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Somatic Mutator activity expression is dependent on the strength of *Cy* trans-active signals in maize *

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Summary. Two receptor element alleles (*vp-rcy* and *bz-rcy*) that respond to the trans-active element (*Cy*) controlling Mutator activity were used to analyze the strength of trans-active signals from *Cy* elements derived from a Mutator active line. Evidence is presented that the Mutator population tested consists mainly of a class of weak *Cy* elements designated as *Cy*:*Mu*. When *Cy*:*Mu* element are present in only a few copies, the strength of the combined transposition signal is weak. It is only when these active elements have a high copy number that the overall transposition signal is sufficiently strong enough to elicit a high frequency of transposition events. This study seeks to investigate the nature of the trans-active signal from *Cy*:*Mu* elements. The implication of these results for molecular studies is discussed.

Key words: Mobile elements – *Cy*:*Mu* – *Cy* – Transactive signals

Introduction

"Mutator activity" was identified as a higher than normal mutation rate in a specific maize line. The original line exhibited a 30-fold greater than normal mutation rate, and all related lines containing this trait are called Mutator lines (Robertson 1978). By definition, Mutator activity is a phenotypic phenomenon based on mutation rate and not a discrete genetic entity; thus, early genetic analyses of Mutator were unlike those used for other transposable element systems. Numerous studies have attempted to identify the mechanism of the Mutator-induced high mutation rate. Early genetic studies using quantitative approaches concentrated on Mutator activity transmission and its general behavior. One of the features revealed by these studies was that, upon outcrossing, 90% of the progeny exhibited Mutator activity (Robertson 1978). Mutator activity occurs late in plant development (Robertson 1980). This activity can be deactivated by inbreeding for or outbreeding away from the Mutator trait (Robertson 1978, 1983; Bennetzen 1985).

Molecular studies have shown that Mutator activity is closely related to a class of transposable elements called *Mu* (Bennetzen 1984; Bennetzen et al. 1984). Several *Mu* elements have been identified, with the 1.4-kb *Mu1* element being the most prevalent (Bennetzen 1984; Alleman and Freeling 1986). At present there is no evidence that any of these isolated *Mu* elements can code for their own transposition. It is most probable that most or all of the *Mu* elements identified thus far are not active, but do respond to an active trans-element (Bennetzen 1985). Therefore, *Mu* elements have similar properties to other known maize transposable element systems, where an inactive element (receptor element), such as *Ds* or *I*, can transpose in the presence of an active element (regulatory element), *Ac* and *En*, respectively.

Genetic and molecular experiments (Schnable and Peterson 1988, 1989; Scheffler and Peterson 1985, 1986) have shown that Mutator activity, including the *Mu1* element located at the *a1-Mum2* allele, is under the control of a classical two-element system (*Cy-rcy*), as defined by McClintock (1955) and Peterson (1965). The active element (*Cy*) behaves as a near-Mendelian unit and regulates the transposition of the receptor element, *rcy* (Schnable and Peterson 1988, 1989). At least two receptor mutants have been identified; one is at the *Vp1* locus, [*vp-rcy* (Scheffler and Peterson 1985)], and the other is located in the *Bz1* locus, [*bz-rcy* (Schnable and Peterson

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1984)]. Transposition of the *rcy* element away from the locus can be monitored by each of the reporter alleles, and this is visualized by the expression of colored spots on the kernel, indicating the restoration of the activity of the locus. The original isolate of the *bz-m805037* allele that is conditioned by a *Cy bz-rcy* interaction as well as the recently revealed *Cy*-controlled *a2-m668219* allele (Peterson 1978, 1988) were from a maize population that was not related to Robertson's active Mutator lines (Schnable and Peterson 1986).

Previous genetic and molecular studies on Mutator lines have been limited by the lack of well-defined receptor element alleles. In this paper, the activity and behavior of *Cy* elements, which we have designated as *Cy*:*Mu* elements to distinguish them from the original isolates of *Cy* elements that originated from unrelated Mutator lines and from those derived from a Mutator active line, are discussed with respect to their interaction with two reporter alleles, *vp-rcy* and *bz-rcy*.

Materials and methods

Two loci involved in anthocyanin production in the aleurone tissue were used in these experiments. The first is *Bzl*, which codes for UFGT and is responsible for purple anthocyanin, whereas the recessive allele (*bz*) gives rise to bronze coloration (Coe and Neuffer 1977). The other locus used in these studies is *Vpl*, which is also needed for purple anthocyanin in the aleurone, but the normal recessive allele (*vp-s*) prevents the formation of color and the onset of developmental arrest (Coe and Neuffer 1977). A second recessive allele (*vp-MC*) was provided by D. S. Robertson, Iowa State University, and is similar to *vp-s*, except that the kernels are often dormant.

For both loci, a recessive mutable allele exists with an *rcy* insert. Both alleles (*bz-rcy* and *vp-rcy*) respond to *Cy* or *Cy*:*Mu* trans-active elements. *Cy*:*Mu* represents *Cy* elements derived directly from a Mutator line. *Cy* is known to be the active element responsible for Mutator activity (Schnable and Peterson 1986, 1988).

Determination of spotting patterns

The spotting pattern is dependent on the time, frequency, and tissue specificity of excision of the element at the locus. The spotting pattern of *vp-rcy* + *Cy*:*Mu* is characterized by single-cell spots, with variation among isolates for the frequency of spots. Single-cell spots indicate that the excision event took place some time after the last chromosomal replication of the spotted cell. Because *vp-rcy* is basically constant for timing of excision, different spotting patterns were classified on a scale of frequency of spots (Fig. 1).

Most mutable loci and their derivatives can be categorized for their spotting pattern on a single-kernel basis. In this study, the spotting pattern of *vp-rcy* and *bz-rcy* was determined by examining the whole ear for the predominant pattern. If there was no predominant spotting pattern, the patterns occurring with the highest frequency were recorded. This method was used to avoid misclassification due to color caused by light induction or diffusion associated with the *vp-rcy* allele. Spotting caused by light induction on a single kernel can sometimes be distinguished from excision events upon very close examination, but the evaluation is not always reliable, nor is it feasible in a large-scale study. Some spotted cells, due to excision events, exhibit diffusion of color to adjacent cells. This diffusion creates an optical illusion by making the kernel appear as though it had a higher frequency of spots.

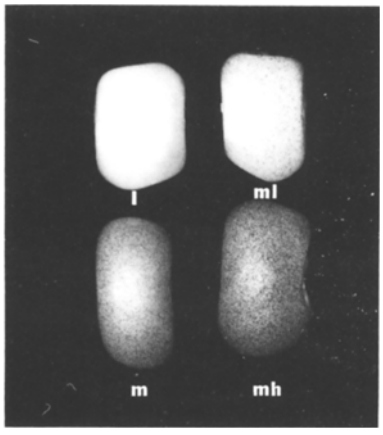

Fig. 1. A scale that represents the frequency of spots on a spotted viviparous kernel. The letter designations *l* (low), *ml* (medium-low), *m* (medium), and *mh* (medium-high), represent the frequency of spots on a kernel. All spots on the viviparous kernels were single celled

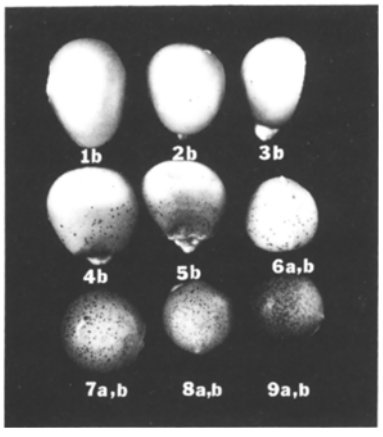

Fig. 2. A representation of some of the *bz-rcy* spotting patterns. The scale of 1 – 9 represents the frequency of spots on a kernel, and the letters indicate the predominant size of the spots, with *b* being the largest and *a* the smallest. *ab* is intermediate between *a* and *b* and *a,b* indicates both types are present

The scale to determine the spotting pattern of *bz-rcy* is based on the frequency of spots, reported as a number from one to nine (with nine being the highest frequency), and size of the spots (Fig. 2). Sizes of spots are recorded as a, ab, or b, with b being the largest. A spotting pattern recorded as 9a, b would indicate that the kernel has a value of nine for the frequency of spots and that there is a mixture of a- and b-sized spots.

Results

Kernel-spotting pattern is dependent on the number of Cy:*Mu elements*

To determine the relationship between the spotting pattern (Fig. 1) and the frequency of spotted kernels on an

Table 1. Progeny of the spotted bronze F_1 (Fig. 3) crossed with *vp* and *bz* testers. Clrd – colored round; spvp – spotted and viviparous; clvp – colorless and viviparous; bzrd – bronze round; spbz – spotted and bronze; spbz sh – spotted, bronze, and shrunken; bzsh – bronze and shrunken

Spotted bronze F_1 plant no. 1985	By *vp1* source			Onto *sh bz*			
	Clrd	spvp[a]	clvp[a]	bzrd	spbz rd	spbz sh	bzsh
1. 2219-3	94	45	60	108	64	1	181
2. 2219-6	213	89	102	140	66	0	0
3. 2219-10	188	148	74	312	184	0	0
4. 2220x-1	232	245	0	2	69	2	75
5. 2220z-1	107	121	0	4	127	1	120
6. 2220z-3	133	144	0	2	35	0	28
7. 2220z-5	180	181	0	3	63	1	139
8. 2221w-2	295	80	0	20	166	9	172
9. 2221z-2	209	176	0	9	153	2	164
10. 2221z-3	156	163	0	2	40	0	47
11. 2222v-1	145	138	0	21	200	0	0
12. 2222v-3	151	148	0	7	200	0	0
13. 2222v-5.5	67	68	1	6	116	7	126
14. 2222v-7	155	143	2	3	38	3	34
15. 2222w-1	208	60	3	42	81	7	106
16. 2223x-2	283	96	9	9	172	0	174
17. 2223y-7	90	45	44	113	32	0	178
18. 2225z-7	379	34	83	296	20	0	0
19. 2226z-2	141	54	132	68	9	0	0
20. 2226z-4	284	25	67	96	23	0	74
21. 2227y-4	180	167	8	37	220	7	245

[a] May be dormant due to the *vp-MC* allele

ear, crosses were made to include two reporter alleles (*vp-rcy* and *bz-rcy*) in order to determine their independent response to the same *Cy*:*Mu* elements. To develop these appropriate genotypes, a *Cy* tester (*bz-rcy*) was crossed by plants grown from colored kernels [(*Sh bz-rcy/sh bz*)/*Sh Bz*; *vp-rcy/Vp Cy*:*Mu*] (Fig. 3, cross 1). Note that the use of parentheses to enclose genotypes indicates that either genotype is possible. Spotted bronze kernels from the progency of cross 1 were selected (the spotted bronze F_1, Fig. 3) and used in further crosses. These selected kernels [(*Sh bz-rcy/sh bz*)/*Sh bz-rcy*: (*vp-rcy/Vp*)/*Vp Cy*:*Mu*)] contained the *Cy*:*Mu* element, a *bz-rcy* receptor allele, and were either homozygous or heterozygous for *Vp*. Only the *Vp* heterozygous kernels were used for further analysis to assure the presence of *Cy*:*Mu*, *bz-rcy*, and *vp-rcy* in the same plant. As indicated in Fig. 3, plants derived from these selected kernels were simultaneously crossed as females with a *vp* allele (*vp-Mc* or *vp-s*; crosses 2 and 3) and as males onto a *bz* allele (cross 4) in the manner shown, to elicit the mutability expression of both *vp-rcy* and *bz-rcy* with the same

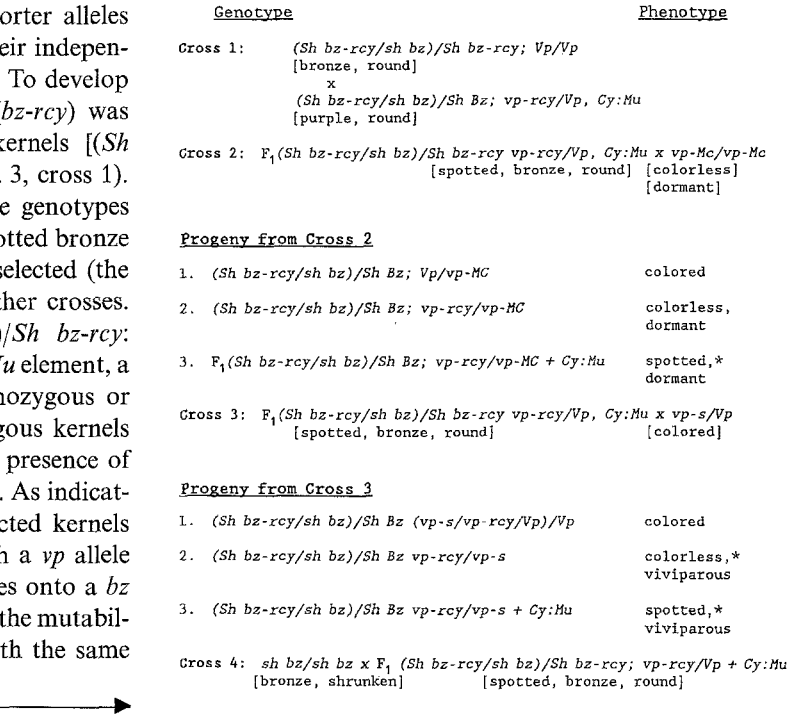

Fig. 3. Crosses 1, 2, 3, and 4 with the genotype and phenotype of the parents and, where indicated, a portion of their expected progeny. Genotypes in *asterisks* indicate that either is possible in such a selection. Phenotypes represented by *asterisks* are phenotypic classes used to calculate the percentage of spotted kernels for each cross

Table 2. Spotting pattern, % of spotted, and the number of *Cy:Mu* among the progeny of the spotted bronze F_1 (Fig. 3) crossed with *vp* and *bz* testers

Spotted-bronze F_1 plant no.	Parent		x *vp1* tester			Onto *bz* tester		
	Spotting pattern	No. of *Cy:Mu*	% of spotted	Spotting pattern	No. of *Cy:Mu*	% of spotted	Spotting pattern	No. of *Cy:Mu*
1	2	3	4	5[a]	6	7	8[b]	9
1. 2219-3	3ab	1	42.9	ml	1	37.2	3ab	0–1*
2. 2219-6	3ab	1	46.6	ml	1	32.0	3ab	0–1*
3. 2219-10	3ab	1	66.7	m	1–2*	37.1	3b	0–1*
4. 2220x-1	6a,b	6	100	m	6–10	97.2	6a,b	4–7
5. 2220z-2	9ab	6	100	m	6–10	96.9	7a,b	4–6
6. 2220z-3	9ab	6	100	mh	6–10	94.9	9a,b	3–6
7. 2220z-5	9ab	6	100	mh	6–10	98.2	7–8a,b	5–7
8. 2221w-2	9ab	6	100	mh	5–10	89.2	9a,ab	3
9. 2221z-2	9ab	6	100	ml–mh	6–10	94.4	9a,ab	4–5
10. 2221z-3	9ab	6	100	ml–mh	6–10	95.2	8a,b	3–6
11. 2222v-1	9ab	5	100	mh	6–10	90.5	9ab	3
12. 2222v-3	9ab	5	100	mh	6–10	96.6	8ab	4–5
13. 2222v-5.5	9ab	5	98.6	mh	4–8	95.1	9ab	4–5
14. 2222v-7	9ab	5	98.6	mh	5–8	92.6	9ab	3–5
15. 2222w-1	6a,b	5	95.1	mh	3–5	65.9	7a,b	1, 2*
16. 2223x-2	5a,b	3–4	91.4	mh	3–4	95.0	9ab	4–5
17. 2222y-7	4ab	3–4	50.6	ml	1	22.1	2ab	0–1*
18. 2225z-7	4b	3	29.0	1–ml	0–1*	6.3	3–4b	0–1*
19. 2226z-2	3ab	1–2*	29.1	1	0–1*	11.71	1ab	0–1*
20. 2226z-4	3ab	1–2*	27.2	1	0–1*	18.6	3ab	0–1*
21. 2227y-4	( )	2	95.4	m–mh	4–5	85.6	9a,b	3

* The number of active *Cy:Mu* elements is estimated to be in range given, but statistically the number is not an integer as defined by χ^2 analysis

[a] The letter designations – l (low), ml (medium-low), m (medium), and mh (medium-high) – represent the frequency of spots on a kernel. All spots on the viviparous kernels are single celled. ml–mh or 1–ml indicates the frequency ranges between these values

[b] The scale of 1 – 9 represents the frequency of spots on a kernel, and the letters indicate the predominant size of the spots, with b being the largest and a the smallest. ab is intermediate between a and b and a,b indicates both types are present

Cy:Mu source. Thus, for each spotted-bronze F_1 tested (crosses 2, 3, and 4), the direct comparison includes two testcross ears, one for *vp-rcy* and one for *bz-rcy*. The two ears were grouped as a set of sibs for analysis.

The ears resulting from each cross (Fig. 3) were classified for the predominant kernel spotting pattern, and then individual kernels were counted to determine the frequency of each phenotype present on an ear (Tables 1 and 2; phenotypic frequencies of F_1 are not shown). Progeny genotypes derived from the two testcrosses are listed in Fig. 3. The percentage of viviparous kernels with spots was determined. Likewise, from all the bronze, round kernels, the percentage of spotted (ignoring the possible 2% crossovers between *sh1* and *bz-rcy*) was determined (Table 2). The percentage of spotted kernels, frequency of spots per kernel (recorded as spotting pattern), and the estimated number of *Cy:Mu* elements (Schnable and Peterson 1986, 1988, 1989) segregating on the ear are recorded in Table 2.

In view of the graded series of the intensity of spotting [spotting pattern (Figs. 1 and 2)] and the varied frequencies of spotted kernels arising out of the testcrosses with both the *vp-rcy* and *bz-rcy* alleles (Table 2, columns 4 and 7), a relationship was sought between these two parameters (spotting pattern versus frequency of spotted kernels). There are several relationships that can be observed, and these are illustrated in Fig. 4a–d. Rows 9 and 10 of Table 2 are omitted from these relationship tests because the *vp-rcy* spotting pattern does not fit into a distinct class.

First, when the *vp-rcy* spotting pattern of an ear (Table 2, column 5) is directly compared with the frequency of *vp-rcy* spotted kernels (Table 2, column 4), a positive relationship is seen (Fig. 4a). Herein, as the frequency of spotted kernels increases, the pattern of spots on the ear becomes denser (more spots). A similar relationship is seen (Fig. 4b), when the same comparison is made for the *bz-rcy* allele pattern (Table 2, column 8) and the frequency of *bz-rcy* spotted kernels (Table 2, column 7). Thus, both receptor alleles have a similar direct relationship between these two parameters in response to *Cy:Mu* element(s) (Fig. 4a and b), in that a high/low percentage of spotted kernels is associated with a high/low spotting pattern on these ears.

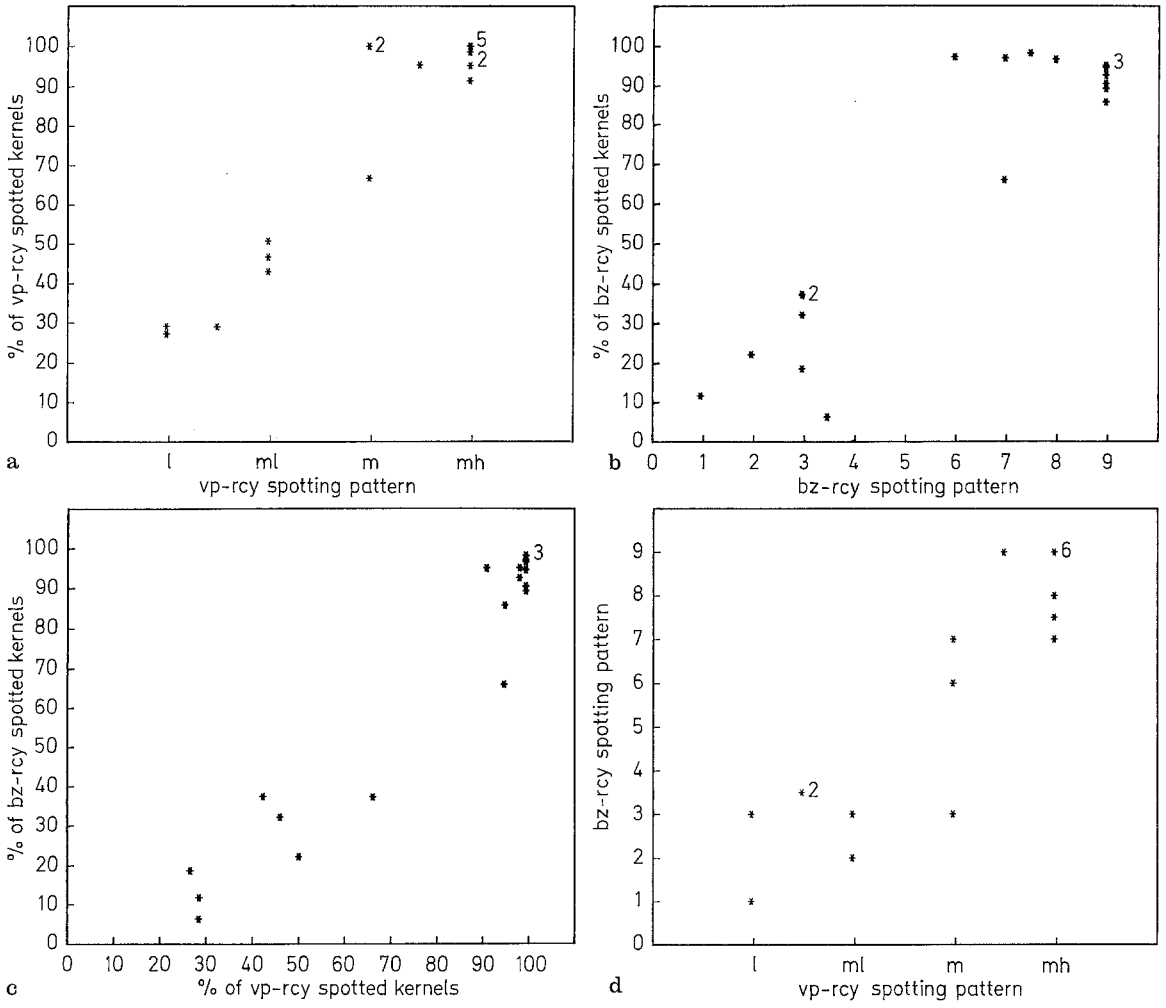

Fig. 4. **a** The coordinates of a *vp-rcy* test ear are determined by its frequency of spots on a kernel (spotting pattern) and the percentage of spotted kernels on an ear (columns 4 and 5 from Table 2). Each star represents one test ear, except when a number is listed indicating the number of overlapping test ear results. **b** A similar graph to 4a but using the *bz-rcy* test ear results (columns 7 and 8 from Table 2) **c** To compare the percentage of spotted kernels of the *vp-rcy* and *bz-rcy* sib testcross ears, each ear was used as a coordinate to plot a common point for the two sib ears (columns 4 and 7 from Table 2). Each star represents one set of sibs, except when a number is listed indicating the number of overlapping test results. **d** A similar graph to 4c but using the spotting patterns (frequency of spots per kernel) of the *vp-rcy* and *bz-rcy* sib testcross ears (columns 5 and 8 from Table 2)

Further comparisons can be made when the two alleles are directly compared to determine if they have similar or different responses to *Cy*:*Mu*. When the frequencies of spotted kernels of both alleles from the sib ears are compared (Table 2, columns 4 and 7), the following relationship is seen (Fig. 4c). As *bz-rcy* spotted-kernel frequency increases, so does the frequency of *vp-rcy* spotted kernels. A similar relationship is seen (Fig. 4 d) when the spotting pattern of both alleles are compared (Table 2, columns 5 and 8). This last association is predictable because of the correlated responses seen in the other tests of relationships between *bz-rcy* spotting and *vp-rcy* spotting. These comparisons clearly illustrate that a high frequency of spotted *vp-rcy* kernels is accompanied by a high frequency of *bz-rcy* kernels on its corresponding sib ear. The same can be said for the pattern relationship, which is an expression of the number of spots per kernel.

Because spotting is dependent on the presence of a *Cy*:*Mu* element, a low or high frequency of spotted kernels is indicative of the presence of only a few or many active *Cy*:*Mu* elements, respectively (Table 2, columns 6 and 9). Thus, because there is a relationship between frequency and pattern (Fig. 4a and b), it follows that spotting pattern (density of spots) is directly dependent on the number of active *Cy*:*Mu* elements.

Comparison of the parental values for the spotting pattern and the estimated number of *Cy*:*Mu* to the testcross progeny shows that the parental spotting pattern is heritable (Table 2, comparison of *bz-rcy* columns 2 and 8), except in lines 5, 16, 17, and 19 in Table 2. The

number of *Cy*:*Mu* is reasonably similar in all related plants (columns 3, 6 and 9), except in lines 8, 11, 15, 17, 18, and 21 of Table 2.

Discussion

Timing of excision events: a function of receptor component

Cy:*Mu* was tested against two receptor alleles, *vp-rcy* and *bz-rcy*, to provide a genetic explanation for "Mutator activity". By using two different reporter alleles containing the receptor (*rcy*) for *Cy*:*Mu*, specific interactions of each receptor element with *Cy*:*Mu* can be detected. These *rcy* elements express differences in the timing of excision. With the spotting of viviparous kernels, the spots were in all instances predominantly single celled. With bronze kernels, the spots ranged from an a to b spot size ("Materials and methods") and, in some instances, a matrix of different sizes of spots was found on kernels with a high or low frequency of spots (column 8 of Table 2). Because the *Cy*:*Mu* source is a constant component for each set of sib ears, the size of spots is a response of the resident *rcy* element. It can be concluded that the timing of excision is determined by the condition of the receptor element and, in these tests, the regulatory element *Cy*:*Mu* has no influence on the timing of somatic mutability, except for providing the trans-active function for transposition to take place.

Frequency: a function of number of Cy:*Mu*

In contrast, the percentage of spotted kernels and the frequency of spots on a kernel are comparable for both receptor alleles in their response to *Cy*:*Mu*. An ear with a high percentage of spotted kernels (*bz-rcy* or *vp-rcy* spotting) is predominantly populated with individual kernels having a high frequency of spots. As the percentage of spotted kernels decreases on an ear, there are fewer spots on individual kernels. The results reported here show that there is a relationship between the frequency of spots on a kernel and the percentage of spotted kernels on an ear. Similar, but not as detailed, observations have been made for the Mutator-induced *bz2-mul* allele (Walbot 1986), indicating that this phenomenon is not unique to the *rcy* elements used in this study.

Using Chi-square analysis, via the method described by Schnable and Peterson (1986), on the number of spotted kernels on an ear, the number of active *Cy*:*Mu* elements in the testcross ears was estimated (Table 2, columns 6 and 9). A low percentage of spotted kernels on an ear indicates that there are few active *Cy*:*Mu* elements present. Because the percentage of spotted kernels (estimated number of *Cy*:*Mu*) and spotting pattern are positively correlated, this implies that the frequency of spots on a kernel is dependent on the number of active *Cy*:*Mu* elements in the kernel. As the dosage of *Cy*:*Mu* elements increases within an ear, more spotted kernels are found on an ear and there are more *Cy*:*Mu* elements in the spotted kernels, resulting in kernels with a greater number of spots. Thus, the frequency of spots is due to a positive dosage interaction of the *rcy* element with the *Cy*:*Mu* elements, which is similar to those observed in other transposable-element systems. This is unlike the *Ac*/*Mp* effect where there is an inverse dosage effect with an increasing dosage of these elements (Brink and Nilan 1952).

Unfortunately, due to the lack of the necessary tester stocks, comparisons of dosage effects between male and female contributions could not be made. Previous tests of male versus female dosage effects on the interaction of *vp-rcy* with this *Cy*:*Mu* population have shown that there may be a slight difference in the number of spots per kernel, but the difference is so small that the spotting pattern classification for both crosses is the same. It has not been determined if the difference is due to *vp-rcy* or *Cy*:*Mu* dosage (B. Scheffler, personal observation).

[In Table 2 (columns 3, 6, and 9) there are several instances where the statistically determined number of active *Cy*:*Mu* elements does not have an integer value. These aberrant ratios can be detected only when the maximum number of elements is three or less, because the sensitivity of the χ^2 analysis is dependent on the total number of kernels (Schnable and Peterson 1986; Steel and Torrie 1980).]

Because a low copy number of *Cy*:*Mu* elements gives rise to a low percentage of spotted kernels, resulting in the aberrant ratios, and low frequency of spots on a kernel, most of the *Cy*:*Mu* elements present in the Mutator line tested have poor penetrance and, therefore, can be classified as weak elements, which is in agreement with previous studies (Schnable and Peterson 1986; Walbot 1986).

Chandler and Walbot (1986) have shown that kernels derived from plants exhibiting aberrant ratios for the *bz2-mul* mutable phenotype often have modified *Mu* elements within the genome. The nonspotted kernels (lack of somatic activity) almost always have methylated *Mu* elements, while the spotted kernels may or may not have methylated *Mu* elements. When methylated elements are present in the spotted kernels, unmethylated elements are always detected. In contrast, when all possible *bz2-mul* kernels on an ear are spotted, there are rarely any modified *Mu* elements in the genome. These results lend support to the conclusion that most *Cy*:*Mu* elements have weak activity. In that, as the number of *Cy*:*Mu* elements decreases, there should be less *Cy*:*Mu* gene product to protect all of the defective *Mu*(*rcy*) elements from methylation. It is reasonable to assume that some *Mu* elements are more susceptible to methylation than others. The

cause of this may be position effect or that some elements have a modified structure that does not interact very well with the *Cy*:*Mu* product.

Cy:*Mu versus Cy effects and its relation to Mutator activity: an hypothesis on the strength of the trans-active signal of Cy versus Cy*:*Mu*

Studies on the *Cy* regulatory element derived from a non-Mutator population show that, if dosage effects occur with this element, they are not as pronounced as that seen with *Cy*:*Mu* (Schnable 1986). A logical explanation for the difference in behavior of *Cy* and *Cy*:*Mu* is that they represent two regulatory-element states that differ. Based on the previous observation, most of the *Cy*:*Mu* elements in this study have weak activity in comparison to *Cy*. Because of this weak activity they are more susceptible to dosage effects, whereas the stronger-acting *Cy* is not. Evidence supporting this difference in behavior is based on the observation that the original isolate of *Cy* behaves in a near-Mendelian manner and gives rise to kernels with a fairly high frequency of spots (Schnable and Peterson 1986). During 5 years of observation of *Cy*:*Mu* behavior, a plant with a single *Cy*:*Mu* has never been positively identified. Since single *Cy*:*Mu* elements are usually weak, they are difficult to follow as they frequently give rise to aberrant ratios and produce kernels with few spots. There is no indication that strong *Cy*:*Mu* elements are absent in Mutator lines. However, if they are present, their relative frequency in the population is low compared with weak *Cy*:*Mu* elements.

The confounding effects of weak *Cy*:*Mu* elements may be one of the reasons that Mutator *Cy*:*Mu* has not been amenable to classical transposable element genetics. The weak activity of the *Cy*:*Mu* elements also explains several significant aspects of the Mutator studies. In most Mutator lines, if the *Mu1* copy number falls below ten, Mutator activity is not detected (Chandler et al. 1985). As illustrated in these tests, as the dosage of *Cy*:*Mu* decreases, the rate of somatic transposition events (kernel spots) decreases, and the rate of germinal transposition events would also be expected to decrease, thus decreasing the rate at which new mutations occur, which may be lower than what is classified as Mutator activity. In addition, Mutator activity could be influenced by the movement of other transposable elements. This indicates that the measurement of Mutator activity (mutation rate) is insufficient to be an accurate monitor of transposition of *Cy*:*Mu* and *rcy* elements. In comparison, somatic transposition is a sensitive assay because it measures the movement of one element away from a locus and, in addition, it can also detect the presence of several trans-functional regulatory elements.

The combined transposition of *Cy*:*Mu*(*Cy*), *Mu1*, and other *rcy* elements generates the high mutation rate that defines Mutator activity. Original Mutator lines generally have 10–50 copies of *Mu1* (Bennetzen 1984). Given that a high percentage of *Cy*:*Mu* elements have weak transposition signals, one possible explanation for the high copy number of *Mu1* elements may be that, in the lines tested, *Mu1* elements are needed in large numbers to achieve the high mutation rate associated with Mutator activity. It is not unexpected that Mutator lines with a low copy number of *Mu1* elements (Schnable and Peterson 1989; Schnable et al. 1989) should be found. In these cases, the *Cy*:*Mu* elements have stronger activity and/or other *rcy* elements would be the prominent contributors to the mutation rate. The molecular isolation of known *Cy* and *Cy*:*Mu* elements is needed to clarify these and other aspects of Mutator phenomena.

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