CHANGE IN STATE FOLLOWING TRANSPosition OF A
REGULATORY ELEMENT OF THE ENHANCER
SYSTEM IN MAIZE¹

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ABSTRACT

From an original A2 allele (colored aleurone), a mutable allele, a2-m-4 1629, that changes from a2 to A2 is described. Mutability is expressed as a very distinct pattern limited to the last cell division.—The mutability of a2-m-4 1629 is autonomously controlled by an En at the a2 locus. This En, inactive on standard a testers for En, is partially active on a2-m-1, an a2 tester for En, and expresses varied levels of activity from limited to nearly full suppression of the a2-m-1 color phenotype.—When the En of the a2-m-4 1629 allele transposes from the a2 locus, it behaves, at the new position, like a standard En in triggering a2-m-1, a-m-1 and a-m(r), which express colored spots on a colorless background. The activity of En is therefore different following the change in chromosome location. This finding supports the “position” hypothesis that has been proposed to explain diverse patterns observed among controlling elements. In this case mutation is related to the terminal cell state and not to tissue differences as shown with some phase-variation regulatory elements.

CONTROLLING elements refer to transposable elements in maize that affect gene expression (McClintock 1951). In this report, a distinction is made between two types of controlling elements: a receptive element, that is located at the locus under consideration, and a second element that triggers the receptive element. The former is identified as a receptor element and functions to block partially or completely the expression of a locus under control. The second element regulates the expression of the controlled allele by triggering a mutation event that leads to altered gene expression; this is identified as a regulatory element. Mutability leading to the expression of diverse patterns is dependent on the response of a receptor element at the locus to a separable or an autonomously located regulatory element (Ac, Spm-En, or Dt), which triggers mutations (a→A). The resultant patterns are heritable.

Controlling element systems in maize can be characterized in a number of ways (Peterson 1970a; Fincham and Sastry 1974). A significant and persistent feature is the presence of numerous mutability patterns (McClintock 1964, 1965, 1967, 1968; Peterson 1961); these describe the state of the locus

FIGURE 1.—An array of pattern types observed among controlling-element systems. (Large spots indicate early occurring mutation events and small spots indicate late occurring events.)

(A.) An ear culture of a-m(pu-pu) from the cross a-m(pu-pu)/a sh X a sh/a sh showing pale and colorless germinal mutants. (B.) An ear culture of a-m from a-m Sh/a sh X a sh/a sh showing a uniform, late pattern; germinal mutations to colorless and colored kernels are evident. (C.) En-flow expression from the cross a-m(r)/a sh, En-flow X a sh/a sh. D, E, and F. Individual a-m kernels showing different frequencies of mutation events.
and are dependent on the timing of the mutation event and the frequency of the event. Various combinations of early or late timing and low or high frequencies result in a wide array of pattern types. Examples of these are illustrated in Figure 1. Derivative, changed patterns can subsequently arise (Figure 2).

In her initial studies with the Ac-Ds system, McClintock (1951) described in detail varied patterns at the loci examined for controlling element activity. Similar pattern expressions have been identified in several other systems in maize controlled by Dt (Nuffer 1961; Doerschug 1973) or Spm-En (McClintock 1964, 1967; Peterson 1960, 1961, 1967). Distinctive patterns occur with different dosages of a controlling element (McClintock 1951; Brink and Nilan 1952; Greenblatt and Brink 1962; Greenblatt 1966; Kedharnath and Brink 1958).

In plants other than maize, similar states of mutability are frequently observed. In Antirrhinum, pattern types that show widely different spot frequencies have been described in the pal series (Fincham and Harrison 1967; Harrison and Fincham 1964, 1968) and the nivea series (Harrison and Carpenter 1973). Some, such as a low-frequency mutation line of mutable pal, respond with a greatly enhanced mutation rate under low-temperature con-

![Figure 2](image_url)

**Figure 2.**—A and B. Individual a2-m/a2 bt kernels illustrating sectors showing the change from a low to a high rate of mutability.
ditions. Similar patterns have been described in Nicotiana by Deshayes (1973) and Sand (1975).

The extremes in pattern expression observed in maize-controlling elements result from differential timing of mutation in the ontogenetic development of the endosperm. When only a few cells are present, as in the early developing endosperm, mutation events result in large sectorials (Figure 1A). When mutations occur late in ontogeny, the mutant sectors will be smaller in size (Figures 1B, D). The most extreme expression occurs when mutability is limited to the products of the last cell division; one-celled spots (Figure 3 and 4) appear, as with a2-m-4 1629, an independently originated mutable allele, the subject of this report. A preliminary note on a2-m-4 1629 has appeared (Peterson 1973).

**Figure 3.**—An individual a2-m-4 1629 kernel: genotype a2-m-4 1629/a2 bt showing 1-celled spots.

**Figure 4.**—Close up view of spots in the aleurone cells of an a2-m-4 1629/a2 bt kernel showing 1-celled spots.
MATERIALS AND METHODS

The mutable allele, \(a2\-m\-4\) 1629

The mutable allele, \(a2\-m\-4\) 1629, arose in 1963 in an isolation plot at one of 7 confirmed and tested mutable alleles in a sampling of approximately 1.6 million gametes. Isolation plot procedures utilized are as described for the isolation of \(a2\-m\-1\) 1511 (Peterson 1963, 1968, 1976; Fowler and Peterson 1974). (The \(a2\) allele is a colorless allele located at the \(A2\) locus on chromosome 5 and is one of 4 major genes responsible for anthocyanin coloration). The \(a2\-m\-4\) 1629 allele originated as an individual kernel on a plant that was \(AA\ A2\ A2\ En\) (Peterson 1975). (\(A\) is a gene determining anthocyanin coloration; \(En\) is a regulatory element present in this source material which triggers receptive alleles such as \(a\-m(r)\) (a colorless \(a\) allele, that responds \((r)\) to \(En\); with \(En\) it gives purple colored sectors on a colorless background in the aleurone tissue of the kernel).

Assay of spot sizes

The count of spots included 20 kernels of the \(a2\-m\-4\) 1629 phenotype (Figure 3) from 24 ear progenies (13A:11B, Table 1) of the cross \(a2\-m\-4\) 1629 \(Bt\) \(a2\ bt\) (\(Bt\)-round; \(bt\)-brittle, non-round, shrunken kernels). Individual kernels were placed on a clay mount with the embryo side of the kernel facing the examiner. With a scaled grid in the eyepiece at 20\(\times\) magnification, a 2mm\(^2\) area of the crown of the kernel was surveyed in each sampling. At this magnification, the individual cells of the aleurone could be clearly distinguished.

All the spots within the designated section of the grid were included in the count. The grid was divided into 4 sections. Spots in the aleurone layer of the crown in the upper left and lower right parts of the grid were alternatively counted. Thus, for each kernel one of these two areas or \(\frac{1}{4}\) of the grid-marked portion of the crown area was counted. The sampling of alternate areas presumably ensured the recording of a representative sample of spots on the crown. The size of individual spots of color arising from the mutation of \(a2\) to \(A2\) was also determined. Spots of color include dark aleurone cells in the center of a spot surrounded by lighter colored cells, the latter showing the interaction and flow of anthocyanin-producing materials into the surrounding cells. Spot size, however, is determined by the number of dark-colored cells only.

Establishment of \(a2\-m\-4\) 1629 in the \(En\) controlling element system

When a new mutable allele arises, crosses are made to determine the relationship of the newly arisen allele to a controlling-element system (Peterson 1961, 1965). Either \(a\-m(r)\) or \(a\-m-1\) (Table 3) is used to test a possible relationship to the \(En\) system \([(\text{relevant genotype: } a\-m(r) \text{ or } a\-m-1, A2 A2)]\). The allele \(a\-m(r)\) is colorless in the absence of \(En\) but shows spots of color on a colorless background in the presence of \(En\). The cross and the progeny expected in the

![Figure 5.—A sector of an ear culture illustrating the progeny of \(a2\-m\-1/a2\-m\-4\) 1629 \(\times\) \(a2\ bt/\ a2\ bt\); the dark kernels are \(a2\ m\ 1\).
case of a positive or a negative relationship are shown in Table 3A. If a2-m-4 1629 mutability is related to the En system (PETERSON 1965), all mutable-a selections (Table 3, A1) would be expected to yield a2-m-4 1629 in a testcross of the F1 progeny while colorless or pale selections would not. On the other hand, if mutability is not associated with the En system, no such relation will be found.

The a2-m-1 tester for En

This tester is similar to a-m-1. Both a2-m-1 and a-m-1 express anthocyanin coloration in the aleurone in the absence of En (Figure 5). In the presence of En coloration is suppressed and a colorless background results. When a mutation event takes place spots of anthocyanin coloration appear. The a2-m-1 allele expresses much stronger coloration than the a-m-1 (pale) used in the original En relationship test (PETERSON 1965).

RESULTS

Limitation of mutation events to a specific stage in the development of the aleurone

Timing of a2-m-4 1629 mutability in the aleurone layer of the kernel results in nonrandomness in the distribution of classes of different spot sizes. Distribution of mutant sector size (Table 1) is not consistent with a distribution based on random events. If mutations occurred randomly in the developing aleurone, there would be a relationship in the distribution of the different sized classes of spots and these would occur in predictable ratios. The spots that originated from mutations which arose one cell generation before the terminal division will be 2-celled, those which arose two generations before will be 4-celled, three generations before, 8-celled and so on. Odd-numbered-celled classes (3, 5, 7) may indicate that some cells stop dividing before the end of aleurone development. [A more detailed description of expectations to account for frequencies of odd-numbered cell types and for non-uniformity in the cell-division process will be presented in another report—PETERSON, NOURI and SERSLAND. (Briefly, the

<table>
<thead>
<tr>
<th>Number of mutant cells</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5 or more</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obs</td>
<td>5321</td>
<td>317</td>
<td>77</td>
<td>69</td>
<td>56</td>
</tr>
<tr>
<td>Exp</td>
<td>2660</td>
<td>1330</td>
<td>665</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Exp: expected number based on 1* and theoretical expectation (discussed in text); Obs: observed number.

* Total number of one-celled spots from 24 ear progenies (13A, 11B).
† This group includes spot sizes greater than 5. The frequency of A (.009) and B (.013) is such that it is not significant to the illustration of synchrony.
‡ A and B represent two populations that will be considered in detail in another report (PETERSON, NOURI and SERSLAND, unpublished).
relationship in the distribution of the different sized classes of spots, \( f(2) = (.5)f(1)f(3) = (.5)(.5)f(1) \), etc. and in general \( f(i) = \frac{1}{2^{i-1}} f(i) \) where \( i = 2,3,4 \) cell numbers per mutant spot and \( f(i) \) denotes the frequency of the class with \( i \) mutant cells.]

It is evident that the \( a2-m-4 \) allele exhibits a very distinct synchrony in mutation, one that is mainly limited to the product of the last cell division.

**Control of mutability of \( a2-m-4 \) and its relation to the \( En \) system**

Mutability of the \( a2-m-4 \) allele resides at the \( a2 \) locus and is therefore autonomously controlled. This is demonstrated by outcrossing the allele to a colored tester and subsequently backcrossing the \( F_1 \) progeny as in the following series of crosses: \( A2/A2 \times a2-m-4/a2-m-4 \) gives rise to colored kernels (\( A2/a2-m-4 \)), backcrosses of these by \( a2/a2 \) give rise to the progenies shown in Table 2A. The appearance of \( a2-m-4 \) mutability among the noncolored class, as well as the low and irregular frequencies of the colorless kernels among the progeny, indicates autonomous control of mutability (Peterson 1960). (An alternative consideration that the frequencies of the colorless kernels could be accounted for by the segregation of several \( En \) cannot be substantiated because of the absence of \( En \) among the required number of sib-colored kernels of the ear progenies listed in Table 2A.) Exceptional colorless types are the result of changes from \( a2-m \) because some of the tested exceptions carry \( En \). Further, from the \( A2/a2-m \) heterozygote, the derived colorless kernels must arise from \( a2-m \), since with independent tests of the \( A2 \) allele, colorless types are not found.

**TABLE 2**

_Samples of progenies from the cross \( A2/a2-m-4 \times a2/a2 \)_

<table>
<thead>
<tr>
<th>Progenies</th>
<th>Colored</th>
<th>( a2-m-4 ) 1629-type mutability</th>
<th>Colorless*</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>not</td>
<td>38</td>
<td>6</td>
<td>44</td>
</tr>
<tr>
<td>2</td>
<td>counted</td>
<td>68</td>
<td>1</td>
<td>69</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>90</td>
<td>18</td>
<td>108</td>
</tr>
</tbody>
</table>

* \( 4/25 \) shown to possess \( En \) in crosses with \( a2-m(r) \), an \( En \) tester.

_B. Progenies from the cross of \( a2-m-4 1629/a2-m(r) \times a2/a2 \)_

<table>
<thead>
<tr>
<th>Progenies</th>
<th>( a2-m-4 ) 1629-type mutability</th>
<th>Colorless*</th>
<th>Coarse</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>166</td>
<td>188</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>186</td>
<td>182</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>160</td>
<td>147</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>149</td>
<td>124</td>
<td>6</td>
</tr>
</tbody>
</table>

* Some of the colorless may be derived from \( a2-m-4 1629 \) changes as seen in Table 2A.
Another means of demonstrating the autonomy of the \(a2-m-4 1629\) allele is by \(a2-m(r)\) [an \(a2\) allele that responds to \(En\) although the \(a2-m(r)\) allele does not respond to the factor controlling \(a2-m-4 1629\) mutability] as shown in the following series of crosses: \(a2-m-4 1629/a2-m-4 1629 \times a2-m(r)/a2-m(r)\); select the \(a2-m-4 1629\) phenotype [expected \(a2-m-4 1629/a2-m(r)\)]; the heterozygote, \(a2-m-4 1629/a2-m(r)\), is backcrossed by \(a2/a2\) and these progeny are shown in Table 2B. The near equality in distribution of the \(a2-m-4 1629\) phenotype and colorless types among the progeny is a demonstration of the autonomous control of \(a2-m-4 1629\) mutability (Table 2B). Some of the colorless types may be derived from changes of \(a2-m-4 1629\).

Some coarse-mutable types arise among the progeny of the crosses listed in Table 2B. These were tested and subsequently shown to possess an independent \(En\) [the pattern is that of an \(En\) on \(a2-m(r)\)]. Similarly, four of the colorless types listed in Table 2A from the cross \(A2/a2-m-4 1629 \times a2/a2\) also contain \(En\). The \(En\) in this instance is also shown to segregate independently. (These colorless types appear to be nonresponsive to \(En\) that arose from the \(a2-m-4 1629\) allele).

The frequency of their occurrence suggests (Peterson 1970b) that these independent \(En\) are newly arisen from the parental \(a2-m-4 1629\) source (\(A2/a2-m-4 1629\)). This, coupled with evidence to be presented on the relationship of \(a2-m-4 1629\) to \(En\), suggests that the independent \(En\) arose from \(a2-m-4 1629\). Interestingly, the \(En\) arising in the \(a2-m-4 1629\) stocks generates a coarse pattern with \(a2-m(r)\) yet does not trigger discernible mutability of \(a2-m(r)\) in its autonomous position, i.e. when in \(cis\) with \(A2\) based on the \(a2-m-4 1629\) phenotype from the heterozygote \(a2-m-4 1629/a2-m(r)\).

The identity of \(En\) at \(a2-m-4 1629\) will be taken up in consideration of Table 4. Additional evidence for the change in \(En\) activity from transpositions from the \(a2-m-4 1629\) allele comes from the isolation of independent \(En\) among sib \(a2\) \(bt/a2\) \(bt\) kernels from the cross \(a2-m-4 1629/a2\) \(bt \times a2\) \(bt/a2\) \(bt\). These kernels also generate a coarse pattern in crosses with \(a2-m(r)\). Further, the possibility that coarse types arise from changes in state of the \(a2-m-4 1629\) alleles is excluded since state changes to coarse or to other forms (other than to colorless) have not been observed among numerous testcrosses of the \(a2-m-4 1629\) allele.

In order to establish whether \(a2-m-4 1629\) is part of the \(En\) controlling element system, the \(a2-m-4 1629\) allele was crossed with \(a-m(r)/a-m-1\) a tester for \(En\). In the testcross of the selections from the progeny arising from the backcross of the \(F_1\), the \(a2-m-4 1629\) allele appears among colorless and pale \(a\) selections and in addition the null \(a2\) allele appears among the mutables (Table 3B). Lack of correlation between \(a\) mutability and \(a2-m-4 1629\) suggests that the promoter of mutability associated with \(a2-m-4 1629\) will not trigger the standard \(a\) tester alleles for \(En\) [\(a-m(r)\) or \(a-m-1\)] although, as will be shown later, \(En\)-like activity can be located at the \(a2-m-4 1629\) allele.

In view of this result, another test was made with the \(a2-m-1\) tester for \(En\). In crosses of the \(a2-m-4 1629\) allele to \(a2-m-1\), (\(a2-m-4 1629\ Bt/a2\) \(bt \times a2-m-1/ a2-m-1\)), the progeny included kernels of full color (Figure 5), those of lighter
### TABLE 3

*Expected and observed phenotypes and genotypes of crosses and progeny showing a relationship or nonrelationship of a newly arisen mutable allele to the En system*

The cross:  
\[ a-m(r)/a-m(r) \times A/A \]
\[ A2 Bt/A2 Bt \times a2-m-4 1629 Bt/a2 bt \]  
(colorless, En tester) \( \sim \) (a2 mutable)

\[ F_1 \]
\[ A/a-m(r) \times A2 Bt/a2-m-4 1629 Bt \times A2 Bt/A2 Bt \]
\[ \sim \] (pale, En tester)
\[ (colored) \]

\( a \) progeny test crossed by \( A/A \) a2 bt/a2 bt (see B)

<table>
<thead>
<tr>
<th>A. Expected</th>
<th>Expected a2 progeny*</th>
</tr>
</thead>
<tbody>
<tr>
<td>a selections</td>
<td>If related to ( En )</td>
</tr>
<tr>
<td></td>
<td>( a2-m )</td>
</tr>
<tr>
<td>1. Mutable</td>
<td>all</td>
</tr>
<tr>
<td>2. Colorless or pale no dots†</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Observed</th>
<th>Presence of ( En )</th>
<th>Observed a2 genotypes ($)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a selections</td>
<td></td>
<td>( A2/a2-m ) Bt</td>
</tr>
<tr>
<td>Mutant a-( m(r)/a-m(r) )</td>
<td>+</td>
<td>30</td>
</tr>
<tr>
<td>or a-( m(r)/a-m-1 )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pale a-( m(r)/a-m-1 )</td>
<td>-</td>
<td>61</td>
</tr>
<tr>
<td>Colorless a-( m(r)/a-m(r) )</td>
<td>-</td>
<td>37</td>
</tr>
</tbody>
</table>

(Phenotypes of parents, \( F_1 \) and tester in parenthesis) (See footnotes †,‡,§ for explanation of phenotypes).

* Reference only to \( a2-m-4 1629 \).
† Indicates a null, colorless allele.
‡ Explanation of genotypes and phenotypic responses.
\( a-m(r) \)—a colorless aleurone allele that responds to \( En \) by showing spots of color on a colorless background.
\( a-m-1 \)—a pale aleurone colored allele which is pale-colored and nonvariegated in the absence of \( Spm \) or \( En \) and variegated in the presence of \( Spm \) or \( En \) (colored spots on a colorless background); represents a tester for the presence of \( Spm \) or \( En \).
\( a-m(r)/a-m-1 \) = pale phenotype.
§ The \( A2/A2 \) not indicated since they provide no information: decision on genotypes based on the following kernel phenotypes and associated genotype: colored-\( A2 \), round mutable-\( a2-m \) Bt, non-round colorless, \( a2 bt \), non-round colorless \( a2-m \) Bt and round colorless, \( a2 bt \).
∥ Either \( a2 bt \) or \( A2 Bt \) depending on the \( F_2 \) that was crossed.

colored pale background with spots of color (Figure 6Aa,B,C) and colorless kernels with spots of color (Table 4, line 7). The class with pale background varied from uniform pale to scattered pale coloration in the aleurone. Each of these phenotypes was selected and subsequently testcrossed by \( a2 bt/a2 bt \) to determine the genotype. In most instances (23 of 30) (Table 4), the kernels that were light pale with spots of color (Figure 6) were confirmed to be \( a2-m-1/ a2-m-4 1629 \). Most of the kernels (31 of 39) designated no spots-dark color, were confirmed to be \( a2-m-1/a2 bt \). These results support the conclusion that \( a2-m-4 1629 \) can partly, although not completely, suppress the \( a2-m-1 \) allele and therefore acts like a weak \( En \). In association with the \( a2-m-4 1629 \) allele it does not have the complete suppressive potency of a standard \( En \). Dilute coloration on the
TABLE 4

Tests for En: Progeny from the cross:

<table>
<thead>
<tr>
<th>Phenotypes selected</th>
<th>Genotypes determined in crosses × a2 bt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a2-m-1/a2-m+</td>
</tr>
<tr>
<td>1. Very fine spots-pale bkgd†</td>
<td>5</td>
</tr>
<tr>
<td>2. No spots-dark color</td>
<td>1</td>
</tr>
<tr>
<td>3. Very fine spots-pale bkgd</td>
<td>6</td>
</tr>
<tr>
<td>4. No spots-dark color</td>
<td>0</td>
</tr>
<tr>
<td>5. Very fine spots-pale bkgd</td>
<td>8</td>
</tr>
<tr>
<td>6. No spots-dark color</td>
<td>0</td>
</tr>
<tr>
<td>7. Very fine spots-cl bkgd</td>
<td>4</td>
</tr>
<tr>
<td>8. Very fine spots-pale bkgd</td>
<td>4</td>
</tr>
<tr>
<td>9. No spots-dark color</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>30</strong></td>
</tr>
</tbody>
</table>

* indicates the a2-m-4 1629 allele.
† (bkgd = background; cl = colorless; fine = late 1-celled spots of color).

Where not indicated, Bt is the designated allele at the Bt locus.

Determination of individual genotypes: a2-m-1 = colored, round; a2-m = mutable, round; a2 bt colorless, nonround (shrunken); a2-m(nr) = colorless, round.

kernels (Figure 6) is an indication of incomplete expression of the En of a2-m-4 1629. The type of coloration illustrated is an example of the variable potency of the a2-m-4 1629 En, which is probably modified by the physiological state of the endosperm. Assignment of such differences due to genetic background are excluded in view of the uniformity of this genetic material.

There are exceptions. In one ear progeny, four of the selections had very fine spots with a colorless background and these four cases were confirmed to be a2-m-4 1629/a2-m-1 (Table 4, line 7) and represent full En standard suppression. In some instances, partial suppression was not evident since dark-colored selections were confirmed as a2-m-4 1629/a2-m-1 [Table 4, lines 2 (1 of 9) and 9 (2 of 11)]. This indicates lack of the expression of suppression ability of a2-m-4 1629 among these three selections. The presence of a2-m-1/a2-m(nr) among the progeny shows the propensity of the a2-m-4 1629 to mutate to the colorless, non-responding a2-m(nr) types (nr=nonresponsive to En) (lines 1, 3, 4, 6, 9, Table 4). This is a very frequent change [9/39(9+30)]. An independent test shows approximately 11% changed (Table 2). Colorless types also appear in other tests among the homozygotes, a2-m-4 1629/a2-m-4 1629.

These results show that the a2-m-1 allele (a tester for En) is affected by a2-m-4 1629, but not as strongly as it is by a standard En, and provide support for the hypothesis that a2-m-4 1629 is an En-related mutable allele.
FIGURE 6.—Partial suppression of the \(a_{2-m-1}\) allele by \(a_{2-m-4}\) 1629: (A) and (B) Individual kernels of \(a_{2-m-4}\) 1629/\(a_{2-m-1}\). (C) An ear culture from the cross \(a_{2-m-4}\) 1629/\(a_{2\,bt}\) \(\times\) \(a_{2-m-1}\); the lighter colored kernels are \(a_{2-m-4}\) 1629/\(a_{2-m-1}\) and the dark kernels are \(a_{2-m-1}/a_{2\,bt}\).

DISCUSSION

In the maize two-unit mutable systems, activity of regulatory elements (such as \(En\)) trigger changes in receptor elements (such as \(I\), (PETERSON 1961, 1965) that result in mutations manifest as spots of color in aleurone tissue or stripes in leaves. Interaction of the elements takes place at a "controlled" allele (in this
case, $a2-m-4\ 1629$) and, in situations where it can be accurately documented as with $Mp$ in mutable pericarp (Brink 1954; Brink and Nilan 1952; Greenblatt 1974), the dominant phenotype is associated with the removal of the inhibitory element from the controlled locus. (In the case of $P-rr$, the dominant phenotype, red pericarp, results from the removal of $Mp$ from $P-Mp$.) The receptor element, $I$, is considered to be located within the controlled allele, as has been demonstrated for other systems at $wx$-controlled alleles (Nelson 1968). The regulatory element may be situated at the controlled allele, as with $a2-m-4\ 1629$, and therefore considered autonomous (Peterson 1960, 1970), or it may be inherited independently. $En$ triggers the mutation event; the timing of the event, however, maybe dependent on both the receptor element, $I$, and the regulatory element, $En$. Changes expressed as pattern types (Mitchellock 1951, 1965; Nuffer 1961; Peterson 1961, 1966) are a consequence of the interaction of these two elements.

The $a2-m-4\ 1629$ allele expresses a unique, synchronous pattern. The precise timing evident with $a2-m-4\ 1629$ is a result of interplay between the regulatory element, receptor element, and the physiological state of the cell. As has been demonstrated, mutation of $a2-m-4\ 1629$ is generally restricted to the cell products of the ultimate division of individual aleurone cells. This is unlike $En$-crown and $En$-flow (Figure 1C), where specific areas of the aleurone (namely, the central portion of the crown and the basal portion of the kernel, respectively) are sites of $En$ activation (Peterson 1966). Thus, $En$ activation of both $En$-flow and $En$-crown is triggered by the state of the tissue. The $a2-m-4\ 1629$ mutation, however, is a consequence of the state of the individual cell and not limited in its expression to different parts of the aleurone as in $En$-flow and $En$-crown. In view of the homogeneity of the dispersion of the different celled classes among a wide array of crosses it can be stated that the background genotype does not appear to cause a discernible amount of variation with respect to the expression of $a2-m-4\ 1629$. The control of mutability of $a2-m-4\ 1629$ must reside with the mutable allele itself in response to the cellular environment.

There is another difference between these allelic states. $En$-flow and $En$-crown expression are strictly $En$ activated (Peterson 1965). This cannot be demonstrated for $a2-m-4\ 1629$ and the lateness of the timing event for this allele suggests that the receptor element, $I$, is responsible (or alternatively, the position of $En$ at the $a2$ locus results in such an effect.) There are two sources of supporting evidence for this. First, $a2-m-4\ 1629$ does not respond to the introduction of other $En$. Only very late types of spotting appear irrespective of the introduced $En$. (Peterson unpublished.)

Secondly, derivative types of $En$, which arise from $a2-m-4\ 1629$ ear progenies show a striking change in activity following transposition of $En$ from the $A2$ locus. The $En$ element of $a2-m-4\ 1629$ affects the adjacent $A2$ in cis but not the $A$ in trans in the $En$ testers $a-m-1$ and $a-m(r)$. There is an observable effect in trans position on the $a2-m-1$ tester (Figure 7). For $a2-m-4\ 1629$, the $En$ effect at the $A2$ locus results in late synchronized timing. When the $En$ element transposes from $A2$, the $En$ acquires full trans activity on $En$-responsive alleles.
The effect of an En, derived from a \( a2-m-4\) 1629 transposition, on \( a2-m(r)\); the mutability shows a random timing when compared to the uniformity of the \( a2-m-4\) 1629 allele (Figure 3).

This change in the pattern expression generated by En that accompanies the transposition of En from the \( A2\) locus to another position supports the "position" hypothesis (Peterson, unpublished) that has been proposed to explain the diverse patterns observed among controlling elements (Figure 1,A to F).

The \( a2-m-4\) 1629 expression does not seem to be related to tissue synchrony of the mutation event based on uniform physiology of the tissue, which is the case in phase variation (Peterson 1966). With \( a2-m-4\) 1629, the stimulant for the mutation event is the individual "cell state" after the last cell division. It is to be expected that cells about to discontinue dividing would be in a physiological state different from cells continuously and actively dividing. It is interesting that the individual mutation event is triggered uniformly throughout all regions of the crown of the kernel. Controlling elements, in this instance, may be exposing a "terminal cell state".

It has been hypothesized that the event associated with mutation induction of \( a2-m-4\) 1629 is related to the terminal cell state. Enzyme synthesis is not continuous throughout the cell cycle and some genes are transcribable only at certain times in the cell cycle (Mitchison 1969; Robbins and Sharff 1966). Certain substances, especially those produced in the G_2 period (Cummins and Rusch 1968) are responsible for a continuation of the mitotic cycle. Other activities leading to the G_0 period cause triggering of events and result from activation or inactivation of portions of the genome which finally appear as qualitative or quantitative changes in protein synthesis (Cosgrove 1971). Cosgrove's studies with Trypanosomatids support the model proposed by Britten and Davidson (1969) which suggests that a battery of producer genes shut down protein synthesis for certain activities.

The triggering of events associated with mutable loci is also highly sensitive to temperature differences. Temperature effects are striking although not necessarily unidirectional; some mutability is enhanced at low temperatures (Harrison and Fincham 1964), and in some systems, the frequency of mutation events
is increased by higher temperature (Peterson 1958; Sand 1957, 1962). This would indicate that a variable set of processes influences the mutation-inducing event. Therefore, the cell state as well as its environment influences mutation induction.

LITERATURE CITED


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