/45	63 (26.9)	62 (26.5)	0 (0.0)	109 (46.6)	1	0.10
10. 81 2012-1/1950	33 (25.6)	27 (20.9)	6 (4.6)	63 (48.8)	1	0.86
11. 81 2012-3t/2131[d]	51 (41.5)	6 (4.9)	1 (0.8)	65 (52.8)	3	0.65
12. 81 2012-9/1951	108 (27.8)	99 (25.4)	3 (0.8)	179 (46.0)	1	0.83
B. Cross = *sh bz/sh bz* × *Sh bz-m805137/sh bz*						
13. 81 3463x/4410-3	47 (37.6)	15 (12.0)	2 (1.6)	61 (48.8)	2	0.17
14. 81 3463x/4410-6	72 (32.0)	67 (29.8)	3 (1.3)	83 (36.9)	1	0.43
15. 81 4141-6t/4410-7	61 (46.9)	15 (11.5)	1 (0.8)	53 (40.8)	2	1.79
16. 81 3463x/4410-8	38 (24.2)	43 (27.4)	1 (0.6)	75 (47.8)	1	0.14
17. 81 3463x/4410-9t	89 (33.3)	51 (19.1)	3 (1.1)	124 (46.4)	—[e]	

[a] Only ears with greater than 100 kernels are included.

[b] Minimum number of independently segregating *Cy* to obtain a nonsignificant χ^2 value at the 0.05 level (based on round phenotypic classes only).

[c] χ^2 value associated with segregation ratio expected for the segregation of the number of independent *Cy* elements indicated in the previous column.

[d] Ears in which the fraction of spotted kernels was greater than expected if two independent *Cy* elements were segregating. For two of the five exceptional ears (80g37-2/44 and 80g37-6/45) the only ratio that fits the χ^2 test is the expectation for the segregation of three *Cy*. For the remaining three ears, the ratios of spotted to nonspotted fit more than one expected ratio (but in all three cases the ratios suggest that three or more *Cy* are present).

[e] The observed ratio is significantly different at the 0.05 level from the expected ratios for all possible numbers of independently segregating *Cy*.

exist for the autonomously mutable *a-m(papu)* (PETERSON 1970).

To test for independently segregating *Cy* elements, plants grown from nonspotted round and sib nonspotted shrunken kernels selected from ears segregating for mutability were intercrossed. If independently segregating regulatory elements are responsible for the diverse ratios observed among the first-generation ears (Figure 1 and Table 2), more than half of the nonspotted shrunken selections should contain at least one regulatory element (*Cy*), and the noncrossover nonspotted round selections should (based on the close linkage between *Bz* and *Sh*) carry *bz-rcy*. Assuming these assumptions were correct, more than half of the progeny ears resulting from this intercross should have segregated for bronze mutability. This was indeed the case. Representative data from these crosses are shown in Table 3 demonstrate that mutability at *bz-m805137* is indeed dependent upon the interaction of two classes of elements, the *cis*-located receptor element, *rcy*, at the *bz* locus, and independently segregating regulatory elements, *Cy*. Mutability at *bz-m805137* therefore represents an interaction between *bz-rcy* and *Cy*. Phenotypes are conditioned as follows:

bz-rcy, no *Cy* = stable bronze

bz-rcy, *Cy* = fully colored spots on a bronze background.

Cy increases: In the test for independently segregating regulatory elements, approximately half of the shrunken selections from two of the families which had only one *Cy* segregating (80g37-1 and 80g37-3), elicited mutability (Table 3). This result indicates that half of the shrunken selections carried one *Cy*, and half lacked *Cy*, as expected. Family 80g37-6, which had three *Cy* segregating, produced progeny with one, two, and three *Cy* (Table 3), which also agrees with the expectation. Family 80g37-7, however, with two *Cy* segregating, produced one unexpected progeny that exhibited a ratio suggestive of three *Cy* (Table 3). Such a result is not in agreement with Mendelian segregation.

Further evidence on non-Mendelian segregation

TABLE 3

Results of crosses designed to test for the presence of a two-element (nonautonomous) control of mutability

Family[a]	No. of *Cy* in family[b]	Presence of mutability[c]	Frequency distribution of No. of *Cy* segregating on progeny ears[d]			
			1 *Cy*	2 *Cy*	3 *Cy*	—*
80g37-1	1	+ (10/17)	12	0	0	2
80g37-3	1	+ (7/18)	3	0	0	3
80g37-6	3	+ (6/9)	2	1	1	0
80g37-7	2	+ (7/14)	3	0	1	2

Paired selections (nonspotted bronze round and shrunken) for the reconstitution of mutability test were drawn from four First Generation Ears (cross 5, Figure 1) and were intercrossed as follows: *Sh bz-rcy/sh bz* +/+ X *sh bz/sh bz Cy*/+.

[a] A family is composed of all the progenies derived from a single ear arising from cross 5, Figure 1.

[b] Estimated from Table 2.

[c] Test for a two-element control of mutability: (+) designates the reconstitution of mutability in the indicated cross. (+) demonstrates that mutability requires the interaction of two independent factors. Parenthetical values represent the number of *sh bz/sh bz* + *Cy* males which contained *Cy* within a family/total in the family.

[d] Minimum number of independently segregating *Cy* to obtain a nonsignificant χ^2 value at the 0.05 level (based on the ratio of spotted round:nonspotted round). Only ears with greater than 100 kernels are included. Some males were used in more than one cross. For these two reasons the number of ears in this frequency distribution exceeds the total number of positive males in the previous column.

* Phenotypic ratio is significantly different (at the 0.05 level) from the expected for all possible numbers of *Cy*. In six of seven cases the lack of fit from the expected ratio for one *Cy* was due to an excess of nonspotted kernels.

TABLE 4

Determination via testcrosses (cross 6, Figure 1) of the No. of *Cy* in spotted progeny from five exceptional first-generation ears (cross 5, Figure 1 and note *d* in Table 2)

Exceptional ear	No. of progeny with	
	Two or less *Cy*	More than two *Cy*
80g37-2/44	5	20
80g37-6/45	7	7
80g38-3/45	5	10
80g38-5/45	7	7
81 2012-3t/2131	8	3

Ears with ambiguous ratios were grouped with the two or less *Cy* class.

was obtained from the first-generation ears (Table 2). If the two *Cy* elements present in the confirmation ear followed Mendelian segregation spotted kernels from the confirmation ear (Table 1) would be expected to contain one and two *Cy* elements in a 2:1 ratio. However, five ears (note *d* in Table 2) resulting from testcrosses of progeny from the confirmation ear (cross 5, Figure 1), exhibited a higher percentage of spotted kernels than would be predicted by the segregation of two *Cy* elements. These exceptional ratios were not the result of coupling linkage between *Cy* and *bz-rcy* (our unpublished data), but they could be explained by the presence of more than two *Cy* elements in the plants that bore these ears. If these five exceptional ratios are due to the segregation of more than two *Cy* elements in the affected plants, a fraction of the spotted kernels from these exceptional ears should themselves carry three or more *Cy* elements. To confirm that these exceptional ratios are in fact the result of the segregation of more than two *Cy*, the number of *Cy* elements present in spotted round selections from these five ears was determined by testcrossing spotted selections by *bz sh* (cross 6 in Figure 1) to generate second-generation ears.

The results of these testcrosses (Table 4) are consistent with the segregation of three or more *Cy* in the five exceptional ears: some spotted kernels from each of the five ears carried more than two *Cy*. Reconciliation of the finding that the confirmation ear carried only two *Cy* while five of 17 of its progeny carried three or more *Cy*, requires that increases in the number of *Cy* occurred between the time of ear initiation on 80 5137 and the times of ear initiation on its progeny which contained more than two *Cy*. These increases in *Cy* numbers will be referred to as first-generation increases.

The unexpectedly high number of second-generation ears carrying more than two *Cy* (Table 4) provides evidence for second-generation increases in *Cy* numbers, *i.e.*, after ear initiation on a first-generation plant and prior to ear initiation on its second-generation progeny. Two of the five exceptional first-generation ears (37-2 and 37-6) contained three *Cy*, based on segregation ratios (note *d* in Table 2). Based on Mendelian segregation of unlinked *Cy* elements only one-seventh of the progeny from such plants were expected to carry more than two *Cy*. The frequency of progeny carrying more than two *Cy* is significantly different from this expectation in both instances (Table 4). The excess is likely a result of increases in the number of *Cy* in some progeny. Third-generation increases were also observed (our unpublished data).

Estimation of the rate of *Cy* increase in main-stalk ears: Given that the number of *Cy* elements increased in at least some lines, it was important to determine whether it is possible to maintain lines carrying a single *Cy* and at what rate a plant carrying a single *Cy* gives rise to progeny carrying two or more *Cy*.

Eight first-generation ears exhibited ratios of spotted:nonspotted kernels which demonstrated that these ears were segregating for a single *Cy* element (Table 2). The rate at which one *Cy* gives rise to two *Cy* was estimated by testing spotted bronze round selections from the first six of these first-generation (one-*Cy*) ears for the number of *Cy* they carried (via testcrosses to produce second-generation ears; cross 7 in Figure 1). Any ear from one of these testcrosses on which the

TABLE 5

Rate of *Cy* increase in one-*Cy* lines: summary of the results of testing 95 gametes in six primary families (crosses 7–9, Figure 1)

Primary family	Progeny with: No increase	Progeny with: Increase	Percent of progeny with *Cy* increases	Confidence limits[a] (%)
A. Results grouped by and pooled over primary families:				
80g37-1	8	0	0	
80g37-3	17	9	34.6	
80g37-4	5	1	16.6	
80g38-6[b]	33	4	10.8	
81 2012-1	8	0	0	
81 2012-9	10	0	0	
Pooled total[c] (without/ 80g37-3)	64	5	7.8	2.58–17.3
80g37-3 alone	17	9	34.6	17.2–55.7
B. Results within the generations of primary family 80g36-6:				
Generation				
2	14	1		
3	14	1		
4	5	2		
Total[d]	33	4		

Ears with ambiguous ratios were grouped with the no increase class in order to provide a conservative estimate of the rate of *Cy* increase.

[a] It is 95% certain that the true rate of increase in the number of *Cy* in one-*Cy* lines lies within this interval. These values were calculated by interpolation within Tables 5 and 6 of MAINLAND *et al.* (1956).

[b] Pooled over generations, see Part B of this table.

[c] The fact that the homogeneity χ^2 value (as described by STEELE and TORRIE 1980) was large enough to rule out homogeneity suggests that the rate of increase in the number of *Cy* in primary family 80g 37-3 may be higher than in the other primary families. For this reason data from this primary family were partitioned out. After the removal of data associated with primary family 80g 37-3 the homogeneity χ^2 value was too small to rule out homogeneity (3.52 with 4 d.f.), hence data were pooled over the remaining primary families.

frequency of spotted kernels is not statistically different than 36.75% or more (Table 1) is expected to carry two (or more) *Cy* and represents a change from one *Cy* to two (or more) *Cy* that occurred prior to ear initiation. Further data on the rate of *Cy* increase were obtained in the 80g38-6 primary family (a primary family is composed of all the descendants of a single first-generation ear) when spotted bronze round selections from a second-generation one-*Cy* ear were backcrossed to *sh bz* (cross 8 in Figure 1) to produce third-generation ears, on which it was possible to estimate the number of *Cy* present. One of these third-generation ears which carried a single *Cy* was itself progeny tested for *Cy* number via cross 9 (Figure 1).

The results of this series of testcrosses are summarized in Table 5. Fourteen of the 95 tested gametes gave rise to ears carrying two or more *Cy* (Table 5A). These 14 represent changes from one *Cy* to two (11 individuals) or more (3 individuals) *Cy*. Table 5 also contains an estimate of the rate of *Cy* increase. It is important to note that only upper main-stalk ears were included in Table 5; tiller and lower main-stalk ears were excluded. Hence the rate of *Cy* increase is being estimated in upper main-stalk ears only.

The homogeneity test described in Table 5A (note *c*) yielded a χ^2 value large enough to rule out homogeneity, *i.e.*, there is evidence that the rate of *Cy* increase is not uniform among primary families. Since the common parent of all the primary families (80 5137/5621 in Table 1) contained two *Cy*, which conceivably could differ in the rate at which they multiply, the same *Cy* is not necessarily present in each of the primary families (each of which received only one *Cy* from the common parent). Most of the heterogeneity was contributed by primary family 80g37-3 (in which the rate of *Cy* increase is 34.6%, Table 5A). Hence it may be appropriate to partition out the results of this family. When this is done the rate of *Cy* increase in the remainder of the population is found to be approximately 8% (Table 5A).

Estimation of the rate of *Cy* increase in tiller ears: In certain instances a lack of correspondence was observed between the estimates of the number of *Cy* in a main-stalk ear and the corresponding tiller ear(s). (A tiller is a basal branch.) This can be explained by an increase in *Cy* numbers in a plant sector encompassing the tiller ear initials, but not the main-stalk ear initials (or vice versa). These are similar to plants discordant for *Spm* copy number recorded by MCCLINTOCK (1957, 1958).

Four such instances were recorded out of the ten plants for which both a main-stalk ear and a tiller ear were available. All four discordant plants experienced a *Cy* increase in the tiller ear; in no instances did an increase in *Cy* numbers occur in a main-stalk ear and not in the corresponding tiller ear.

Of the ten plants studied, three belong to primary family 80g37-3 and were excluded from further analysis because the *Cy* in this primary family appears to differ from the *Cy* in the rest of the one-*Cy* primary families (see previous section). Of the remaining seven, three exhibited tiller sectors of *Cy* increase. This rate (3/7) is significantly different from the rate of *Cy* increase observed in main-stalk ears (5/69, Table 5A). Hence it appears that the rate of *Cy* increase in one-*Cy* lines is higher in tillers than in main stalks, however it should be noted that this conclusion is based on a small sample.

Non-Mendelian losses of *Cy* activity: Sixteen ears and three plants were isolated that were sectored for *bz-rcy/Cy* mutability. These sectors were evidenced by the appearance of regions of stable bronze expression in tissue that would otherwise be expected to exhibit mutability due to the interaction of *bz-rcy* and *Cy*. Additionally, 16 discordant plants were recovered. Discordant plants exhibit a lack of correspondence between the endosperm phenotype of the planted kernel (spotted) and the endosperm phenotypes of the progeny kernels (nonspotted) (Figure 2). Crosses were in all instances established such that heterofertilization (SPRAGUE 1929, 1932), could not account for the discordant phenotype.

Each of these 25 loss-of-mutability events is due to the absence of either the regulatory element function or the receptor function. To determine the basis for these losses of mutability two tests were performed on progeny kernels arising from within the loss sector. These tests are described in MATERIALS AND METHODS.

The absence of regulatory element function (Cy losses) accounted for 10/16 ear sectors, 2/3 plant sectors and 13/16 discordant plants. The remainder were shown to represent losses of receptor function, *i.e.*, mutations from *bz-rcy* to *bz-n(rcy)*.

Twenty of the 25 cases of *Cy* loss arose in a potential population of 936 individuals (not all planted kernels yielded ears) in 25 primary families. The percent loss of *Cy* activity over the pooled samples is therefore at least 2.14 (with confidence limits of 1.47–3.37%). It is proper to pool across primary families because there is no statistical evidence to suggest that primary families differ in their capacity to undergo *Cy* loss events.

When the rate of *Cy* loss is plotted against the number of *Cy* present in the parental ear a trend is observed. Although not statistically significant, parental plants that carried a single *Cy* produce more *Cy* loss progeny than parental plants that carry more than one *Cy*. The rates of *Cy* loss in progeny are 2.75%, 2.68%, 1.64% and 0.80% for parents with 1, 2, 3, and more than 3 *Cy*. This means that progeny that are most likely to carry a single *Cy* (those from one-*Cy* parents) may be most likely to undergo a *Cy* loss event. Conversely, progeny that are most likely to carry more than a single *Cy* (progeny from parents which themselves carried more than one *Cy*) may be less likely to exhibit *Cy* loss events.

DISCUSSION

The 1979 isolation plot was established with the intention of obtaining *En* insertion mutants at *Bz*. It is worth noting that even though each of the females in the plot contained at least one *En* (at the *A* locus), the only *bz-m* isolated was not *En*-related (SCHNABLE and PETERSON 1988), but was instead responsive to *Cy*, a previously undescribed regulatory element. *Cy* is abundant in the progenitor TEL population (SCHNABLE and PETERSON 1986) but its presence there had remained undetected until the discovery of a suitable responsive allele.

Three lines of evidence indicate that *Cy* behaves as a near-Mendelian factor. 1) Only 25 of 263 crosses exhibited ratios of spotted to nonspotted that did not conform to any Mendelian ratio (our unpublished data). 2) It was possible to maintain one-*Cy* lines for four generations. 3) Ears that exhibit few if any nonspotted kernels (*e.g.*, line 7 in Table 2) can be explained either by *bz-rcy* mutability being inherited in a non-Mendelian fashion (like the *Mutator* phenomenon) or by the Mendelian segregation of many discrete *Cy* elements. That the latter interpretation is correct is demonstrated by the recovery of one-*Cy* lines from ears that had very few nonspotted kernels (our unpublished data).

If *Cy* behaved as a true Mendelian factor, a plant heterozygous for one *Cy* should give rise to only two classes of progeny: those with one *Cy* and those with none. Although the transmission of *Cy* elements usually follows this pattern, some exceptional progeny carry two or more *Cy*, while others that receive a *Cy* element later lose it; these phenomena are termed *Cy* increase and *Cy* loss respectively.

Cy increases can be explained by either of two phenomena: activation of previously silent elements or transposition-mediated replication. Transposable element activations are rare events in other systems (MCCLINTOCK 1950; DOERSCHUG 1973; DEMPSEY 1985; NEUFFER 1966; FEDOROFF 1986; PAN and PETERSON 1986) and no *Cy* activations have occurred in *bz-rcy* lines that lacked *Cy* (unpublished observation). Therefore it is likely, but not certain, that the majority, if not all, of the *Cy* increases are the result of transposition-mediated *Cy* replication.

Two kinds of transposition, replicative transposition and excision-dependent transposition (BRINK and NILAN 1952; GREENBLATT and BRINK 1962, 1963; GREENBLATT 1966, 1968, 1974, 1984), can result in transposable element replication. However, although replicative transposition could result in *Cy*-increases, because it does not involve excision of the parental element, it could not generate *Cy* losses.

The second kind of transposition, excision-dependent, involves the excision of a transposable element and its subsequent insertion elsewhere. This transposition mechanism has been well documented and studied using *Mp* at the *P-VV* allele. BRINK and NILAN (1952), GREENBLATT and BRINK (1962, 1963) and GREENBLATT (1966, 1968, 1974, 1984) have demonstrated that after *P-VV* replication one of the daughter *Mp* elements occasionally excises. If the excised *Mp* element inserts into an as yet unreplicated chromosome segment one daughter cell from a mitotic division will have two *Mp* elements (CHEN, GREENBLATT and DELLAPORTA 1987). Hence, excision-dependent transposition is also potentially capable of generating *Cy*-increases.

About 40% of the *Mp* excisions from *P-VV* result in ear sectors that lack an *Mp* (GREENBLATT and BRINK 1962, 1963; GREENBLATT 1968). Although it is not clear how these sectors arise the important point is that some daughter cells from a parental cell that underwent an excision-dependent transposition do not carry *Mp* (GREENBLATT and BRINK 1962, 1963; GREENBLATT 1968). Hence, one distinctive difference between elements that transpose via a replicative mechanism versus those which, like *Mp*, utilize excision-dependent mechanisms is that the latter class of element should exhibit occasional transposition-related losses.

Cy losses could arise via either of two mechanisms: deactivation or as a consequence of excision-dependent transposition. Proof that the observed *Cy* losses are transposition-dependent would demonstrate that *Cy* transposes in an excision-dependent manner. The finding that *Cy* losses are more likely to occur in the progeny of one-Cy plants suggests that *Cy* loss is not the result of a general deactivation of all the *Cy* elements present in a genome, but rather occurs to each element independently of the others. This is compatible with excision-based losses. It may be possible to further the argument that *Cy* losses are related to transpositions by analyzing altered linkage behaviors of derivatives of the *Cy* element that was recently mapped to chromosome *5L* (PETERSON 1988).

The findings that *Cy* increases can best be explained by transpositions and excision-dependent transpositions may account for the observed *Cy* losses, provide suggestive evidence for the ability of *Cy* to undergo excision-dependent transposition. This does not preclude the possibility that some *Cy* losses are the result of inactivations or that *Cy* is also capable of replicative transposition in addition to excision-dependent transposition. This latter point is significant because of the relation between *Cy* and *Mutator* and because *Mu* elements are thought to engage in replicative transposition (ALLEMAN and FREELING 1986).

The rate of *Cy* increase varies among one-*Cy* lines. Because the common parent of all the one-*Cy* lines contained two *Cy*, one-*Cy* lines derived from that common parent would be expected to carry only one of these *Cy* elements. This fact can be used to explain the heterogeneity among the rates of *Cy* increase among one-*Cy* lines by assuming the two *Cy* elements differ in the rate or timing at which they transpose. Such differences have been observed among various isolates of the *En/Spm* system (MCCLINTOCK 1961).

Another possibility is that the more active form of *Cy* is actually two or more closely linked *Cy*. Although such a complex would be nearly indistinguishable from a single *Cy* in segregation patterns, it would be expected to yield more transpositions to independent insertion sites.

The difference in rates of *Cy* increase between mainstalks and tillers is presumably a consequence of a higher absolute amount of transposition in the latter tissue, which may reflect a higher rate of transposition per cell cycle. Such a phenomenon would be consistent with the ability of at least one other regulatory element (*En-v*) to be differentially affected by main-stalk versus tiller environments (FOWLER and PETERSON 1978). The appearance of these sectored plants also demonstrates that *Cy* transposition at least often follows fertilization. This is in contrast to *Mu1* elements—which appear to transpose prior to fertilization (ALLEMAN and FREELING 1986). Supporting evidence regarding the timing of *Cy* transposition comes from the highly active *Cy* in primary family 80g37-3 which has given rise to nine cases of *Cy* increase (34%, Table 5). A rate of transposition prior to or during meiosis in a plant carrying a single copy of *Cy80g37-3* sufficient to result in 34% of the spotted progeny carrying two *Cy* would substantially distort the ratio of spotted to nonspotted kernels on the resulting ear. Because such a distortion was not observed on the ear (80g37-3/45, Table 2) which gave rise to these nine instances of *Cy* increase it can be concluded that the transpositions which generated these *Cy* increases occurred postmeiotically.

Other factors may influence the rate of *Cy* increase. All five instances of *Cy* increase that appear in Table 2 arose in ears as opposed to tassels (Table 2, part A *vs.* part B). This may reflect a difference in the rate of *Cy* increase in different tissues. Alternatively, because four of the five instances of *Cy* increase in Table 2 arose on plants grown in a winter nursery ('80g ears) as opposed to the Ames summer nursery ('81 ears), and because all of the winter nursery plants are in part A of Table 2, the imbalance between parts A and B of Table 2 may reflect the role of the external environment on the rate of *Cy* increase. A number of investigators have shown that altered external environments can affect transposition (EYSTER 1926; PETERSON 1958; RHOADES 1941; VAN SCHAIK 1955). Unfortunately neither of these hypotheses can be tested from existing data.

Two to three percent of the gametes from plants homozygous for *Ac* carry one of several *Ac* constitutions, each of which must arise via transposition (McCLINTOCK 1949). In contrast, 9% of the gametes derived from plants carrying one *Cy* eventually produce ears which yield evidence of transposition prior to ear initiation. The 9% value is the sum of the rate of *Cy* increase (7.8%) and the rate of *Cy* loss discordant plants (1.2%). By using this lower value for the rate of *Cy* loss (which excludes *Cy* losses that arose as ear and plant sectors) the data from the two studies (*Ac* and *Cy*) can be more reasonably compared. However, one unavoidable difference between the two studies is that while the *Ac* gametes were studied as kernels, the genetic constitution of the *Cy* gametes was necessarily assayed by the ears to which these gametes eventually gave rise. This longer interval in the *Cy* study which would allow for more transpositions may account for the higher rates observed.

Although regulatory elements of many of the maize transposable element systems, including *Ac*, *Uq*, *Dt*, *En*, and *Bg* (McCLINTOCK 1949; FRIEDEMANN and PETERSON 1982; DOERSCHUG 1976; NOWICK and PETERSON 1981; SALAMINI, BREMENKAMP and MAROTTA 1982), have been observed to exhibit increases and/or losses, rates have been established for only *Spm* (which is genetically and molecularly homologous to *En*). Out of 249 spotted selections from ears carrying one *Spm*, McCLINTOCK (1956) obtained rates of *Spm* increase and loss of 10.4% and 3.2%; the comparable figures for *Cy* are 7.8% and 2.14%. Hence, *Cy* (excluding the *Cy* in primary family 80g37-3) and *Spm* appear to be similar in the rate at which they multiply and are lost. In contrast, the *Cy* in primary family 80g37-3 multiplies at a much more rapid rate than the *Spm* element described by McCLINTOCK.

It is significant that although the *Cy* system and *Mutator* show a certain degree of functional homology (SCHNABLE and PETERSON 1988), the inheritance of *Cy* elements differs markedly from that of *Mu* elements. Although *Cy* elements exhibit some deviations from Mendelian inheritance as a result of *Cy* increases and *Cy* losses, they can still be tracked as single dominant genetic units. In contrast to the results of ALLEMAN and FREELING (1986) who found that each *Mu1* transposes once each generation (as demonstrated by the maintenance of *Mu1* copy numbers), a much smaller fraction of *Cy* elements appear to transpose in any given generation. This difference in rates of increase may partially reflect the selective pressure for high rates of transposition to which *Mutator* has been exposed (BENNETZEN *et al.* 1987). However, it should be noted that BENNETZEN *et al.* (1987) have observed a substantially lower transposition rate for *Mu1* (25% new bands in outcross progeny in contrast to the 50% noted by ALLEMAN and FREELING 1986) which, although still higher, is more in agreement with that seen for the more active form of *Cy*; the rate of 34% *Cy* increase observed for *Cy80g37-3* is expected to be equivalent to 17% new bands because the rate of *Cy* increase was derived from spotted kernels which comprise only 50% of the progeny.

In contrast to the situation with *Mu1* observed by ALLEMAN and FREELING (1986) the rate of *Cy* increase in one-Cy lines is not sufficient to maintain the parental copy number on average in outcross progeny. However, because the rate of *Cy* increase is higher (7.8% or 34.6%) than the rate of *Cy* loss in the progeny of one-Cy plants (2.75%), it appears likely that once *Cy* enters a population its gene frequency will tend to increase even in the absence of position selection pressure. This is particularly true in that the genetic test for *Cy* increase has the inherent limitation that it can not reveal the appearance of new *Cy* elements closely linked to the parental position. And because *Ac* and *En* (NOWICK and PETERSON 1981), at least, have been shown to preferentially transpose to linked locations this genetic test may have underestimated the true rate of *Cy* transposition.

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