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A genetic study on the activation of quiescent *Uq* transposable elements in *Zea mays* L.

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A genetic study on the activation of quiescent *Ug* transposable elements in *Zea mays* L.

by

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GENERAL INTRODUCTION

Transposable elements, also known as mobile elements or jumping genes, are segments of DNA capable of moving in the genome. They were first described in maize by Barbara McClintock more than four decades ago (McClintock, 1946). When active, these transposable elements cause unstable gene mutations and gross structural rearrangements in chromosomes (McClintock, 1951, 1956a, 1956b, 1978).

In maize, transposable elements have been classified into either the autonomous or the nonautonomous elements (McClintock, 1950). The autonomous elements are not only able to transpose by themselves but also able to transactivate other nonautonomous elements to move. In contrast, the nonautonomous elements can not transpose by themselves. Thus, the trans-acting autonomous elements are also defined as regulators and the nonautonomous elements as receptors. Interactions between regulator and receptor elements are found to be highly specific. It is this specificity that defines a maize two-element transposable system (Fincham and Sastry, 1974; Peterson, 1981). A receptor element can be transactivated only by the regulatory element belonging to the same system.

Thus far, at least nine two-element systems have been defined (for review, see Peterson, 1987). These include Dt-dt

(Rhoades, 1936, 1938), Ac-Ds (McClintock, 1946), En/Spm-I/dSpm (Peterson, 1953, McClintock, 1954), Fcu-cu (Gonella and Peterson, 1977), Ug-rug (Friedemann and Peterson, 1982), Bg-o2-m(r) (Salamini, 1981), Mrh-mrh (Rhoades and Dempsey, 1982), Mut-mut (Rhoades and Dempsey, 1982), and Cy-rcy (Schnable and Peterson, 1986). In addition, there have been several other transposable elements that are functionally related to some of the nine systems. Mp (Brink and Nilan, 1952) and Ac2 (Rhoades and Dempsey, 1982) are related to the Ac-Ds system, while Spf (Singh et al., 1975) is related to the Fcu-cu system, and Mutator (Robertson, 1978) is related to the Cy-rcy system.

Of these nine systems, the Ug transposable element system has been established from unstable a-alleles in progenies of Barley-Stripe-Mosaic-Virus (BSMV) treated corn plants by Sprague and McKinney (1966, 1971) (Friedemann and Peterson, 1982). This system consists of the regulatory Ug elements and the rug receptor elements. Thus far, rug receptor elements have been found to reside within the A1 locus (the a-rug allele) (Friedemann and Peterson, 1982; Sprague, 1986) and the C1 locus (the c-rug31, c-rug65, c-rug66, and c-rug67 alleles) (Caldwell and Peterson, 1989). The two loci in association with other loci, condition maize-plant and -seed aleurone pigmentation. Generally, maize kernels of either a-rug/(a-rug or a^{o}), no-Ug or c-rug/(c-rug or c^{o}), no-Ug genotype have colorless aleurones due to rug-insertional inactivation of the

A1 (or C1) anthocyanin gene. However, if an active Ug coexists and provides the necessary transposition function, excision of the rug element away from either maize locus may occur thereby restoring the normal gene function. As a result, these maize kernels will have spotted aleurones (colored spots on a colorless background).

Unlike the negative dosage-effect in the Ac-Ds system (McClintock, 1951), the Ug regulatory elements have a positive dosage-effect (Friedemann and Peterson, 1982; Pan and Peterson, 1990). When only one copy of Ug element is present, a fine-low spotting pattern is expressed. If two or three copies of Ug are present, a coarse-high pattern results (Friedemann and Peterson, 1982). No dosage effect of the rug receptor element was observed at a-rug (Friedemann and Peterson, 1982). However, this may not be the case for the rug receptors at the c-rug alleles (E. E. O. Caldwell, Department of Genetics, Iowa State University, Personal communication).

The Ug regulatory elements are ubiquitous. When the a-rug or c-rug alleles have been used as "reporter alleles", active Ug elements have been found in numerous maize lines, testers, and breeding populations (Peterson and Salamini, 1986; Peterson and Friedemann, 1983; Peterson, 1986). The Ug regulatory elements are also diverse. Unique spotting patterns on identical a-rug or c-rug reporter alleles can be

triggered by various active Ug elements (Pereira and Peterson, 1985; Caldwell and Peterson, 1989). Although it is not possible, thus far, to determine whether these diverse patterns of Ug activity are due to their structural differences, various Ug elements have nevertheless been allocated to at least 3 maize linkage groups (Pereira and Peterson, 1985).

The present genetic study focuses on the Ug-rug transposable element system in maize. There have been several significant findings in this study. These include:

1) the discovery of quiescent Ug element sequences in the genomes of maize inbred lines originally lacking Ug activity;
2) the isolation and genetic characterization of five spontaneously germinally activated new Ug elements including Ug2, Ug3, Ug4, Ug5 and Ug6;
3) that all these five newly activated germinal Ug elements are independent of the original standard Ug (or Ug1) but are either allelic or linked on one linkage group; and finally,
4) the isolation and genetic characterization of another activated Ug element upon 5-aza-2'-deoxycytidine treatment, and of a dominant miniature mutant, Mn::Ug, which is tagged by this activated Ug.

Explanation of dissertation format

In presenting these research findings, I have chosen the alternative format for my dissertation. There are four sections, all of which are either published or submitted to international scientific journals for publication.

Section I describes sectors of Ug-a-rug spotting in otherwise colorless maize aleurone layers of a-rug/a-rug/a-rug, no active Ug genotype. Genetic evidence is presented to indicate that the Ug activity in these spotted sectors is an expression of spontaneously activated quiescent Ug elements. This activation can occur even in the absence of any unusual treatment such as BBF, BSMV, or tissue culture regimes.

Section II reports the isolation and genetic characterization of five spontaneously germinally activated new Ug elements (Ug2, Ug3, Ug4, Ug5, and Ug6), all showing stronger activities than Ug1. Genetic proof for the authenticity of these five new Ug isolates is also presented.

Section III summarizes the results of allelism tests among the Ug1 and the newly activated Ug2, Ug3, Ug4, Ug5, and Ug6 elements. The unique spotting phenotypes elicited by these Ug elements on identical standard a-rug reporter alleles are illustrated, either alone or in pairs. Genetic data are presented to show that all the five new Ug elements are independent of Ug1, but themselves are clustered into one

maize linkage group, either allelic or linked.

The last section, Section IV, reports the first experiment involving 5-aza-2'-deoxycytidine in maize and describes the isolation and genetic characterization of the Mn::Ug mutant. Genetic evidence is presented to prove that this dominant miniature mutant is tagged by an activated, originally quiescent, Ug element, very likely upon treatment with 5-aza-2'-deoxycytidine. Genetic data are also presented to show the independence of Mn::Ug from three other recessive miniature mutants, mn1, mn2 and mn7690.

SECTION I

SPONTANEOUS ACTIVATION OF QUIESCENT *Ug* TRANSPOSABLE ELEMENTS DURING ENDOSPERM DEVELOPMENT IN *Zea mays* L.

SPONTANEOUS ACTIVATION OF QUIESCENT *Ug* TRANSPOSABLE ELEMENTS DURING ENDOSPERM DEVELOPMENT IN *Zea mays* L.[1]

Running title: Activating transposable elements

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ABSTRACT

This study addresses the question of the activation of quiescent *Ug* transposable elements in maize breeding lines. When exposed to various genotypes of assorted maize inbred lines lacking any active *Ug* element, the standard *a-rug* allele reports *Ug* activity in the form of sectors of colored spots in otherwise colorless aleurone tissue. This activation of quiescent *Ug* elements occurs randomly during the growth of the endosperm. It is concluded that there are components in the genome that enhance the rare activation of quiescent *Ug* elements. Further, it seems that this activation occurs in the absence of any stress-inducing treatment, but that normal growth conditions provide sufficient stimulus for such activation.

Key words: Activation-*Ug* transposable element-*Zea mays* L.

INTRODUCTION

Transposable elements in maize were initially observed in corn lines of families that were undergoing a cycle of chromosome bridge, break, and fusion (BBF) (McClintock, 1951b). Though transposable elements were observed earlier in genetic nurseries (Emerson, 1914; Rhoades, 1936), they were subsequently found in naturally occurring populations (Peterson, 1987). Other reports have emphasized the role of a stress environment that triggers the activity of quiescent elements. This was most notable in the Barley Stripe Mosaic Virus (BSMV) treatments of corn plants by Sprague and McKinney (1966, 1971) that uncovered unstable a-alleles later identified as belonging to the Ubiquitous (Ug) transposable element system (Friedemann and Peterson, 1982; Peterson and Friedemann, 1983). More recently, active elements have been recovered from lines originating from genotypes that were under a tissue culture regime that is considered a stress environment (Burr et al., 1987; Peschke et al., 1985).

Of all the transposable elements currently under study in maize, the Ug transposable element is one of two that is most pervasive and persistent in numerous maize breeding populations and several genetic testers (Mrh is the other) (Peterson and Salamini, 1986; Peterson and Friedemann, 1983; Peterson, 1986). Although found in the BSSS (Iowa Stiff Stock

Synthetic) breeding populations (Peterson, 1986; Peterson and Salamini, 1986), active Ug elements are not detectable in the BSSS-derived inbred lines and other commercial inbreds tested (Cormack and Peterson, 1987; Pan and Peterson, 1986, 1987; Peterson, 1986; Peterson and Friedemann, 1983; Peterson and Salamini, 1986). Their disappearance in the derived inbreds has been hypothesized to be due to strong selective pressure against variability.

It was early observed that among kernels of the standard a-rug allele, occasional spots appeared at a low frequency even in the absence of an active Ug element. This became more apparent in outcrosses in which the a-rug allele is acting, in this case, as a "reporter allele", for the presence of an active Ug element in assorted inbred lines. What was revealed in these outcrosses was an unusual type of mutability that consistently appears as a single spot of varied size (1-24 colored cells) in an otherwise colorless aleurone layer. The genetic basis of this one-spot mutable phenotype was assumed not to be due to the presence of a very weak Ug element that was activated, but more likely to be due to a nonheritable instability of the rug receptor element at the A locus (Peterson and Friedemann, 1983). Changes in the state of the rug element have been found in two other studies, giving rise to several derivatives such as a-rug(lo), nonresponsive colorless, nonresponsive pale (Pereira and Peterson, 1985),

and A(m), a stable self-colored allele (Sprague, 1986) in the presence of a standard Ug element.

In this paper, we report on the activation of quiescent Ug elements in four maize inbred lines, B70, C103, C123, and 187-2, that have not received any unusual treatment. It is our contention that quiescent mobile elements are continuously being activated even in the absence of any unusual treatment such as BBF, BSMV, or tissue culture regimes. The genetic basis of this activation as revealed by sectoring in the maize aleurone tissue has been determined and its possible mechanism is discussed.

MATERIALS AND METHODS

Crossing scheme to test Ug element activity in inbred lines

A description of gene symbols and genetic terms used in this paper are given in Peterson and Friedemann (1983).

Random seed samples of the four maize inbred lines (B70, C103, C123 and 187-2) were obtained from W. A. Russell (Iowa State University, Ames, Iowa). Ug element activity in these inbreds was tested in a general crossing scheme (Fig. 1). The total number of individual gametes from the original seed samples of B70, C103, C123 and 187-2 tested were 6, 7, 5 and 6, respectively.

The expected genotypic and phenotypic frequencies of the first backcross generation (BC_1) progeny kernels (Fig. 1) depend upon the Ug content in the inbred line studied (Table 1). In the first case where no active Ug element is present, 50% of the BC_1 progeny kernels are expected to be colored or mottled, while 50% are colorless. When only one active Ug element is present in the F_1 of a cross of inbred lines to the a-rug tester (Table 1, case II), then the BC_1 progeny kernels consist of 50% colored or mottled, 25% colorless, and 25% fully spotted. If two nonallelic active Ug are present in the F_1 (Table 1, case III), the expected frequency will be 50%

Figure 1. General crossing scheme to test the distribution of the Ug transposable element in maize inbred lines

The mottling is a characteristic feature of the r allele in the triploid endosperm when it is derived from the female in a cross yielding an R/r/r allelic condition while all other color related genes are dominant. A question mark implies that the Ug content in each inbred line was not known at the time of test; a "+" indicates no active Ug is present. BC_1 is the first backcross generation.

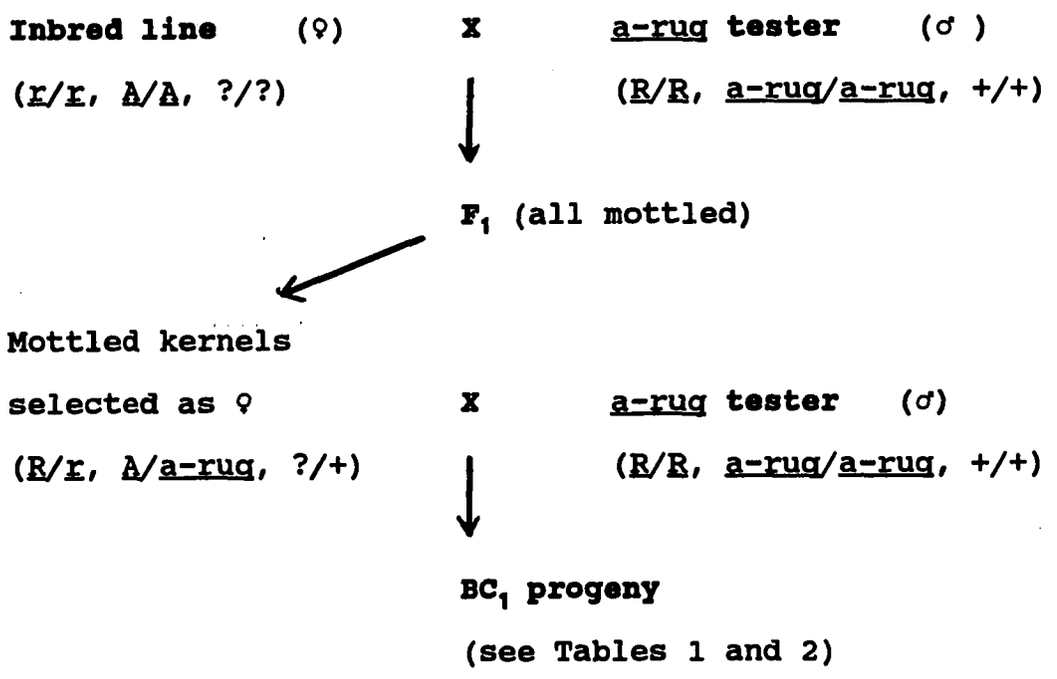

Inbred line (♀)
(r/r, A/A, ?/?)
X
a-rug tester (♂)
(R/R, a-rug/a-rug, +/+)
F1 (all mottled)
Mottled kernels
selected as ♀
(R/r, A/a-rug, ?/+)
X
a-rug tester (♂)
(R/R, a-rug/a-rug, +/+)
BC1 progeny
(see Tables 1 and 2)

Table 1. Expected genotypic and phenotypic frequencies of BC_1 (Figure 1) progeny kernels under three possible cases[a]

Genotype	Phenotype	Frequency[b]		
		Case I	Case II	Case III
A/a-rug, +/+ or Ug/+	Colored and mottled	0.5	0.5	0.5
a-rug/a-rug, +/+	Colorless	0.5	0.25	0.125
a-rug/a-rug, +/Ug	Spotted	0	0.25	0.375

[a]All kernels carry at least one copy of R.
[b]Case I, when inbred line does not have any active Ug element; case II, when inbred line has one active Ug element; and case III, when inbred line has two unlinked Ug elements.

colored or mottled, 37.5% spotted and 12.5% colorless.

Determination of sector size, location, and the approximate number of aleurone cells within each sector

The area of each of the sectors (for example, Figure 2, B and C) was determined by a two-step procedure. In the first step, black and white photomicrographs of the sectors were taken on a Leitz Macro-Dia Setup at a magnification of X 6. Enlarged prints of the sectors were made so that print magnification for all photomicrographs of the sectors was X 5. Generally, one photograph was taken to cover each small- and medium-sized single sector located on one side of a maize kernel. For sectors covering more than one side of a kernel, two or more overlapping photographs were taken and a composite image was made. The second step was to determine directly the area of each of the 29 single sectors (listed in Table 4) on photomicrographs by a computer simulation program. An Apple IIE computer and Houston Instruments digitizer pad were used in conjunction with a modified program for measuring the areas.

In order to determine the number of cells per given area, three random areas within the spotted sector shown in Figure 3B that have a countable number of cells were selected. Their areas were measured and combined. The average area for a

Figure 2. Sectors of colored spotting of varying sizes and locations in the aleurone layers of 12 BC_2 kernels

The colored spots represent somatic a-rug --> A events triggered by a Ug element that has been activated in different cells at various stages during development of the endosperm tissue. Kernels A through G were isolated from backcrosses of no-spot BC_1 selection with the a-rug tester; kernels H and I were from backcrossing few-spotted BC1 type with the a-rug tester; and kernels J through L were generated in the backcrosses between sectored BC_1 and the a-rug tester.

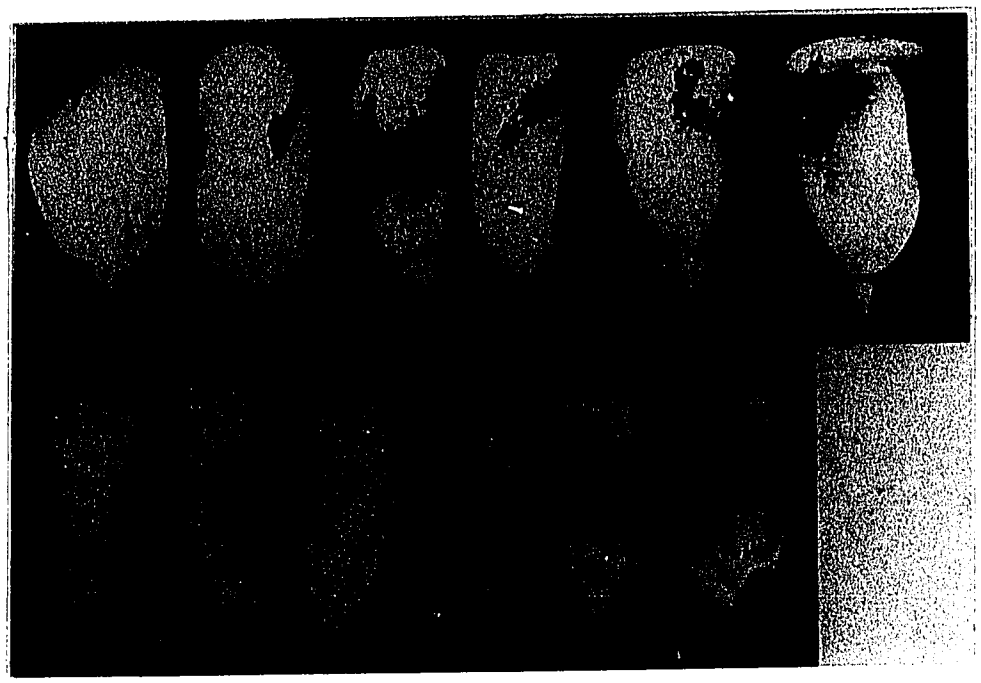
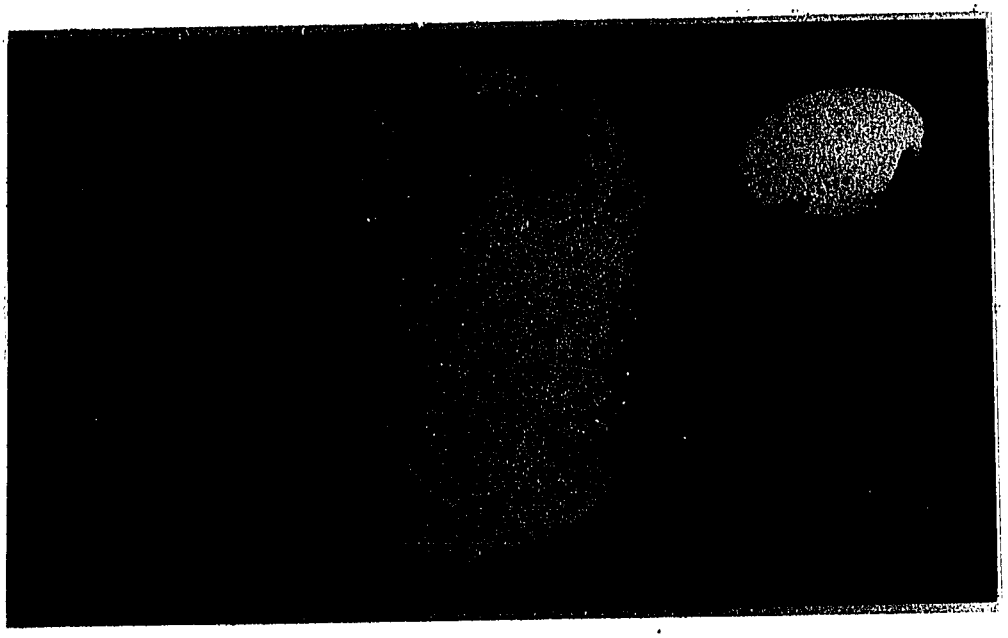


Figure 3. Ug/a-rug spotting phenotype

(A). A kernel homozygous for a-rug is fully spotted when an active Ug element is present in the genome. (B and C). Two BC_1 colorless kernels of a-rug/a-rug genotype lacking an active Ug element show a single sector of color spots upon activation of a quiescent Ug element during endosperm development.



single aleurone cell was determined to be 6.05 X 10^{-4} mm^2 by dividing the combined area by the total number of cells from the three areas chosen. This value was in turn used to derive the total number of aleurone cells within each of the 29 single sectors.

It is obvious that the accuracy of the data obtained is affected by at least two factors: the curvature of the maize aleurone layer and the arbitrary peripheries of the single sectors. Nevertheless, these two factors were discounted in this study because our main interest is to indicate the various sizes and locations of sectors of Ug activation in the aleurone tissue.

Testing of the sectored kernel

From 200 BC_1 progeny ears (Table 2), 29 single sectored kernels were isolated (Table 4). From these kernels, 25 plants were successfully grown. In order to determine the r allele content we test-crossed their first ears by r-g (or r) line, and their second ears or tiller ears, if available, by the standard a-rug line. The latter cross identifies the Ug content. In addition, each of the 25 plants was crossed several times to an a-rug line in order to verify the absence of Ug. Plants were also grown from randomly selected few-spot and no-spot sib kernels (Table 2), and were tested by

reciprocal crosses with the a-rug line in order to uncover any sectoring behavior among these few-spot and no-spot selections. The mean frequencies of sectored kernels generated in the resulting individual BC_2 progeny ears were calculated. The statistical analysis utilized included an unbalanced split-plot analysis of variance blocked on individuals with kernel type (Table 3) as main plot effects and sex as a subplot effect (Cochran and Cox, 1957).

RESULTS

All four inbred lines lack an active Ug element

The test for the presence of an active Ug element in the four maize inbred lines (B70, C103, C123 and 187-2) are summarized in Table 2. The BC_1 progeny populations derived from each of the four inbreds (Figure 1) could be classified into two phenotypic classes (colored and mottled kernels as one class, and colorless kernels as another class) at a ratio that did not significantly deviate from 1:1 (see X^2 values in Table 2 and case I in Table 1). No fully spotted kernels, as would be expected for cases II and III in Table 1, were found among the 37,434 colorless kernels. This indicates that none of the four maize inbred lines possesses a genetically active Ug element.

Though an active Ug was not present, there was Ug activity. There were 278 colorless kernels with 1 to 5 small (from 3 to 5 cells in diameter) spots (defined as few-spotted or few-spot) and 29 colorless kernels with a single sector of color spotting (defined as sectored) (Figure 3, B and C) among the 37,434 colorless kernels (Table 2). These exceptional kernels occurred in the BC_1 colorless progenies at frequencies less than 1% for the few-spotted and less than 0.1% for the

Table 2. Phenotypic classification of the BC_1 progeny ears produced from the testcross of the F_1 (inbred line X a-rug tester) by a-rug tester

Inbred line	No. of ears	No. of kernels	Phenotype classification (%)[a] Colored & mottled	Colorless: no-spot	Colorless: few-spotted	Colorless: sectored	Colorless: Total	$(X^2)^b$
B70	46	16,797	8,317 (49.6)	8,456 (50.4)	23 (0.14)	1 (0.006)	8,480	1.58^{ns}
C103	63	24,734	12,265 (49.5)	12,380 (50.1)	86 (0.35)	3 (0.01)	12,469	1.68^{ns}
C123	37	12,375	6,236 (50.4)	6,116 (49.4)	18 (0.15)	5 (0.04)	6,139	0.79^{ns}
187-2	54	20,483	10,137 (49.5)	10,175 (49.7)	151 (0.74)	20 (0.10)	10,346	2.11^{ns}
Grand total		74,389	36,955	37,127	278	29	37,434	

[a] Few-spotted indicates a colorless aleurone layer with 1 to 5 color spots; sectored indicates a colorless aleurone layer with sector of color spots, size of sectors varies in different sectored kernels.

[b] A X^2 value for case I in Table 1; ns = not significant at 0.05 level.

sectored.

Sectoring is due to the action of an activated Ug on the a-rug allele

Because the sectoring resembles the mottling phenotype associated with the R alleles when it is in the R/r/r/ condition in the endosperm (Coe and Neuffer, 1977) (Only the R allele could be implicated, the other color alleles are fully dominant), it was necessary to clarify the R status in these crosses. Twenty-five plants grown from the BC1 sectored kernels were testcrossed (Table 2 and 3). Analysis of the resulting BC_2 (second backcross generation) progeny ears showed the following results:

1. Crosses of these 25 plants by r-g (or r) tester showed that 14 plants were R/R, and 11 were R/r (data not shown). That sectoring was occurring in plants that were R/R eliminates the role of R-mottling in this sectoring phenomenon. Further, in the a testcross, all were proven homozygous for the a-rug allele, producing only colorless progeny when backcrossed either by or onto the a-rug tester. The homozygosity of the a-rug allele in all the sectored kernels tested is supportive of the data showing that R-mottling does not contribute to the sectoring. There is, therefore, support for our proposal that the sectoring is an

Table 3. Number and mean frequency of sectored kernels on colorless BC_2 progeny ears produced from the reciprocal backcrosses with a-rug tester of plants grown from three types of BC_1 sib kernels

BC_1 kernel type	No. of plants tested	Parental type[a]	No. of progeny ears	No. of colorless progeny kernels: not-sectored	sectored	spotted	Total	Mean frequency of sectored kernels per individual BC_2 ear[b]
Sectored	25	M	15	3,382	16	0	3,398	0.00495 ± 0.00088
		P	67	16,205	30	1	16,236	0.00167 ± 0.00040
Few-spot	70	M	78	29,136	46	2	29,184	0.00152 ± 0.00035
		P	107	32,137	62	0	32,199	0.00166 ± 0.00032
No-spot	147	M	155	59,833	55	2	59,890	0.00102 ± 0.00025
		P	102	27,084	23	1	27,108	0.00072 ± 0.00035

[a]M, maternal; P, paternal.
[b]Estimated least squares mean frequency and standard error by a split plot analysis model of Cochran and Cox (1957).

expression of the interaction of a-rug and an activated Ug. Further support is derived from a comparison of the mutability pattern of the sectors with that of a standard Ug on an a-rug reporter allele (Fig. 3, A compared to B and C). Given that the only reporter allele present was a-rug, it follows that this sectoring can only be a consequence of the mutability of the a-rug allele induced by a quiescent Ug that has become active and not due to R-mottling.

2. Single fully spotted kernels were rare (only one, but currently not verified) in the BC_2 progenies. This indicates that in none of these 25 sectored kernels had a Ug element become activated in its germinal tissue and that Ug-like was confined to the areas of individual sectors in the aleurone tissue and therefore was not heritable.

3. Sectored kernels were regenerated in the BC_2 progenies when these 25 plants were backcrossed reciprocally with the a-rug line (Table 3; Fig. 2, Kernels J through L). There was a difference in the progenies of the reciprocal crosses in the frequency of sectors. When the 25 plants were used as maternal parents, 16 out of the 3,398 colorless progeny kernels had a spotted sector at a mean frequency of 0.00495 per individual BC_2 progeny ear. On the other hand, when these same plants were used as a male parent, 30 out of 16,236 colorless progeny kernels were sectored at a mean frequency of 0.00167 (Table 3). In addition, 4 sectored kernels were

recovered on 4 selfed ears from 4 different tillers (data not shown). Note that the a-rug genotypic constitution is identical in both of these crosses.

Basis for the few-spot and no-spot progeny types

Similar results were obtained for the few-spot and no-spot BC_1 sib kernels. Although their R content was not determined because of labor and space limitations, all these sib kernels were proven to be homozygous for a-rug and to lack an active Ug element (data not shown). These few-spot and no-spot BC_1 kernels did generate sectored kernels similar to those previously discussed in reciprocal backcrosses to the a-rug line (Table 3; Fig. 2, Kernel A through I). However, the mean frequency of generating sectored kernels per individual BC_2 progeny ear was lower for the few-spot sib kernels (0.00152 as maternal and 0.00166 as paternal) and was the lowest for the no-spot sibs (0.00102 as maternal and 0.00072 as paternal) (Table 3).

Analysis of variance using an unbalanced split plot model (Cochran and Cox 1957) has indicated highly significant differences among the three BC_1 kernel types (sectored, few-spot and no-spot) and among kernel-type by sex interactions in generating BC_2 sectored kernels (data not shown). Comparisons made between the six least squares mean

frequencies in Table 3 have shown that 0.00495 is the only value that is highly significantly different from the other five values (data not shown). In other words, when plants grown from the BC_1 sectored kernels were backcrossed by the a-rug tester, they produced sectored kernels at a higher frequency per individual BC_2 progeny ear than either their few-spot or no-spot sibs. This was not apparent when they were used as pollen donors. On the other hand, the few-spot and no-spot sibs did not differ significantly in either sex in their mean frequencies of producing BC^2 sectored kernels, a situation similar to the results of a previous study in which the one-spot and no-spot sibs were compared (Peterson and Friedemann 1983).

It appears, therefore, that the three classes, no-spot, few-spot, and sectored, are part of the same genotypic composition for a-rug in that each can generate a similar array of phenotypes, though differing in the frequency of each of the types.

Morphological features of spotted sectors

Morphological studies on the 29 single sectors indicated three features. First, they are located at various sites in the aleurone layer (Fig. 3). Second, each of the sectored areas vary in size, with areas varying from 0.05 mm^2 to

32.82 mm^2. The number of cells within these sectors were from as few as 82 to as many as 54,257 (Table 4). Third, they displayed a spotting pattern very similar to the one triggered by a standard a-rug/Ug interaction (Fig. 3).

An examination of the number of cells per given area provides an opportunity to determine the timing of activation events. If it is assumed that if activation is a random event, then the incidence of Ug activation leading to a sector should be correlated with the population of cells at a given time. It is found that there are more activation events later in endosperm development as the population of cells increases. Most of the recognizable activation events occur between the 7th and 13th cell generation (Fig. 4) following the fusion of the polar nuclei with the sperm. Of course, there are later events (after the 13th cell generation) but these could not be clearly defined for measurement. It can be concluded, however, that with more cells, there is a greater chance for Ug activation supporting our contention that Ug activation is a random event.

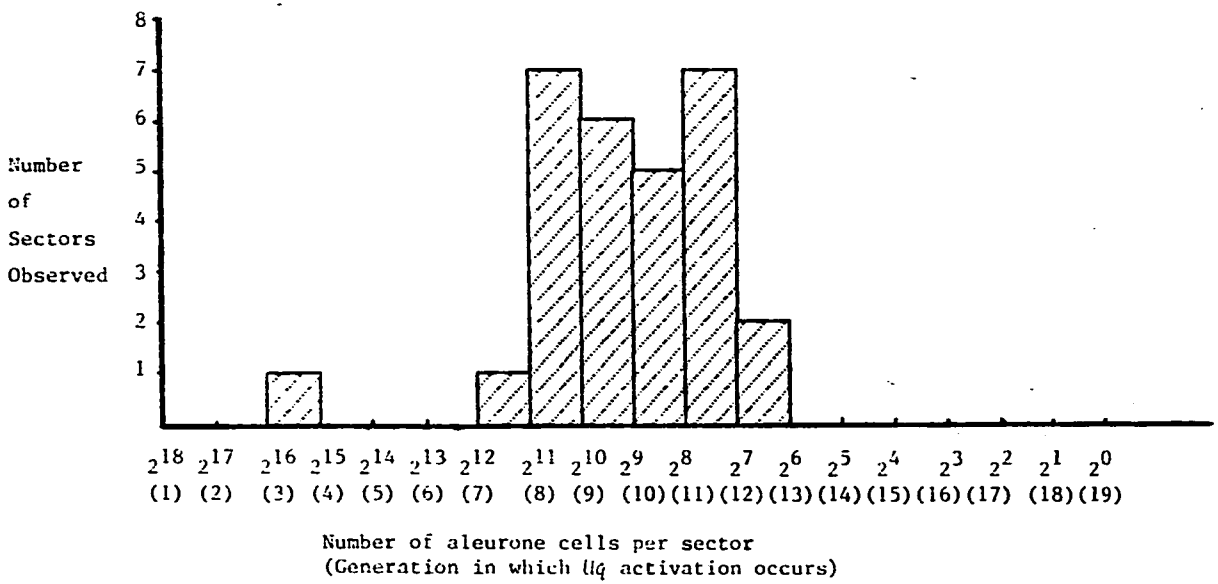
Table 4. Relative area of, and number of cells in, the sector of spotting in colorless aleurone layer of 29 individual sectored kernels generated in BC_1 progeny ears[a]

Kernel No.	Source (progeny ear)	Area of sector(mm^2)	No. of cells in sector
1	822603-22/2712	0.36	595
2	822608-23/0322	0.25	410
3	822610-22/0322	0.29	480
4	822611-22/2719	0.91	1,500
5	822612-24/2703	0.15	250
6	862613-22/3665	0.90	1,490
7	822613-23/2717	0.49	810
8	822613-27/2711	0.11	180
9	822614-22/2718	0.32	530
10	822614-26/2702	0.32	530
11	822614-27/2720	0.15	250
12	822614-29/2717	0.63	1,040
13	822614-29/2717	0.05	80
14	822615-23/2720	0.09	150
15	822615-23/2720	0.11	180
16	822616-21/2710	0.11	180
17	822616-26/2720	0.74	1,225
18	822616-25/2710	0.17	280
19	822616-27/2709	0.05	80
20	822616-28/2709	32.82	54,260
21	822616-29/3665	0.32	530
22	822616-30/2718	0.98	1,620
23	822617-23/2710	0.67	1,110
24	822617-28/2709	0.22	365
25	822620-23/2716	0.19	315
26	822620-28/2709	0.70	1,160
27	822620-28/2709	2.03	3,355
28	822622-28/2719	0.13	215
29	822623-24/0322	0.41	680

[a]The origins of these 29 sectored kernels are identified in Table 2.

Figure 4. Histogram showing the activation of Ug leading to sectored kernels

This class occurs more frequently between 7th and 13th cell generation of endosperm growth. Even more frequently, activation events occur after generation 13. They, however, usually produce the few-spotted phenotype (Table 1), and, therefore, are not included here. This graph is based on a mature maize aleurone layer consisting of 160,000 cells (Stadler, 1944). In order to produce an aleurone layer of this size, approximately 18 cycles of cell divisions are needed after fertilization.


Number of Sectors Observed
8
7
6
5
4
3
2
1
2^{18} 2^{17} 2^{16} 2^{15} 2^{14} 2^{13} 2^{12} 2^{11} 2^{10} 2^{9} 2^{8} 2^{7} 2^{6} 2^{5} 2^{4} 2^{3} 2^{2} 2^{1} 2^{0}
(1) (2) (3) (4) (5) (6) (7) (8) (9) (10) (11) (12) (13) (14) (15) (16) (17) (18) (19)
Number of aleurone cells per sector
(Generation in which *lig* activation occurs)

DISCUSSION

Since the establishment of the a-rug, Ug maize transposable element system (Friedemann and Peterson, 1982), a number of studies have been conducted to search for a genetically active Ug regulatory element in various cycles of the Iowa Stiff Stalk Synthetic (BSSS) populations (Peterson, 1986; Peterson and Salamini, 1986); in several maize genetic testers (Peterson and Friedemann, 1983); in the Rhoades-Dempsey High-loss line (Peterson, 1985b); in maize inbreds, commercial lines and/or varieties (Cormack and Peterson, 1987; Peterson, 1986; Peterson and Friedemann, 1983; Peterson and Salamini, 1986). The general method used in these studies involves crosses of the Ug-responsive a-rug tester to the tested maize material followed by either selfing or, usually, backcrossing of the resulting F_1 progeny with the a-rug tester. The availability of four other Ug-reacting c-rug alleles (Caldwell and Peterson, 1989), however, has offered a more convenient assay that directly tests Ug activity in maize populations or lines since most carry at least one recessive c allele at the C locus (Cormack and Peterson, 1987; Pan and Peterson, 1987; Peterson, 1986).

With the relative absence of the other elements in these same populations, one could consider that active Ug elements are quite pervasive. They have been found among a very small

sample of plants drawn from three cycles of the heterogenous BSSS populations, i.e., BSSSC3, BSSSC5 and BS13(S)C2 (Hallauer et al., 1983). Yet, they have not been found within the limits of sampling in the original cycle (BSSSC0) and several other cycles tested (Peterson, 1986), but are present in one of the original lines contributing to the development of BSSS (Karazawa and Peterson, 1987; J. Cormack, Agronomy Department, Iowa State University, personal communication). Active Ug is also found in the Rhoades-Dempsey High-loss line (Peterson, 1985b) and in a number of maize genetic testers (Peterson and Friedemann, 1983). Of greater interest is that there is an absence of a genetically active Ug element in either BSSS-derived maize inbreds or other maize commercial inbred lines, except in one study where less than 1% of the progeny kernels showed one spot consisting of 1 to 24 color aleurone cells (Peterson and Friedemann, 1983).

Results reported in this paper are in good agreement with the previous studies. Testing of a larger number of plants from four maize inbreds (B70, C103, C123 and 187-2) with the a-rug tester did not uncover fully spotted kernels in their BC_1 progeny population, indicating that none of these four inbreds contains a genetically active Ug element (Table 2). On the other hand, less than 1% of the progeny kernels exhibited the few-spot type mutability, and less than 0.1% of the progeny kernels had single sectors of color spots (Table

2; Fig. 3). Moreover, in further backcrossing with the a-rug line, all the progeny types whether no-spot, few-spot, or sectored do give rise to progeny with sectors of spots (Table 3). This indicates that each of the kernel types has the potential to give sectors which is indicative of the presence of quiescent Ugs in all the progeny, differing only in the frequency and times of activation. That these sectors of spotting result from an interaction between the a-rug allele and an activated Ug element is strongly supported by several lines of evidence: (1) All these sectored kernels had a a-rug/a-rug, R/R or R/r genotype containing no active Ug. (2) These sectors of spotting were indistinguishable from the standard Ug/a-rug spotting pattern. Differences in sector size simply reflect the timing of Ug activation events. Larger sectors are caused by earlier activation that contributes to a greater number of cells during ontogeny of the aleurone (Figure 4). Later-occurring activations affect a smaller amount of tissue, expressing smaller sectors in the aleurone. The various locations of these sectors in the aleurone layer can be related to one original dividing endosperm cell in which a Ug element is being activated. (3) Since none of these sectored kernels had a Ug activated in its germinal tissue, the Ug activity seen in these sector areas was not heritable.

It is well known that several factors (both internal and

external) can cause "genomic stress" (McClintock, 1984) of the maize plants which in turn activates a number of maize transposable elements in both somatic and germinal tissues. These factors include chromosome breakage during the bridge-breakage-fusion (BBF) cycle (McClintock, 1945, 1950, 1951b; Doerschug, 1973) and during tissue culture (Burr, Archer and Burr, 1987; Peschke et al., 1985), physical and chemical mutagens (Stadler, 1944; Neuffer, 1966; Walbot, 1986), and aberrant ratio after viral infection (Dellaporta et al., 1984; Friedemann and Peterson, 1982; Mottinger et al., 1984; Peterson, 1985a; Sprague and McKinney, 1966, 1971; Sprague, 1986).

In three of these studies, activation of the Dt transposable element often resulted in a few-spotted or a sectored aleurone layer of a-dt/a-dt/a-dt composition that otherwise lacked an active Dt (McClintock, 1950, 1951a; Doerschug, 1973). Sectors of mutability have also been found for several regulatory elements that undergo phase changes and phase variations (Fedoroff, 1986; McClintock, 1958, 1964, 1965; Peterson, 1966; Schnable and Peterson, 1986). One particular example is the reversible changes between an active phase and an inactive phase of an inserted Ac element at wx-m7 and their effects on the a-m3 allele that has a Ds inserted at the A locus (McClintock 1964, 1965). According to several molecular studies, no differences were found between active

and inactive Ac elements at wx-m7 in terms of both size and position of insertion (Dellaporta and Chomet, 1985). But a shift from an active phase to an inactive phase was found to be associated with modification of the Ac DNA sequences through cytosine methylation (Dellaporta and Chomet, 1985). DNA methylation is also correlated with loss of activity of another maize transposable element, the Robertson's Mutator or Mu, in a number of Mu-loss lines (Chandler and Walbot, 1986).

It seems that the factors discussed except for demethylation can be dismissed as playing a role in restoring Ug activity seen in the spotted sector areas in a-rug/a-rug/a-rug, no Ug aleurone tissue. Whether demethylation is the underlying mechanism of Ug activation cannot be tested at present until a germinal event of a similar type of activation is rescued. The standard a-rug allele originated from a maize line several generations after infection with a RNA virus (Peterson, 1985a). Upon selfing or sibbing, plants of the standard a-rug line often produce few-spotted kernels at very low frequencies (Peterson and Friedemann, 1983; Y.-B. Pan, Department of Genetics, Iowa State University, personal observation), but never sectored kernels (Pan and Peterson, 1986). In contrast, outcrossing of the standard a-rug tester with two other testers (a^{o} sh2, no Ug and C sh bz) and with several maize inbred lines has consistently produced a-rug/Ug type of sectors of different

sizes at frequencies less than 1% (Y.-B. Pan, Department of Genetics, Iowa State University, unpublished results). It appears, therefore, that there are components in the genomes of these inbred lines and the two testers that enhance the sectoring phenotype. This is deduced from the significantly higher incidence of sectored kernels arising from BC_1 sectored kernels (Table 3). Further, the significant difference between the two progenies from reciprocal crosses in the mean frequency of sectored kernels per individual BC_2 ear when the a-rug constitution was equal in each of the crosses does implicate the background genotype as enhancing Ug activation. In addition, the lowered frequencies of sectored types among the few-spot and no-spot classes would support the contention that the background genotype of these classes might have less of the sectoring-inducing components. This same observation also eliminates the a-rug allele as a contributing factor in the activation of Ug.

As a result of this study, we conclude that the presence of the spotted sectors in an otherwise colorless aleurone layer is due to the activation of a quiescent Ug element that triggers the mutability at the a-rug receptor locus. This activation occurs at any time and in any dividing cell during endosperm development. Further, the genomes of the 4 maize inbred lines, perhaps cytoplasms too, provide certain

conditions that stimulate the activation of quiescent Ug elements.

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SECTION II

SPONTANEOUS GERMINAL ACTIVATION OF QUIESCENT *Ug* TRANSPOSABLE ELEMENTS IN *Zea mays* L.

SPONTANEOUS GERMINAL ACTIVATION OF QUIESCENT *Uq* TRANSPOSABLE ELEMENTS IN *Zea mays* L.[1]

Running title: Activating transposable elements

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ABSTRACT

The spontaneous germinal activation of quiescent Uq transposable elements is reported. Thirty-nine spotted exceptions were observed at a rate of about 2 X 10^{-4} from 687 colorless ears produced from the cross of a-rug/a-rug (colorless or occasionally sectored) X an a-rug tester (colorless). All exceptions had spotting patterns distinct from the pattern of our original standard Uq (Uq1)-a-rug spotting.

From these spotted exceptions five new Uq elements (Uq2, Uq3, Uq4, Uq5, and Uq6) have been isolated. Genetic evidence for the Uq nature of the five germinal isolates is presented. First, each of the five spotted exceptions was homozygous for the a-rug reporter allele. Second, four new Uq isolates (Uq2, Uq3, Uq4, and Uq5), after being reconstituted into a a sh2/a sh2 (no Uq) line, could transactivate the standard a-rug allele and continue to produce their distinct spotting phenotypes. Third, these five new Uqs are also capable of transactivating the c-rug65 and c-rug67 alleles, However, the transactivation of c-rug is generally weaker than that of a-rug.

key words: Activation-Uq transposable element-Zea mays L.

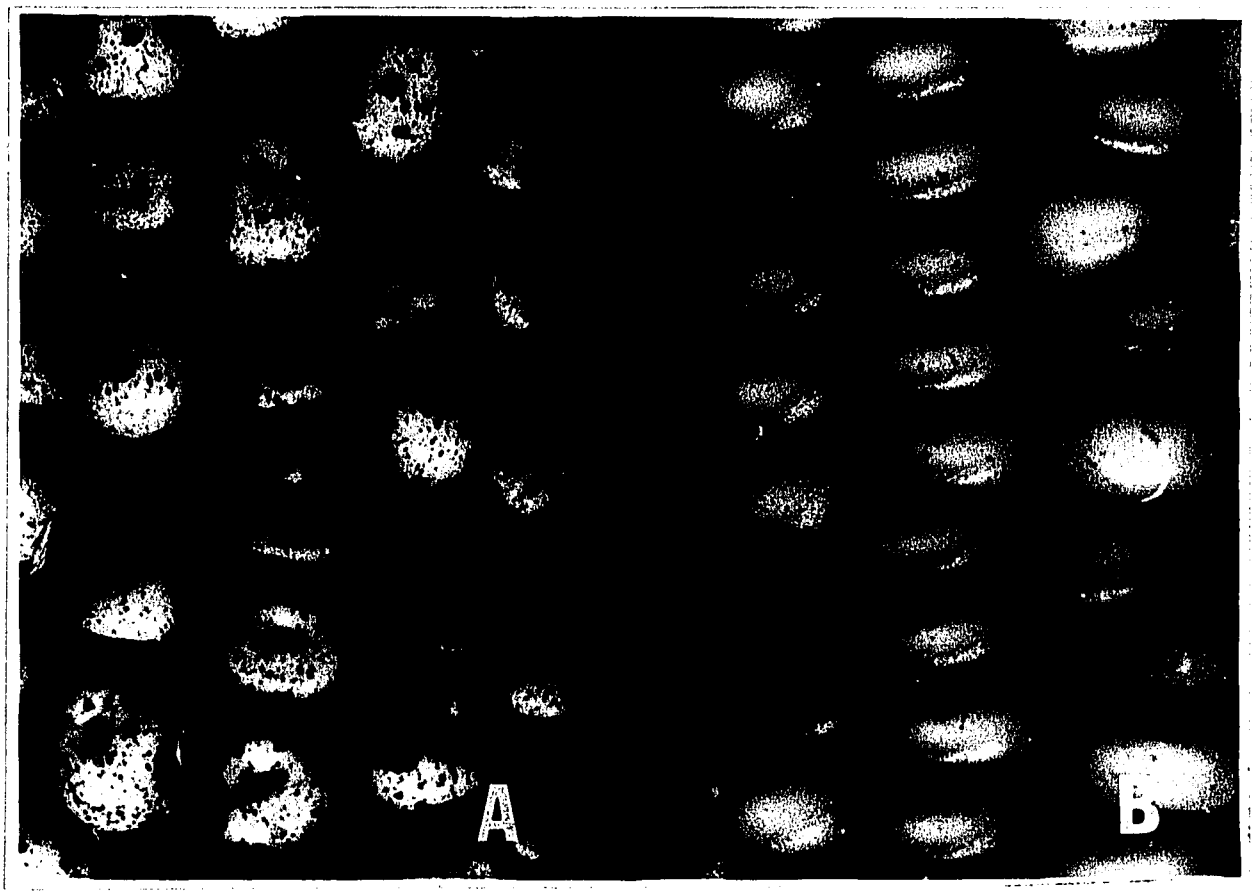
INTRODUCTION

The *Ug-rug* system (Friedemann and Peterson,1982) appears to be the most pervasive transposable element in maize and corn breeding populations. It is one of the nine established "two-element" transposable element systems in maize (Peterson, 1987). Thus far, *rug* receptor elements have been found to reside within two maize loci, i.e., *A1* (Friedemann and Peterson, 1982; Sprague, 1986) and *C1* (Caldwell and Peterson, 1989) that in association with other loci, condition maize-plant and -seed aleurone pigmentation. Without an active *Ug* element, both *a-rug* and *c-rug* are stably recessive; and maize seeds with a genotype either of *a-rug/a-rug* or *a°* or of *c-rug/c-rug* or *c°* are colorless. When an active *Ug* is present, however, with either the *a-rug* or the *c-rug* reporter allele in the same genome, kernel spotting occurs (Fig. 1), which indicates the excision of the *rug* elements away from either maize locus and the restoration of normal functioning of the maize gene. Unlike the negative dosage-effect in the *Ac-Ds* system (McClintock, 1951a), a positive dosage-effect operates in the *Ug-rug* system (Friedemann and Peterson, 1982).

When the *a-rug* or *c-rug* has been used as "reporter alleles," active *Ug* elements have been found in various maize lines, testers, and populations (Peterson and Salamini, 1986; Cormack et al., 1988). Even in maize inbred lines lacking

Figure 1. Spotting phenotype of Ug1 (the standard Ug), a-rug interaction

Ear A is from the cross (a-rug/a-rug, Ug1/Ug1) X (a-rug/a-rug, +1/+1) (see Table 1 for symbol explanations). Ear B is from the reciprocal cross (adapted from Friedemann and Peterson, 1982).


A
B

active Ug elements, the presence of quiescent Ug is obvious because Ug activity has been detected as spotted sectors of a-rug/a-rug/a-rug aleurone cells (Pan and Peterson, 1988). Because such aleurone sectors of Ug-a-rug spotting have never been found in self-pollinated plants or in sibling progeny of the a-rug tester line (Pan and Peterson, 1986), we have proposed that the Ug activity in aleurone cells of the spotted sectors arise from the transiently activated quiescent Ug sequences in the genomes of the maize inbred lines (Pan and Peterson, 1988).

In this paper, we report the isolation and genetic characterization of five such quiescent Ug elements. These new Ugs arose spontaneously through germinal activation in crosses of a-rug/a-rug (either colorless or sectored: see Pan and Peterson, 1988) X an a-rug tester (colorless). Each cross was found to elicit a readily distinguishable spotting pattern in association with the standard a-rug reporter allele. These five new Ugs were found to be able to transactivate c-rug65 and c-rug67, two other reporter alleles of Ug (Caldwell and Peterson, 1989). Finally, we present genetic proof for the authenticity of these five new Ug germinal isolates.

MATERIALS AND METHODS

Maize stocks, testers, and gene symbols

Table 1 lists the maize stocks, testers, and gene symbols used in this study.

Generation and isolation of putative new Ugs-a-rug spotted exceptions

Plants were grown in 3 Ug-activation plots from 67 colorless, 39 few-spot, and 213 sectored BC_2 sib kernels (Pan and Peterson, 1988). All sib kernels had the following genotype: a-rug/a-rug, no active Ug. These plants were hand-pollinated by an a-rug tester line (a-rug/a-rug, C Sh Bz/C Sh Bz, or C sh bz) (Table 2). Meanwhile, all the male tester plants used were self-pollinated. Progeny ears produced from both outcross and selfing were harvested and examined for exceptional spotted kernels. Because of the lack of an additional genetic marker in the a-rug male tester, only 39 individually spotted exceptional kernels were selected exhibiting spotting patterns distinct from the 3-4a or b (occasionally with 1-2c spots) pattern of Ug1 (1 dose)-a-rug spotting (ear B in Fig. 1) (Fig. 2: also refer to Reddy

Table 1. List of maize stocks and genetic testers

Maize Stock:

colorless, few-spot, and sectored BC_2 siblings (Pan and Peterson, 1988, Genetics): selected from backcrossed populations of four maize inbred lines (C103, C123, B70, and 187-2) by the standard a-rug tester. Genomes of these populations are shown to have quiescent Ug sequences spontaneously activated in spotted sectors of aleurone cells of a-rug/a-rug/a-rug.

Genetic testers:

Unless specified in the description, all genes for the anthocyanin pathway are homozygous dominant. All phenotypes refer to the maize kernels.

standard a-rug line: colorless round, homozygous for a-rug Sh2, responds only to active Ug to yield a spotted round phenotype;

a-rug/a-rug, C Sh Bz or C sh bz/C Sh Bz line: colorless round, homozygous for a-rug, responds only to active Ug to yield a spotted round phenotype. The line is the colorless round selection of the BC_1 population between a C sh bz line and the standard a-rug line (used as the recurrent parent);

ao sh2, no Ug line: colorless shrunken, homozygous for ao and sh2;

ao sh2, Ug1 line: colorless shrunken, homozygous for ao, sh2 and Ug1. Interacts with a-rug to yield a specific spotting phenotype (Fig. 1);

c-rug65 line: colorless round, homozygous for c-rug, Sh1 and Wx, responds only to active Ug to yield a spotted round phenotype;

c-rug67 line: colorless round waxy, homozygous for c-rug, Sh1 and wx, responds only to active Ug to yield a spotted round phenotype;

a-m1 sh2 line: colored shrunken, homozygous for a-m1 and sh2, responds only to active En to yield a spotted phenotype;

Table 1. (Continued)

a-m(r)h/a-mdt line: colorless round, F_1 progeny of a-m(r)h and a-mdt, responds only to active Mrh or Dt to yield a spotted phenotype;

Line C: a derivative of line W22, is homozygous for all dominant anthocyanin genes except pr/pr.

Gene Symbols:

Uq1: the standard Uq element (Friedemann and Peterson, 1982);
Uq2: the new Uq element isolated from spotted exception 870621y;
Uq3: the new Uq element isolated from spotted exception 870801u;
Uq4: the new Uq element isolated from spotted exception 870829u;
Uq5: the new Uq element isolated from spotted exception 870829y;
Uq6: the new Uq element isolated from spotted exception 870834y;
Uq*: stands for any new Uq element in case concerned;
+1, +2, +3, +4, +5, +6: refers to the absence of Uq1, Uq2, Uq3, Uq4, Uq5, and Uq6 in its allelic site, respectively.

Terms:

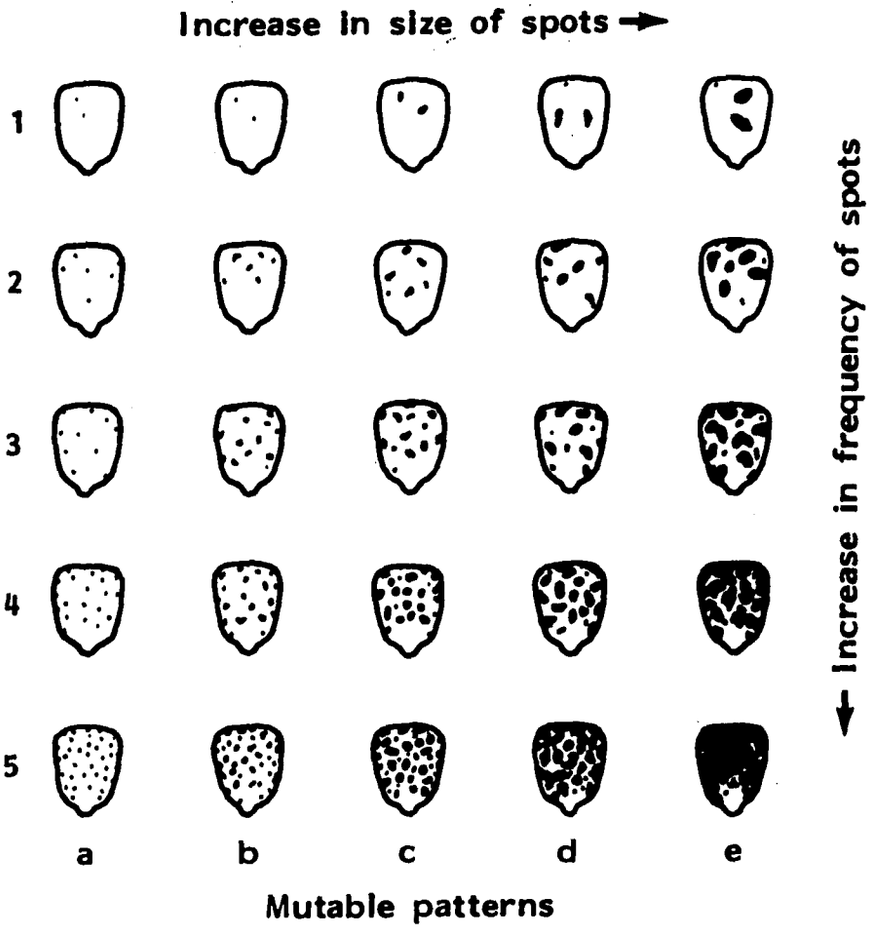
Quiescent: refers to an element in the genome that has the potential for transactivity but for some cause (methylation?) is inactive until some process activates the element.

Table 2. Frequency of spontaneous germinal activation events of quiescent Ug in the crosses of colorless or few-spot or sectored (a-rug/a-rug, no active Ug) X a-rug tester (see Pan and Peterson, 1988 for definitions)

Plot and No. of plants	# of ears	kernel classification			frequency of spotted
		not-spotted[a]	spotted	total	
colorless 67	68	18,785	5	18,790	2.6×10^{-4}
few-spot 39	81	27,148	4	27,152	1.5×10^{-4}
sectored 213	538	144,299	30	144,329	2.0×10^{-4}
319	687	190,232	39	190,271	

[a]This class includes colorless, few-spot and sectored kernels.

Figure 2. Standardized diagrammatic array of spotting patterns used to classify kernel types in maize genetic analysis (adapted from Reddy and Peterson, 1984)


Increase in size of spots →
1
2
3
4
5
a
b
c
d
e
Mutable patterns
← Increase in frequency of spots

and Peterson, 1984, for identification of pattern classes). Because of space and labor limitations, of these 39 exceptions, only five (870621y, 870801u, 870829u, 870829y, and 870834y) were utilized in further genetic experiments.

Testing the heritability of the five spotted exceptions and their Ug-a-rug nature

To confirm their heritability, each of the five spotted exceptions was crossed as female by an a° sh2 (no Ug) tester (Type I in Table 3). Each was also crossed as male to an a-rug tester (Type II in Table 3). We used the a° sh2 (no Ug) tester for two reasons: (1) to separate the two homologous a alleles in each of the spotted exceptions; and (2) to isolate the putative new Ug element in each spotted exception into an a° sh2/a° sh2 genetic background. This isolation could be accomplished by selfing the spotted round F_1 progenies from the confirmation tests. The authenticity of each new Ug could be established if the colorless shrunken F_2 progenies produced spotted ears when tested on the standard a-rug tester plants, thereby showing similar if not identical spotting patterns to that of the confirmation ear.

i. Test 1: Identification of a-rug homozygosity in the spotted exceptions

In each spotted exception selected, one a-rug allele needed to have been derived from the BC_2 progenies from the female plants in our Ug activation plots. Absence of a useful additional genetic marker in the a-rug male tester, however, made it difficult to determine whether the second a allele was also a-rug. If it were a-rug, then all the colorless round sibs from the confirmation ears (Table 3, type I, by a° sh2, (no Ug) tester) would have been of the same genotype, i.e., a-rug Sh2/a° sh2, no Ug. When these colorless round sibs are tested by a verified a° sh2/a° sh2, Ug1/Ug1 tester, all should produce progeny ears segregating for 50% spotted round and 50% colorless shrunken. In contrast, if the second a allele is not a-rug (that is, if it has come from a contaminated a(?) pollen), then the colorless sibs from the type I confirmation ears should have two genotypes, either a-rug/ao sh2, no Ug or a(?)-Sh2/ao sh2, no Ug. When these colorless round sibs are tested by the same verified a° sh2/a° sh2, Ug1/Ug1 tester, at least one out of seven progeny ears should segregate 50% colorless round and 50% colorless shrunken at a probability of 99% (Sedcole, 1977), in addition to those segregating 50% spotted round and 50% colorless shrunken.

Table 3. Genetic analysis of the confirmation tests of the five spotted exceptions

Confirmation ear[a]	Source of[b]	Spotting pattern	Kernel classification[c] cl-rd	spotted[d]	Cl-rd	total	(X^2)[e]
Type I: by a° sh2, (no Ug) tester							
870621y/0709	Ug2	heavy	266	266	7	539	0.07^{ns}
870801u/0819	Ug3	varies	314	74(6)	0	388	142^{**}
870829u/0731	Ug4	varies	257	216(12)	0	485	1.62^{ns}
870829y/0731	Ug5	varies	245	228(10)	1	474	1.77^{ns}
870834y/0732	Ug6	varies	288	39(244)	11	582	0.04^{ns}
Type II: on a-rug tester							
870712/0621y	Ug2	6-7bc/>10a	93	95	1	189	0.02^{ns}
870824/0801u	Ug3	----------diseased----------					
870726/0829u	Ug4	varies	133	120(3)	2	258	0.19^{ns}
870712/0829y	Ug5	varies	148	118(4)	0	270	2.31^{ns}
870813/0834y	Ug6	5-6c	220	196	0	416	1.27^{ns}

[a]Spotted pattern of the five original exceptions: **870621y** = 6-7bc in 10a spotted background; **870801u** = 4b in 4a background; **870829u** = 4a or b; **870829y** = 3b or c in 5a background; **870834y** = 5-6c.

[b]Ug3, Ug4, Ug5 and Ug6 were isolated from the colored-like spotted kernels.

[c]Abbreviations used in Tables: **Cl** = colored; **cl** = colorless; **spt** = spotted; **rd** = round; **sh** = shrunken.

[d]Number in parenthesis is the number of colored-like spotted kernels out of the total spotted.

[e]A X^2 value for 1 (spt + Cl-rd) : 1 cl-rd segregation; ns = not significant at 0.05 level; ** = significant at 0.01 level.

Table 4. Phenotypic classification of the progeny ears derived from the crosses of the colorless round selections from the confirmation ears (see Table 3) X *a° sh2/a° sh2*, *Ug1/Ug1*

Confirmation ear	# of cl-rd tested	classification of progeny ears[a] I	II
870621y/0709	11	0	11
870801u/0819	12	0	12
870829u/0731	13	0	13
870829y/0731	11	0	11
870834y/0732	13	0	13

[a]Class I: ears segregating 50% colorless round and 50% colorless shrunken; Class II: ears 50% spotted round (*Ug1-a-rug* type) and 50% colorless shrunken.

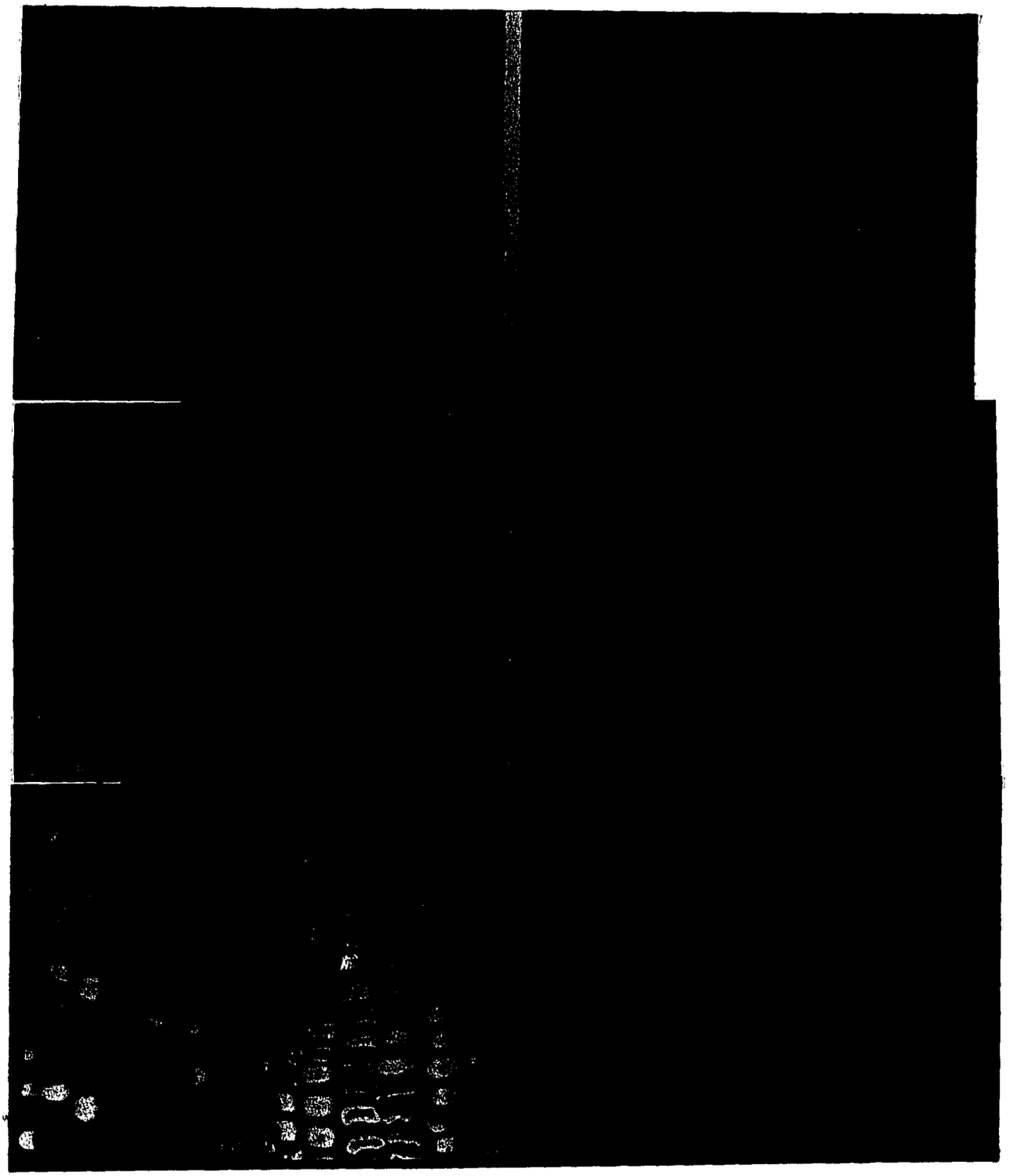
To test this hypothesis, 14 colorless round kernels from the confirmation ear (type I) of each spotted exception (Table 4) were randomly selected and were testcrossed as female by an a° sh2/a° sh2, Uq1/Uq1 male tester. The tester itself was crossed as male simultaneously onto standard a-rug tester plants to verify the homozygosity of Uq1. All progeny ears were examined for the frequency and pattern of spotted kernels.

ii. Test 2: Identification of the Ug element triggering spots

Spotted round kernels of a unique pattern were selected from each Type I confirmation ear in Table 3. For exceptions 870801u, 870829u, and 870829y, the selections had a heavy spotting and were nearly full-colored (Fig. 3, 2a, 3a, and 4a). These were either self-pollinated to give the F_2 generation (for 870621y, 870801u, 870829u, and 870829y) or outcrossed as male to the a-m1 sh2 tester (for 870834y). Colorless shrunken F_2 progenies, 32, 11, 10, and 11 for exceptions 870621y, 870801u, 870829u, and 870829y, respectively, were crossed simultaneously as male to several tester lines containing element-reporting alleles. These included the a-m1, a-mrh/a-mdt, and a-rug tester lines testing for active En, Mrh/Dt, and Ug elements, respectively. For

Figure 3. Spotting phenotypes of Uq, a-rug interactions

1a: a-rug tester X a° sh2/a° sh2, Uq2/Uq2; **2a:** a-rug tester X a° sh2/a° sh2, Uq3/Uq3; **3a:** a-rug tester X a° sh2/a° sh2, Uq4/+; **4a:** a-rug tester X a° sh2/a° sh2, Uq5 +/ + Uq5; **5a:** a-m1 sh2/a-rug Sh2, Uq6/+ X a° sh2, no Uq tester;
1b through 5b: a-rug/a° sh2, +/+ (colorless round selection from the confirmation ears of the new Uq mutants: **1b:** Uq2; **2b:** Uq3; **3b:** Uq4; **4b:** Uq5; **5b:** Uq6) X a° sh2/a° sh2, Uq1/Uq1.



870834y, 14 colored shrunken and 28 colored round outcross progenies were crossed by the a-rug tester and the a° sh2 (no Ug) tester, respectively, to test for the authenticity of the activated Ug. In addition, the spotted round selections from the confirmation ear 870834y/0732 were also crossed as male to Line C, a W22 inbred colored line. Thirteen colored round progenies of this cross were randomly selected and self-pollinated. These selfed ears were examined for frequency and pattern of spotted kernels.

Test for the ability of the new Ugs to transactivate c-rug65 or c-rug67 reporter alleles

Each of the five spotted exceptions was crossed as male to either the c-rug65 or the c-rug67 tester. Seven colored or mottled F_1 progenies for each exception were planted in the greenhouse and backcrossed to either c-rug tester. The BC_1 progeny ears were examined for the frequency and pattern of spotted kernels.

RESULTS

Generation and isolation of spotted exceptions conditioned by putative activated Ug elements

A total of 687 colorless progeny ears (190,271 kernels) were harvested from the 319 plants in the 3 Ug activation plots containing the a-rug reporter allele (Table 2). In addition to the few-spotted and sectored progeny kernels occurring at frequencies of about 1.6 X 10^{-3} in a previous experiment (Pan and Peterson, 1988), a total of 39 fully-spotted exceptions were selected, each exhibiting a distinctly different spotting pattern from the 3-4a or b (occasionally with 1-2c spots) pattern expected of the standard Ug1 (1 dose)-a-rug spotting (Fig. 1, ear B) (see Fig. 2 and Reddy and Peterson, 1984 for spotting pattern identification). Among these, five were found from the colorless plot, four from the few-spot plot, and 30 from the sectored plot, with frequencies of 2.6 X 10^{-4}, 1.5 X 10^{-4}, and 2.0 X 10^{-4}, respectively.

Because of space and labor limitations, only five of the 39 spotted exceptions that expressed decidedly distinct phenotypes were pursued for further genetic testing (Table 3). These were designated 870621y, 870801u, 870829u, 870829y, and 870834y, respectively. Following confirmation tests with either the a^{o} sh2 (no Ug) (producing Type I ears) or the a-rug

(producing Type II ears) testers, all but 870801u produced progeny ears segregating 50% spotted round and 50% colorless round kernels (Table 3). An aberrant ratio was observed on the confirmation ear 870801u/0819, which had an excess of colorless round progenies. As will be discussed, this aberrant ratio was very likely due to reinactivation or to loss of the Ug3 element.

On the other hand, variation in spotting pattern was observed for both types of confirmation ears derived from exceptions 870801u, 870829u, 870829y, and 870834y (Table 3). In addition to kernels showing the original spotting pattern (footnote a in Table 3), there were heavy, near full colored spotted kernels. These colored-like kernels were selected and used as sources of Ug3, Ug4, Ug5, and Ug6 (footnote b in Table 3).

All five spotted exceptions were homozygous for the standard a-rug reporter allele

Results from test 1 (testing Ug1 against the reporter alleles in each of the five spotted exceptions) are summarized in Table 4. At least 11 out of the 14 colorless round selections from each confirmation ear (type I) of the five spotted exceptions have been crossed by verified a° sh2/a° sh2, Ug1/Ug1 tester plants. The homozygosity of Ug1 in these

tester plants was demonstrated by their yielding approximately 100% spotted kernels on standard a-rug tester plants (data not shown). Evidently, all progeny ears derived from crosses of the colorless selections X a° sh2/a° sh2, Ug1/Ug1 belonged to class II and segregated for 50% spotted round and 50% colorless shrunken kernels (photographs of sample ears are shown in Fig. 3, 1b through 5b). In addition, regardless of the source of the colorless round selection, all tests yielded the same spotting phenotype as that of the Ug1-standard a-rug interaction (comparing ear B in Fig. 1 with ears 1b through 5b in Fig. 3). The results from these tests provided definitive evidence for the homozygosity of the standard a-rug allele in each of the five original spotted exceptions in Table 3. These results also indicated that the unique spotting pattern (Table 3, footnote a) manifested by each spotted exception was not due to changes in the a-rug reporter allele but to the effect of different Ug elements.

The unique spotting phenotypes in five original spotted exceptions can be reconstituted by the interaction between the isolated new Ug elements and the standard a-rug allele

Test 2 was conducted under the assumption that the five exceptional spotted phenotypes were the product of the interaction between the standard a-rug allele and the new germinally activated Ug elements. It would then be expected that approximately 3/4 of the F_2 colorless shrunken progenies contained at least one active copy of the corresponding newly-activated Ug, whereas the remaining 1/4 would have none. This new Ug, in combination with the standard a-rug reporter allele, would impart its particular spotting pattern.

The results from **Test 2** verified this proposal (Table 5). First, 22, 8, 7, and 9 of the colorless shrunken (a° sh2/a° sh2) F_2 progenies derived from the exceptions 870621y, 870801u, 870829u and 870829y, respectively, were demonstrated to contain at least one new Ug element (Tables 5, 6a through 6d). These new Ug elements were designated Ug2, Ug3, Ug4, and Ug5, respectively. Second, the particular spotting pattern for each new Ug on interaction with the a-rug allele was reconstituted: One dose of Ug2 elicited a pattern of 6-7bc spots in 10a spotting background (1a in Fig. 3), in interaction with the standard a-rug allele. One dose of Ug3

Table 5. Summary data from the reconstitution tests[a] on new Ugs (Uq*s)

Uq* element	Uq* content in the colorless shrunken F_2 selections				
	+/+	Uq*/+	Uq*/Uq*	Uq* +/+ Uq*	Total
Uq2	10	20[b]	2[c]	0	32
Uq3	3[d]	7[e]	1	0	11
Uq4	3	6	0	1	10
Uq5	2	8	0	1	11

[a]The tests included multiple-crosses of the a-rug, a-m1, a-mdt/a-mrh tester lines (as female plants) by the colorless shrunken F_2 selections.

[b]One out of the 20 F_2 also had an active En.

[c]Self-pollination of these two F_2 plants failed to set ears.

[d]Two out of the three F_2 also had an active En.

[e]The Uq3 element in one F_2 was found to be either lost or inactive in its main stalk but to be active in its tiller; in addition, five F_2 plants also had an active En.

Table 6a. The reconstituted *Uq2* element in *a° sh2/a° sh2* genetic background transactivates specifically the standard *a-rug* reporter allele

Progeny	Kernel classification			*Uq2*	Reaction to other reporter alleles	
ear[a]	cl-rd[b]	spotted	total	status	*a-m1*	*a-mdt/a-mrh*
880355/0206-2	174	163	337	*Uq2*/+2	-	-
880359/0206-3	216	248	464	"	-	NA[c]
880354/0206-9	101	129	230	"	-	-
880357/0206-10	230	241	471	"	-	NA
880329/0206-11	116	107	223	"	-	NA
880326/0206-13t	246	221	467	"	-	NA
880357/0206-14	56	42	98	"	-	-
880353/0210-2	82	77	159	"	-	-
880350/0210-5	35	42	77	"	-	-
880351/0210-7	0	100%	100%	*Uq2/Uq2*	-	-
883648/0210-8	267	254	521	*Uq2*/+2	NA	-
880353/0210-9	73	64	137	*Uq2*/+2	-	-
880349/0210-11	0	100%	100%	*Uq2/Uq2*	-	-
880356/0210-12	77	67	144	*Uq2*/+2	-	-
880329/0210-13	207	198	405	"	-	NA
883648/0213-3	202	197	399	"	-	-
883649/0213-4	98	125	223	"	*En*/+	NA
880321/0213-6	42	27	69	"	NA	-
883648/0213-7	207	174	381	"	NA	NA
880350/0213-8	91	92	183	"	-	-
880348/ "	69	182	251	"	-	-
880354/ "	117	172	289	"	-	-
883649/ "	17	25	42	"	-	-
883648/0213-9	206	234	440	"	NA	NA
880354/0213-10	154	159	313	"	NA	-

[a]Derived from the crosses of *a-rug* tester X colorless shrunken F_2 plants (*a° sh2/a° sh2*, *Uq2/Uq2* or +2); data for other nine F_2 plants containing no *Uq2* are not shown.

[b]cl-rd = colorless round.

[c]NA = cross not available.

Table 6b-1. The reconstituted Uq3 element in a° sh2/a° sh2 genetic background transactivates specifically the standard a-rug reporter allele

Progeny	Kernel classification			Uq3	Reaction to other reporter alleles	
ear[a]	cl-rd[b]	spotted	total	status	a-m1	a-mdt/ a-mrh
8803227/0217-1	0	336	336	Uq3/Uq3	-	-
880329/0217-4t	235	117	352	Uq3/+3	En/En	-
883812/0217-5	169	212	381	"	-	NA
880347/0217-7	23	4	27	"	En/+	-
880346/0217-8	455	0	455	+3/+3[c]	"	-
880330/0217-8t	129	282	411	Uq3[c]/+3	"	-
880330/0217-9t	376	9	385	Uq3[d]/+3	"	-
883812/0217-11	167	152	319	Uq3/+3	NA	NA
880330/0217-13t	117	8	125	Uq3[d]/+3	En/En	-

[a]Derived from the crosses of a-rug tester X a° sh2/a° sh2, Uq3/Uq3 or +3.

[b]cl-rd = colorless round.

[c]The Uq3 element activity in plant 880217-8 had been totally lost in the main stalk but had two linked Uq3 elements in its tiller.

[d]Crosses with main stalk were not available, but Uq3 activity almost lost completely in these two F_2s.

Table 6b-2. The reconstituted Ug3 element in a-m1 sh2/a-rug Sh2 genetic background retains its specific ability to transactivate the standard a-rug reporter allele

Progeny ear[a]	Kernel classification[b]					T.E. content	
	cl-rd	Cl-rd	Cl-sh	spt	total	Ug3	En
880218y-1t/0310	0	136	233	105	474	Ug3/+3	+/+
y-2t/0331	0	124	253	118	495	"	"
y-4t/0331	0	153	207	78	438	Ug3[c]/+3	+/+

[a]Derived from the crosses of (a-m1 sh2/a-rug Sh2 Ug3/+3) X a-m1 sh2 tester.

[b]See footnote c in Table 3 for abbreviations.

[c]An excess of colored round over spotted indicates a partial loss of Ug3.

Table 6c. The reconstituted *Uq4* element in *a° sh2/a° sh2* genetic background transactivates specifically the standard *a-ruq* reporter allele

Progeny ear[a]	Kernel classification: cl-rd	spotted: heavy	spotted: 2-5b	total	Uq4 status	Reaction to other reporter alleles: a-m1	a-mdt/ a-mrh
880346/0219-1	86	22	98	206	*Uq4*/+4	-	-
880327/ "	169	25	96	290	"	-	-
880351/0219-2	202	0	37	239	*Uq4*[b]/+4	-	-
880359/0219-3	245	31	100	376	"	-	-
880346/ "	402	3	134	539	"	-	-
880346/0219-4	54	28	17	99	*Uq4*/+4	-	-
880352/0219-6	70	27	270	367	*Uq4 +4/ +4 Uq4*	-	-
880346/ "	9	11	132	152	"	-	-
880360/ "	80	151	69	300	"	-	-
880328/0219-7	229	104	54	387	*Uq4*[b]/+4	-	NA
880317/0219-8	164	0	107	271	"	-	-

[a]Derived from the crosses of *a-ruq* tester X *a° sh2/a° sh2*, *Uq4*/+4 or *Uq4 +4/+4 Uq4* (plant 880219-6 had linked *Uq4* elements, more likely as a result of secondary transposition).

[b]Partial loss or inactivation of *Uq4* activity in these crosses.

Table 6d. The reconstituted Uq5 element in a° sh2/a° sh2 genetic background transactivates specifically the standard a-ruq reporter allele

Progeny ear[a]	Kernel classification: cl-rd	spotted: heavy	spotted: 2-5b	total	Uq5 status	Reaction to other reporter alleles: a-m1	a-mdt/ a-mrh
880359/0221-1	276	27	31	334	Uq5[b]/+5	-	-
880330/0221-1t	162	0	32	194	"	-	-
880326/0221-2	188	42	102	332	Uq5/+5	-	NA
883649/0221-3	204	36	153	393	"	NA	NA
883649/0221-6	250	0	227	477	"	NA	NA
880329/0221-8	3	61	235	299	Uq5 +5/ +5 Uq5	-	NA
880324/0221-10	139	10	83	232	Uq5[b]/+5	-	NA
880355/0221-11	307	0	16	323	"	NA	-
880347/ "	338	0	56	394	"	"	-
880358/0221-11t	125	0	24	149	"	"	-
880328/ "	137	0	38	175	"	"	-
880348/0221-12	237	0	145	382	"	-	-
880327/0221-12t	204	24	132	360	"	NA	NA
880327/0221-13	146	0	123	269	"	-	NA
880348/0221-16	33	4	21	58	"	NA	-

[a]Derived from the crosses of a-ruq tester X a° sh2/a° sh2, Uq5/+5 or Uq5 +5/+5 Uq5 (plant 880221-8 had linked Uq5 elements, more likely as a result of secondary transposition).

[b]Partial loss or inactivation of Uq5 activity in these crosses.

elicited a homogeneous colored-like spotting pattern (2a in Fig. 3). The spotting pattern of one dose of Uq4 or Uq5 was found, however, to continue to vary, with a majority of 4ab for Uq4 and 3bc in 5a background for Uq5 and with a minor portion of colored-like spotting for both Uqs (3a and 4a in Fig. 3). Uq4 and Uq5's elicitation of two different spotting patterns on the same a-ruq allele has persisted in later experiments, a phenomenon likely related to the relative position of the element, as a result of secondary transposition (Y.-B. Pan, Department of Genetics, Iowa State University, unpublished results).

Neither active Mrh nor Dt elements were detected in these tested colorless shrunken F_2 progenies (Tables 6a through 6d). They all produced colorless ears on a-mrh/a-mdt tester plants (data not shown). The En content, however, was found to differ among pedigrees. No active En element was present in the colorless shrunken F_2 progenies in the Uq4 and Uq5 pedigrees (Table 6c, 6d). One Uq2-related colorless shrunken F_2 (out of 32 tested) and 7 Uq3-related colorless shrunken F_2 progenies (out of 11 tested) were found to contain active En (Tables 6a and 6b-1); yet, this typical En-a-m1 type spotting was consistent in all cases, regardless of pedigree or of new Uq content. Therefore, these En elements were unrelated to the newly-activated Uq2 and Uq3 elements.

There were three tests for Uq6, the putative Uq element that controlled the 5-6c spotting pattern in 870834y. In the first and second tests, colored shrunken and colored round siblings from the cross a-m1 sh2/a-m1 sh2 tester X a-rug Sh2/a° sh2, Uq6/+6 were used. Only one plant was grown from the 14 colored shrunken (a-m1 sh2/a° sh2, Uq6 or +6/+6) progenies and it did not contain Uq6. When the colored round selections were crossed by the a° sh2, no Uq line, however, 10 out of the 22 progeny ears (Table 7) were found to segregate 50% colored shrunken : 25% colored-like spotted round (unique for Uq6-a-rug) : 25% colorless round, a ratio indicative of a genotype of a-rug Sh2/a-m1 sh2, Uq6/+6 for these colored round selections. The other 12 colored round progenies were found to have no Uq6. Following crosses with the a° sh2, no Uq tester, they segregated 50% colored shrunken and 50% colorless round (Table 7).

In the third test, 13 colored round F_1 selections from the cross of a colored line, "Line C" X spotted round kernels from 870834y/0732 (Table 3, Type I, Uq6) were self-pollinated and yielded similar results. Although 11 F_1 progenies did not have Uq6 and their F_2 progenies segregated approximately for 3/4 colored : 1/4 colorless, 2 F_1 progenies had Uq6 and segregated approximately for 12/16 colored, 3/16 spotted and 1/16 colorless in their F_2 (data not shown).

Table 7. Segregation pattern of the progeny kernels derived from the cross (a-m1 sh2/a-rug Sh2, Ug6 or +6/+6) X a° sh2, no Ug tester

Progeny ear[a]	Classification of progeny kernels[b]					
	Cl-sh	Cl-rd	heavy-spt-rd	cl-rd	total	$(X^2)^c$
1	277	0	157	140	574	1.70^{ns}
2	176	0	77	67	320	3.82^{ns}
3	286	11	129	155	581	0.91^{ns}
4	148	0	67	91	306	4.09^{ns}
5	161	0	40	132	333	51.20^{**}
6	185	0	38	147	370	64.22^{**}
7	71	1	22	50	144	10.15^{*}
8	164	0	87	95	346	1.31^{ns}
9	22	0	12	12	46	0.09^{ns}
10	78	0	30	27	135	3.40^{ns}
11-22	50%	0	0	50%	100%	

[a]Ug6 is not present in progeny ears #11 through 22.
[b]See footnote c in Table 3 for abbreviations.
[c]A X^2 for 50% colored shrunken : 25% spotted round : 25% colorless round; ns = not significant at 0.05 level, * = significant at 0.05 level, ** = significant at 0.01 level; the excess of colorless round progenies in ears #8, 9 and 10 was due to partial loss or inactivation of Ug6.

These results, in conjunction with the homozygous a-rug in the original spotted exception 870834y (Table 4), provide adequate evidence that Ug6 was indeed the independent element controlling the heavy spotting pattern in 870834y.

In conclusion, results derived from both Test 1 and Test 2 clearly demonstrated that the unique spotting patterns of the five spotted exceptions, exhibited in Fig. 3, 1a through 5a, represent the phenotypic expression of the interactions between these different new Ug elements and the same standard a-rug allele.

Ug2, Ug3, Ug4, Ug5 and Ug6 transactivate the c-rug65 and c-rug67 reporter alleles

Five, 6, 6, 8, and 10 BC_1 progeny ears (c-rug tester as the recurrent parent) were produced from each of the original Ug2, Ug3, Ug4, Ug5, and Ug6 spotted exceptions, respectively (Table 8). The presence of spotted ears among these BC_1 progeny ears in all Ug categories (2 spotted ears in Ug2, 2 in Ug3, 3 in Ug4, 3 in Ug5, and 3 in Ug6) (Table 8) indicates that all these five new Ugs are capable of transactivating the c-rug alleles. The frequencies of Ug-c-rug spotted kernels, however, were generally lower than the expected 25% in Ug3, Ug4, Ug5, and Ug6 pedigrees (Table 8). This indicates that

these four new *Ug* elements may have less potency in transactivating the *c-rug* reporter alleles.

Table 8. The newly-activated Ug elements transactivate the c-rug65 and c-rug67 alleles

Progeny	Progeny kernel classification[b]				Ug* status	
ear[a]	Cl-rd	cl-rd	spt-rd	total	Ug*/+	+/+
1-3	50%	50%	0	100%		x
4	38	16	22	76	x	
5	112	58	55	225	x	
6	50	33	9	92	x	
7	1	1	1	3	x	
8	40	45	1	96	?	
9-11	50%	50%	0	100%		x
12-14	50%	50%	0	100%		x
15	5	3	2	10	x	
16	95	65	36	196	x	
17	104	95	11	210	x	
18-22	50%	50%	0	100%		x
23	16	32	2	50	x	
24	112	72	27	211	x	
25	36	25	9	70	x	
26-32	50%	50%	0	100%		x
33	123	118	15	256	x	
34	16	17	7	40	x	
35	116	89	51	256	x	

[a]Progeny ears were derived from crosses of (A/a-rug, C/c-rug, Ug* or +/+) X c-rug tester. Ug* implies: Ug2 in progeny ears #1 through 5; Ug3 in ears #6 though 11; Ug4 in ears #12 through 17; Ug5 in ears #18 through 25; and Ug6 in ears #26 through 35.

[b]See footnote c in Table 3 for abbreviations.

DISCUSSION

Both active and inactive forms of maize transposable elements are known to exist in the maize genome. Phase variation (McClintock, 1965; Peterson, 1966) or cyclic events of activation and inactivation have been reported in three maize transposable elements, namely En/Spm (McClintock, 1958; Peterson, 1966), Ac (McClintock, 1964, 1965), and Dt (Doerschug, 1973). In addition, activation of quiescent transposable elements can occur either spontaneously such as in Ug (Pan and Peterson, 1988) and Cy (Schnable and Peterson, 1986) or in the presence of another weakly active element such as Spm/En (Fedoroff, 1989).

Both internal and external environmental factors, also known as "genomic stress or shock" (McClintock, 1984) factors, have been found to stimulate the activation of maize transposable elements. These factors include chromosome breakage (McClintock, 1945, 1950, 1951b; Doerschug, 1973), tissue culture (Burr et al., 1987; Peschke et al., 1985), physical and chemical mutagens (Stadler, 1944; Neuffer, 1966; Walbot, 1986), viral infection (Dellaporta et al., 1984; Friedemann and Peterson, 1982; Mottinger et al., 1984; Peterson, 1985; Sprague and McKinney, 1966, 1971; Sprague, 1986), and demethylation (discussed in Otto and Walbot, 1990).

In this study, we have demonstrated the origin of newly-

activated Ug elements. One advantage of our experiments was that maize stocks known to be homozygous for the Ug-reporting a-rug allele and to have quiescent Ug sequences were available. These included the colorless, few-spot, and sectored BC_2 sib kernels available from our previous Ug activation experiment (Pan and Peterson, 1988).

As expected, in crossing with the a-rug tester, BC_2 sibs produced only colorless ears. Nevertheless, individual fully-spotted exceptional kernels were found among these colorless ears, at frequencies between 1.5 - 2.6 X 10^{-4}. Because there was no additional genetic marker in the a-rug tester (the male tester plants were confirmed to contain no active Ug by self-pollination) in our Ug activation plots, two precautions were adopted. The first was to limit our selection to those exceptional kernels showing a decidedly different spotting pattern from the 3-4a or b (occasionally with 1-2c spots) pattern of Ug1 (1 dose)-a-rug interaction. The second was to carry out rigid genetic tests to determine if the selected spotted exceptions were homozygous a-rug (i.e. all the pollen was a-rug) and had verified new Ug elements.

A total of 39 spotted exceptions was selected based on their non-Ug1 phenotypes (Table 2). A series of genetic tests were conducted for five of these original selections. Actual results from test 1 indicated that both alleles at the a locus in the five selected spotted exceptions were standard a-rug.

In addition, we have successfully reconstituted in test 2 the unique spotting phenotypes of Ug2, Ug3, Ug4, and Ug5 in ao sh2/ao sh2 background with the standard a-rug allele (Fig. 3, 1a through 4a; Table 5; Tables 6a through 6d). This demonstrates their independent origin.

None of these five new Ug elements were found to interact with a-m1, a-mdt, or a-m(r)h, the reporter alleles for En, Dt, and M(r)h, respectively. These results suggested that all the five original spotted exceptions, although each exhibited a spotting pattern (Table 3) distinct from that of Ug1-standard a-rug, had two copies of the standard a-rug reporter allele and that the various unique spotting phenotypes exhibited by these exceptions were under the effect of different new Ug isolates.

The authenticity of Ug6, which conditioned the colored-like phenotype in spotted exception 870834y has been established based on two facts: (1) a segregation ratio of 50% colored shrunken : 25% colored-like spotted round : 25% colorless round in progeny ears derived from the testcrosses in which colored-like seeds (a-m1 sh2/a-rug Sh2, Ug6 or +6/+6) were crossed by the a^{o} sh2, no Ug tester (5a in Fig. 3, Table 7); and, (2) the a-rug homozygosity in 870834y.

Additional proof for the authenticity of these germinal Ug isolates has been derived from their ability to transactivate the Ug1-responsive c-rug65 and c-rug67 alleles.

It should be noted that Uq3 was extracted from the six colored-like heavy spotted derivatives among the 4b-c/4a spotted class on the confirmation ear 870801u/0819. This colored-like spotting was found to be heritable (Table 6b). On the other hand, Uq5 (Uq4 somehow behaves similarly to Uq5) was also extracted from the 10 colored-like spotted derivatives among 228 2-4b/6-7aa spotted class on the confirmation ear 870829y-3/0731. But in this case, the colored-like phenotype was not stably transmitted. In a testcross of such colored-like individuals with the a-rug tester, only a portion of the spotted progenies was colored-like; whereas the majority was 2-4b/6-7aa.

That two distinct spotting phenotypes could be produced by the same new Uq element on the same a-rug allele may be a result of the relative positions of the element in maize chromosomes. This has been ascribed to a "position effect" (Peterson, 1977). It is possible that these new Uq elements could have undergone cycles of secondary transposition by which their chromosome positions were altered. There have been several cases in which linked copies of Uq3 (Table 6b-1, plant 880217-8t), Uq4 (Table 6c, plant 880219-6), and Uq5 (Table 6d, plant 880221-8) were observed. In addition, inactivation or loss of new Uq element activity causing an aberrant ratio of greater-than-expected colorless versus too few spotted was not uncommon. These events probably occurred

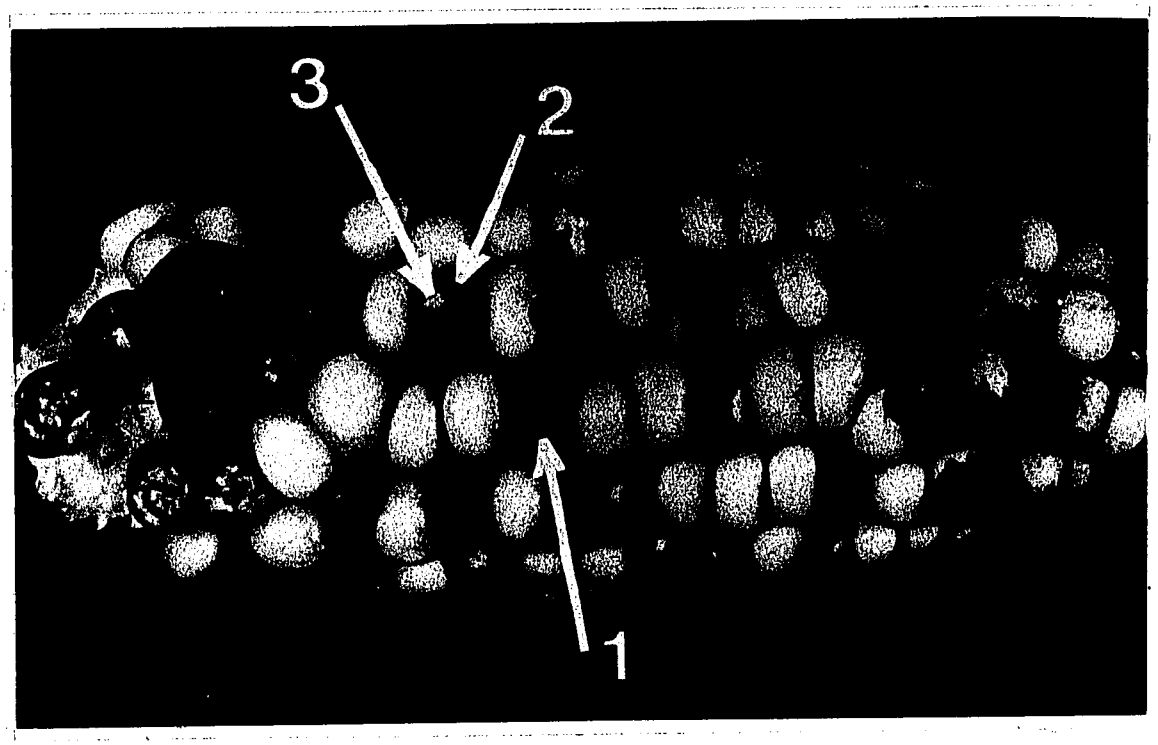
because abortive secondary transposition lead to the loss of the element.

The colored-like heavy spotting exhibited by genetic combinations of the standard *a-rug* reporter allele with any of the five new *Uq* isolates (Fig. 3, 1a through 5a) may indicate either a much earlier or a more frequent, and thus a stronger, transposition function of these new *Uq*s in comparison with that of *Uq1*. One example is shown in Fig. 4. The large colored sectors, as well as the colored revertants indicate early excisions; whereas the colorless sectors suggest that a transpositional loss or reinactivation of the *Uq2* element may have occurred. Therefore, we can make use of this unique property of the isolated new *Uq* elements in *Uq* tagging experiments (for example, Pan and Peterson, 1989a).

Why then are these transposable elements maintained in a quiescent state? Maize transposable elements are known to play an important role in the generation of gene mutations and in the creation of genetic variability (reviewed by Peterson, 1986; also by Gierl et al., 1989). In nature, however, their active transposition into maize house-keeping genes, and perhaps into other genes as well, may be harmful or even lethal to the individual. Such an individual is less fit and often selected against. One example is given by the recessive *Uq*^{10aa}*-SS* (Smaller Seed) mutant (Pan and Peterson, 1989b). It has been observed that the presence of 2 homozygous *Uq*^{10aa}*-SS*s

Figure 4. Spotting phenotypes of *Ug2*-*a-rug* interactions

The ear shown is derived from the cross (*a*° *sh2*/*a*° *sh2*, *Ug2*/+2) X *a-rug* tester and segregates for 50% (spotted plus few colored ?) : 50% colorless. Arrows indicate the colored-revertant (not yet confirmed) (1) and the spotted-with-colored (2) or -colorless (3) sectors.


3
2
1

in the genome of a maize individual is required to allow expression of the 10aa spotting. This homozygosity, however, causes a loss of embryo viability and is very likely lethal to the individual (Y.-B. Pan, Department of Genetics, Iowa State University, unpublished results). One biological solution to the maintenance of these elements in such individuals is to quench the transposable elements via DNA methylation. This seems an especially promising selective approach for corn inbreeding programs, which normally anticipate a reduced level of genetic variability in the maize-line development process (Peterson, 1986; 1988).

DNA methylation involves a de novo methylation function and a maintenance methylation function. Although de novo methylation is a slow process and often requires several plant generations (Fedoroff, 1989; Otto and Walbot, 1990), loss of methylation can be rapid due to the absence or failure of the maintenance methylation function (Otto and Walbot, 1990). Such a demethylation procedure would provide a ready access of elements for the variation-producing function. Changes in DNA-sequence methylation level of maize transposable elements have been correlated to the activity of elements such as Mu (Chandler and Walbot, 1986), Ac (Schwartz and Dennis, 1986; Kunze and Starlinger, 1989; Chomet et al., 1987), and Spm/En (Banks et al., 1988).

Because Ug has not yet been cloned, it is not possible to

test whether the disappearance or loss of Ug activity in the developmental process of maize inbred lines (Cormack et al., 1988; Pan and Peterson, 1987) is also correlated with DNA methylation. The frequent occurrence without any stress-factor effect on spontaneous activation of quiescent Ug elements has suggested that certain demethylation components may be involved in this activation process (Pan and Peterson, 1988). These components may act alone in genomes of the maize inbred lines or act along with other components from the a-rug tester stock to promote transient demethylation. Whether this is the underlying mechanism of Ug activation, however, needs to be tested further when a Ug probe becomes available.

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SECTION III

NEWLY ACTIVATED GERMINAL Uq ELEMENTS IN MAIZE ARE CLUSTERED ON ONE LINKAGE GROUP THAT IS INDEPENDENT OF THE STANDARD Uq ELEMENT

NEWLY ACTIVATED GERMINAL Ug ELEMENTS IN MAIZE ARE CLUSTERED ON ONE LINKAGE GROUP THAT IS INDEPENDENT OF THE STANDARD Ug ELEMENT[1]

Running Title: Cluster of activated Ug elements

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ABSTRACT

Allelism tests between the standard Ug element (Ug1) and five newly activated germinal Ug elements (Ug2, Ug3, Ug4, Ug5 and Ug6) show that these new Ug elements are independent of Ug1. Gametes, which either contain one Ug, or contain various combinations of two different and phenotypically distinguishable Ug elements, have been constructed with or without the a-rug reporter allele. Genetic analyses of their progenies (the standard a-rug tester line is the other parent) have indicated: 1) Each Ug element, when present alone, has the capacity of full activity expression except in cases where a secondary transposition or loss of activity has occurred; 2) all five new Ug elements are found to be independent of Ug1; and 3) these newly originated Ugs are clustered on one linkage group. Ug2 is allelic to Ug4, Ug3 is allelic to Ug5, whereas Ug6 is linked to both allelic pairs. A putative linkage map of these Ug elements is presented.

Key words: Ug Allelism-Ug cluster-Zea mays L.

INTRODUCTION

The standard Ug (now designated Ug1 by Pan and Peterson, 1990)-rug transposable element system was identified by Friedemann and Peterson (1982) in G. F. Sprague's Aberrant Ratio lines (Sprague and McKinney, 1966) that were derived from wheat stripe mosaic virus infection. It is one of the 9 established "two-element" (regulator-receptor) transposable element systems in maize (reviewed by Peterson, 1987). The rug receptor element has been identified at the A1 (the a-rug allele) (Friedemann and Peterson, 1982; Sprague, 1986) and the C1 maize genes (the c-rug alleles) (Caldwell and Peterson, 1989). In the absence of an active Ug regulatory element, a-rug/(a-rug or a^0) or c-rug/(c-rug or c^0) maize kernels have colorless aleurones. Nevertheless, if an active Ug element coexists in the same genome, maize kernels of the same a or c genotype will have spotted aleurones (colored spots on a colorless background), indicating excisions of the rug elements away from either maize locus, thereby restoring the normal functioning of the anthocyanin genes.

There have been a few cases where a-rug and c-rug respond differently to a particular Ug element, such as Ug13 (Caldwell and Peterson, 1989) and Mn::Ug (Pan and Peterson, 1989a). These observations indicate that structural differences may be present between the rug inserts at a-rug and c-rug. Both alleles, however, have been used extensively as Ug-specific

"reporter alleles" (Peterson, 1986) in detecting Ug element activity in various maize populations, breeding lines as well as genetic testers (Friedemann and Peterson, 1982; Peterson and Friedemann, 1983; Pan and Peterson, 1987; Peterson and Salamini, 1986; Cormack et al., 1988).

One significant finding from these Ug distribution studies is the pervasiveness and diversity of the Ug regulatory elements. Active Ug elements have been found in many maize populations, genetic testers and lines (Caldwell and Peterson, 1989; Cormack et al., 1988; Pereira and Peterson, 1985; Peterson and Salamini, 1986). These active Ug elements from various sources trigger distinguishable spotting patterns when interacting with the standard a-rug or c-rug reporter alleles.

Thus far it is not possible to determine whether these diverse patterns of Ug activity are due to structural differences. Nevertheless, various Ug elements were identified to at least 3 maize linkage groups in the study of Pereira and Peterson (1985). Ug13 is found to be independent of the standard Ug (Ug1). Ug13 triggers a unique flow type of spotting with a-rug (Pereira and Peterson, 1985) but with the c-rug alleles (Caldwell and Peterson, 1989) expresses a distinguishable fine spotting pattern (requires magnification to detect). Another Ug element from the C sh bz wx Ac maize line elicits on a-rug a coarse high pattern with spots in many pale sectors and is found to be independent of both Ug1 and

Uq13 (Pereira and Peterson, 1985). In all these cases, it is the same transactive functional element monitored on different receptors (reporter alleles).

One other significant finding from these Uq distribution studies is the spontaneous Uq activation (both somatic and germinal), in maize inbred lines originally lacking Uq activity. Using the standard a-ruq as a Uq-reporter allele, quiescent Uq elements were detected as spotted sectors on a-ruq/a-ruq/a-ruq aleurones in these inbred lines (Pan and Peterson, 1988). Continued backcrossing of these maize stocks with the a-ruq tester produced a number of exceptional spotted kernels that were heritable. From these spotted exceptions, 5 new Uq elements (Uq2, Uq3, Uq4, Uq5, and Uq6) were isolated (Pan and Peterson, 1990). These new Uqs have shown stronger activities than Uq1 even with the same reporter allele. Upon interaction with the standard a-ruq allele, each of these new Uqs elicits a unique heavier spotting pattern than that of the Uq1-a-ruq pattern. In addition, each new Uq element is also able to transactivate (though weakly) the c-ruq65 and c-ruq67 reporter alleles.

In this paper, we present the results from allelism tests among Uq1, Uq2, Uq3, Uq4, Uq5, and Uq6 elements. We illustrate the unique spotting phenotypes of these Uq elements, either alone or in pairs, in interacting with a-ruq. We demonstrate that all the five newly activated germinal Uq

elements are independent of Uq1. We also present genetic evidence that these five new Uq elements are clustered on one linkage group.

MATERIALS AND METHODS

Source of different Ug elements

The standard Ug element (Friedemann and Peterson, 1982) is now identified as Ug1 (Pan and Peterson, 1990). Ug2, Ug3, Ug4, Ug5 and Ug6 are verified new germinal isolates from spontaneous activation events (Pan and Peterson, 1990). The particular sources of these Ug elements used in this study are listed in Table 1. Ug1 is from a verified a° sh2/a° sh2, Ug1/Ug1 line. Ug2, Ug3, Ug4, and Ug5 were from verified reconstitution a° sh2/a° sh2 lines in our previous study (Pan and Peterson, 1990). Each line contains only one new Ug element, either homozygous or heterozygous. These lines were crossed as male to the standard a-rug tester (Table 1). From these crosses, spotted progeny kernels (general genotype: a-rug Sh2/a° sh2, Ug/+) were selected for the allelism tests. On the other hand, Ug6 was selected as pale spotted kernels from the cross a-m1 sh2/a-m1 sh2 tester X a-rug Sh2/a° sh2, Ug6/+6 (Table 1). An illustration of the distinctive spotting phenotypes of these 6 different Ug elements on the a-rug reporter allele is shown in Fig. 1.

Table 1. Source of different Ug elements included in the diallel cross

Ug element	Source: cross[a]	Source: genotype of the spotted selections[b]
Ug1	880358/0122-2	a-rug Sh2/a° sh2, Ug1/+1
Ug2	880320/0206-9	a-rug Sh2/a° sh2, Ug2/+2
Ug3	880327/0217-1	a-rug Sh2/a° sh2, Ug3/+3
Ug4	880328/0219-7	a-rug Sh2/a° sh2, Ug4/+4
Ug5	880329/0221-8[c]	a-rug Sh2/a° sh2, Ug5/+5
Ug6	880336/0236z-5	a-rug Sh2/a-m1 sh2, Ug6/+6

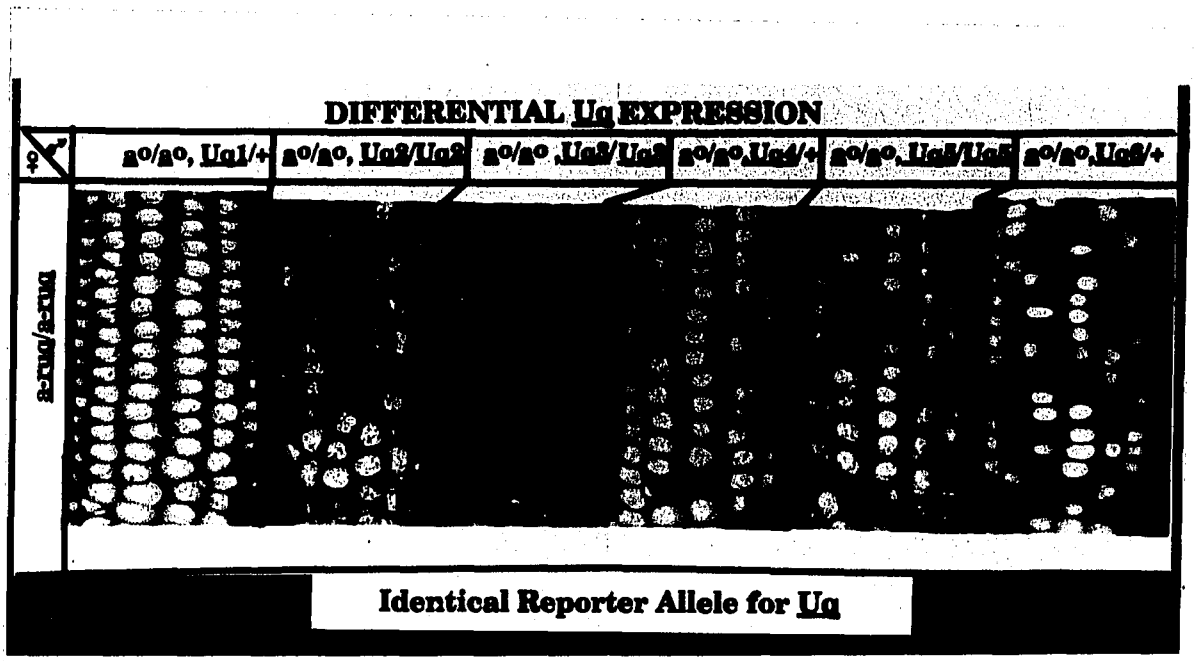
[a]Except for Ug6, the general genotype of the crosses was (a-rug Sh2 tester) X (a° sh2/a° sh2, Ug/Ug or +); for Ug6, it was (a-m1 sh2 tester) X a-rug Sh2/a° sh2, Ug6/+6.

[b]From such crosses, each group of spotted selection only contains one kind of Ug element shown, while all other kinds of Ug elements are absent (symbols not shown).

[c]The plant 880221-8 contains two copies of Ug5 which are more likely to be closely linked rather than allelic (see footnote a in Table 6d in Pan and Peterson, 1990).

Figure 1. Distinct spotting phenotypes of one dose of different *Ug* elements in the standard *a-rug* reporter allele

All ears shown were derived from the cross (*a-rug*/*a-rug*, +/+) X (*a°_sh2*/*a°_sh2*, *Ug*/*Ug* or +).


DIFFERENTIAL Uq EXPRESSION
♀
g°/g°, Uq1/+
g°/g°, Uq2/Uq2
g°/g°, Uq3/Uq3
g°/g°,Uq4/+
g°/g°, Uq5/Uq5
g°/g°,Uq6/+
mus/mus
Identical Reporter Allele for Uq

A diallel cross among Ug1, Ug2, Ug3, Ug4, Ug5, and Ug6 and tests for their linkage relationships

A diallel cross has been made among the 6 groups of spotted selections from the indicated source in Table 1. The genotypes of these 6 selected groups are a-rug Sh2/a° sh2, Ug1/+1, a-rug Sh2/a° sh2, Ug2/+2, a-rug Sh2/a° sh2, Ug3/+3, a-rug Sh2/a° sh2, Ug4/+4, a-rug Sh2/a° sh2, Ug5/+5, and a-rug Sh2/a-m1 sh2, Ug6/+6, respectively. From these diallel crosses, at least 14 colorless shrunken kernels were randomly selected from each cross combination except when Ug6 was involved (Table 2). With Ug6, twelve colored shrunken kernels were selected at random. For all the Ug combinations, at least 15 spotted kernels were selected but, in most cases, selections were not random. In general, selections favored the kernels with a heavier spotting pattern. Both types of selections, either random or nonrandom, were grown and testcrossed by the standard a-rug tester for Ug segregation analysis.

Because the randomized selections, i.e., colorless shrunken or colored shrunken kernels, include each of the four kernel types (Types 1, 2, 3, and 4) (Table 2) with an equal frequency, crossing of these kernel selections to the standard a-rug tester plants would in turn produce the same four types

Table 2. Expected Ug composition and frequencies of the four progeny types derived from the diallel cross (Table 1) among genotypes carrying either independent Ug elements (Ug1 and Ug2 as an example) or linked Ug elements (Ug2 and Ug3 as an example)[a]

Parental Ug composition[b]	Gamete type (frequency)	Progeny selections (Ug genotype & frequency)[c] Kernel	Ear	combination	gametic genotype & frequency
Ug1/+1, +2 +3/+2 +3	a. +1, +2 +3 (0.5)	type 1	type 1	(a X c)	+1/+1, +2 +3/+2 +3 (0.25)
	b. Ug1, +2 +3 (0.5)	type 2	type 2	(b X c)	Ug1/+1, +2 +3/+2 +3 (0.25)
		type 3	type 3	(a X d)	+1/+1, Ug2 +3/+2 +3 (0.25)
+1/+1, Ug2 +3/+2 +3	c. +1, +2 +3 (0.5)	type 4	type 4	(b X d)	Ug1/+1, Ug2 +3/+2 +3 (0.25)
	d. +1, Ug2 +3 (0.5)	type 1	type 1	(c X e)	+1/+1, +2 +3/+2 +3 (0.25)
		type 2	type 2	(d X e)	+1/+1, Ug2 +3/+2 +3 (0.25)
+1/+1, +2 Ug3/+2 +3	e. +1, +2 +3 (0.5)	type 3	type 3	(c X f)	+1/+1, +2 +3/+2 Ug3 (0.25)
	f. +1, +2 Ug3 (0.5)	type 4	type 4	(d X f)	+1/+1, Ug2 +3/+2 Ug3 (0.25)

[a]It is normally expected that the diallel cross among the seven genotypes produce progenies, of which 3/4 are a-rug Sh2/-, and 1/4 are a^{o} sh2/a^{o} sh2 (or a-m1 sh2).

[b]All have a-rug Sh2/a^{o} sh2 and all are spotted.

[c]All kernel selections are colorless or colored shrunken in combination with a^{o} sh2/a^{o} sh2 (or a-m1 sh2); however, in combination with a-rug Sh2/-, type 1 is colorless or colored round, types 2, 3 and 4 are spotted round.

Figure 2. **Expected classes of gametes and their Ug composition produced by the type 4 progeny kernel selections**

P = parental; R = recombinant; r = linkage value; see footnote a in Table 4 for phenotype abbreviations.

type 4 kernel selection	Gamete class (frequency)	Phenotype on a-rug tester
i. Independent Ugs		
example:	+1, +2 +3 (0.25)	cl-rd
Ug1/+1, Ug2 +3/+2 +3	Ug1, +2 +3 (0.25)	Ug1-spt-rd
(Table 2)	+1, Ug2 +3 (0.25)	Ug2-spt-rd
	Ug1, Ug2 +3 (0.25)	Ug2-spt-rd
ii. Linked Ugs		
example:	+1, Ug2 +3 (0.5-r/2) (P)	Ug2-spt-rd
+1/+1, Ug2 +3/+2 Ug3	+1, +2 Ug3 (0.5-r/2) (P)	Ug3-spt-rd
(Table 2)	+1, Ug2 Ug3 (r/2) (R)	Ug3-spt-rd
	+1, +2 +3 (r/2) (R)	cl-rd

of progeny ears. Type 1 ears do not have any Ug element. Type 2 ears consist of one Ug element that is relatively less active. Type 3 ears consist of the other relatively more active Ug element. Type 4 ears include both Ug elements (Table 2). In the case of nonrandomized (or spotted) selections, however, type 1 progeny ears normally would be avoided (Table 2). The frequency of type 2 progeny ears would be, if not totally absent, much lower than the frequency of either type 3 or type 4 progeny ears simply due to the biased selection.

In addition, in the case of randomized selections, each of the four progeny types will be selected at frequencies not significantly different from 25% (Table 2). Due to the nature of the diallel cross, these frequencies should not be controlled by the linkage relationship of the Ug elements involved. Therefore, it is not the frequency of type 1 ears (all kernels are colorless) but the frequency of colorless kernels (ones containing neither of the two Ugs) on type 4 ears that determines the linkage relationship between any two Ug elements (Fig. 2). If the two Ug elements are not linked and therefore are independent of each other, this frequency should not differ significantly from 25% (Fig. 2). On the other hand, if this frequency is significantly lower than 25%, the two Ug elements in combination are said to be genetically

linked with a linkage value that is two times the frequency of the colorless kernels on type 4 ears.

The linkage test between Ug1 and Ug2 serves as an example for independent Ug elements. Type 4 progeny ears are derived from the cross of standard a-rug tester as female parents either by a° sh2/a° sh2, Ug1/+1 and Ug2/+2 (included in the colorless shrunken selections in Table 2, see footnote) or by a-rug/-, Ug1/+1 and Ug2/+2 (included in the spotted selections in Table 2, see footnote). It is obvious that independent assortment between Ug1 and Ug2 would yield four kinds of male gametes with an equal frequency of 25%: (Ug1, Ug2), (Ug1, +2), (+1, Ug2) and (+1, +2) (Table 2 and Fig. 2). Upon crossing to the a-rug tester, approximately 25% of the progeny kernels are expected to be colorless round (a-rug/-, +1/+1, +2/+2); 25%, Ug1-spotted (a-rug/-, Ug1/+1, +2/+2); and 50%, Ug2-spotted (a-rug/-, either +1/+1, Ug2/+2 or Ug1/+1, Ug2/+2) (Fig. 2 and 2a in Fig. 3).

The linkage test between Ug2 and Ug3 serves an example for linked Ug elements (Table 2 and Fig. 2). Type 4 progeny ears are derived from the cross of standard a-rug tester as the female parent either by a° sh2/a° sh2, Ug2 +3/+2 Ug3 (included in the colorless shrunken selections in Table 2, see footnote) or by a-rug/-, Ug2 +3/+2 Ug3 (included in the spotted selections in Table 2, see footnote, and note that these two

Uq elements are linked in repulsion). These type 4 selections produce the following 4 classes of male gametes: two are parental (Uq2 +3 and +2 Uq3), and two are recombinant (Uq2 Uq3 and +2 +3) (Table 2 and Fig. 2). For the parental classes, the expected frequency is greater than 25% but less than 50%. It is, however, less than 25% for the recombinant classes (Fig. 2). Upon crossing to the a-rug tester, significantly less than 25% of the progeny kernels are colorless round (a-rug/-, +2 +3/+2 +3) and significantly more than 75% are spotted (a-rug/-, Uq2 +3/+2 +3 or +2 Uq3/+2 +3 or Uq2 +3/+2 Uq3) (Fig. 2). The Uq2-spotted kernels can be phenotypically distinguished from either Uq3-spotted or Uq2 Uq3-spotted kernels (Fig. 4, 2a). The spotting pattern of Uq2 Uq3, however, resembles that of +2 Uq3 alone. This resemblance makes a subclassification between these two spotted classes impossible.

In general, whenever possible, subclassification among different Uq*-spotted kernels is made. When this is not possible, however, the spotted kernels on type 4 progeny ears are counted as one class.

Determination of linkage values and construction of a putative linkage map

In the present allelism tests, there usually are several type 4 kernel selections (both random and nonrandom) for any particular pair of Ug elements that have been tested by crossing to the a-rug tester. As a result, more than one individual linkage value is available for any particular pair of linked Ug elements. In order to determine the linkage value between these new Ugs, homogeneity tests were performed on these sets of individual linkage values by the procedure of Nowick and Peterson (1981). After eliminating the odd values from each set, mean linkage values are computed and used in the construction of a putative linkage map of these Ug elements.

RESULTS

Genetic identity of each Ug element

The genetic identity of each Ug element is assessed by two criteria: 1) spotting pattern, and 2) capacity of eliciting spotting, on interaction with the a-rug reporter allele. The spotting phenotype of a Ug element on a-rug is expressed in the maize aleurone tissue. It is controlled by both timing and frequency of somatic excision of the rug receptor away from the A1 locus, and is, therefore, directly regulated by that Ug. Capacity of eliciting a-rug spotting, however, reflects the strength of Ug transposition function and is measured, in this study, by the frequency of spotted kernels in progeny ears segregating for one particular Ug element.

The distinctive spotting phenotypes on a-rug of the 6 Ug elements (Ug1, Ug2, Ug3, Ug4, Ug5 and Ug6) illustrated in Fig. 1 are consistent and reproducible. Generally this spotting becomes more intense in accordance with the following Ug order: Ug1 < Ug4 = (?) Ug5 < Ug2 < Ug3 < Ug6. On many type 4 progeny ears simultaneously segregating 2 different Ug elements, spotted kernels of the Ug in lower order can be phenotypically distinguished from the rest of the spotted kernels (Figs. 3 and 4). Nevertheless, due to a similarity

Table 3. Capacity of eliciting spots on a-rug of different Ug elements and their effect on the rate of germinal reversion from the crosses a-rug/a-rug tester X types 2 and 3 kernel selections from the diallel cross (either Cl-sh, cl-sh, or spt-rd)[a]

Gamete type[a] (as male)	Ug element	# of type 2 & 3 ears[b] Total	(1:1)[ns]	(1:1)[*]	Frequency of reversion[c]
From randomized selection:					
cl-sh	Ug1	18	18 (100%)	0,0	0
cl-sh	Ug2	21	19 (90%)	2,0	0
cl-sh	Ug3	13	13 (100%)	0,0	0
cl-sh	Ug4	15	14 (93%)	1,0	0
cl-sh	Ug5	10	10 (100%)	0,0	0
Cl sh	Ug6	22	20 (91%)	2,0	NA
	Total	99			
From nonrandomized selections:					
spt-rd	Ug1	11	11 (100%)	0,0	3.4×10^{-3}
spt-rd	Ug2	33	31 (94%)	0,2	1.7×10^{-3}
spt-rd	Ug3	24	23 (96%)	1,0	1.8×10^{-3}
spt-rd	Ug4	38	32 (84%)	3,3	2.0×10^{-3}
spt-rd	Ug5	29	22 (76%)	2,5	6.3×10^{-4}
spt-rd	Ug6	27	25 (93%)	2,0	2.0×10^{-2}
	Total	162[d]			

[a]cl-sh = colorless shrunken (a^{o} sh2/a^{o} sh2, only one copy of Ug); Cl-sh = colored shrunken (a-m1 sh2/a^{o} sh2, Ug6/+ only); spt-rd = spotted round (a-rug/a-rug or a^{o}, one copy of Ug only).

[b]Under column 1:1[ns] is the number and frequency (in parenthesis) of type 2 & 3 progeny ears segregating 50% spotted and 50% colorless kernels; under column 1:1[*] are two numbers of type 2 & 3 progeny ears not segregating 50% spotted and 50% colorless kernels. The number on the left represents cases where the reciprocal crosses are missing, while the number on the right represents cases where the reciprocal ears do not segregate 50% spotted and 50% colorless kernels either (data shown in Table 3).

[c]Derived from (total number of colored kernels)/(total number of progeny kernels).

[d]This plus the 28 spotted round selections listed in Table 5 equal to a total of 190.

Table 4. Exceptional loss or secondary transposition of *Uq* acitivity in types 2 and 3 spotted round selections (see Table 2)

*Uq** element	Progeny classification[a] spt-rd	cl-rd	Cl-rd	total	(X^{2})[b]
Uq2 (secondary transposition):					
893916/3850-10	195	40	2	235	100.9**
3850-10/3912	194	33	19	246	130.2**
3916/3850-13	287	38	0	325	189.2**
3850-13/3912	37	2	0	39	29.6**
Uq4 (partial or total loss)					
893928/3832-10	94	132	0	226	6.1*
3832-10/3932	12	41	0	53	14.8**
0660/3815-9	88	287	0	375	104.5**
3815-9/3956	0	254	0	254	126**
0660/3815-13	160	260	1	421	23.8**
3815-13tself	179	110sh,66rd	0	355	7.3*
Uq5 (partial loss)					
893925/3838-8	122	295	0	417	54.0**
3838-8/3925	10	40	0	50	16.8**
3922/3844-5	114	157	0	271	6.5*
3844-5/3916	140	179	0	319	4.5*
3752/3846-3	130	219	0	349	22.2**
3846-3/3911	135	195	0	330	10.5*
3918/3846-8	53	321	0	374	190.6**
3846-8/3913	128	308	0	436	73.5*
3915/3846-13	121	286	0	407	66.1**
3846-13/3916	189	251	0	440	8.5**

[a]Derived from reciprocal crosses between the *a-rug* tester line and *a-rug*/-, *Uq**/+. The new *Uq* elements in these crosses underwent either secondary transposition to increase copy number or partial or total loss event. Abbreviaitons: Spt-rd = spotted round; cl-rd = colorless round; Cl-rd = colored round.

[b]A X^{2} test for 1 spt-rd (plus Cl-rd) : 1 cl-rd segregation ratio. *, ** = significant at 0.01, 0.05 levels, respectively.

Table 5. Inactivation or loss of Ug activity in tassel tissue of plants grown from types 2 & 3 spotted round selections (see Table 2)

Ug* element	Progeny classification[a] spt-rd	cl-rd	Cl-rd	total	(X^2)b
Ug1					
893959/3842-1	213	170	0	383	4.61^{*}
3842-1/3920	7	2	0	9	1.78^{ns}
Ug2					
893917/3848-13	80	162	0	242	27.1^{**}
3843-13/3913	288	283	0	571	0.03^{ns}
3917/3848-14	58	36	0	94	4.69^{*}
3848-14/3913	179	191	0	370	0.33^{ns}
3915/3850-4	249	195	3	447	7.02^{**}
3950-4/3913	85	92	0	177	0.20^{ns}
3921/3852-5	109	163	0	272	10.3^{**}
3852-5/3911	176	151	0	327	1.76^{ns}
0658/3804-9	168	225	0	393	7.98^{**}
3804-9/3747	302	314	0	616	0.20^{ns}
Ug3					
893931/3802-2	197	136	3	336	11.8^{**}
3802-2/3937	10	12	0	22	0.05^{ns}
3751/3802-6	153	197	1	351	5.03^{*}
3802-6/3937	42	48	0	90	0.28^{ns}
Ug4					
893928/3832-4	227	298	0	525	9.30^{**}
3832-4/3929	69	79	0	148	0.55^{ns}
3935/3815-8	114	160	0	274	7.39^{**}
3815-8/3956	2	2	0	4	0.00^{ns}
3936/3816-4	117	151	0	268	4.06^{*}
3816-4/3938	49	45	1	95	0.17^{ns}
3655/3830-5	23	43	0	66	5.47^{*}

[a]Derived from reciprocal crosses between the a-rug tester line and types 2 & 3 spotted kernel selections (a-rug/-, Ug*/+). The new Ug elements in these crosses undergo partial activity loss. See footnote a in Table 4 for abbreviations.

[b]A X^2 test for 1 spt-rd (plus Cl-rd) : 1 cl-rd segregation ratio (except self). ns = not significant, and *, ** = significant at 0.01, 0.05 levels, respectively.

Table 5. (Continued)

Ug* element	Progeny classification[a] spt-rd	cl-rd	Cl-rd	total	(X^{2})[b]
3830-5/3924	44	57	0	101	1.43^{ns}
3455/3811-3	198	246	0	444	4.98*
3811-3/3935	30	38	0	68	0.72^{ns}
3930/3811-9	54	81	1	135	5.01*
3811-9/3957	244	240	1	465	0.42^{ns}
Ug5					
893923/3826-13	101	155	0	256	11.0**
3826-13/3934	160	163	0	323	0.01^{ns}
3752/3854-3	225	275	0	500	4.80*
3854-3/3910	238	243	0	481	0.03^{ns}
3925/3836-8	132	281	0	413	53.0**
3836-8/3925	7	6	0	13	0.00^{ns}
3460/3838-1	208	302	0	510	17.0**
3838-1tself	92	31sh,31rd	1	155	1.71^{ns}
3920/3844-2	177	217	0	394	3.86*
3844-2/3917	139	146	1	286	0.09^{ns}
3924/3844-4	101	142	1	244	6.23*
3844-4/3917	177	168	0	345	0.19^{ns}
Ug6					
893654/3804-1	186	228	0	414	4.06*
3804-1/3936	256	256	0	512	0.00^{ns}
0658/3804-8	230	187	0	417	4.23*
3804-8/3747	224	227	1	452	0.00^{ns}
3930/3811-4	241	122	13	376	45.6**
3811-4/3956	134	123	6	263	0.97^{ns}
3455/3812-1	165	135	16	316	6.41*
3812-1tself	134	69sh 46rd	23	272	0.60^{ns}
3642/3812-3	280	242	17	539	5.41*
3812-3/3935	47	52	0	99	0.16^{ns}
3921/3844-9	107	68	16	191	15.3**
3844-9/3916	206	240	2	448	2.15^{ns}
3917/3844-11	152	124	11	287	5.03*
3844-11/3918	225	219	3	447	0.14^{ns}
3456/3846-14	157	207	7	371	4.75*
3846-14/3910	190	205	1	396	0.43^{ns}

in spotting phenotypes given either by the Ug in higher order alone or by both Ugs, further classification among the rest of the spotted kernels is very difficult. For example, on ears segregating both Ug1 and Ug2 elements, it is relatively easy to distinguish the number of Ug1-a-rug spotted kernels (Fig. 3, arrow in panel 2a). However, it is very difficult, if not impossible, to distinguish Ug2-a-rug spotted kernels from (Ug1 plus Ug2)-a-rug spotted kernels. In the present study, whenever possible, these different spotting phenotypes have been used as identification tags for each Ug element in Ug segregational analysis.

Testcrosses between the a-rug tester and both types of selections (randomized vs. nonrandomized) have revealed that a total of 99 randomized selections (colorless or colored shrunken) and 190 nonrandomized selections (spotted) contained a single Ug copy and produced types 2 and 3 progeny ears (Fig. 1 and Table 3). Among these, 18 colorless shrunken and 11 spotted selections had only one copy of Ug1. Correspondingly, 21 and 33 had Ug2, 13 and 24 Ug3, 15 and 38 Ug4, 10 and 29 Ug5, and 22 and 27 Ug6, respectively.

Upon genetic analysis with appropriate progeny tests, more than 90% of the types 2 and 3 progeny ears derived from the randomized selections segregate for 50% spotted : 50% colorless (or plus colored if Ug6 is involved) and have

nonsignificant X^2 values at 0.05 level (Table 3 under the column 1:1^{ns}). Similar results were derived from the nonrandomized (or spotted) selections, although this frequency is reduced to 84% for Ug4 and to 76% for Ug5 (Table 3 under the column 1:1^{ns}). Ten spotted selections (2 for Ug2, 3 for Ug4 and 5 for Ug5), listed in Table 3 as the number on the right under the column 1:1*, gave aberrant segregation ratios other than 1:1 in either reciprocal cross with a-rug (Table 4). In addition, a few of the spotted selections (1 for Ug1, 2 for Ug3, 5 for Ug2, 6 for both Ug4 and Ug5, and 8 for Ug6) are not included in Table 3 because of their significantly different performance in reciprocal crosses as shown in Table 5. When used as males, these selections produce ears segregating either too many or too few colorless kernels. In contrast, the segregation is found to be normal when the same selections are used as females. This reciprocal discrepancy is probably due to partially inactivation or loss of the relevant Ug element in the maize tassel tissue.

An additional factor that is correlated to the genetic identity of each Ug is its effect on the germinal reversion rate of the a-rug allele (Table 3). It is interesting that no germinal revertants of a-rug have been observed among the type 3 and type 4 progeny ears derived from the randomized selections (genotype of a^{o}/ao or a-m1, Ug/+). In contrast,

germinal revertants of a-rug occur at a rate of about 0.002 in progenies derived from the spotted selections containing any single copy of Ug1, Ug2, Ug3 or Ug4 (Table 3). The lowest rate of about 0.0006 is found to be associated with Ug5, and the Ug6 spotted selection has the highest reversion rate of 0.02 (Table 3). Since the only difference between the randomized and the nonrandomized sib selections from the diallel cross is the absence or presence of the a-rug reporter allele, it seems likely that germinal reversion events at a-rug take place more likely prior to fertilization, probably during DNA replication or meotic divisions.

Ug1 is independent of the 5 new Ug elements

Of the 25, 20, 22, and 14 colorless shrunken selections tested for Ug1 vs. Ug2, Ug1 vs. Ug3, Ug1 vs. Ug4 and Ug1 vs. Ug5 combinations, respectively, 6, 7, 6, and 6 are shown to contain both Ug elements and produce type 4 progeny ears in crossing to the a-rug tester plants (Table 6-1). In addition, 1 out of the 9 colored shrunken selections tested had both Ug1 and Ug6 element and yielded a type 4 progeny ear.

Photographs of portions of sample ears are shown in Fig. 3 to illustrate the segregation of Ug1 (lightly spotted) (arrows in panels 2a, 3a, 4a, 5a, and 6a) vs. new Ug or new Ug

Table 6-1. Independent segregation of Uq1 from each of the five new Uq elements in crosses (a-ruq/a-ruq, +1/+1, +/+) tester line X (a° sh2/a° sh2 or a-m1 sh2, Uq1/+1, new Uq*/+)

Uq pair cross	Progeny classification[a] spt-rd Uq1	spt-rd Uq*	cl-rd	Cl-rd	total	$(X^2)^b$
Uq1 vs. Uq2						
893914/3847-3	17	93	70	0	180	17.8^{**}
3911/3847-11	53	142	67	0	262	0.02^{ns}
3918/3847-12	18	63	32	0	113	0.50^{ns}
3929/3847-14	77	157	78	0	312	0.00^{ns}
3930/3847-15	47	89	44	0	180	0.01^{ns}
3916/3849-10	60	133	58	0	251	0.38^{ns}
Uq1 vs. Uq3						
893923/3839-3	71	158	78	0	307	0.01^{ns}
3657/3839-4	110	191	100	0	401	0.00^{ns}
3925/3839-8	96	184	103	0	383	0.63^{ns}
3960/3839-11	41	103	65	0	209	3.83^{ns}
3930/3841-1	14	37	14	0	65	0.25^{ns}
3658/3841-2	48	103	56	0	207	0.36^{ns}
3931/3860-1	47	99	47	0	193	0.02^{ns}
Uq1 vs. Uq4						
893931/3831-3	51	80	43	0	174	0.00^{ns}
3924/3831-4	86	153	79	0	318	0.00^{ns}
3920/3831-6	59	130	75	0	264	1.46^{ns}
3958/3831-13	68	81	45	0	194	0.25^{ns}
3926/3833-1	115	230	124	0	469	0.44^{ns}
3659/3833-3	104	150	95	0	349	0.80^{ns}

[a]See footnote a in Table 4 for abbreviations. The colored-round progenies have a genotype of a-m1/a-ruq, neither Uq1 nor Uq6.

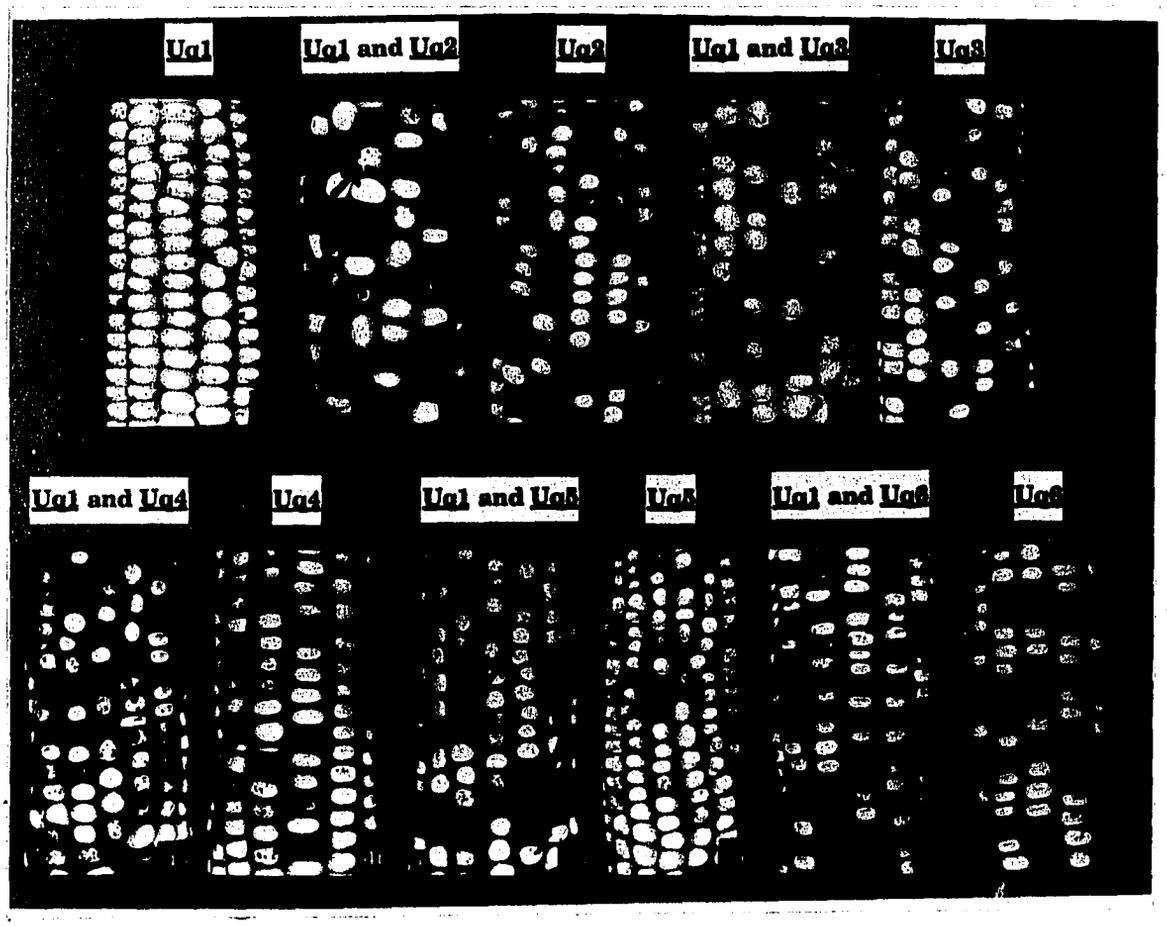
[b]A X^2 test for 3 spotted (Uq presence) : 1 colorless (Uq absence); ns = not significant at 0.05 level; * = significant at 0.05 level.

Table 6-1. (Continued)

Uq pair cross	Progeny classification[a] spt-rd Uq1	spt-rd Uq*	cl-rd	Cl-rd	total	$(X^2)^b$
Uq1 vs. Uq5						
893660/3825-1	68	157	69	0	294	0.29^{ns}
3660/3825-2	123	268	126	0	517	0.08^{ns}
3928/3825-8	51	67	46	0	164	0.66^{ns}
3937/3825-10	49	101	60	0	210	1.24^{ns}
3959/3825-15	68	126	55	0	249	0.98^{ns}
3936/3825-16	101	131	99	0	331	3.99^{*}
Uq1 vs. Uq6						
893644/3856-5t	95	187	50	62	394	0.66^{ns}

Figure 3. Photographs showing an independent segregation of Uq1 and each of the 5 new Uqs

Panels 1, 2b, 3b, 4b, 5b and 6b showing portions of ears segregating Uq1, Uq2, Uq3, Uq4, Uq5, and Uq6, respectively; panels 2a, 3a, 4a, 5a and 6a showing portions of ears segregating Uq1 and Uq2, Uq1 and Uq3, Uq1 and Uq4, Uq1 and Uq5, and Uq1 and Uq6, respectively. Arrows indicate Uq1-spotted kernels on ears segregating both Uq1 and one new Uq.


Ua1
Ua1 and Ua2
Ua2
Ua1 and Ua3
Ua3
Ua1 and Ua4
Ua4
Ua1 and Ua5
Ua5
Ua1 and Ua6
Ua6

plus Uq1 (heavily spotted). Segregation analysis (Table 6-1) indicates that all but 2 (893914/3847-3 and 893936/3825-16) of these type 4 progeny ears segregate for 3/4 spotted (Uq presence) : 1/4 colorless (Uq absence) with X^2 values not significant at 0.05 level. Ears 893914/3847-3 and 893936/3825-16 have too many colorless kernels and have to be excluded from the data pool after a homogeneity test.

That Uq1 is independent of any of the 5 new Uq elements is confirmed by the results obtained from testing the spotted selections having pairs of Uq elements (Table 6-2). Again with few exceptions, most of these spotted selections (3 for Uq1 vs. Uq2, 11 for Uq1 vs. Uq3, 7 for Uq1 vs. Uq4, 8 for Uq1 vs. Uq5, and 7 for Uq1 vs. Uq6) segregate for 3/4 spotted (Uq present) and 1/4 colorless (Uq absent) progenies in the crosses to the a-rug tester (Table 6-2). In addition, four of the 6 exceptional spotted selections, i.e., 893848-7 and -9, and 893834-4 and -10, give a 3/4 Uq : 1/4 no Uq segregation through their female gametes.

The 5 new Uq elements are either allelic or linked to one another

One, 1, 9, 5, and 3 randomly selected colorless shrunken kernels are found to contain different diallelic pairs (except

Table 6-2. Independent segregation of Ug1 from each of the five new Ug elements in crosses (a-rug/a-rug, +1/+1, +/+) tester line X (a-rug Sh2/a-rug Sh2 or a° sh2 (a-m1 sh2 for Ug1 vs. Ug6) , Ug1/+1, new Ug*/+)

Ug pair: cross	Progeny classification[a] spt-rd Ug1	spt-rd Ug*	cl-rd	Cl-rd	total	$(X^2)^b$
Ug1 vs. Ug2:						
893913/3848-1	76	164	77	3	320	0.10^{ns}
3913/3848-3	52	120	48	14	234	0.13^{ns}
3917/3848-7	83	54	96	11	244	26.0^{**}
3847-7/3912	55	108	64	9	236	0.46^{ns}
3917/3848-9	130	75	117	8	330	12.0^{**}
3848-9/3913	142	240	141	8	531	0.60^{ns}
3916/3848-12	51	108	53	12	224	0.15^{ns}
Ug1 vs. Ug3						
893959/3840-1	34	42	30	0	106	0.45^{ns}
3921/3840-2	85	170	96	1	352	0.85^{ns}
3459/3840-4	181	264	166	2	613	1.31^{ns}
3914/3840-8	23	58	14	0	95	4.80^{*}
3921/3840-11	99	194	101	0	394	0.05^{ns}
3923/3840-9	100	225	116	0	441	0.33^{ns}
3915/3842-2	76	141	78	0	295	0.25^{ns}
3659/3842-5	93	181	106	0	380	1.55^{ns}
3458/3842-6	125	278	154	1	558	1.87^{ns}
3923/3842-11	14	19	8	0	41	0.40^{ns}
3923/3842-13	81	205	112	3	401	1.68^{ns}
3922/3901x-5	72	179	87	2	340	0.04^{ns}
Ug1 vs. Ug4						
893928/3832-1	79	122	65	3	269	0.06^{ns}
3928/3832-2	59	131	60	0	250	0.09^{ns}
3928/3832-8	34	76	39	0	149	0.06^{ns}
3929/3832-12	128	190	121	0	439	1.40^{ns}
3929/3832-14	71	137	70	0	278	0.00^{ns}

[a]See footnote a in Table 4 for abbreviations. The colored round progenies have a genotype of either A/a-rug, Ugs(?) if these are germinal revertants or plus a-m1/a-rug, no Ug under the Ug1 vs. Ug6 case.

[b]See footnote b in Table 6-1.

Table 6-2. (Continued)

Uq pair: cross	Progeny classification[a] spt-rd Uq1	spt-rd Uq*	cl-rd	Cl-rd	total	$(X^2)^b$
Uq1 vs. Uq4						
3926/3834-1	96	132	93	2	323	2.28^{ns}
3927/3834-4	126	228	147	0	501	4.81^{*}
3834-4/3924	19	35	20	0	74	0.07^{ns}
3927/3834-9	97	210	124	1	432	2.97^{ns}
3927/3834-10	126	213	143	2	484	5.09^{*}
3834-10/3929	108	229	109	3	449	0.09^{ns}
3927/3834-11	63	113	64	0	240	0.27^{ns}
3927/3834-13	98	193	103	0	394	0.21^{ns}
Uq1 vs. Uq5						
893935/3826-3	62	106	51	3	222	0.38^{ns}
3935/3826-4	36	77	34	15	162	1.19^{ns}
3935/3826-5	49	96	55	0	200	0.54^{ns}
3929/3826-7	68	125	70	2	265	0.21^{ns}
3935/3826-8	101	186	87	4	378	0.69^{ns}
3937/3826-9	99	273	144	6	522	1.73^{ns}
3938/3826-10	255		101	0	356	1.98^{ns}
3939/3826-11	100	137	88	0	325	0.64^{ns}
Uq1 vs. Uq6						
893913/3857-6	9	31	9	6	55	1.75^{ns}
3915/3857-10	126	253	152	22	553	1.69^{ns}
3753/3857-12	73	197	92	3	365	0.00^{ns}
3918/3859-2	82	86	13	16	198	11.3^{*}
0658/3859-4	50	156	30	52	286	0.47^{ns}
3912/3859-5	64	227	35	52	378	2.38^{ns}
3915/3859-8	60	133	31	40	264	0.01^{ns}
3754/3859-9	256		76	4	336	0.89^{ns}

Uq3 vs. Uq4) among Uq2, Uq3, Uq4 and Uq5 elements, respectively. In addition, 12, 1, 8 and 1 randomly selected colored shrunken kernels have both Uq6 and Uq2, or Uq3, or Uq4, or Uq5, respectively. Segregation of these new Uq pairs in these randomized selections are analyzed in the type 4 progeny ears produced from crosses with a-rug tester. The results are shown in Tables 7 and 8, and photographs of portions of sample ears are shown in Fig. 4.

In contrast to their independence relationship to Uq1, these five new Uq elements are found to be either allelic or linked to one another. In most of the randomized selections, segregation ratios of different pairs of new Uq elements are found to be highly significantly different from 3/4 with Uq (spotted phenotype) : 1/4 without Uq (colorless or plus colored phenotype if Uq6 is involved) (X^2 values in Tables 7 and 8). There are only a few selections, mostly in association with Uq4 or Uq5, that segregate 3/4 with Uq : 1/4 without Uq as demonstrated by their nonsignificant X^2 values. However, it is not clear whether this is due to the partial loss of these new Uq's activity (Tables 4 and 5) or due to a secondary transposition of these Uq to unlinked sites.

Following homogeneity tests on the linkage values shown in Tables 7 and 8, average linkage values between Uq2 & Uq5, Uq2 & Uq6, Uq3 & Uq5, and Uq4 & Uq6 are computed as 32.3 ± 6.6,

Table 7. Segregation pattern of the progenies derived from the crosses between (a° sh2/a° sh2, 2 new Ug elements in repulsion) (as male) and the a-rug tester

Ug pair: cross	Progeny classification[a] spt-rd[b] a	(a+b)	b	cl-rd(%)	Cl-rd	total	$(X^2)^c$
Ug2 vs. Ug3							
893958/3801-10		389		102 (20.6)	4	495	4.86*
Ug2 vs. Ug4							
893656/3905-5	196		74	113 (29.5)	0	383	3.91*
Ug2 vs. Ug5							
893914/3851-5		358		78 (17.9)	0	436	11.4**
3749/3851-10		371		93 (20.0)	0	464	5.82*
3918/3851-13		209		49 (19.0)	0	258	4.65*
3644/3851-14	128		114	49 (16.8)	0	291	9.91**
3753/3853-1		345		48 (12.2)	0	393	33.6**
3644/3853-4		228		43 (15.9)	0	271	11.6**
3935/3953-11	130		71	15 (6.9)	0	216	36.6**
3644/3853-13	91		277	43 (10.5)	0	411	45.6**
3921/3853-15		321		41 (11.3)	0	362	35.4**
Ug3 vs. Ug5							
893919/3835-4		93		0 (0.0)	0	93	29.7**
3919/3835-5		267		0 (0.0)	0	267	87.7**
3917/3837-11		266		2 (0.8)	0	268	82.8**
3957/3837-12		313		0 (0.0)	0	313	103**
3956/3837-13	187		30	91 (29.5)	0	308	3.64ns
Ug4 vs. Ug5							
893928/3823-1	96		156	86 (25.4)	0	338	0.06ns
3927/3823-4	32		75	34 (24.1)	0	141	0.02ns
3923/3828-2		336		53 (13.6)	0	389	26.2**

[a]See footnote a in Table 4 for abbreviations.

[b]Class a has a spotting phenotype of the first Ug element; class b has a spotting phenotype of the second Ug element; (a + b) indicates a difficulty in subclassification of the spotted class.

[c]A X^2 testing if the 2 new Ug elements are independent, ns = not significant, *, ** = significant at 0,05, 0.01 levels.

Table 8. Segregation pattern of the progenies derived from the crosses between (a° sh2/a-m1 sh2, Uq* +6 / + Uq6) (as male) and the a-rug tester

Uq pair: cross	Progeny classification[a] spt-rd[b] a	(a+b)	b	(cl-rd + Cl-rd) (%)	total	$(X^2)^c$
Uq6 vs. Uq2						
893933/3803-1		385		43 + 32 (16.5)	460	18.1**
3752/3803-3	176		90	11 + 25 (11.9)	302	26.9**
3660/3803-8		424		21 + 27 (10.2)	472	54.6**
0658/3803-10		378		15 + 24 (9.4)	417	53.6**
3956/3805-3t		407		29 + 33 (13.2)	469	34.1**
3934/3805-3		438		22 + 23 (9.3)	483	62.5**
3934/3805-4		403		24 + 29 (11.6)	456	42.8**
3458/3821-2		304		21 + 30 (14.4)	355	20.8**
3641/3821-3		501		24 + 21 (8.2)	546	80.9**
3929/3821-4		299		23 + 27 (14.3)	349	20.6**
3936/3821-5		233		29 + 31 (20.5)	293	2.96ns
3457/3821-6		268		18 + 11 (9.8)	297	36**
Uq6 vs. Uq3						
893936/3817		138		5 + 2 (4.8)	145	30.4**
Uq6 vs. Uq4						
893749/3809-2		316		2 + 1 (0.9)	319	97.2**
3658/3809-8		327		3 + 3 (1.8)	333	94.3**
3750/3810-5		457		7 + 5 (2.6)	469	125**
0659/3809-10	339		66	2 + 6 (2.0)	408	114**
3936/3809-1	109		44	23 + 28 (25.0)	204	0ns
3936/3809-13	178		48	43 + 51 (29.4)	320	3.50ns
3936/3810-1	232		63	2 + 75 (20.7)	372	3.44ns
3747/3810-11	239		107	35 + 60 (21.5)	441	2.63ns
Uq6 vs. Uq5						
893913/3843-4		171		1 + 1 (1.2)	173	51.2**

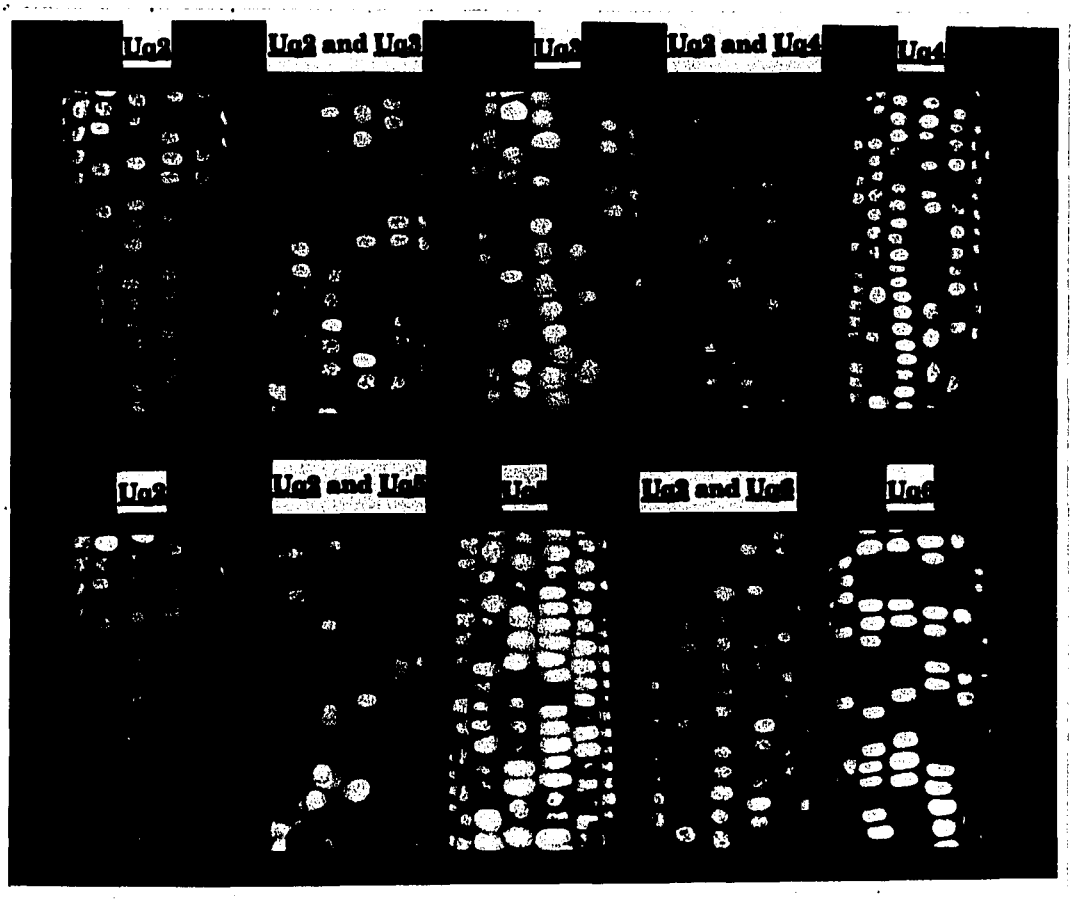
[a]See footnote a in Table 4 for abbreviations.

[b]Class a has a spotting phenotype of Uq6; class b has a spotting phenotype of the other new Uq element; (a + b) indicates a difficulty in subclassification of the spotted class.

[c]See footnote c in Table 7.

Figure 4. Photographs of portions of ears showing segregation of either linked or allelic new Ugs

Panels 1, 2b, 3b, 4b, and 5b showing ears segregating Ug2, Ug3, Ug4, Ug5, and Ug6, respectively; panels 2a, 3a, 4a, and 5a showing portions of ears segregating Ug2 and Ug3, Ug2 and Ug4, Ug2 and Ug5, and Ug2 and Ug6, respectively. Generally, there are two features: 1). In most cases, each new Ug elicits a distinguishable spotting phenotype on a-rug allele; 2). If a kernel (a-rug/a-rug or a^{o}) has 2 different Ugs, it expresses the heavier spotting phenotype of the more active Ug (thus a positive dosage effect) (Friedemann and Peterson, 1982).


Uα2
Uα2 and Uα3
Uα2
Uα2 and Uα4
Uα4
Uα2
Uα2 and Uα5
Uα5
Uα3 and Uα5
Uα6

23.7 ± 5.3, 0.4 ± 0.8 and 3.6 ± 1.3 at sample sizes of 7, 10, 4, and 4, respectively. Compatible data are derived from the spotted or nonrandomized selections segregating these new Uq pairs (Tables 9 and 10). From homogenized samples of 5, 18, 9, and 3, the average linkage values for the Uq2 vs. Uq5, Uq2 vs. Uq6, Uq3 vs. Uq5, and Uq4 vs. Uq6 are 30.6 ± 4.0, 23.2 ± 5.4, 0.4 ± 0.5, and 3.6 ± 1.8, respectively.

In addition, average linkage values for several other new Uq pairs are also derived from the data shown in Tables 9 and 10. Based on homogenized sample sizes of 5, 6, 4, 3, pairs of Uq2 vs. Uq3, Uq2 vs. Uq4, Uq4 vs. Uq5, and Uq3 vs. Uq6 have linkage values of 35.3 ± 7.8, 0.4 ± 0.4, 1.1 ± 1.3, and 4.0 ± 0.2, respectively.

According to the independence of these new Uq elements from Uq1 and the average linkage values among these new Uq elements themselves, a putative Uq linkage map is constructed (Fig. 5). In summary, Uq1 is located on a separate linkage group, whereas the 5 new Uq elements are clustered to one another in the order shown. Uq4 may have several sites: One site is very likely allelic to Uq2, another site is approximately 4 map units away from Uq6 (not shown in Fig. 5), still another possible site is on a third linkage group, about 1.1 map units from the secondary site of Uq5. The primary site for Uq5 is

Table 9. Segregation pattern of the progenies derived from the crosses between (a-rug Sh2/a° sh2 or a-rug Sh2, new Ug elements in repulsion) (as male) and the a-rug tester

Ug pair: cross	Progeny classification[a] spt-rd[b] a	(a+b)	b	cl-rd (%)	Cl-rd	total	(X^2)[c]
Ug2 vs. Ug3							
890659/3802-1		376		99 (20.7)	3	478	4.46*
0659/3802-3	165		218	82 (17.6)	1	466	13.6**
3750/3802-8	88		163	55 (18.0)	0	306	7.69*
3958/3802-10	146		234	101 (20.8)	4	485	4.29*
3750/3802-11		369		49 (11.2)	19	437	43.6*
Ug2 vs. Ug4							
893933/3815-1		298		0 (0.0)	0	298	98.0**
3933/3815-2		155		0 (0.0)	0	155	50.3**
3933/3815-5		376		2 (0.5)	0	378	119**
3934/3815-12		363		1 (0.3)	0	364	117**
3936/3815-15		354		1 (0.3)	0	355	114**
3938/3816-1		360		0 (0.0)	0	360	119**
Ug2 vs. Ug5							
893914/3852-1		156		31 (16.6)	0	187	6.63*
3912/3854-5		282		39 (12.2)	0	321	27.6**
3660/3908-1		309		55 (15.1)	1	365	18.7**
3910/3908-4		121		22 (15.4)	0	143	6.55*
3919/3908-7		221		47 (17.4)	2	270	7.9**
3911/3854-9		61		23 (27.4)	0	84	0.14ns
3911/3854-13		224		79 (26.1)	0	303	0.13ns
3935/3908-2		72		24 (25.0)	0	96	0ns
3911/3908-6	139		59	51 (20.4)	1	250	2.58ns
3911/3908-8	153		24	43 (19.4)	2	222	3.46ns
Ug3 vs. Ug5							
893925/3836-2		264		1 (0.4)	0	265	84.4**
3918/3836-5		199		38 (16.0)	0	237	9.69**
3918/3836-7		312		0 (0.0)	0	312	103**
3926/3836-12		352		0 (0.0)	0	352	116**
3918/3836-13		158		0 (0.0)	0	158	51.3**

[a]See footnote a in Table 7.
[b]See footnote b in Table 7.
[c]See footnote c in Table 7.

Table 9. (Continued)

Uq pair: cross	Progeny classification[a] spt-rd[b] a	(a+b)	b	cl-rd (%)	Cl-rd	total	$(X^2)^c$
Uq3 vs. Uq5*							
3917/3838-3		318		2 (0.6)	0	320	100^{**}
3925/3838-5		462		0 (0.0)	0	462	153^{**}
3924/3838-9		539		3 (0.6)	1	543	172^{**}
3924/3838-11		269		1 (0.4)	0	270	86^{**}
3956/3838-12		336		0 (0.0)	0	336	111^{**}
Uq4 vs. Uq5*							
893751/3824-3	58		69	50 (28.2)	0	177	1.18^{ns}
3938/3824-4	42		118	38 (18.9)	3	201	3.66^{ns}
3934/3824-6	60		158	83 (26.9)	7	308	0.73^{ns}
3928/3824-7	46		131	52 (22.4)	3	232	0.70^{ns}
3653/3824-12	93		207	79 (20.7)	2	381	3.47^{ns}
3653/3824-13	136		279	127 (22.8)	14	556	1.27^{ns}
3922/3830-4	223		183	2 (0.5)	0	408	129^{**}
3655/3830-8		404		1 (0.2)	1	406	131^{**}
3921/3830-12	128		91	0 (0.0)	0	219	71.7^{**}
3922/3830-3		207		3 (1.4)	0	210	61^{**}

Table 10. Segregation pattern of the progenies derived from the crosses between a-rug Sh2 or a° sh2/a-m1 sh2 or a-rug Sh2, +· Uq6/Uq* +6 (spotted round) (as male) and the a-rug tester

	Progeny classification[a]						
Uq pair: cross	spt-rd[b] a	(a+b)	b	cl-rd (%)[c]	Cl-rd	total	(X^2)[d]
Uq6 vs. Uq2							
893933/3804-3	302			33 (9.9)	0	335	40.2**
3655/3804-4	205		173	44 (10.4)	1	423	47.3**
3752/3804-6	232		192	55 (11.4)	2	481	46.5**
3933/3804-10	385			54 (12.3)	0	439	37.1**
3653/3804-11	250		141	48 (10.9)	0	439	45.6**
0658/3804-13	157		157	36 (10.3)	1	351	39.9**
3753/3804-14	190		193	28 (6.8)	0	411	71.5**
3754/3806-3	216		140	54 (13.0)	6	416	31.4**
3652/3806-5	181		118	46 (12.9)	11	356	27.1**
3652/3806-6	267		154	70 (14.2)	2	493	30.1**
3753/3806-9	434			94 (17.5)	8	536	15.5**
3455/3822-4	236			21 (8.1)	1	258	38.2**
3748/3822-6	169		78	42 (14.2)	6	295	17.7**
3938/3822-7	183		175	28 (7.3)	0	386	63.9**
3458/3822-10	445			24 + 27 (10.3)		496	56.5**
3460/3822-12	259			36 (12.0)	5	300	26.4**
3934/3822-13	154			26 (14.0)	6	186	11.5**
3460/3822-14	514			77 (12.9)	7	598	46.2**
Uq6 vs. Uq3							
893459/3818-1	326			7 (2.1)	0	333	91.9**
3935/3818-5	344			7 (2.0)	2	353	88.6**
3458/3818-8	358			7 (1.9)	0	365	103**

[a]Class a has a spotting phenotype of Uq6; class b has a spotting phenotype of the other Uq element; (a + b) indicates a difficulty in subclassification of the spotted class.

[b]See footnotes b in Table 7.

[c]In case the number under the colored round class is equal or greater than than that under the colorless round class, the two numbers are then pooled and the frequency of both classes is computed.

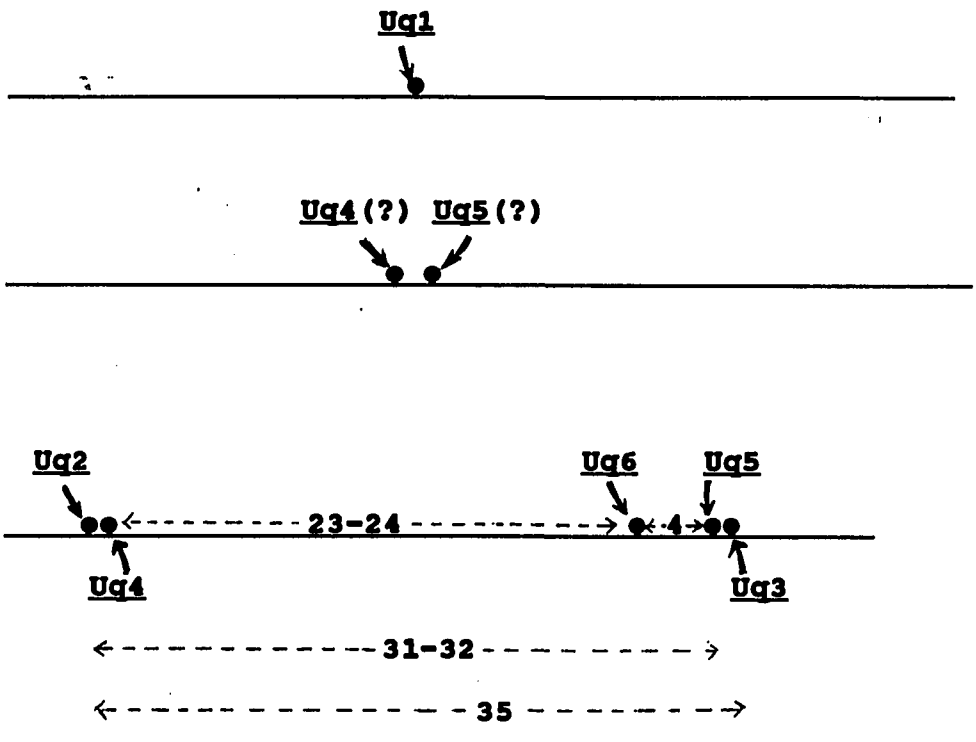
[d]See footnote c in Table 7.

Table 10. (Continued)

Uq pair: cross	Progeny classification[a] spt-rd[b] a	(a+b)	b	cl-rd (%)[c]	Cl-rd	total	(X^2)[d]
Uq6 vs. Uq4							
893651/3811-7		367		5 + 4 (2.4)		376	101**
3651/3811-10		377		36 (8.6)	6	419	59.3**
3930/3811-11		278		79 + 85 (37.1)		442	35.2**
3931/3812-6	184		67	2 (0.8)	0	253	77.8**
3931/3812-9	202		100	2 + 5 (2.3)		309	84**
Uq6 vs. Uq5							
893919/3844-10		164		0 (0.0)	0	164	53.3**
3920/3844-12		115		14 (10.8)	1	130	13.3**
3911/3846-1		239		67 (21.5)	6	312	1.88ns
3921/3846-10		129		63 (32.3)	3	195	5.95*

Figure 5. A putative linkage map of the standard Ug element (Ug1) and the five spontaneously germinally activated new Ugs elements, i.e., Ug2, Ug3, Ug4, Ug5, and Ug6

The one linkage group with Ug4(?) and Ug5(?) is more hypothetical and needs further genetic tests. In addition, other possible sites for Ug4 and Ug5 (see text) in the new Ug linkage group are not shown.


Uq1
Uq4(?) Uq5(?)
Uq2
Uq6
Uq5
23-24
4
Uq4
Uq3
31-32
35

very likely allelic to Uq3. Finally, Uq6 is linked to both allelic pairs.

DISCUSSION

The Ug-rug two-element system is the most pervasive among the nine transposable element systems in maize (for review, see Peterson, 1987). Using either a-rug or c-rug reporter alleles, active Ug elements have been uncovered in many maize genetic testers, lines and breeding populations (Cormack et al., 1988; Karazawa and Peterson, 1987; Peterson, 1985; Pereira and Peterson, 1985; Peterson and Friedemann, 1983; Peterson and Salamini, 1986). Even in maize inbred lines originally lacking apparent Ug activity, exceptional Ug activity often appears in both the somatic and the germinal tissues from activated quiescent Ug sequences. This activation process can be either spontaneous (Pan and Peterson, 1988, 1990) or inductive (Pan and Peterson, 1989a, 1989b).

Recently, we have successfully isolated six genetically verified new Ug elements (Mn::Ug, Ug2, Ug3, Ug4, Ug5, and Ug6) from such activating processes (Pan and Peterson, 1989a, 1990). Allelism tests involving Ug1 and five of these new Ug elements (not including Mn::Ug) were completed in the present study, facilitated by their reproducible unique spotting patterns on a-rug (Fig. 1).

One significant finding from the present study is that all

the five new Uq elements are independent of Uq1 (Fig. 3, Tables 6-1 and 6-2) and are therefore not located on maize chromosome 2 (Pereira and Peterson 1985). Moreover, these five new Uq elements are themselves clustered. Uq2 is allelic to Uq4, Uq3 is allelic to Uq5, and Uq6 is linked to these two allelic Uq pairs. An additional unlinked site has been found for both Uq4 and Uq5, based on their segregational analysis (Fig. 5). Although these additional sites could have been generated by secondary transposition of Uq4 and Uq5, it is also possible that these sites are not true because the linkage values showing independence are due to inactivation or loss of these two Uq elements. This possibility is strong because Uq4 and Uq5 showed the highest probability of "off-ratio" transmission when either was present alone (Table 3).

That all the new Uqs are clustered is not surprising. Both the genetic and molecular evidence available suggest a nonrandom distribution of the maize transposable elements following excision (Nowick and Peterson 1981; Johns et al. 1990). Often, preferential regions on certain chromosomes can be found. In their transpositional study of the En transposable element, Nowick and Peterson (1981) discovered that the distributions of primary and secondary transposed Ens on chromosome 3L were not random but showed some regional preference. By using the 0.7 kb EcoRI-HindIII internal fragment of the Ac element as a probe, Johns et al. (1990) in

their RFLP analysis experiment found that the 24 Ac-like sequences in the four maize recombinant inbred parental lines (Burr et al. 1988) were distributed in a non-random fashion, with many of these sequences located in three clusters on chromosomes 4. In addition, secondary clusters of Ac-like sequences were also found on chromosomes 1 and 2 (Johns et al. 1990). Because the five new Uq elements were independently isolated from spotted exceptions originally lacking Uq activity, it is more likely that either these elements were directly activated from a cluster of quiescent Uq sequences or their clustering was a consequence of a "founder" effect (Johns et al. 1990).

Another significant finding of the present study is the heterogeneity of the Uq transposable elements. Various Uq elements, either naturally residing (Caldwell and Peterson 1989; Cormack et al. 1988; Pereira and Peterson 1985; Peterson 1987; Sprague 1986) or germinally activated (Pan and Peterson 1989a, 1989b, 1990), elicit distinctive spotting phenotypes with the a-rug reporter alleles (for examples, Plate 1 in Pereira and Peterson 1985; Fig. 3 in Pan and Peterson 1990). When these a-rug reporter alleles are isolated and reconstituted with a genetically verified Uq1, all alleles prove identical in producing a similar type of Uq1-a-rug spotting pattern (Pan and Peterson 1989a, 1990). Therefore, the unique spotting patterns given by these a-rug alleles with

various Uq elements must reflect heterogeneity among the Uq elements.

A spotting pattern of a-rug is determined by both the timing and the frequency of rug-excision events, which in turn are controlled by when and how much Uq-specific transposition functions is transcribed. Notably the a-rug "substrate" for Uq transposase function is constant; the variable is the Uq. Generally, a heavier, near full-color-like a-rug spotting implies earlier and more rug excision events and, therefore, a stronger Uq transposase function. In contrast, a lighter and less frequent a-rug spotting indicates a weaker Uq transposase function.

It is not known, however, whether these differences in Uq transposition functions are due to different Uq structures (the composition hypothesis, McClintock 1950, 1958) or to their various chromosomal locations (the position hypothesis, Peterson 1976), or both. The Ac7 and Ac9 express different phenotypes but have the same size (Muller-Neumann et al. 1984; Pohlman et al. 1984). We observed that once in their allelic site, Uq4 elicited a spotting phenotype similar to that of Uq2 (Fig. 4, panel 3b), and Uq5 elicited a spotting phenotype similar to that of Uq3, although each retained its two spotting patterns (Fig. 1) when present alone or in pairs with Uq2 (Fig. 4, 2b) and Uq3 at nonallelic sites. These observations provide direct evidence that expression of one

Ug's transposition function can be altered by its relative location in the genome. But, a more direct answer to the question of what causes weak and strong Ug transposition functions awaits the successful molecular cloning and characterization of the Ug element. Unfortunately, except for the Mn::Ug (Pan and Peterson 1989a) and perhaps Ug^{10aa}-Smaller Seed (Pan and Peterson 1989b), no Ug-controlled maize mutants are available at a cloned gene. Because the exact chromosomal location for both Mn::Ug and Ug^{10aa}-Smaller Seed is unclear, either mutant is not a suitable material for Ug cloning experiment.

On the other hand, both a-rug and c-rug receptor alleles have been cloned and sequenced (Antionio G. Pisabarro, Max-Planck-Institut fur Zuchtungsforschung, Koln, Federal Republic Germany, personal communication). Nevertheless, even the rug receptors' being cloned does not guarantee that Ug can be cloned by using rug as a probe. There are numerous examples in which receptor elements are not sequence-related to their functional regulator elements. There is genetic evidence that, based on their different response to Ug13 (Caldwell and Peterson 1989), Mn::Ug (Pan and Peterson 1989a), Ug3, Ug4, and Ug5 (Pan and Peterson 1990), the rug receptors in a-rug and c-rug alleles may differ structurally. Moreover, there is molecular evidence that some nonautonomous elements, like Ds1 (Dellaporta and Wessler 1984; Sutton et al. 1984), may not be

deletion derivatives of their autonomous element, Ac (Muller-Neumann et al. 1984; Pohlman et al. 1984).

Finally, the putative Uq linkage map shown in Figure 5 includes the following points. First, the probability for each Uq element to fully express its activity has been determined to be at least 90% in randomly selected types 2 and 3 kernels and at least 80% in nonrandomly selected types 2 and 3 kernels. This finding indicates that in at least 8 out of 10 crosses to a-rug, segregation of each of the six particular Uq elements (when present alone and heterozygous), is done in Mendelian fashion and yields 50% spotted and 50% colorless progenies without undergoing secondary transposition or activity loss events. Second, in our previous study (Pan and Peterson 1990), crosses of individuals, two, with homozygous reconstituted Uq2/Uq2, and one, with Uq3/Uq3, to the a-rug tester produced 100% spotted progenies (Table 6a, lines 10 and 13; Table 6b-1, line 1 in Pan and Peterson 1990), again, a fully active expression. Third, each set of individual linkage values for any pair of two new Uq elements was homogenized before a mean linkage value for this particular Uq pair was established.

In summary, the small percentage of colorless round progeny kernels on type 4 ears segregating Uq pairs of Uq3 vs. Uq6 and Uq5 vs. Uq6 are caused true crossover recombinants rather than by activity loss of either Uq. The genetic evidence supports

our description of the linkage map of these five new Uq elements.

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SECTION IV

TAGGING OF A MAIZE GENE INVOLVED IN KERNEL DEVELOPMENT BY AN ACTIVATED *Uq* TRANSPOSABLE ELEMENT

TAGGING OF A MAIZE GENE INVOLVED IN KERNEL DEVELOPMENT BY AN ACTIVATED *Uq* TRANSPOSABLE ELEMENT[1]

Running title: Tagging the miniature gene

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ABSTRACT

A quiescent Uq transposable element has been activated in a maize plant treated with 5-aza-2'-deoxycytidine. This activated Uq activity cosegregates with a heritable dominant miniature (Mn) kernel phenotype, indicating its physical association with a maize miniature locus (Mn::Uq). The Mn::Uq mutant is dominant in producing a miniature seed phenotype of variable size and in reducing seedling vigor in the early growth stage. Genetic experiments indicate that the Mn::Uq mutant also affects the activity of the male gametophyte, whereby pollen germination is inhibited, thus lacking pollen tube growth resulting in the male nontransmissibility of this mutant. Proof for the Uq element in this mutant is derived by its ability to transactivate the standard a-rug reporter allele to yield spotted aleurone tissue. However, the Mn::Uq mutant does not transactivate a normally Uq-responsive c-rug67 allele, suggesting a structural difference between the rug receptors at the A1 and C1 loci. The Mn::Uq mutant is not allelic to three other recessive miniature mutants, namely, mn1, mn2, and mn7690.

Key words: Mn::Uq-(5-aza-2'-deoxycytidine)-Zea mays L.

INTRODUCTION

DNA modification, particularly methylation, is inversely correlated with gene activity (for review, see Doerfler, 1983). In maize, the loss of activity of transposable elements, such as Mu1 (Chandler and Walbot, 1986), Ac (Chomet et al., 1987), and Spm (Banks et al., 1988) is due to an increased level of DNA methylation of these elements. Recently, spontaneous activation events of quiescent Ug transposable elements have been repeatedly observed in both the somatic (Pan and Peterson, 1988a) and the germinal tissues (Pan and Peterson, 1990) of several maize inbred lines originally lacking any Ug activity.

The Ug/rug system (Friedemann and Peterson, 1982) is one of the nine two-component (the regulator and the receptor) transposable element systems known in maize (for review, see Peterson, 1987). The activity of the Ug regulator element is detected by excision events of the rug receptor elements at either the A1 (the a-rug reporter allele) or the C1 (the c-rug reporter allele) loci. The excision events can be seen as colored spots on a colorless aleurone layer when the kernel genotype contains the a-rug allele and Ug or the c-rug allele and Ug. In the absence of an active Ug element, these kernels are colorless and without spots.

Because there is evidence that there is no alteration in the rug receptor element in those exceptional gametes showing germinal Ug activity (Pan and Peterson, 1990), these newly arisen Ug activities likely originated through demethylation of highly methylated quiescent Ug element sequences in the genomes of these maize inbred lines. An experimental approach to enhance such an activation process is to use the DNA nucleotide analog 5-aza-2'-deoxycytidine. A number of studies have shown that the treatment of animal cells with nucleotide analogs such as 5-aza-cytidine or 5-aza-2'-deoxycytidine can activate previously dormant genes by reducing or eliminating methylation in their DNA sequences (for review, see Doerfler, 1983).

This report summarizes one such experiment in maize and presents the following results:

1) A quiescent Ug element has been activated upon treatment with 5-aza-2'-deoxycytidine in a maize plant grown from a a-rug/a-rug, sectored kernel. A sectored kernel is one that is essentially colorless but with a sector of spots. The size of the sector varies. In this case, the sector of spots is the phenotype of an interaction between the a-rug allele and an activated Ug element in the cells underlying the sector (see Figs. 2, 3 in Pan and Peterson, 1988a).

2) The coinduction of a heritable dominant Mn mutant, very likely, by the transposition and reinsertion of the activated Ug element into a locus causing miniature phenotype and identified as Mn::Ug.
3) The genetical characterization of this Mn::Ug mutant.
4) Recovering of revertants from the Mn::Ug mutant.
5) The allelic relationship of the Mn::Ug to mn1, mn2 and mn7690.

MATERIALS AND METHODS

Source material and the 5-aza-2'-deoxycytidine treatment

The treatment included 35 one-week-old maize seedlings with another set of 15 seedlings of the same age as the control. All 50 seedlings were derived ffom a-rug/a-rug, sectored kernels known to lack an active germinal Ug element (Pan and Peterson, 1988a). Treatment was conducted by submerging the freshly emerging roots of the germinating seedlings in a 30 μM 5-aza-2'-deoxycytidine solution for 72-90 hours. After the treatment, the plants were transferred into the greenhouse and grown for another 2 weeks before being transplanted into the field. During this period, five seedlings died; others had brown root tips and folded first leaves. These seedlings gradually recovered and grew to maturity.

At the flowering stage, each of the 30 treated and the 15 control plants were crossed reciprocally with a-rug tester. At maturity, all progeny ears were harvested and analyzed to uncover germinal Ug activity.

Testing of transmission of the Mn::Ug mutant

Two generations of progeny tests on the Mn::Ug mutant have been conducted to confirm that this mutant is dominant and not male transmissible. Ten colorless normal-sized and 50 spotted miniature kernels (10 for each of the five arbitrarily defined size classes, a, b, c, d, and e, as shown in Fig. 2) were collected from the ear 866248U/a-rug (ear A in Fig. 1) and were grown into plants. These plants were crossed reciprocally with either a a° sh2/a° sh2 (no Ug) line or a c-rug tester. Either of the two Ug-responsive c-rug testers, c-rug65 or c-rug67 (Caldwell and Peterson, 1989), was used. In addition, crosses of a few miniature-derived plants (as male) on two other genotypes (r-g tester and Hy inbred line) were also made to test the male transmission of the Mn::Ug mutant. The spotted miniature and the mottled F_1 progenies derived from these crosses were tested in another generation.

To characterize the physical basis of the male nontransmissibility of the Mn::Ug mutant, fresh mature pollen samples were collected from plants that had either a homozygous normal (+/+) or a heterozygous miniature (Mn::Ug/+) genotype. The pollen samples were first stained by I_2-KI and examined directly under a microscope. In addition, the pollen samples were allowed to germinate on a in vitro germination

media (Saini and Dube, 1986) to detect if there is difference in pollen germinaiton.

Testing that Mn::Ug is indeed a Ug-induced mutant

To prove that the element that has been activated is indeed a Ug element, two approaches were taken. First, to show that the a-rug allele was present among the colorless normal sib kernels of ear 866248U/a-rug, 24 were tested by crossing to a a° sh2/a° sh2, Ug1/Ug1 line (the Ug1 composition of each individual plant used in this line was determined by crossing the same plant to a standard a-rug tester).

The second genetic approach was to isolate the activated Ug element of the original Mn::Ug mutant away from the a-rug receptor allele and to introduce it into a a° sh2/a° sh2, no Ug line. This would allow a test of the ability of this activated Ug element to transactivate the rug receptor elements at either A1 or C1 to excise. The procedure involved the crossing of the spotted miniature kernels of ear A shown in Fig. 1 by a a° sh2/a° sh2, no Ug line. From this cross, spotted miniature F_1 seeds were collected and were either self-pollinated or backcrossed by the same a° sh2/a° sh2, no Ug line. The shrunken miniature seeds were selected from either the F_2 or the BC_1 progeny. These shrunken miniature seeds were

germinated, transplanted, and crossed by the standard a-rug tester. At harvest, the progeny ears were analyzed for Mn::Ug activity.

Recovering revertants from the Mn::Ug mutant

Five hundred fifty-three spotted miniature kernels of e-size class were selected from 12 progeny ears derived from either crosses of (a-rug/a-rug, Mn::Ug/+) X (a^{o} sh2/a^{o} sh2, no Ug) tester or selfing of (a-rug/a-rug, Mn::Ug/+) listed in Table 3. From these kernels, 461 plants were grown. These plants were hand-pollinated by the standard a-rug tester. At harvest, plants showing a segregation of miniature on either hand- or open-pollinated ear (note that the dominant Mn::Ug mutant phenotype always expresses no matter what the pollen source is) were discarded. Only when the miniature phenotype was not apparently present were both hand- and open-pollinated ears harvested. Further confirmation tests were done with the spotted kernels of an apparently normal size from hand-pollinated ears. Plants grown from these spotted kernels were self-pollinated to varify if they were true revertants of the Mn::Ug mutant.

Allelism tests among Mn::Ug, mn1, mn2, and mn7690

Plants grown from spotted miniature (Mn::Ug/+) were crossed by three other miniature lines, mn1/+ (Lowe and Nelson, 1946), mn2/mn2 (Bryce, 1974; Van Horn, 1968), and mn7690/mn7690 (source: O. E. Nelson Jr., Department of Genetics, University of Wisconsin). These three miniature genes are recessive and are equally transmitted through both male and female gametes. All F_1 progenies were colored, of which about 50% were miniature and another 50% were normal. Both the miniature and the normal F_1 seeds from each cross were selected and were grown into plants. These plants were backcrossed by their corresponding homozygous miniature lines. At maturity, all backcross progeny ears were harvested and analyzed. Expected BC_1 progeny classes and their frequencies under three different allelic conditions were shown in Table 1.

Table 1. Expected phenotypic classes and their frequencies in the F_1 and BC_1 generations under three different linkage relationships between the Mn::Ug mutant and each of mn1, mn2, and mn7690[a]

Miniature pair	Classification of (F_1)[b]	Classification of (BC_1)[c] progeny ears		
		allelic	linked	independent
Mn::Ug vs. mn1	1. Cl-mn (50%); Mn::Ug/+, +/(mn1 or +)	all miniature (1/2 BC_1 ears); 50% mn + 50% normal (1/2 BC_1 ears).	(> 3/4 mn) + (< 1/4 normal) (1/2 BC_1 ears); 50% mn + 50% normal (1/2 BC_1 ears).	3/4 miniature, + 1/4 normal (1/2 BC_1 ears); 50% mn + 50% normal (1/2 BC_1 ears).
	2. Cl-normal (50%); +/+, +/(mn1 or +);	all normal (1/2 BC_1 ears); 50% mn + 50% normal (1/2 BC_1 ears).	all normal (1/2 BC_1 ears); 50% mn + 50% normal (1/2 BC_1 ears).	all normal (1/2 BC_1 ears); 50% mn + 50% normal (1/2 BC_1 ears).
Mn::Ug vs. mn2	1. Cl-mn (50%); Mn::Ug/+, +/mn2;	all miniatures	(> 3/4 mn) + (< 1/4 normal)	3/4 miniature, + 1/4 normal
	2. Cl-normal (50%); +/+, +/mn2;	50% mn + 50% normal	50% mn + 50% normal	50% mn + 50% normal
Mn::Ug vs. mn7690	1. Cl-mn (50%); Mn::Ug/+, +/mn7690;	all miniatures	(> 3/4 mn) + (< 1/4 normal)	3/4 miniature, + 1/4 normal
	2. normal (50%); +/+, +/mn7690;	50% mn + 50% normal	50% mn + 50% normal	50% mn + 50% normal

[a]Only the miniature genes and their phenotypes are concerned.
[b]There are two classes of F_1: Cl-mn (colored miniature) and Cl-normal (colored normal), each at a 50% frequency with its expected genotype indicated. The F_1

progenies are derived, respectively, from the following crosses: (Mn::Ug/+, +/+) X (+/+, mn1/+) or X (+/+, mn2/mn2) or X (+/+, mn7690/mn7690). Note that Mn::Ug is dominant and mn1, mn2, mn7690 are recessive.

[c]The BC_1 progenies are derived, respectively, from the following crosses:
1). For Mn::Ug vs. mn1: (Mn::Ug/+, mn1 or +/+) X (+/+, mn1/mn1).
2). For Mn::Ug vs. mn2: (Mn::Ug/+, mn2 or +/+) X (+/+, mn2/mn2).
3). For Mn::Ug vs. mn7690: (Mn::Ug/+, mn7690 or +/+) X (+/+, mn7690/mn7690).
Different classes of BC_1 progeny kernels are expected depending upon whether the pair of miniature genes is allelic, linked, or independent of each other. The three columns are exclusive to one another.

RESULTS

Activation of Ug and induction of the Mn::Ug mutant

All the control plants as well as the twenty-eight treated plants produced normal type of colorless ears (data not shown). However, one treated plant, 866247x-3, was totally sterile. Another treated plant, 866248U, produced two distinct types of ears in the reciprocal cross (Table 2, Fig. 1).

When plant 866248U was crossed by an a-rug tester plant (this tester plant was neither a miniature mutant nor did it show Ug activity based on a normal type of colorless ear from the self-pollination of this plant), an ear bearing 279 kernels resulted that could be classified into 136 colorless plumpy and 143 spotted miniature-like (Table 2). These two classes of kernels were evenly distributed on the ear (Fig. 1, ear A). In addition, the spotted miniature-like kernels could be further subclassified arbitrarily into five groups (a, b, c, d, and e), all of which had a reduced size in comparison with the colorless plumpy seeds (Fig. 2, variable penetrance). In contrast, when plant 866248U was crossed as a pollen parent to a standard a-rug tester, only a normal type of ear bearing 328 colorless plumpy kernels was produced (Table 2, Fig. 1,

Table 2. Original reciprocal crosses between the Mn::Ug mutant and the a-rug tester

Cross[a]	Progeny classification[b]				
	spotted		colorless		
	norm	mn	norm	mn	total
866248U X a-rug	0	147	132	0	279
a-rug X 866248U	0	0	328	0	328

[a]The mutant plant 866248U was treated with 5-aza-2'-deoxycytidine. It had a genotype of a-rug/a-rug, Mn::Ug/+. The a-rug tester had a genotype of a-rug/a-rug, +/+.

[b]Norm = normal kernel size; mn = miniature.

Figure 1. Phenotype of the Mn::Ug mutant showing that Mn::Ug is a dominant mutant and is transmitted maternally only

Ear A is derived from the cross (a-rug/a-rug, Mn::Ug/+) X (a-rug/a-rug, +/+) tester; and ear B is from the reciprocal cross. Ear C is derived from the cross of (a-rug/a-rug, Mn::Ug/+) X (c-rug67/c-rug67, +/+), and ear D is from the reciprocal cross.

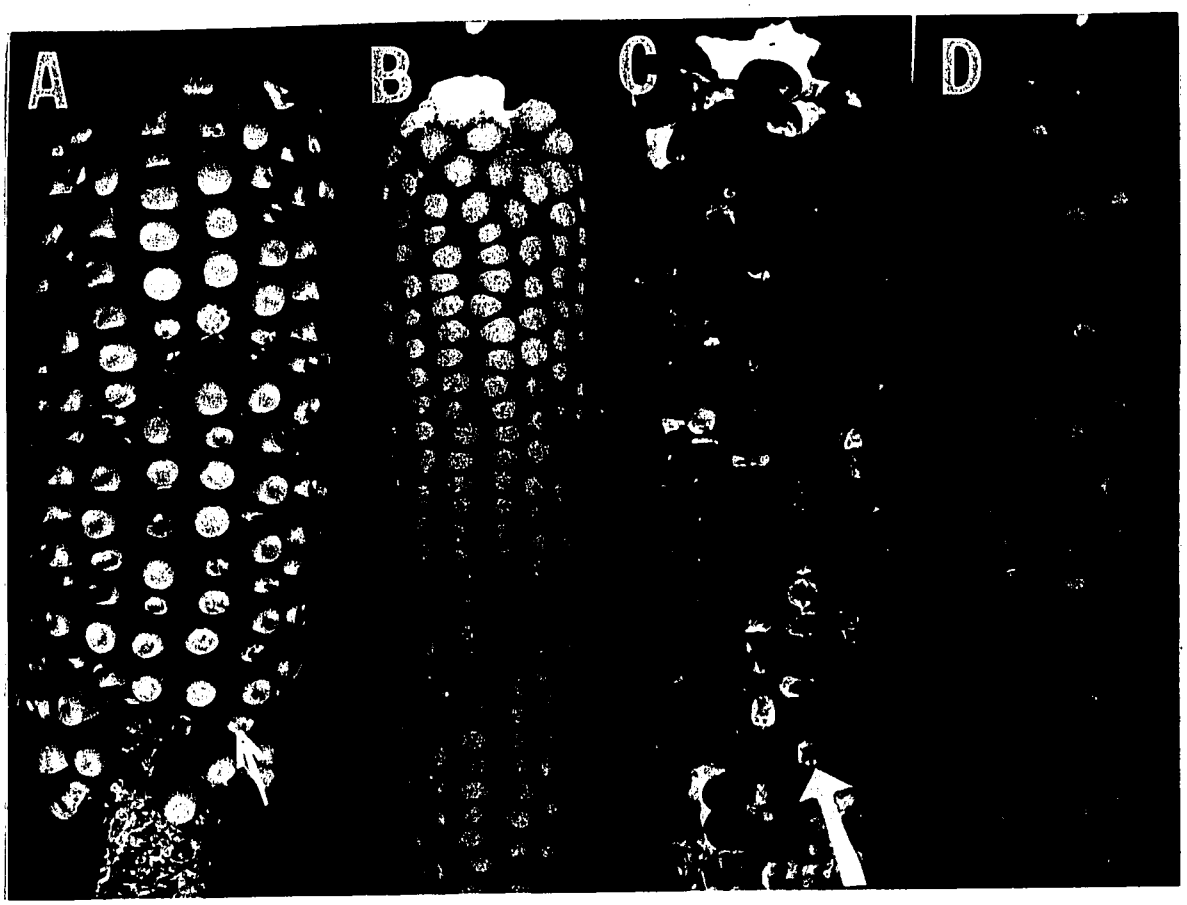
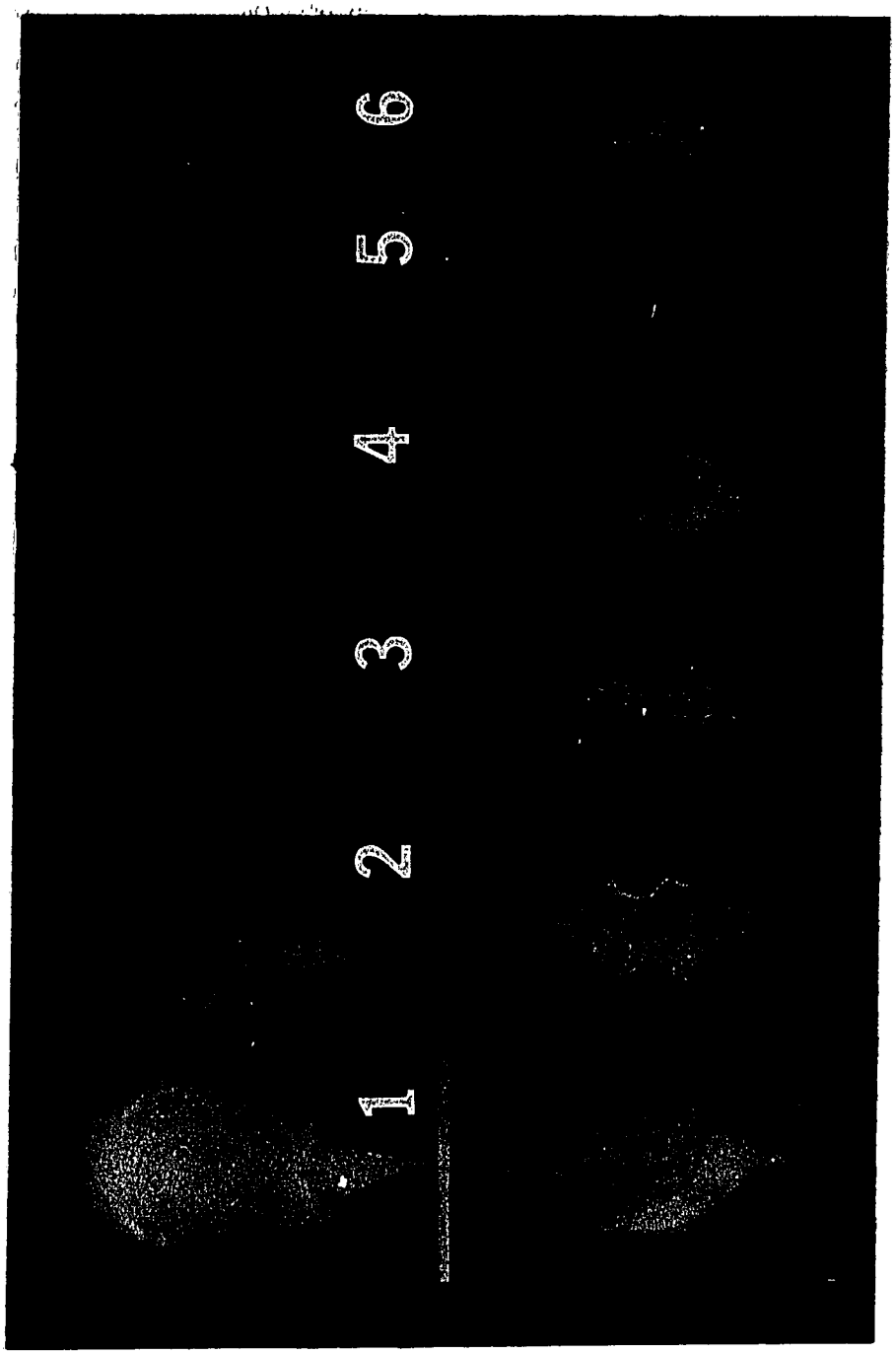

A
B
C
D

Figure 2. Sample kernels from ear A shown in Fig. 1

Both the abgerminal (top row) and the germinal sides (bottom row) are shown. Kernel 1 is colorless normal-sized, kernels 2 through 6 are spotted miniature with an arbitrary size of e, d, c, b, and a, respectively. The white bar represents 0.5 cm.


1
2
3
4
5
6

ear B).

From these observations and the data to be presented, three conclusions can be drawn: (1) A quiescent Ug element had been activated in plant 866248U, (2) this activated Ug cosegregates with a miniature-like mutant that acted dominantly in both embryo and endosperm development, and (3) this cosegregating Ug and miniature-like mutant is not male transmissible.

The Mn::Ug mutant is dominant and not male transmitted

From the two generations of progeny tests, the following results are observed: First, from the progenies of crosses with the 10 colorless normal sibs, neither the miniature nor the activity of Ug was present regardless of the type of cross (Table 3). Second, in every F_1 progeny ear from the crosses in which the spotted miniatures were used as maternal parents (Table 3, under columns by a^o sh2, no Ug and by c-rug), both the miniature and the Ug activity were segregating; nevertheless, both were missing from the progenies when the same spotted miniatures were used as paternal parents (Table 3, under columns on a-rug and on c-rug). In addition, both the miniature and Ug were not present in all the mottled progenies derived from the cross of either the r-g/r-g tester or inbred Hy (genotype r/r, A/A) X spotted miniature (genotype

Table 3. A summary data from two generations of Mn::Ug progeny tests

Progeny type[a]	by a° sh2 (no Ug)		by c-rug		on a-rug		on c-rug	
	mn	Ug	mn	Ug	mn	Ug	mn	Ug
spt-mn (1)	30/30[b]	30/30	19/19	?/?	0/33	0/33	0/42	?/?
spt-mn (2)	35/35	35/35	/	/	0/43	0/43	/	/
cl-norm	0/10	0/10	0/4	?/?	0/6	0/6	0/11	?/?
mottled (1)	0/34	0/34	/	/	0/43	0/43	/	/
mottled (2)	/	/	0/7	0/7	/	/	/	/

[a]Sources for the progeny types tested: Spt-mn (1) (spotted miniature) and cl-norm (colorless normal) were from the ear 866248U/a-rug; spt-mn (2) was from the cross of spotted miniature X a° sh2, (no Ug) tester; mottled (1) was from the cross of r-g/r-g tester X spotted miniature; and mottled (2) was from the cross of inbred Hy X spotted miniature.

[b]Values below the bar represent the total number of progeny ears analyzed, whereas values above the bar are the number of ears that segregate miniature or Ug; symbol / indicates that such cross is not available, and ? indicates that Ug activity can not been assessed due to colored aleurones.

R/R, a-rug/a-rug, Mn::Ug). Third, the seed size difference shown among the five arbitrarily defined groups of spotted miniatures was due to the variable expressivity of the Mn::Ug mutant because when crossed by the same a sh2/a sh2 (no Ug) tester, each of these five seed size groups gave progenies that segregated for all sizes of spotted miniature (Table 4, Fig. 3, variable penetrance).

Like several other maize genes that are expressed in both sporophytic and gametophytic phases (Demerec, 1924; Brink and MacGillvray, 1924; Freeling, 1976; Gorla et al. 1986; Clark and Sheridan, 1988; Nelson, 1978; Ottaviano et al. 1988), this dominant Mn::Ug mutant phenotype was expressed, not only in the sporophytic tissues affecting both the normal development of the maize seeds (embryo and endosperm) and early seedling growth, but also in the male gametophytes (the pollen grains) to affect their normal function.

No apparent difference was observed between Mn::Ug/+ and +/+ genotypes in terms of pollen grain size, shape, and I_2-KI staining quality (Figs. 4a, 4b). However, after one hour germination on in vitro germination media, it was observed that about half of the pollen population taken from the heterozygous miniature mutant (Mn::Ug/±) failed to grow pollen tubes, although the other half germinated quite well (Fig. 4d). On the other hand, almost all the pollen grains from

Table 4. Variable penetrance of the Mn::Ug mutant by testing spotted miniature kernels of varying sizes

Size & Cross[a]	Progeny classification[b] cl-norm	spt-mn a	b	c	d	e	total	Grand total
size a:								
870455-1/0708*	68	5	10	18	12	18	63	131
-1t self	87	9	34	35	34	21	133	220
-2/0708*	127	6	3	12	30	38	89	216
-3/0708	132	23	22	34	43	13	135	267
-4/0710*	251	3	26	56	57	43	185	436
-5/0708*	228	113	38	34	17	9	211	439
-7t/0403*	192	22	11	14	34	34	115	307
-7t self*	109	4	17	20	36	16	93	202
Mean	**149**	**23**	**20**	**28**	**33**	**24**	**128**	**277**
size b:								
870456-1/0401*	193	5	9	9	25	81	129	322
-2/0708*	225	8	2	34	56	97	197	422
-3/0732*	197	30	35	47	53	65	230	439
-5/0732*	136	10	27	36	23	14	110	246
-6/0404	11	0	2	3	4	3	12	23
-8t self	170	27	23	33	37	19	139	309
Mean	**155**	**13**	**16**	**27**	**33**	**47**	**136**	**291**
size c:								
870457-1/0708*	290	7	18	48	117	48	238	528
-2/0706	19	2	2	4	10	10	28	47
-3/0403*	225	11	11	58	36	29	145	370
-4/0407	118	3	9	14	36	28	90	208
-4t self	72	0	8	18	11	29	66	138
-5/0403	122	14	3	5	17	16	55	177
-6/0401	166	6	9	44	72	25	156	322
-10/0707	15	1	0	2	4	10	17	32
Mean	**128**	**5**	**8**	**24**	**38**	**24**	**100**	**228**

[a]General genotype of the cross is (a-rug/a-rug, Mn::Ug/+) X (a° sh2/a° sh2, +/+, no Ug); 553 spotted miniature kernels of e-size class were selected from the 12 crosses marked with * and were used as source material for revertant isolation.

[b]cl-norm = colorless with normal size; spt-mn = spotted miniature subclassified into a, b, c, d, and e sizes.

Table 4. (Continued)

Size & Cross[a]	Progeny classification[b] cl-norm	spt-mn a	b	c	d	e	total	Grand total
size d:								
870458-1/0708	75	26	13	10	14	6	69	144
-2/0707	133	64	20	27	22	11	144	277
-3/0402	57	2	2	8	25	16	53	110
-3t self	255	4	16	34	79	89	222	477
-4/0708	272	4	7	16	85	106	218	490
-5/0404	28	2	0	4	22	8	36	64
-6/0708	183	40	25	44	35	11	155	338
-6t self	104	13	9	17	24	18	112	216
Mean	**138**	**19**	**12**	**20**	**38**	**33**	**126**	**264**
size e:								
870459-1/0710	129	5	5	26	55	59	150	279
-2/0707	80	38	7	29	26	4	94	174
-3/0704	324	19	31	67	96	33	246	570
-3t self	203	6	10	27	93	46	182	385
-5/0709	205	12	4	20	104	73	213	418
-6/0709	162	16	18	26	33	8	101	263
-8t self	165	4	5	35	80	29	153	318
-9t self	85	1	6	18	34	8	67	152
Mean	**169**	**13**	**11**	**31**	**65**	**32**	**151**	**320**

Figure 3. Variable penetrance of the Mn::Ug mutant

The five arbitrary seed size groups of the Mn::Ug mutant (a, b, c, d, and e as shown by sample kernels 6, 5, 4, 3, and 2 in Fig.2, respectively) produce all five seed size groups of offspring when crossed by a a^{o} sh2/a^{o} sh2, no Ug tester (or when selfed, but data not shown). However, the frequency for each group varies within as well as among the five seed size groups tested.

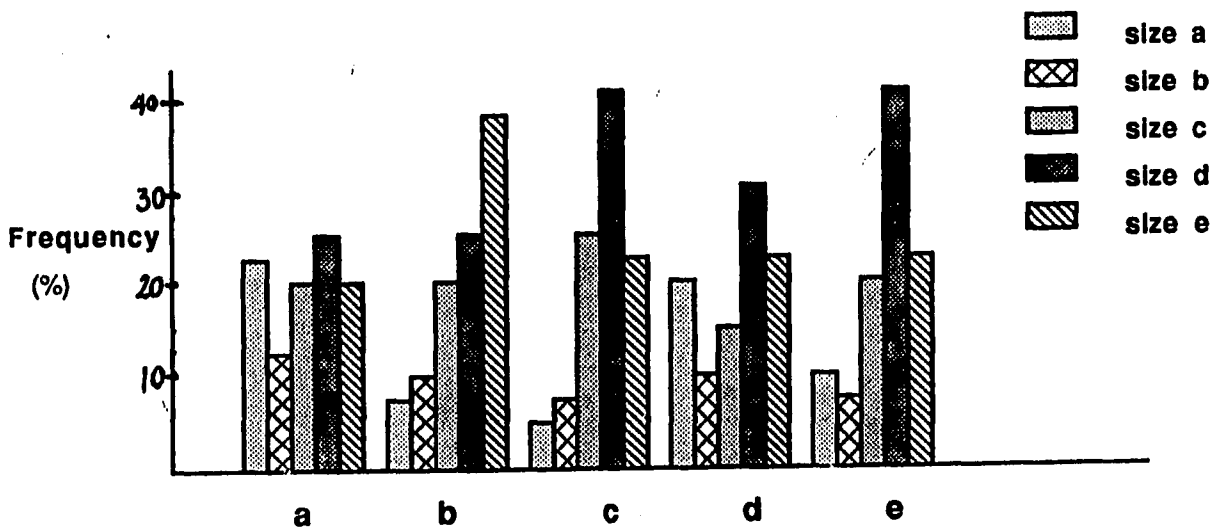
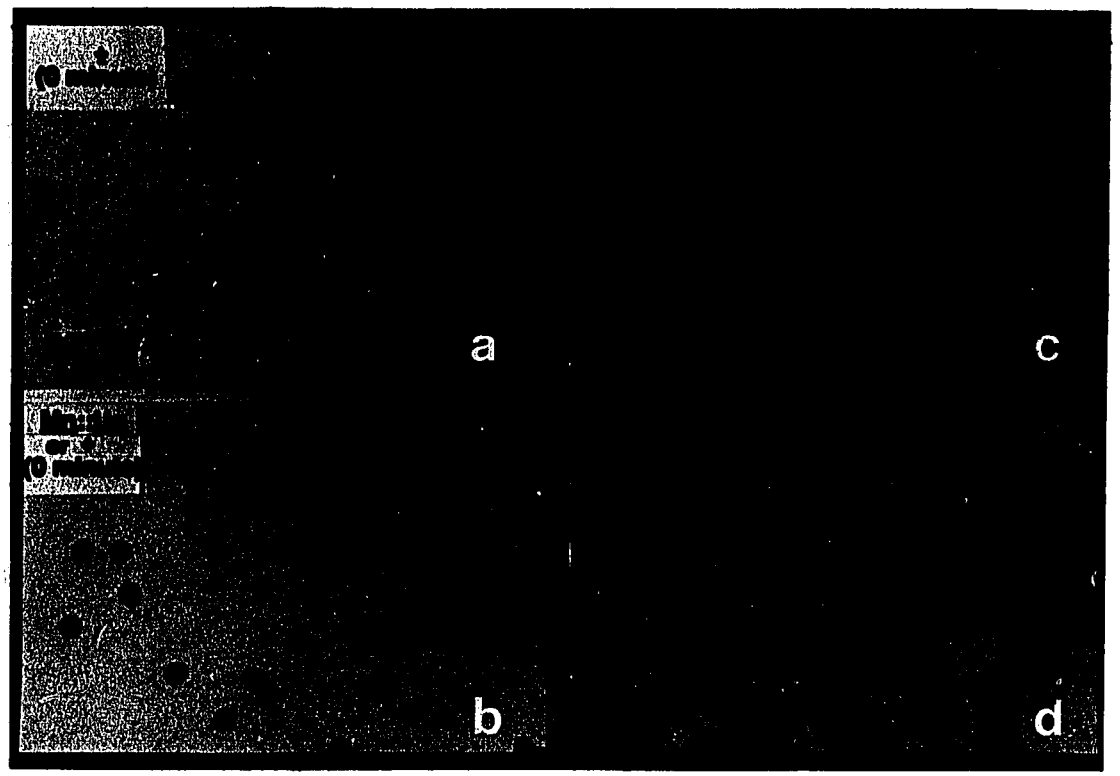

size a
size b
size c
size d
size e
Frequency
(%)
40
30
20
10
a
b
c
d
e

Figure 4. The Mn::Uq pollen fails to germinate

Arrow-Mn: the Mn::Uq pollen grain.
Arrow +: Pollen tube of nonmutant pollen (see panel d and text). Panels a, b do not have the same scale of magnification as that of c, d.


a
c
b
d

homozygous normal sib plants (+/+) germinated (Fig. 4c) (data not shown). Therefore, the male nontransmissibility of the mutant is due to the failure of the Mn::Uq pollen to germinate. It seems that the normal allele of the miniature locus encodes a certain type of product that plays an essential role, not only in maize embryo and endosperm development, but also in pollen germination and tube growth. This function is disrupted in the miniature mutant because of the Uq insertion into the miniature locus.

The Mn::Uq mutant is indeed under the control of the activated Uq element

All the 24 colorless normal sib kernels from ear A (Fig. 1) were homozygous a-rug/a-rug. In the crosses by a verified a° sh2/a° sh2, Ug1/Ug1 line, they all produced progeny ears bearing almost 100% Ug1-a-rug spotted normal kernels (data not shown). In addition, 50 colorless normal F_1 sib kernels, derived from a cross of (spotted miniature from ear A in Fig. 1) X (a° sh2/a° sh2, no Uq) tester were also tested by the same a° sh2/a° sh2, Ug1/Ug1 line. This gave rise to ears that segregated approximately 50% Ug1-a-rug spotted normal and 50% colorless shrunken kernels (data not shown). These frequencies of Ug1-a-rug spotted normal kernels could be observed only if plant 866248U had a a-rug/a-rug genotype.

In the second genetic approach, F_2 progenies derived from the spotted miniature F_1 segregated for spotted miniature, colorless normal, colorless shrunken kernels in the same ratio to that of the BC_1 progenies (data not shown). This ratio was indicative of an independent mode of transmission for the A1 and Mn::Ug loci. In addition, about half of the shrunken kernels were miniature.

Progeny ears produced from the crosses of these colorless shrunken miniature by a-rug tester only segregated two classes of kernels: either colorless normal or spotted miniature in a 1:1 ratio (Table 5). Neither spotted normal nor colorless miniature was observed. Since these kernels had a a-rug/a° genotype, the spotted miniature phenotype provides definitive genetic proof that the activated element does activate the introduced a-rug allele. Therefore, the element which cosegregates with the Mn gene is indeed a Ug element.

Further support for these assertions is derived from the genetic analysis of 30 progeny ears that were derived from the cross of the spotted miniature of genotype a-rug/a-rug Mn::Ug/+ by the a° sh2/a° sh2, no Ug line. All ears segregated for two classes of seeds, namely, the colorless normal and the spotted miniature in a 1:1 ratio. In no case was a colorless miniature seed confirmed (data not shown). In addition, nine self-pollinated ears from the tillers of these plants derived from spotted miniature also segregated colorless normal-sized

and spotted miniature seeds in a 1:1 ratio (data not shown). This, in combination with results from several other tests involving a-m1 sh2 (reporter for En) and r tester lines (data not shown), has indicated that the activated Ug element is at the miniature locus.

However, this activated Ug differs from Ug1 in that it does not transactivate the Ug-responding c-rug67 or c-rug65 allele (Caldwell and Peterson, 1989). When the colored miniature kernels derived from the cross (the original Mn::Ug mutant X c-rug67/c-rug67, +/+ tester, ear C in Fig. 1) were backcrossed by the c-rug65 tester, the BC_1 progeny segregated for colored-normal-sized, colored miniature, colorless-normal-sized, and colorless miniature in a ratio not significantly different from 1 : 1 : 1 : 1 (Table 6). There were no spotted miniatures (c-rug/c-rug, Mn::Ug/+) as would be expected if Mn::Ug transactivated either c-rug allele. This differential interaction between the same Ug element and the two reporter alleles (the standard a-rug and c-rug65 or c-rug67) is suggestive that the rug receptors at these two maize loci might differ structurally. The segregation ratio also indicates that the Mn::Ug mutant is independent of the C1 locus.

Table 5. Transactivation of the a-rug allele by a reconstituted Mn::Ug mutant

Progeny Ear[a]	Progeny classification[b] spotted mn	spotted norm	colorless mn	colorless norm	total	$(X^2)^c$
1	3	0	0	4	7	0^{ns}
2	11	0	0	13	24	0.04^{ns}
3	49	0	0	51	100	0.01^{ns}
4	10	0	0	6	16	0.56^{ns}
5	4	0	0	8	12	0.75^{ns}
6	19	0	0	30	49	2.04^{ns}
7	4	0	0	10	14	1.78^{ns}
8	32	0	0	35	67	0.06^{ns}
9	29	0	0	19	48	1.69^{ns}
10	11	0	0	22	33	3.03^{ns}
11	13	0	0	18	31	0.52^{ns}

[a]Derived from the cross (a° sh2/a° sh2, Mn::Ug/+) (colorless shrunken miniature) X (a-rug/a-rug, +/+) tester. The small number of seeds per ear is due to a Reduced-Seed-Set (RSS) effect of the cross (Sukhapinda and Peterson, 1983).

[b]See footnote b in Table 1.

[c]A X^2 value for 1 spt-mn : 1 cl-norm. ns = not significant at 0.05 level.

Table 6. The *c-rug65* and *c-rug67* alleles are not transactivated by the *Mn::Ug* mutant

Progeny Ear[a]	Kernel classification[b] colored mn	colored norm	colorless mn	colorless norm	spotted	total	$(X^2)^c$
1	31	33	27	27	0	118	0.92^{ns}
2	23	27	23	24	0	97	0.44^{ns}
3	6	5	10	9	0	30	2.27^{ns}
4	10	14	12	13	0	49	0.71^{ns}
5	15	14	13	13	0	55	0.20^{ns}
6	10	8	10	9	0	37	0.29^{ns}

[a]Derived from the cross (*C*/*c-rug67*, *Mn*::*Ug*/+) X (*c-rug65*/*c-rug65*, +/+) tester.
[b]See footnote b in Table 1.
[c]A X^2 value for 1 cl-norm : 1 cl-mn : 1 cl-norm : 1 cl-mn. ns = not significant at 0.05 level.

Isolation of putative revertants from Mn::Ug

During harvest season, both hand- (first ear) and open- (second ear) pollinated ears were screened for the segregation of spotted miniature kernels. Those which apparently did so were discarded. On the other hand, hand-pollinated ears on 17 different plants did not show miniature kernels, which were not present on their open-pollinated ears either. Both types of ears were harvested and underwent genetic analysis.

Based on the genetic analysis, nine putative revertants of Mn::Ug were selected (Table 7). The poor seed setting of the hand-pollinated ears was likely due to the inadequate live pollen grains supplied by multi-pollination. From these crosses, spotted kernels were selected and were grown in the greenhouse. All were self-pollinated. At maturity, 4 out of the 9 selections segregated spotted miniature and therefore were discarded. In addition, three selections (893636-1/2504, 893620-2/3750, and 893625-1/3460) did not give miniature progeny but retained Ug activity, and other 2 selections (893632-1/3457 and 893634-1/1121) did not have either miniature or Ug. These putative revertants were designated tentatively at rev-1, rev-2, rev-3, rev-4, and rev-5, respectively (Table 8). The spotting patterns expressed by the Ug elements in rev-1, rev-2 and rev-3 look different from

Table 7. Isolation of revertants from Mn::Ug (first-round selection)

Cross[a] (1989 summer)	Progeny classification[b] norm Cl	norm spt	norm cl	mn	Total	mn in open-pollinated ear[c]
3613/3749	1	10	14	1(?)	26	n.a.
3615-1/3747	0	5	0	0	5	likely no
3620-2/3750	0	1	17	0	18	no
3625-1/3460	0	2	5	0	7	no
3628-1/3456	0	12	15	0	27	likely yes
3631-1/3456	0	9	11	0	20	likely yes
3632-1/3457	0	2	0	0	2	no
3634-1/1121	0	24	24	0	48	no
3636-1/2504	1	2	28	0	31	no

[a]Genotype of the cross: (a-rug Sh2/a° sh2, Mn::Ug/+) (spotted miniature with e-size) X a-rug tester.

[b]Abbreviations: Cl = colored; spt = spotted; cl = colorless; norm = normal size; mn = miniature.

[c]Yes or no = presence or absence of mimiature in open-pollinated sib ear; n.a. = not available.

Table 8. Isolation of revertants from Mn::Ug (second-round selection)

Putative revertant #	1989 summer cross[a]	ear #	Selfed progeny classification[b]				
				norm			
			spt	cl -rd	cl -sh	mn	Total
rev-1	893636-1/2504	1	140	2	58	0	201
		2	41	6	13	0	62
rev-2	893620-2/3750	1	21	89	30	0	141
rev-3	893625-1/3460	1	50	134	49	0	234
rev-4	893634-1/1121	1	1	141	0	0	143
		2	8	234	0	0	244
		3	0	58	0	0	58
		4	0	49	15	0	64
		5	1	213	86	0	299
		6	0	82	37	0	119
rev-5	893632-1/3457	1	0	13	2	0	15

[a]Spotted kernels from source cross were grown into plants. These plants were selfed.

[b]See footnote b in Table 6 for abbreviations. In addition, rd = round, sh = shrunken.

the Mn::Ug spotting pattern. However, because these are selfed ears, a clear comparison can not be made. Further testing by outcross is needed to ascertain the particular spotting phenotype of the Ug elements in these three putative revertants (Table 7).

The Mn::Ug mutant is independent of mn1, mn2 and mn7690

Upon backcrossing to their corresponding homozygous mn lines, about half of the colored normal F_1s from Mn::Ug vs. mn1 and all the colored normal F_1s from Mn::Ug vs. mn2 as well as from Mn::Ug vs. mn7690 produced BC_1 progeny ears segregating 50% miniature and 50% normal kernels (data not shown). The other half of the colored normal F_1s from Mn::Ug vs. mn1, when crossed by a homozygous mn1/mn1 line, produced BC_1 progeny ears having only normal kernels. These are what should be expected (Table 1), because none of these normal F_1 progeny contains the Mn::Ug mutant.

When the colored miniature F_1s were backcrossed as maternal parents (due to the male nontransmissibility of Mn::Ug) by their corresponding mn lines, the following results were observed: First, in the case of Mn::Ug vs. mn1, there were two types of BC_1 progeny ears. One type of ears segregated 50% miniature and 50% normal kernels indicating the absence of the mn1 allele in their F_1 parents (data not shown). The

other type of ears segregated 3/4 miniature and 1/4 normal kernels (Table 9). For both Mn::Ug vs. mn2 and Mn::Ug vs. mn7690, however, all the BC_1 progenies ears segregated 3/4 miniature and 1/4 normal kernels (Tables 10 and 11). Although in the case of Mn::Ug vs. mn7690, there were a few BC_1 ears of which the segregation ratio of miniature vs. normal kernels was significantly different from 3 : 1, this aberrant ratio was, however, due to the variable penetrance, this case towards normal, of the Mn::Ug mutant.

In summary, these results clearly indicate that the Mn::Ug mutant is independent of all the three recessive miniature mutants: mn1 (in linkage group #2), mn2 (in linkage group #7), and mn7690 (location unknown). Since Mn::Ug segregates independently from either A1 or C1, it is concluded, therefore, that the Mn::Ug mutant is not in the maize linkage groups #2, 3, 7, and 9. The exact location of this mutant, however, is still unkown.

Table 9. The *Mn::Ug* mutant is independent of *mn1*

BC_1 progeny ear[a]	Progeny kernel classification: miniature	normal	total	($X^{2)b}$
893501-21/3517-4	213	105	318	10.9^{**}
893503-30/3518-1	24	10	34	0.16^{ns}
-31/3517-1	100	33	133	0.03^{ns}
-32/3518-1	268	103	371	1.37^{ns}
-33/3516-1	347	125	472	0.48^{ns}
-34/3518-1	231	95	326	2.76^{ns}
893505-4/3516	126	53	179	1.79^{ns}
-21/3517-4	341	101	442	0.98^{ns}
893507-21/3517-3	160	60	220	0.49^{ns}

[a]Derived from the cross (*Mn::Ug*/+, *mn1*/+) X (+/+, *mn1*/*mn1*).

[b]A X^2 test for a (3 miniature : 1 normal) ratio of independent segregation; ns = not significant at 0.05 level.

Table 10. The Mn::Uq mutant is independent of mn2

BC_1 progeny ear[a]	Progeny kernel classification: miniature	normal	total	(X^{2})[b]
893523-1/3520-2	158	54	212	0.01^{ns}
-2/3520-2	239	94	333	1.68^{ns}
-21/3519-1	224	80	304	0.21^{ns}
-24/3519-1	300	122	422	3.23^{ns}
-25/3520-2	123	46	169	0.33^{ns}
893525-1/3520-2	230	83	313	0.31^{ns}
-4/3520-2	102	32	134	0.04^{ns}
-6/3520	108	40	148	0.23^{ns}
-22/3520-2	180	66	246	0.35^{ns}
-23/3520-2	303	104	407	0.04^{ns}
-24/3519-1	127	50	177	0.83^{ns}
893527-1/3520-2	148	61	209	1.74^{ns}
-4t/3520-2	120	39	159	0.00^{ns}
-5/3520	131	47	178	0.12^{ns}
-21/3520-2	284	114	398	2.63^{ns}
893537-1/3520-1	243	81	324	0.00^{ns}
-5/3519-2	248	76	324	0.33^{ns}
-21/3519-2	57	26	83	1.45^{ns}
-24/3519-3	387	148	535	1.88^{ns}
893539-21/3520-3	110	38	148	0.01^{ns}
-22/3520	175	76	251	3.45^{ns}
-23/3520	136	49	185	0.15^{ns}
-24/3520-1	203	68	271	0.00^{ns}
-25/3520	150	41	191	1.09^{ns}
-26/3520	169	63	232	0.47^{ns}

[a]Derived from the cross (Mn::Uq/+, mn2/+) X (+/+, mn2/mn2).
[b]See footnote b in Table 9.

Table 11. The Mn::Uq mutant is independent of mn7690

BC_1 progeny ear[a]	Progeny kernel classification: miniature	normal	total	($X^{2})^{b}$
893546-21/3542-1	24	8	32	0.00ns
-7t/3542-1	40	22	62	3.10^{ns}
893548-1t/3542-1	10	7	17	1.59^{ns}
-3/3542-1	18	5	23	6.39^{*}
-4/3542-1	22	10	32	0.38^{ns}
-7t/3542-2	80	85	165	61.5^{**}
-21/3542-1	225	85	310	0.84^{ns}
-22/3542-1	138	76	214	12.1^{**}
893550-2/3542-1	20	21	41	13.7^{**}
-4/3542-1	86	34	120	0.54^{ns}
-4t/3542-1	325	103	428	0.15^{ns}
-5/3542-1	39	12	51	2.88^{ns}
-6/3542-1	21	7	28	0.00^{ns}

[a]Derived from the cross (Mn::Uq/+, mn7690/+) X (+/+, mn7690/mn7690).

[b]A X^2 test for a (3 miniature : 1 normal) ratio of independent segregation; ns = not significant at 0.05 level, and *, ** = significant at 0.05, 0.01 level, respectively.

DISCUSSION

Numerous kernel mutants are known in maize (Neuffer et al., 1968; Neuffer and Sheridan,1980). Among these, one type has been called miniature (Wentz, 1924; Lowe and Nelson, 1946; Kermicle, 1978; Bryce, 1974; Van Horn, 1968). The miniatures, as characterized by their reduced-size embryos and endosperms, differ from another type of kernel mutants, the defective kernel mutants (Jones, 1920; Mangelsdorf, 1923; Garber and Wade, 1924; Sheridan and Neuffer, 1980) in that the miniatures are generally germinable under appropriate conditions and can grow to plants that are approximately equal in vigor, size, and maturity to the normal, although with a slower growth rate at an early seedling stage.

We report in this paper the induction and genetic characterization of a dominant maize miniature mutant. This mutant, designated Mn::Ug, affects the normal development of the maize endosperm tissue, thereby reducing the maize kernel size to varying extent. The differentiation and development of the embryo, however, is not likely being affected in the mutant kernel. This is based on the facts that usually the mutant seeds can germinate and that, regardless of its slower growth rate at early stages, the mutant seedlings can grow into vigorous plants.

Thus far, there is no direct evidence indicating that this

mutant was 5-aza-2'-deoxycytidine induced. The identification of another recessive Ug^{10aa}-Smaller Seed mutant from the same treated maize plant, however, may give additional support that the DNA nucleotide analog could have played a role in the induction of both mutants. In addition, there have been numerous cases where dormant prokaryotic or animal genes become active upon treatment with 5-aza-2'-deoxycytidine (for review, see Doerfler, 1983).

The most significant feature of this miniature mutant is that it cosegregates with a germinal Ug activity that was not present before the treatment. Because we started with maize material that showed frequent activation of quiescent Ug elements in somatic tissues, it is very likely that one such quiescent Ug sequence was activated, perhaps through a demethylation process, and transposed into a normal miniature locus, causing the dominant mutation.

There has been genetic proof for this proposal. First, the original plant, 866248U, was proved to be homozygous for the Ug-reporting a-rug allele. Second, the Mn::Ug mutant, once isolated and reconstituted with a standard a-rug line, is able to elicit the same miniature spotting phenotype observed in the mutant ear 866248U/a-rug. Third, we have been able to isolate five putative revertants from 553 e-size (see sample kernel in Fig. 2) miniature spotted kernels. Among these five revertants, three are found to retain the Ug activity (Table

8).

Another interesting feature of the Mn::Ug mutant is that it acts dominantly over the wildtype locus. What the underlying molecular mechanism is for this dominance is not known. In maize, the dominant color-inhibitor C-I allele is shown to be caused by an 3.8 kb insertion (Paz-Ares et al., 1990). This insertion is located at the 3'end of the coding region of the C1 gene and causes a 370 bp deletion of the C1 sequence. As a result, the C-I protein is truncated and has an altered acidic domain. The C-I protein acts more likely as a transcriptional suppressor in being able to bind but not to activate due to the lack of its activator domain (U. Wienand, Max-Planck-Institut für Züchtungsforschung, Köln, Federal Republic Germany, personal communication) thereby providing its dominant function. However, before the Mn::Ug mutant is cloned and molecularly analyzed, we do not know if Mn::Ug acts in a similar way to that of the C-I allele.

Our observations on the Mn::Ug mutant indicate that this mutant affects several developmental processes of the maize plant. It is likely that the wild type miniature locus encodes for a regulatory protein or a kind of growth regulator that is necessary for the endosperm development as well as pollen germination and tube growth. One experimental approach to address this hypothesis is to study the effect of different

plant growth regulator substances on a supplement *in vitro* germination test of the *Mn::Ug* pollen.

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SUMMARY AND DISCUSSION

The Ug-rug transposable element system (Friedemann and Peterson, 1982) is one of the nine known two-element systems (for review, see Peterson, 1987). It consists of the Ug regulator (or autonomous) elements and rug receptor (or nonautonomous) elements. Although none of the Ug elements has been found to be physically associated with a cloned maize locus, the location of the rug receptor elements has been identified to be at two maize anthocyanin genes, i.e., A1 (the a-rug reporter allele) (Friedemann and Peterson, 1982; Sprague, 1986) and C1 (the c-rug31, c-rug65, c-rug66, c-rug67 reporter alleles) (Caldwell and Peterson, 1989).

The Ug regulator elements show a positive dosage effect (Friedemann and Peterson, 1982; Pan and Peterson, 1990). Two or more than two copies of Ug1 elicit a coarse-high spotting pattern on a-rug, whereas one copy of Ug1 gives a fine-low spotting (Friedemann and Peterson, 1982). These Ug elements are heterogenous (Pereira and Peterson, 1985; Pan and Peterson, 1990) and thus far, the most pervasive transposable elements in maize (for review, see Peterson, 1987).

The Ug transposable elements are distributed in maize in either active or quiescent forms. Although active Ug elements can be reported directly by either the a-rug or the c-rug alleles (Cormack et al., 1988; Peterson and Friedemann, 1983;

Pereira and Peterson, 1985), quiescent elements can be detected only upon their infrequent random activation (Pan and Peterson, 1988; 1989a; 1990).

This study addresses the question of the activation, either spontaneous or induced, of such quiescent Ug transposable elements in maize breeding lines. The study was initiated by testing the content of active Ug elements in four maize inbred lines (B70, C103, C123 and 187-2). Crosses were made between the four inbred lines and the standard a-rug tester according to the scheme described by Peterson and Friedemann (1983) (Fig. 1 in section I). These crosses did not uncover a genetically active Ug element in all four lines tested. Activation of quiescent Ug elements, however, was quite obvious in that less than 0.1% of the BC_1 progenies exhibited single sectors of color spots.

This activation has been found to be consistent in continuous backcrossing of three types of BC_1 siblings (colorless, few-spot, and sectored) with the a-rug tester. Several significant findings have been discovered in the present study including:

i) The discovery of quiescent Ug element sequences in genomes of maize inbred lines originally lacking Ug activity. Activation of these quiescent Ug sequences has been detected phenotypically either as sectors of Ug-a-rug (or Ug-c-rug, Y.-B. Pan, Department of Genetics, Iowa State University,

personal observation) spotting (somatic activation) in a colorless aleurone or as exceptional fully Uq-a-ruq spotted (germinal activation) aleurone;

ii) the isolation and genetic characterization of five spontaneously germinally activated new Uq elements, namely, Uq2, Uq3, Uq4, Uq5 and Uq6. Each new Uq element elicits a unique, and heavier a-ruq spotting phenotype. In addition, the interactions between the regulatory elements, Uq3, Uq4, Uq5, or Uq6 and the c-ruq65 or c-ruq67 reporter alleles are weaker in terms of their low spotting patterns and lower-than-expected spotting frequencies;

iii) the finding that all five newly activated germinal Uq elements are independent of the original standard Uq (or Uq1);

iv) the finding that Uq2 is allelic to Uq4, Uq3 is allelic to Uq5, and Uq6 is linked to either allelic pair of new Uq elements;

v) the isolation and genetic characterization of a dominant miniature mutant, Mn::Uq, from a 5-aza-2'-deoxycytidine treated plant containing only quiescent Uq sequences; and finally,

vi) the finding that this Mn::Uq is independent of mn1, mn2, and mn7690, three different recessive miniature genes. The Mn::Uq transactivates a-ruq but neither c-ruq65 nor c-ruq67, suggesting that these ruq receptors elements at A1 and C1 may differ structurally in terms of their size or

nucleotide sequences.

The results from these experiments are in good agreement with the previous studies (Doerschug, 1973; Fedoroff, 1989; McClintock, 1958, 1964, 1965; Peterson, 1966; Schnable and Peterson, 1986). Both active and inactive forms of maize transposable elements are known to exist in the maize genome. Phase variation (McClintock, 1965; Peterson, 1966) or cycle events of activation and inactivation have been reported in three maize transposable elements, namely En/Spm (Fedoroff, 1989; McClintock, 1958; Peterson, 1966), Ac (McClintock, 1964, 1965), and Dt (Doerschug, 1973). Both internal and external environmental factors, also known as "genomic stress or shock" (McClintock, 1984) factors, have been found to stimulate the activation of the maize transposable elements. These factors include chromosome breakage, tissue culture, physical and chemical mutagens, viral infection, and demethylation.

There is molecular evidence indicating that an active transposable element can turn into inactivity by methylating the cytosine residues in its DNA sequences (Banks et al., 1988; Chandler and Walbot, 1986; Dellaporta and Chomet, 1985; Schwartz and Dennis, 1986). One particular example is the reversible changes between an active phase and an inactive phase of an inserted Ac element at wx-m7 and their effects on the a-m3 allele that has a Ds inserted at the A locus (McClintock 1964, 1965). According to several molecular

studies, no differences were found between active and inactive Ac elements at wx-m7 in terms of both size and position of insertion (Dellaporta and Chomet, 1985). But a shift from an active phase to an inactive phase was found to be associated with modification of the Ac DNA sequences through cytosine methylation (Dellaporta and Chomet, 1985). DNA methylation is also correlated with loss of activity of another maize transposable element, the Robertson's Mutator or Mu, in a number of Mu-loss lines (Chandler and Walbot, 1986).

The same mechanism may have played a role in maintaining quiescent Ug sequences in maize inbred lines. Upon crossing with the a-rug tester line, certain components from genomes of the maize inbred lines may enhance such a demethylation process by acting alone or by interacting with other components from the a-rug tester.

Recently, the rug receptor elements at both the standard a-rug and the four c-rug reporter alleles have been cloned and sequenced (Antionio G. Pisabarro, Max-Planck-Institut für Züchtungsforschung, Köln, Federal Republic Germany, personal communication). The rug receptor element, cloned from the standard a-rug, is different from the rug receptor elements cloned from the four c-rug alleles.

The question now is whether these cloned rug elements can be used to clone the Ug regulatory elements. The answer will depend on if there is sequence homology between the regulatory

Ug and the rug receptor elements. If these rug elements represent various deletion derivatives of the Ug regulatory elements, they certainly can be used as probes to clone the Ug regulatory elements by the cosegregation approach. Once the Ug elements are cloned, questions such as whether activated new Ug elements (Ug2, Ug3, Ug4, Ug5, Ug6, and Mn::Ug) are undermethylated versions of their quiescent, genetically inactive copies can be addressed. It also might be possible to explore what the components or factors are that enhance the demethylation process.

In addition, the molecular basis of the heterogeneity of the Ug transposable elements can also be addressed. These various Ug elements, either naturally residing (Caldwell and Peterson, 1989; Cormack et al., 1988; Pereira and Peterson, 1985; Peterson, 1987; Sprague, 1987) or germinally activated (Pan and Peterson, 1989a, 1989b, 1990), elicit distinctive spotting phenotypes on the same a-rug reporter allele. Because the a-rug "substrate" for Ug transposase function is constant, the variable spotting phenotypes, therefore, reflect the differences in the transposase functions of the different Ug elements. In turn, differences in the transposase function can be due either to their structural differences (the composition hypothesis, McClintock, 1950, 1958), or to their different chromosomal locations (the position hypothesis, Peterson, 1976).

However, if the *rug* receptors are not deletion derivatives of their *Ug* elements, these approaches would not be practical. As a matter of fact, there are few cases where receptor elements are not sequence related to their functional regulator elements, one particular example is the *Ds1* receptor element of *Ac*, which shares no sequence homology except the 11 base pair terminal inverted repeats (Pohlman et al., 1984; Sutton et al., 1984). If this is also the case for *rug* and *Ug*, a different strategy must be adopted, which usually involves tagging of a clond maize gene with one *Ug* transposable element. This has been tried with the *Ug1* element, but thus far no success has been made in recovering a *Ug1* tagged maize mutant (Peter A. Peterson, Department of Agronomy, Iowa State University, personal information). This tagging process may be more successful if the new *Ug* elements are used.

There is additional approach for *Ug* cloning that involves the *Mn::Ug* dominant mutant. There has been strong genetic evidence from the present study indicating the physical association of this *Ug* element with the maize miniature locus. There are several reasons for this optimism. The phenotypic effect is on both endosperm development and pollen germination processes in the heterozygous *Mn::Ug* mutant, and there are several putative revertants of *Mn::Ug* available. These attributes make it

possible to directly clone this miniature locus and its Uq insertion by following the "subtraction library" approach (Sommer et al., 1990).

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