Theor Appl Genet (1992) 84:225–236

THEORETICAL AND APPLIED GENETICS

Genetic regulation of somatic mutability of two *Mu*-induced *a1* mutants of maize *

D.S. Robertson ** **and P.S. Stinard**

Department of Zoology and Genetics, Iowa State University, Ames, IA 50011, USA

Received July 29, 1991; Accepted October 9, 1991
Communicated by P.L. Pfahler

Summary. Previous studies of stocks of two *Mutator*-induced mutable *a1* alleles (*a1-Mum2* and *al-Mum3*) gave results consistent with the presence of one or more autonomous elements regulating the expression of mutability. This article reports on the results of studies designed to map these autonomous elements by using a series of *waxy* marked translocations. Linkage of *waxy* with autonomous elements was found for *a1-Mum2* by using the translocations *wx* T2-9d, *wx* T4-9e and *wx* T4-9b. Several different linkage values were found in crosses involving *wx* T2-9d, suggesting that autonomous elements have transposed to different locations on chromosome 2. Linkage of autonomous elements with *waxy* was found for *a1-Mum3* using translocation *wx* T2-9d. Again, several different linkage values were found. Some of these values were the same as those observed for *a1-Mum2*, but some were unique. In some crosses, the number of autonomous elements increased by one or two unlinked elements in addition to the linked element in one generation (i.e. the generation of the cross to the translocation series). Such an increase in number is probably the result of transposition of the original autonomous element to an independent locus while retaining the autonomous element at the original locus. Reduction in the number of autonomous elements is probably the result of the independent assortment in crosses of plants with two or more autonomous elements.

Key words: *Mutator* – Autonomous elements – Mapping

Introduction

The *Mutator* transposable-element system of maize has differed in the past from most other transposable-element systems in lacking a demonstrable autonomous element responsible for activating non-autonomous elements. This system was first characterized by its ability to induce germinal mutation rates 40 fold or more that of non-*Mutator* lines (Robertson 1978). The transmission of this system did not behave in a Mendelian fashion when first discovered. Ninety percent or more of the offspring of a *Mutator* plant have germinal *Mutator* activity. Robertson (1978) and Bennetzen (1984, 1987) observed that such a pattern of inheritance could be accounted for by the pressence of multiple autonomous elements or regulators. The studies of germinal activity were unable to demonstrate the regular transmission of a single autonomous element.

After the isolation, molecular characterization and analysis of the transmission of the *Mu1* element of *Mutator* system, it became obvious that *Mu1* and the similar *Mu1.7* element were not autonomous elements of this system (Alleman and Freeling 1986). At least 12 different elements have been isolated and characterized: *Mu1*, *Mu1.7*, *Mu1-del*, *Mu3*, *Mu4*, *Mu5*, *Mu6*, *Mu7*, *Mu8*, *MuA*, *MuA2*, and *MuR1* (Barker et al. 1984; Chandler et al. 1986; Taylor and Walbot 1987; Oishi and Freeling 1987; Varagona et al. 1987; Talbert and Chandler 1988; Talbert et al. 1989; Schnable et al. 1989; Fleenor et al. 1990; Qin and Ellingboe 1990; Chomet et al. 1991; Quin et al. 1991). Elements *Mu1* through *Mu8*, in the above list, are not autonomous elements, while recent evidence suggests that elements *MuA2* and *MuR1*, and possibly *MuA*, are autonomous elements of the *Mutator* system (Qin and Ellingboe 1990; Qin et al. 1991; Chomet 1991).

* Journal Paper No. J-14569 of The Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No. 2870

** Correspondence to: D. S. Robertson

Because *MuA2* and *MuR1* were isolated from the same *a1-Mum2* stock and their restriction maps are identical, it is very likely that *MuA2* and *MuR1* represent but one element isolated independently by different laboratories.

The isolation of *Mutator*-induced mutable alleles of genes regulating anthocyanin biosynthesis in the aleurone provided material that could be analyzed in a manner similar to those that demonstrated autonomous and nonautonomous elements in other transposable DNA systems. Early attempts to do this with *Mutator*-induced aleurone mutants were frustrated by the lack of consistent Mendelian ratios of mutable and stable kernels in outcross progenies or progenies of self-pollination. Occasional 1:1 (mutable:stable kernels) ratios in outcrosses or 3:1 (mutable:stable kernels) ratios on self-pollinated ears were observed, but these seemed to be ephemeral occurrences not consistently transmitted to future generations.

One factor responsible for the ephemeral nature of these ratios undoubtedly is the ready susceptibility of *Mu* elements to the methylation (modification) of cytosine bases at target sites for 5-methylcytosine-sensitive restriction enzymes (Bennetzen 1985, 1987; Walbot et al. 1985; Chandler and Walbot 1986; Bennetzen et al. 1988; Chandler et al. 1988). The methylation of *Mu* elements in a line with somatic mutability has been shown to hinder somatic excision of *Mu* elements, resulting in stable kernels (Chandler and Walbot 1986; Bennetzen et al. 1988). Such stable mutant kernels are not the result of the loss of an autonomous element but are rather the result of *Mu*-element modification.

Another feature of the *Mutator* system is the possible presence of weak autonomous elements that individually result in only rare excisions of a non-autonomous element from a locus resulting in kernels with one or two reverent sectors, or in many instances, no reverent sectors. The presence of such weak regulators was first suggested by Robertson and Stinard (1989). Scheffler and Peterson (1990) obtained results consistent with the presence of such elements in some lines. These data suggest that these elements could have a cumulative (additive) effects, resulting in a more intense mutability pattern and a higher frequency of mutable kernels correlated with an increased number of weak autonomous elements.

Robertson and Stinard (1989) for the first time presented evidence that heritable ratios consistent with the segregation of one autonomous element could be derived from *Mutator* stocks. Lines could be isolated from these progenies that had only stable *Mu*-induced mutant kernels as well as lines that did not have the *Mu*-induced mutant kernels but carried the autonomous element. When these two lines were intercrossed, somatic mutability was restored in ratios indicative of the presence of a single autonomous element. It was the availability of these lines with a single autonomous element that eventually led to the isolation of the autonomous element (Qin et al. 1991; Chomet et al. 1991).

Schnable and Peterson (1989) and Schnable et al. (1989) demonstrated that *Cy* and *rcy* (autonomous and non-autonomous elements, respectively) were part of the *Mutator* system. This was true at the genetic level because *Mutator* lines induced mutability in the stable *bz-rcy* allele (Schnable and Peterson 1989) and at the molecular level when it was shown that *rcy* was a member of the *Mu* family of elements (Schnable et al. 1989). Thus, the first autonomous element of the *Mutator* system demonstrated genetically was *Cy*. Before the work of Robertson and Stinard (1989), however, individual autonomous elements had not been demonstrated genetically from Robertson's *Mutator* lines.

This report describes the successful mapping of autonomous elements from stocks developed by Robertson and Stinard (1989) and provides some insight into their behavior as they are transmitted from one generation to another.

Materials and methods

The two *Mutator*-induced mutable *a1* mutants, *a1-Mum2* and *a1-Mum3*, were used in these tests. (See Table 1 for the definition of terms and symbols used in this paper.) The mutants were produced in an isolation plot in which the male rows were purple aleurone *Mutator* stocks, and the female rows were of the genotype *a1 a1 sh2 sh2*. The mutants were isolated as yellow plump kernels with purple spotting (i.e. mutable). In the early generations of propagation of these mutants, stable (non-mutable) kernels were observed, but not in ratios that would indicate a Mendelian pattern of inheritance for one or more autonomous element regulating the mutability. After several generations of outcrossing to *a1 sh2* testers, some ears were found that had 1:1 ratios of mutable to stable kernels, expected if a single autonomous element was segregating. Further tests of such ears supported the single autonomous element hypothesis (Robertson and Stinard 1989). The most efficient way to locate an autonomous element to a particular chromosomal position is to perform a cross to a series of *waxy*-linked chromosome nine translocations with their second break-points distributed among the other chromosomes. If the autonomous element is on the non-chromosome-nine chromosome and closely linked to the break-point, linkage will be established between *wx* and the autonomous element. The series of translocations utilized in these tests are shown in Table 2. In addition to the translocations series, crosses also were made with a non-translocation *waxy* stock.

Plants from mutable kernels of *a1-Mum2* and *a1-Mum3* stocks segregating for one or more putative regulators (Table 3) were crossed to the translocations stocks that, in most instances, were homozygous. The germs of the mutable parental kernels were of the putative genotype *a1-Mum sh2/a1 sh2*, A/+. The F_1 plants were outcrossed as males onto the *a1 wx* tester stocks (Table 1). Crosses could be made only in this direction because most of the translocation stocks were homozygous for the recessive *r* allele. If crosses had been made in the reciprocal direction, *R* mottling would have occurred, making mutability classification impossible.

Table 1. Definition of terms and symbols

A1	The dominant allele at the *a1* locus. One of the many complementary genes necessary for the production of anthocyanin-pigments in the aleurome
a1	The standard recessive allele of *A1*, which, when homozygous, prevents the production of anthocyanin pigments in the aleurone
sh2	A recessive allele responsible for kernels with a shrunken or brittle phenotype. The *a1* and *sh2* loci are tightly linked with only 0.2% of recombination between them
a1 sh2	A tester stock homozygous for *a1* and *sh2*
wx	*waxy*, a recessive gene that alters the starch content of the kernel resulting in a waxy phenotype
Wx	The wild-type allele at the *wx* locus (phenotype is starchy)
Autonomous element (A)	A transposable DNA element capable of encoding for its own excision and insertion as well as the excision and insertion of non-autonomous elements (see below). (Alternative terms for this element are regulator or controlling element.)
Non-autonomous element	An element of a transposable DNA system that has lost the ability to transpose on its own, but will transpose in the presence of an active autonomous element in the genome
1:1 ratio	Ratio of mutable to stable *a1* kernels on outcross ears. [In most instances the crosses were putatively (*a1-Mum Sh2/a1 sh2*, A/+) × *a1 sh2* tester. Only the *Sh2* kernels are scored to determine the ratio.]
a1 a1 wx wx	A tester stock homozygous for *a1* and *wx*. All genes other than *a1* required for aleurone color are homozygous

Table 2. Translocations utilized in this study and their breakpoints

Translocation	Breakpoints[a]	
1-9 4995	1L.19	9S.20
1-9 8918	1S.21	9L.20
1-9c	1S.48	9L.22
1-9 8460	1S.13	9L.24
1-9 8389	1L.74	9L.13
1-9 8886	1L.33	9L.23
2-9b	2S.18	9L.22
2-9d	2L.83	9L.27
3-9c	3L.09	9L.12
3-9 8447	3S.44	9L.14
4-9g	4S.27	9L.27
4-9 5657	4L.33	9S.25
4-9 6504	4L.09	9S.83
4-9e	4S.53	9L.26
4-9b	4L.90	9L.29
5-9a	5L.69	9S.17
5-9 4790	5L.34	9L.45
5-9c	5S.07	9L.10
6-9b	6L.10	9S.37
6-9e	6L.18	9L.24
7-9a	7L.63	9S.07
7-9 4363	7 ctr.	9 ctr.
8-9d	8L.09	9S.16
8-9 6673	8L.35	9S.31
9-10b	10S.40	9S.13
9-10 8630	10L.37	9S.28

[a] L and S represent the long arm and short arm of the chromosome, respectively. Decimal values are the positions of breakpoints, as measured from the centromere relative to the total length of the chromosome arm. Positions of breakpoints determined by Longley (1961)

Mutability was measured on the same five-class scale used by Robertson and Stinard (1989). Kernels in classes 3 and 4 were counted as mutable, and those in classes 2 and 1 were counted as stable [see Robertson and Stinard (1989) for the rationale for this classification procedure.] Linkage with *waxy*, and, thus, with the break-point in the other chromosome involved in the translocation with chromosome nine, was determined by standard linkage procedures. (See Fig. 1 for a diagram of the crosses made to determine linkage between autonomous elements and *waxy*.)

In some crosses there was evidence for more than 1 autonomous element. Ratios of 3:1 and 7:1 for mutable to stable classes indicate the segregation of two or three autonomous elements, respectively. For example, in some crosses with *wx* T2-9d, there was evidence of linkage of an autonomous element with *wx* and the presence of 1 or 2 independent autonomous elements. The expected frequencies of phenotypic classes for situations with 1 linked and 1 or 2 unlinked autonomous elements were calculated as shown in Fig. 2 and 3.

There were no outcrosses of F_1 plants with *wx* T9-4b and *wx* T9-4e that had only one autonomous linked to *waxy*. However, there were outcrosses with 1 autonomous element linked to *wx* and 1 or 2 additional independent autonomous elements. The procedure used to calculate the amount of linkage and the frequencies of the expected phenotypic classes in these crosses is shown in Fig. 4.

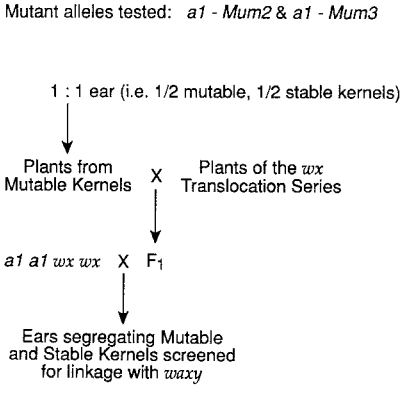


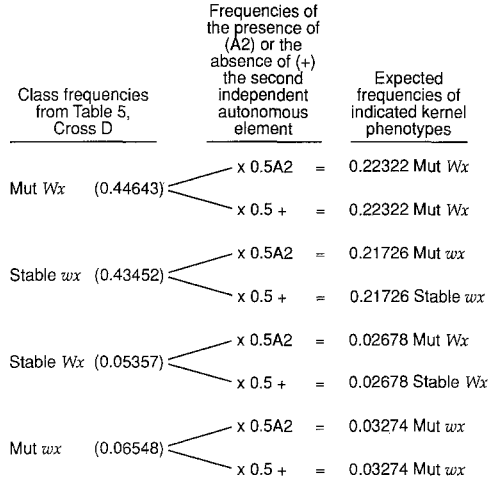
Fig. 1. A diagram of the crosses made to determine linkage between autonomous elements and *waxy*

With but few exceptions, most of the testcrosses had less than the expected 50% *waxy* kernels. A deficiency of the transmission of the *wx* allele is a frequently observed phenomenon when heterozygous *waxy* plants are testcrossed as males. To compensate for the effect of such a reduced transmission of *wx* on recombination values, the *waxy* class was increased in num-

Table 3. Stocks segregating for putative autonomous elements utilized in this study

Stocks crossed to translocations	Ratio of mutable to stable kernels of stocks listed in column 1	Allele	Source of the stock
86-2280-1	1:1	*a1-Mum3*	New 1:1
86-2280-3	1:1	*a1-Mum3*	New 1:1
86-8442-4	1:1	*a1-Mum3*	Robertson and Stinard (1989) Table 5
86-87-8849-1	1:1	*a1-Mum2*	Robertson and Stinard (1989) Table 3
87-4191-4	1:1	*a1-Mum2*	Robertson and Stinard (1989) Table 3
87-4191-8T	60.29% mutable[a]	*a1-Mum2*	Robertson and Stinard (1989) Table 3
87-4195-8	1:1	*a1-Mum3*	Robertson and Stinard (1989) Table 5
87-6261-8	1:1	*a1-Mum2*	Robertson and Stinard (1989) Table 4
88-1173-8	1:1	*a1-Mum3*	New 1:1
88-1179-1	1:1	*Al-Mum3*	New 1:1

[a] When tested by using χ^2 for agreement with a 1:1 ratio, this value was different at the 5% but not at 1% level of significance

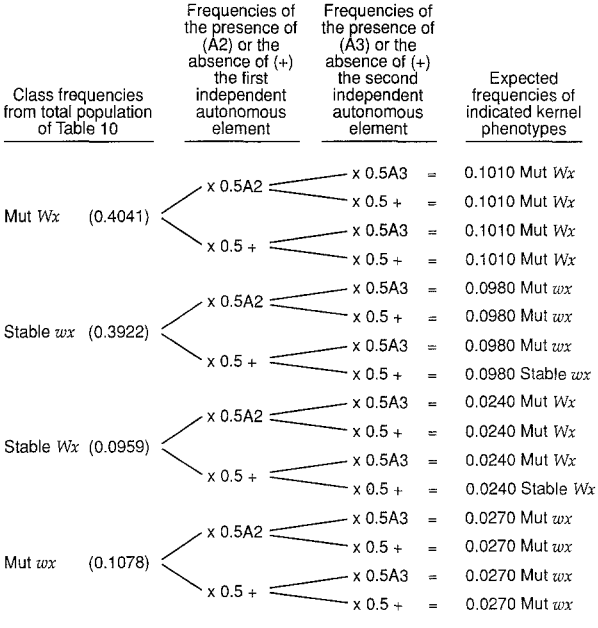


	Parental Classes		Cross-over Classes		
	Mut *Wx*	Stable *wx*	Stable *Wx*	Mut *wx*	Total (Table 6)
Expt. freq. of each class	0.47322	0.21726	0.02678	0.28274	
Observed[a]	393	209	23	207	832
Expected[a]	393.72	180.76	22.28	235.24	832

$X^2 = 7.8266$ $p > 5\%$ and $< 2\%$

[a] The number of kernels observed in each class (Table 5) and expected number of kernels in each class as calculated from the expected frequencies

Fig. 2. Method for calculating expected classes for Table 5. (One linked and one unlinked autonomous element)



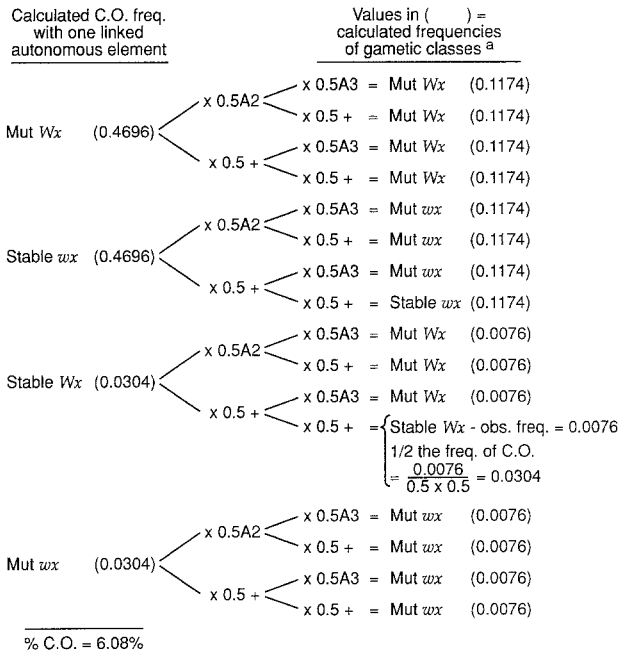
	Parental Classes		Cross-over Classes		
	Mut *Wx*	Stable *wx*	Stable *Wx*	Mut *wx*	Total (Table 11)
Expt. freq. of each class	0.4760	0.0980	0.0240	0.4020	
Observed[a]	40	10	1	31	82
Expected[a]	39.032	8.036	1.968	32.964	82

$X^2 = 1.0972$ $p > 80\%$ and $< 70\%$

[a] The number of kernels observed in each class (Table 10) and the expected number of kernels in each class as calculated from the expected frequencies

Fig. 3. Method for calculating expected classes for Table 10. (One linked and two unlinked autonomous elements)

ber to match the number of starchy kernels observed. The added *waxy* kernels were distributed among the mutable and stable classes in the relative proportions that these classes occurred in the raw data. The numbers of kernels in each class were rounded to the nearest whole kernel. Although this creates an artificial condition in which the number of starchy and *waxy* kernels are equal, a condition rarely observed in the raw data, the resulting recombination values probably approach more closely the true values than those calculated from raw data. These adjusted values and recombination frequencies are designated as "corrected". Seldom do the recombination values determined from the raw data differ by more than 1% or 2% from those calculated by using the corrected values. Thus, even without this correction, the data to be presented still provide overwhelming evidence for the linkage of autonomous elements with various translocations.



	Parental Classes		Cross-over Classes		
	Mut *Wx*	Stable *wx*	Stable *Wx*	Mut *wx*	Total (Table 8)
Expt. freq. of each class	0.4924	0.1174	0.0076	0.3826	
Observed [b]	194	37	3	157	394
Expected [b]	194.01	46.26	2.99	150.74	394

X^2 = observed vs. expected = 1.0711 p > 80% and < 70%

[a] Because the amount of linkage of the linked autonomous element is unknown, it is calculated as shown on the Figure, using the observed frequency of the stable *wx* class (one of the cross-over classes.). Once the frequency of the cross-over class is determined, the expected frequencies of the parental and the cross-over classes can be calculated for the linked autonomous element. With these values the expected frequencies of the other 15 gametic classes can be determined, as shown. From these gametic frequencies, the expected frequencies of the parental and cross-over classes can be determined

[b] The number of kernels observed in each class (Table 7) and the expected number of kernels in each class as calculated from the expected frequencies

Fig. 4. Method for calculating expected classes in Table 7 (cross with one linked plus two unlinked autonomous elements)

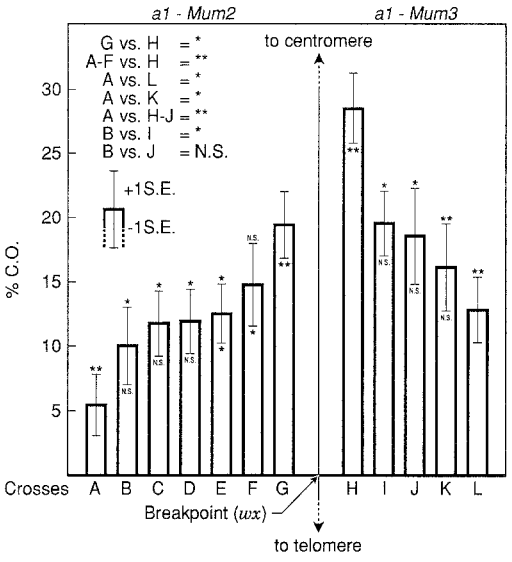


Fig. 5. A graphic presentation of cross-over values and standard errors for individual crosses with one autonomous element linked to *wx* T2-9d. Crosses *A* through *G* are *a1-Mum2* crosses from Table 4, and crosses *H* through *L* are *a1-Mum3* crosses from Table 9. One the *A–G side* of the graph *, ** and *N.S.* found *above* the positive standard error bar represent the significance of the differences of the cross-over values of each cross (*A–F*) with the cross-over value of cross *G* [significant differences at the 5% level (*), at the 1% level (**) and no significant difference (*N.S.*)]. Likewise the significance indicated *below* the negative standard error bar represent the significance of the cross-over differences of crosses *B–G* compared with the cross-over value of cross *A*. The same pattern is followed on the *H–L side*. Values *above* the positive standard error bar indicate the significance of the differences in cross-over values of *I–L* as compared with the cross-over value of cross *H*, and the significance of the differences in the cross-over values for cross *L* compared with the cross-over values of crosses *K–H* are indicated *below* the negative standard error bar

Another consistent feature of the observed ratios is the consistent deficiency in the stable kernels compared with the mutable ones. This discrepancy is eliminated when the correction is made for the *waxy* deficiency because such a correction results in increases in the stable *wx* parental and *wx* mutable cross-over classes. In all instances, whether one, two or three regulators are present, the corrected mutable : stable ratios never differ significantly (i.e. *P* was never less than 0.05) from the expected ratio for the number of autonomous elements segregating.

Because of variations in the maturity of both the *a1-Mum* and the translocation stocks, no single *a1-Mum* plant was crossed to all translocations. Furthermore, only a quarter of the outcrosses to the translocations were by plants that carried both the autonomous element(s) and the *a1-Mum* allele, thus reducing the number of crosses that provided useful information. Both these factors reduced the likelihood of performing a cross that would provide evidence of linkage. The only instances in which linkage was indicated are included in this report.

In Tables 5 and 10, crossing-over values for the sum of all crosses were calculated by the method of Phillips (1969). Heterogeneity chi-squares for Tables 5 and 10 were calculated by using the formula of Brandt and Snedecor as described by Kempthorne (1969).

The standard error of individual crosses was calculated as follows:

$$SE = \sqrt{pq/n}.$$

To determine the significance or lack of significance between the cross-over values of two different crosses, *t*-values were calculated as

$$t = (q1 - q2)/\sqrt{p1\,q1/n1 + p2\,q2/n2}.$$

The significance of the *t*-value was used to determine if the two recombination frequencies were significantly different.

Table 4. Testcrosses of *wx* T2-9d/*a1-Mum2* with *a1 a1 wx wx* showing linkage of *wx* with one autonomous element

	Cross[a]		Parental classes		Cross-over classes		Total	*Wx*/*wx*	% c.o.	SE	Plant crossed to translocation series	Number of autonomous elements in plant crossed to translocation series
			Mutable *Wx*	Stable *wx*	Stable *Wx*	Mutable *wx*						
90-5001-6	A	Obs[b]	43	27	3	1	74	1.6429	5.41		89-4001-2	1
4003-9		Corr[b]	43	44	3	2	92		5.43	±2.36		
90-5012-3	B	Obs	47	37	3	6	93	1.1628	9.68		89-4001-2	1
4005-9		Corr	47	43	3	7	100		10.00	±3.00		
90-5007-4	C	Obs	76	37	5	8	126	1.8000	10.32		89-4001-2	1
4003-10		Corr	76	67	5	14	162		11.73	±2.54		
90-5010-9	D	Obs	75	67	9	10	161	1.0909	11.80		89-3001-9	2
4007-7		Corr	75	73	9	11	168		11.90	±2.50		
90-5004-8	E	Obs	121	79	11	16	227	1.3894	11.89		89-4001-2	1
4004-9		Corr	121	110	11	22	264		12.50	±2.03		
90-5001-5	F	Obs	51	52	10	8	121	1.0167	14.88		89-4001-2	1
4001-1		Corr	51	53	10	8	122		14.75	±3.21		
89-90-4508-9	G	Obs	91	62	25	13	191	1.5467	19.90		89-4001-2	1
3509-2		Corr	91	96	25	20	232		19.40	±2.60		
Totals		Obs	504	361	66	62	993	1.3475	11.73	±1.01		
		Corr	504	486	66	84	1,140		13.15	±0.96		

[a] Letters assigned to the crosses in Fig. 7
[b] Obs, Observed values; Corr, values corrected for the deficiency of *waxy* kernels

Results

In 29 of 78 testcrosses (*a1 a1 wx wx x wx T*/*a1-Mum*) exhibiting mutability, the mutability was linked to *wx*. The *a1-Mum2* stocks showed linkage with T2-9d, T4-9e and T4-9b, whereas the *a1-Mum3* stocks only showed linkages with T2-9d.

The crosses of *a-Mum2* with only one autonomous element linked to *wx* T2-9d (Table 4) showed some variation in the amount of crossing-over; from a low of 5.43% to a high of 19.40% (corrected values). These cross-over values and their standard errors are shown graphically in Fig. 5. The extreme values differ very significantly (at the 1% level). Thus, we know that there are elements at at least two different positions on the chromosome. There are four values that differ significantly (at the 5% level) from the cross with 19.40% crossing-over. Also, there are two values that differ significantly (at the 1% level) from the cross with 5.43% crossing-over. These observations suggest that some of the intermediate cross-over values may represent elements at other positions between the two extremes. A homogeneity χ^2 value for these crosses is significant at the 5% level, confirming that some of the elements are at different locations on the chromosome.

Note that it has been assumed in Fig. 5 that these elements with different cross-over values map in only one direction from the break-point (proximal). There is no reason to believe that this is necessarily true. Some elements could be located about the same distance proximal to the break-point as others are distal, or for that matter, the elements could all map distally. Two-point linkage tests fail to give direction. In Fig. 5, they are shown proximal to demonstrate that, even under this condition, there is evidence that regulators are found at different positions from the break-point. If some of the elements are proximal, then there could be elements at more loci than is indicated by the plot in Fig. 5. If there are several real differences in the percentage recombination, as the statistics suggest, these data indicate that the autonomous element may have transposed from one location on chromosome two to another. Alternatively, the different positions of elements on chromosome two may be due to the 1 or 2 elements in the original 1:1 parents transposing from some other chromosome into different positions on chromosome two during the crosses leading up to the 1:1 stocks that were crossed with the translocation series. Plants grown from kernels of two different 1:1 ears (i.e. 89-4001-2, 89-3001-9) were crossed to *wx* T2-9d. The cross-over value for the 1-autonomous-element outcross of 89-3001-9 is very close to some of the cross-over values observed for the outocrosses of 89-4001-2 (see crosses C, D, E, Table 4 and Fig. 5). These 1:1 ears may have carried the same autonomous element at the identical locus as the result of descent from a common progenitor (i.e. the original *a1-Mum2* mutation event). Alternatively, their common values may be coincidental.

Table 5. Testcrosses of *wx* T2-9d/*a1-Mum2* with *a1 a1 wx wx* showing linkage of *wx* with one autonomous element plus a second independent autonomous elements

	Parental classes		Cross-over classes		Total	*Wx/wx*	Plant crossed to translocation series	Number of autonomous elements in plant crossed to translocation series
	Mutable *Wx*	Stable *wx*	Stable *Wx*	Mutable *wx*				
89-90-4534-6 3537-3	24	11	2	23	60		89-3001-9	2
90-5006-5 4006-3	102	30	9	49	190		89-3001-9	2
90-5006-4 4009-8	97	44	5	39	185		89-3001-9	2
90-5007-1 4011-1	136	73	5	43	257		89-3001-9	2
90-5010-7 4011-4	34	17	2	19	72		89-3001-9	2
Totals	393	175	23	173	764	1.1954		
Corrected totals[a]	393	209	23	207	832			
Expected totals[b]	393.72	180.76	22.28	235.24	832			
χ^2 observed versus expected = 7.826 $P < 5\%$ and $>2\%$ (χ^2 value at 5% is 7.815)								

[a] Corrected for the deficiency of *waxy* kernels

[b] Expected totals calculated by using the cross-over values from the corrected data of cross cross D from Table 5 and assuming independence for the second autonomous elements

Plant 89-3001-9 showed evidence of carrying 2 autonomous elements. The testcross of one F_1 plant with *a1 a1 wx wx* (Table 4) indicates the presence of only 1 autonomous element. Testcrosses of other F_1 plants gave results consistent with the presence of 1 autonomous element linked to *wx* plus an additional 1 or 2 independent autonomous elements (Tables 5 and 6). The results from Tables 4, 5 and 6 demonstrate that the number of autonomous elements can either increase or decrease with a single generation of outcrossing.

Table 5 summarizes the data from crosses with an autonomous element linked to *wx* T2-9d plus a second independent autonomous element. The observed and expected results are significantly different at the 5% level of significance but not at the 1% level. This is the only instance where expected and observed results differ at the 5% level of significance. The expected results were calculated by using the corrected linkage value of cross D (Table 4) because this cross and all the crosses in Table 5 shared the same 1:1 parent plant (89-3001-9). Thus, it is likely that the autonomous element linked to *wx* T2-9d is at the same position in both the F_1 plant with 1 linked autonomous element and the F_1 plants with a second independent autonomous element. At the 1% level of significance, the results support this hypothesis.

Table 6 summarizes the data from crosses with an autonomous element linked to *wx* T2-9d and 2 additional independent autonomous elements. Again, the corrected linkage value of cross D in Table 4 was used to calculate expected values because all but one cross in Table 6 involved plant 89-3001-9. The data do not differ significantly from the expected values. The first cross of Table 6 came from an *a1-Mum2* plant (89-4001-2) that had only 1 autonomous element; yet, now, 3 are observed, 1 linked to *wx* T2-9d and 2 others at independent locations. Plant 89-3001-9 contributed to testcrosses in Tables 4, 5 and 6. This plant carried 2 independent autonomous elements, 1 of which is shown to be linked to *wx* T2-9d (Table 4). In these outcross progeny, however, 1 additional independent element is present.

Two crosses of *wx* T4-9b/*a1-Mum2* gave results consistent with an autonomous element linked to *wx* T4-9b (4L.90, 9L.29) and 1 or 2 additional independent autonomous elements (Table 7). The cross-over value (6.08%) and the chi-square test for the cross with 2 independent autonomous elements is not reliable because there were only three kernels in the starchy, stable class, which was the class used to estimate the amount of crossing-over. However, all classes show close agreement between observed and expected values. Thus, there is a strong indication of linkage of an autonomous element with the *wx* T4-9b break-point, with a cross-over value that is probably not greatly different from the estimated one (6.08%). The linked autonomous element in the crosses with 1 independent element is linked to *wx* with 20.12% recombination. The cross-over value for the two sets of crosses differ at the 1% level of significance. The significance determination, however, was made by as-

Table 6. Testcrosses of *wx* T2-9d/*a1-Mum2* with *a1 a1 wx wx* showing linkage of *wx* with one autonomous element plus two independent autonomous elements

	Parental classes		Cross-over classes		Total	*Wx*/*wx*	Plant crossed to translocation series	Number of autonomous elements in plant crossed to translocation series
	Mutable *Wx*	Stable *wx*	Stable *Wx*	Mutable *wx*				
90-5002-3 4002-7	123	28	4	88	243		89-4001-2	1
90-5006-7 4007-8	154	20	11	79	264		89-3001-9	2
90-5005-4 4008-2	107	30	5	72	214		89-3001-9	2
90-5010-2 4010-2	105	23	2	74	204		89-3001-9	2
Totals	489	101	22	313	925	1.2343		
Corrected totals[a]	489	125	22	386	1,022			
Expected totals[b]	497.32	111.02	13.65	399.98				
χ^2 observed versus expected = 7.4483			$P < 10\%$ and $< 5\%$					

[a] Corrected for the deficiency of *waxy* kernels

[b] Expected totals calculated by using the cross-over values from the corrected data of cross cross D from Table 5 and assuming two additional idependent autonomous elements

Table 7. Testcrosses of *wx* T4-9b/*a1-Mum2* with *a1 a1 wx wx* showing linkage of *wx* with one autonomous element plus one or two independent autonomous elements

	Parental classes		Cross-over classes		Total	*Wx*/*wx*	Plant crossed to translocation series	Number of autonomous elements in plant crossed to translocation series
	Mutable *Wx*	Stable *wx*	Stable *Wx*	Mutable *wx*				
Crosses with one independent autonomous element								
90-5011-8 4013-4	81	49	19	53	202		89-3001-1T	2
98-99-4541-1 3540-7	98	31	1	45	175		89-3001-1T	2
Totals	179	80	20	98	377	1.1180		
Corrected totals[a]	179	89	20	110	398			
Expected totals	178.98	79.48	20.02	119.52	398		(assuming 20.12% c.o.)	
χ^2 observed versus expected = 1.2161			$P > 80\%$ and $< 70\%$					
Crosses with two independent autonomous elements								
89-90-4533-5 3540-6	89	25	1	66	181		89-3001-1T	2
89-90-4539-9 3540-1	105	12	2	79	198		89-3001-1T	2
Totals	194	37	3	145	379	1.0824		
Corrected totals[a]	194	40	3	157	394			
Expected totals	194.01	46.26	2.99	150.74			(assuming 6.08% c.o.)	
χ^2 observed versus expected = 1.0711			$P > 80\%$ and $< 70\%$					

[a] Corrected for the deficiency of *waxy* kernels

suming the 6.08% recombination value is reliable, which is not necessarily so. Three crosses involving *wx* T4-9e (4S.53, 9L.26) and *a1-Mum2* produced plants with 1 autonomous element linked to this translocation plus 1 independent autonomous element (one plant) or 2 independent autonomous elements (two plants, Table 8). For both these situations the observed values do not differ significantly from the predicted values estimated by using the frequency of the stable, starchy classes (0.0719 and 0.0460, respectively) to calculate the expected frequencis.

Table 8. Testcrosses of *wx* T4-9e/*a1-Mum2* with *a1 a1 wx wx* showing linkage of *wx* with one autonomous element plus one or two independent autonomous elements

	Parental classes		Cross-over classes		Total	*Wx/wx*	Plant crossed to translocation series	Number of autonomous elements in plant crossed to translocation series
	Mutable *Wx*	Stable *wx*	Stable *Wx*	Mutable *wx*				
Crosses with one independent autonomous element								
90-5020-8 4015-1	60	29	9	33	131	1.1129	89-3001-4	3
Corrected[a]	60	32	10	37	139			
Expected	59.51	24.76	9.99	42.74	139		(assuming 28.70% c.o.)	
χ^2 observed versus expected = 3.4600			$P > 50\%$ and $< 30\%$					
Crosses with two independent autonomous elements								
89-90-4528-1 3528-2	45	10	6	39	100		89-3001-4	3
89-90-4528-2 3528-4	34	11	2	41	88		89-3001-4	3
Totals	79	21	8	80	188	0.8614		
Corrected totals[b]	79	18	8	69	174			
Expected	79.00	13.75	8.00	73.25			(assuming 36.79% c.o.)	
χ^2 observed versus expected = 1.5602			$P > 70\%$ and $< 50\%$					

[a] Corrected for the deficiency of *waxy* kernels
[b] Corrected for the deficiency of starchy kernels

Table 9. Testcrosses of *wx* T2-9d/*a1-Mum3* with *a1 a1 wx wx* showing linkage of *wx* with one autonomous element

	Cross[a]		Parental classes		Cross-over classes		Total	*Wx/wx*	% c.o.	SE	Plant crossed to translocation series	Number of autonomous elements in plant crossed to translocation series
			Mutable *Wx*	Stable *wx*	Stable *Wx*	Mutable *wx*						
90-847-1 747-6	H	Obs[b]	105	74	32	37	248	1.2342	27.82		88-9009-4	1
		Corr[b]	105	91	32	46	274		28.47	±2.73		
90-847-3 747-7	I	Obs	93	97	30	17	237	1.0789	19.83		88-9009-4	1
		Corr	93	105	30	18	246		19.51	±2.53		
90-842-5 748-7	J	Obs	40	33	14	4	91	1.4595	19.78		88-9009-4	1
		Corr	40	48	14	6	108		18.52	±3.74		
90-845-4 745-3	K	Obs	55	24	4	8	91	1.8438	13.19		88-9009-4	1
		Corr	55	44	4	15	118		16.10	±3.38		
90-6255-6 5254-4	L	Obs	78	83	8	16	185	0.8687	12.97		85-9009-4	1
		Corr	78	72	8	14	172		12.79	±2.55		
Totals		Obs	371	311	88	82	852	1.1679	17.83	±1.31		
		Corr	371	360	88	99	918		20.04	±1.29		

[a] Letters assigned to the crosses in Fig. 7
[b] Obs, Observed values; Corr, values corrected for the deficiency or surplus of *waxy* kernels

Use of these values gives estimates of *wx* to regulator distances of 28.70 and 36.79, respectively. All of the crosses of Table 8 are derived from the same 1:1 *a1-Mum2* plant, which had 3 autonomous elements. Thus, it is possible that the same autonomous element located at the same position relative to the translocation breakpoint is present in the plants with one and two independent regulators. The difference between the two crossover values (8.09%) is not statistically significant.

Evidence for linkage of an autonomous element to *wx* T2-9d was also found in a 1:1 *a1-Mum3* test (Table 9). The corrected crossing-over for the total population was 20.04%, which is significantly greater than that observed for the *a1-Mum2* linkage with the same translocation

(13.15%, Table 4). Some of the individual *a1-Mum3* cross-over frequencies, however, do not differ significantly from those observed in the *a1-Mum2* crosses. Values of between 12% and 20% are found in both sets of testcrosses. The Table 4 recombination values of crosses B, C, D, E and F do not differ significantly at the 1% level of significance from those of crosses L, K and J of Table 9 (see also Fig. 5). The recombination value of cross G in Table 4 is not significantly different at the 1% level from those of crossed I, J and K of Table 9. One *a1-Mum2* cross has a cross-over value considerably less (5.43%) than any found for *a1-Mum3* crosses, and one *a1-Mum3* cross has a value of 28.47%, which is considerably higher than any found for *a1-Mum2*. The *a1-Mum2* and *a1-Mum3* alleles were produced in the same isolation plot in which purple aleurone *Mutator* plants were used as male parents. It is possible that these two mutant alleles arose from a sector in the tassel of a purple *Mutator* plant and, thus, might have had some autonomous elements in common. Analyses of the original isolates of these mutants in their immediate progenies and in later generations indicated that multiple regulators of somatic mutability were present in the genomes of these mutants when they were first isolated. Even if *a1-Mum2* and *a1-Mum3* are not derived from a single mutation event but arose independently, they nonetheless were induced in a common purple aleurone *Mutator* population, and thus, progeny plants carrying the independent mutations could have many autonomous elements in common. Therefore, it is possible that lines carrying the different *a1-Mum* alleles have a common autonomous element on the long arm of chromosome two.

One testcross of a *wx* T2-9d/*a1-Mum3* F_1 segregated for an autonomous element linked to the translocation plus 2 independent autonomous elements (Table 10). The chi-square test in this instance is not reliable because only one starchy, stable kernel was observed. However, the expected frequencies of all classes are in close agreement with the observed values, suggesting that this cross indeed involves 2 independent autonomous elements and a linked autonomous element, with about 9.76% crossing-over with *wx*.

Discussion

These studies and the molecular analyses of Qin et al. (1991) and Chomet et al. (1991) demonstrate that the *Mutator* system has an autonomous element regulating its own transposition and the transposition of other *Mutator* elements. The outcross DR1 utilized by Qin et al. (1991) in the isolation of *MuA2* was derived from the same original 1:1 ear as that used in these linkage studies. Thus, one region where this element can insert is known to be the long arm of chromosome two.

The autonomous elements of the *Mutator* system readily undergo replicative transposition. Plant 89-3001-9 (*a1-Mum2*), with 2 autonomous elements, was outcrossed to the translocation series, followed by testcrossing of the next generation. In three of the progeny of the crosses of this plant to *wx* T2-9d that showed linkage, the number of autonomous elements had increased from the 2 found in the parent to 3 in the testcrosses (Table 6). Also, one sibling plant of 89-3001-9, 89-3001T, with 2 autonomous elements in the parent, went from 2 to 3 in the testcross (Table 7). The *a1-Mum3* plant that was crossed to *wx* T2-9d, 88-9009-4, had only 1 autonomous element. In most testcrosses of the F_1, only 1 was retained. In one cross, however, 2 additional autonomous elements were found (Table 10).

The autonomous elements that have been mapped regulate somatic mutability. Their role, if any, in regulating germ-line activity of the *Mutator* system (i.e. the induction of new mutations) has not been determined. There are many observations of lines with intense somatic mutability, including the mutability observed in lines segregating for 1 or more autonomous elements controlling somatic activity, that do not have germinal activity (Robertson et al. 1988; Robertson and Stinard 1989). However, there are some somatically mutable lines that

Table 10. Results of a testcross of *wx* T2-9d/*a1-Mum3* with *a1 a1 wx wx* showing linkage of *wx* with one autonomous element plus two independent autonomous elements

	Parental classes		Cross-over classes		Total	*Wx*/*wx*	Plant crossed to translocation series	Number of autonomous elements in plant crossed to translocation series
	Mutable *Wx*	Stable *wx*	Stable *Wx*	Mutable *wx*				
90-748-77 / 745-3	40	5	1	16	62	1.9524	88-9009-4	1
Corrected[a]	40	10	1	31	82		(assuming 9.76% c.o.)	
Expected	39.032	8.036	1.968	32.964				
χ^2 observed versus expected = 1.0972			$P > 80\%$ and $> 70\%$					

[a] Corrected for the deficiency of *waxy* kernels

do have germinal activity. Attempts to restore germinal activity to *Mutator* lines that have been continually outcrossed and have lost germinal activity by crossing with stocks with an autonomous element have failed (unpublished results). Although some lines with somatic mutability do have germinal activity, all lines that have been without somatic mutability for at least two generations have also lost germinal activity (Robertson et al. 1988). Thus, there seems to be a direct connection between the absence of somatic activity and the loss of germinal activity. However, no such direct connection exists between the presence of somatic activity and germinal activity. Thus, there is some relationship between the presence or absence of an autonomous element and germinal activity, but the nature of this relationship remains to be established.

Conclusions

(1) Ears that have 1:1 or other ratios of mutable:stable kernels, indicative of the presence of 1 or more autonomous elements regulating somatic mutability, have been shown to be carrying autonomous elements that can be mapped to chromosome locations by using standard linkage tests. In the stocks tested, autonomous elements have been located to the long arm of chromosome two and to the long and short arms of chromosome four.

(2) In different sibling plants from an outcross of a single plant to *wx* T2-9d, several different cross-over values are observed between *wx* and a linked autonomous element, suggesting that the autonomous element on chromosome two may be transposing to different positions on the chromosome.

(3) Autonomous elements regulating somatic mutability of *Mutator*-induced mutants can both increase and decrease in number from one generation to the next. In these studies, the increase in number is probably the result of the transposition of the autonomous element to a locus independent of its original site, plus its retention at the original site. The decrease in number is undoubtedly the result of the independent assortment of unlinked autonomous elements.

Acknowledgements. The authors gratefully acknowledge the National Science Foundation for support of this research by grants PCM 79-23052, PCM 83-02214 and DCB 86-08188.

References

Alleman M, Freeling M (1986) The *Mu* transposable elements of maize: Evidence for transposition and copy number regulation during development. Genetics 112:107–119.

Barker RF, Thompson DV, Talbot DR, Swanson J (1984) Nucleotide sequence of the maize transposable element *Mu1*. Nucleic Acids Res 12:5955–5967

Bennetzen JL (1984) Transposable element *Mu1* is found in multiple copies only in Robertson's *Mutator* maize lines. J Mol Appl Genet 2:519–524

Bennetzen JL (1985) The regulation of *Mutator* function and *Mu1* transposition. UCLA (University of California, Los Angeles) Symp Mol Cell Biol New Ser 35:343–353

Bennetzen JL (1987) Covalent DNA modification and the regulation of *Mutator* element transposition in maize. Mol Gen Genet 208:45–51

Bennetzen JL, Brown WE, Springer PS (1988) The state of DNA modification within and flanking maize transposable elements. In: Nelson O (ed) Plant transposable elements. Plenum Publ, New York London, pp 237–250

Chandler VL, Walbot V (1986) DNA modification of a maize transposable element correlates with loss of activity. Proc Natl Acad Sci USA 83:1767–1771

Chandler VL, Rivin C, Walbout V (1986) Stable non-*Mutator* stocks of maize have sequences homologous to the *Mu1* transposable element. Genetics 114:1007–1021

Chandler VL, Talbert LE, Mann L, Faber C (1988) Structure and DNA modification of endogenous *Mu* elements. In: Nelson O (ed) Plant transposable elements. Plenum Publ, New York London, pp 339–350

Chomet P, Lisch D, Hardeman KJ, Chandler VL, Freeling M (1991) Identification of a regulatory transposon that controls the *Mutator* transposable element system in maize. Genetics 129:261–270

Fleenor D, Spell M, Robertson D, Wessler S (1990) Nucleotide sequence of the maize *Mutator* element, *Mu8*. Nucleic Acids Res 18:6725

Kempthorne O (1969) An introduction to genetic statistics. Iowa State University Press, Ames, Iowa pp 153–154

Longely AE (1961) Breakage points for four corn translocation series ad other chromosome aberrations. Agric Res Ser 34 16:1–29

Oishi K, Freeling M (1987) A new *Mu* element from Robertson's *Mutator* line. In: Nelson O (ed) Plant transposable elements. Plenum Publ, New York London, pp 289–291

Phillips RL (1969) Recombination in *Zea mays* L. I. Location of genes and interchanges in chromosomes 5, 6 and 7. Genetics 61:107–116

Qin M, Ellingboe AH (1990) A transcript identified by *MuA* of maize is associated with *Mutator* activity. Mol Gen Genet 224:357–363

Qin M, Robertson DS, Ellingboe AH (1991) Cloning of the *Mutator* transposable element *MuA2*; a putative regulator of somatic mutability of the *a1-Mum2* allele in maize. Genetics 129:845–854

Robertson DS (1978) Characterization of a *Mutator* system in maize. Mutat Res 51:21–28

Robertson DS, Stinard PS (1989) Genetic analyses of putative two-element systems regulating somatic mutability in *Mutator*-induced aleurone mutants of maize. Dev Genet 10:482–506

Robertson DS, Morris DW, Stinard PS, Roth BA (1988) Germ line and somatic *Mutator* activity; Are they functionally related? In: Nelson O (ed) Plant transposable elements.Plenum Publ, New York London, pp 17–42

Scheffler BE, Peterson PA (1990) Somatic *Mutator* activity expression is dependent of the strength of *Cy* transactive signals in maize. Theor Appl Genet 79:449–456

Schnable PS, Peterson PA (1989) Genetic evidence of a relationship between two maize transposable element systems: *Cy* and *Mutator*. Mol Gen Genet 215:317–321

Schnable PS, Peterson PA, Saedler H (1989) The *bz-rcy* allele of the *Cy* transposable element system of *Zea mays* contains a *Mu*-like element insertion. Mol Gen Genet 217:459–463

Talbert LE, Chandler VL (1988) Characterization of a highly conserved sequence related to *Mutator* transposable elements in maize. Mol Biol Evol 5:519–529

Talbert LE, Patterson GI, Chandler VL (1989) *Mu* transposable elements are structurally diverse and distributed throughout the genus *Zea*. J Mol Evol 29:28–39

Taylor, LP, Walbot V (1987) Isolation and characterization of a 1.7-kb transposable element from a mutator line of maize. Genetics 117:297–307

Varagona M, Fleenor D, Wessler SR (1987) Unique *DS* and *Mu* elements inserted in exons of the *Waxy* gene. In: Nelson O (ed) Plant transposable elements. Plenum Publ, New York London, p 381

Walbot V, Chandler V, Taylor L (1985) Alterations in the *Mutator* transposable element family of *Zea mays*. UCLA (University of California, Los Angeles) Symp Mol Cell Biol New Ser 35:333–347