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Table 1. The effectiveness of propylene oxide in a closed system using non-sterile Petri plates and PDA. Sterilization determined by the inhibition ratio.

Amount of propylene oxide	Time (Hours after exposure) 0	24	48	72
1.0 ml	.009	.470	1.030	1.040
2.0 ml	.009	.001	.010	.047
3.0 ml	.000	.000	.002	.016
4.0 ml	.000	.000	.000	.001
5.0 ml	.000	.000	.000	.000

Table 2. Percentage of 5 ml nutrient broth tubes showing growth after direct application of propylene oxide and incubated at refrigerator conditions, room conditions and 37°C.

Temperature condition	Amount of propylene oxide	Time (Hours of incubation) 24	48	72	96
Refrigerator	0.0 ml	13%	13%	100%	100%
	0.3	14	14	24	96
	0.4	14	14	22	69
Room conditions	0.0	98	100	100	100
	0.3	8	22	50	65
	0.4	12	16	25	50
37°C	0.0	100	100	100	100
	0.3	0	20	30	40
	0.4	3	10	10	10

sterility (Table 2). The apparent arithmatic growth increase as incubation time increased regardless of the amount of propylene oxide used was probably due either to the propylene oxide becoming inert or to a diffusion problem. The 90% sterility obtained with 0.4 ml of propylene oxide at 37°C would be attributed to the kinetic energy of propylene oxide at that temperature.

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Influence of Mutable Genes on Induction of Instability in Maize[1]

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Abstract. Mutable systems possess transposable elements that cause mutability of associated gene loci. The *En* system (composed of *I* and *En*) was used to test the induction of mutability of two selected loci, A_2 and *C* possessing the normal alleles. In a test of over five million gametes one mutable was found at each of the selected sites as well as two others, both dominant and non-allelic to the selected sites.

It appears that this low rate of induction of mutability (as compared to other reports) is a consequence of the non-randomness of site selection; i.e., a certain physical chromo-

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somal continuity abets the transposition of elements to selected sites.

McClintock (1,2) discovered certain genetic elements in maize that regulate the activity of genes and that also regularly change position in the genome without the occurrence of a chromosomal rearrangement. By means of this process, transposition, these elements are relocated to new sites where they in turn control the activity of the gene located at this new position. These transposition events are verified by the observation of simultaneous changes that include the location of the element at a new site and its absence at the old position (2, 3, 4). In addition, McClintock (5) has shown transposition of the *Ac-Ds* two-element system to the A_1 and A_2 loci, and her tests represent clear demonstrations of the induction of instability at previously normal loci.

The elements which are a part of the mutable system (6) to be discussed in this paper are the Enhancer (*En*) and the Inhibitor (*I*). These components of the *En* system have been found to control the mutability of two loci, a_1 and pg^m, and to transpose regularly. As previously reported (7), *I* becomes associated with the locus A_1 and inhibits its expression. (A_1 is one of the genes necessary for anthocyanin coloration in the aleurone layer of the kernal and in other plant parts; a_1 is the colorless allele.) Phenotypically, therefore, A_1I resembles the recessive colorless form a_1. In the presence of a second factor, *En*, which can be variously located, colored sectors appear on a colorless background, and these sectors represent a change in phenotype from a_1 to A_1. These changes to A_1 have been shown to be permanent and stable. In the absence of *En*, the A_1I condition remains stable.

Particular patterns of mutability are characterized by differences in time (manifest in size of mutant areas) and rate (manifest in number of mutant areas) of change (figure 1). These patterns represent differences in the mutable allele itself as recognized by the maintenance of a characteristic expression in successive outcrosses. In an attempt to evaluate the nature of the differences between pattern types and the maintenance of pattern integrity following transposition, tests were made with different patterns to uncover and analyze instability at new sites (A_2 and *C* loci which are on chromosomes 5 and 9, respectively). These loci affect aleurone pigmentation and changes at those loci are readily identifiable in large scale tests. This paper is a report of the induction of changes at the A_2 and *C* loci.

Materials and Methods

Various mutable pattern types heterozygous for $a_1{}^{dt}sh_2$ were

Figure 1. Two different pattern types of a_1 . Left, late in time, medium in frequency. Right, early, medium.

crossed to an anthocyanin color converted inbred W-22 line (containing all the dominant alleles for purple aleurone color). This allowed the procurement of seed with general uniformity, a necessary condition in isolation tests. A varied number of *En* were present in these mutable plants as well as B-chromosomes and an abnormal chromosome 10 (8) in some of the cultures. The genetic constitution of the plants being tested with respect to anthocyanin pigmentation and the sh_2 allele was $A_2A_2CCRRA_1Sh_2a_1{}^mSh_2$ or sh_2 and $A_2A_2CCRRA_1a_1{}^{at}sh_2sh_2$.

Tests for the origin of new instability at the a_2 and c loci were conducted over four years in two different isolation blocks with two female rows alternating with one pollen row. Each of the blocks was differentiated by the pollen parent that was used. In the a_2 block, $a_2a_2btbtyy$ was used as the pollinator whereas in the c block, $ccsh_1sh_1wxwxyy$ was used as the male. The closely linked markers in the pollinator parents helped to identify the original allele that gave rise to the new mutable.

In 1958, 1959 and 1960, each shoot was hand pollinated and bagged according to regular field nursery procedures. In 1961 control of pollination was obtained by the detasseling of female rows.

Each of the harvested ears was examined and exceptional types consisting of variegated kernels on otherwise all purple ears were saved. Over the four year period 1,848 exceptions were tested. By virtue of the pollen parent all a_2 and c changes could be discriminated. (Contamination could readily be dis-

cerned in subsequent tests because of the marked pollen parent that was used.) Many of them were somatic losses of A_1 that simulated the origin of a mutable type by uncovering the a_1^m. The use of sh_2 linked with a_1^m ($a_1^m sh_2$) helped to obviate this difficulty. Mottled types, representing changes at the *r* locus, could be readily discriminated. Many stable changes at the a_2 and *c* loci that were not mutable in the presence of *En* were discarded.

Results and Discussion

Four verified exceptions, independently originated, have been tested in an analysis of their activity relative to the *En* system. One, designated as $V^m mp$-1817, appeared as a coincident event with the isolation of an *r*-stable mutant. $V^m mp$ affects chlorophyll development in the mature plant and is expressed as a virescent with dark green stripes. It is dominant as revealed in the progeny of outcrosses to standard lines and various genetic testers and, in its presence, chlorophyll formation is inhibited after 4-6 weeks. (Thus, the symbol $V^m mp$ for the designation of a virescent-mutable, mature plant which was found in culture 1817.) Mutability in this case is recognized by the appearance of green stripes in a virescent-type background. This lack of complete greening of the background is the result of an inhibitory type of action and the appearance of green stripes seems to represent a change or loss of this inhibition.

Results of crosses of $V^m mp$ plants to an *En* tester ($a_1^{m(r)}$ shows purple sectors in the presence of *En*) indicate that the mutability expressed in $V^m mp$ is not dependent upon *En*. The genetic constitution of the progeny with respect to the genes involved was $V^m mp/+, a_1^{m(r)}/\ a_1 sh_2$. Phenotypically, the mature plants have green stripes in a virescent background although the aleurone is completely colorless. If *En* were present and the cause of $V^m mp$ mutability, the aleurone would also show mutability in the form of colored sectors on a colorless background.

Another isolate in these tests, a full color form that shows losses of color in the kernel, has been found on three independent occasions. (Culture numbers 1831, 1776, and 1898 will serve to identify these mutants.) In crosses to an *En* tester these mutants also failed to show a relation to *En*.

The third mutant, a_2^m-1511, originating from an A_2 allele, changes from the colorless form of a_2, and appears as dots on a colorless background (figure 2). The mutability expression is similar to the patterns observed with a_1^m. Mutability has been found to be independently controlled and tests, though incomplete, indicate that the regulator of mutability is *En*.

The expression of the fourth mutant, c^m-1702, is similar to that

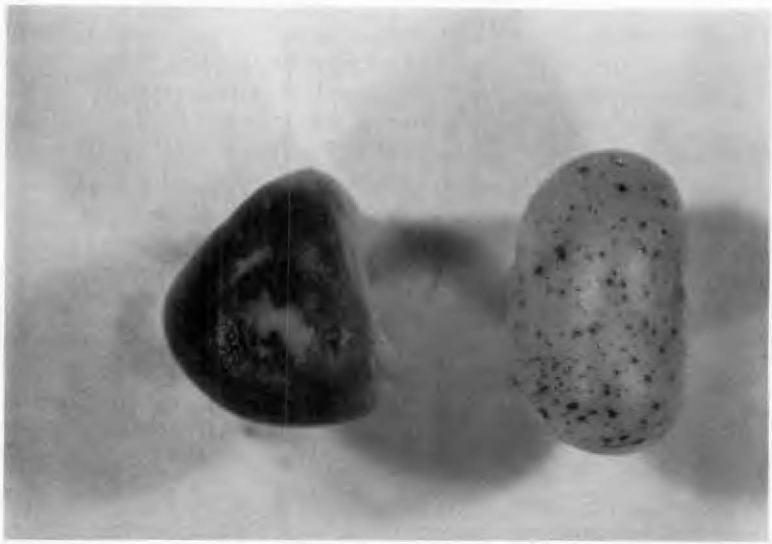

Figure 2. a_2–1511 showing early and late sectoring on the same kernels.

of a_2^m-1511, with colored sectors on a colorless background. It has been confirmed that this new mutant is allelic to *c* (chromosome 9) and originated from an initial *C* allele. The relation of this new mutant to *En* has not as yet been determined. Interestingly, however, both a_2^m-1511 and c^m-1702 stem from the same a_1^m stock (1957 405) crossed to B-chromosome-containing stocks.

These four different mutants appeared in a test of 5,627,651 gametes. Only two, a_2^m-1511 and c^m-1702, have a possible relation to the *En* system. This represents a lower rate of induction of instability than that found in the *Ac-Ds* system (5) where four new mutants were recovered in a test of 191 ears. (With 300 kernels per ear, this would represent the induction of approximately one new mutable in every 15,000 gametes.) A comparison of rates of induction of new sites of mutability between different systems is, however, valid only if the transposition of elements is a randomized event and, thus, a reflection of the rate of activity of a particular system in inducing changes at any locus. The results of certain experiments suggest that the sites of transposition of elements occur in a non-randomized manner.

Tests to determine the location of new sites of transposable elements have been conducted with variegated pericarp $\overline{P^{rr}Mp}$ In a study of transposed Modulator (*tr-Mp*), Van Schaik and Brink (9) found that two-thirds of the new sites of *tr-Mp* were linked with the *P* locus on chromosome-1, whereas these *P*-linked sites would be expected to be the future sites of *tr-Mp* only 9.5% of the time if the transposition phenomena were a random

event. Furthermore, in a study involving thirteen cases of twin sectors (a result of a coincident event recognized to arise from the transposition of *Mp* from the variegated pericarp locus, $P^{rr}Mp$, to a new site yielding a sector with P^{rr} free of the adjacent *Mp* – red kernels – and a light variegated sector ($\overline{P^{rr}Mp + tr\text{-}Mp}$), Greenblatt and Brink (10) found that the new location of *tr-Mp* was related to the old site. Both of these studies indicate that the transposition of elements is not a randomized event but that the future site is highly influenced by physical continuity of the chromosome.

In this context the chance of finding new a_2 and *c* mutables would have been abetted if *En* were present on chromosomes -5 and -9, respectively. In the *Ac-Ds* studies (5) *Ac* was present on the far end of chromosome -5. This may have influenced the induction of changes at the a_2 locus.

A number of studies have shown that elements associated with mutable loci undergo transposition and that, subsequently, instability arises at new sites. This seems to be a common feature of the different mutable systems. Some studies also indicate that the transposition of elements may be increased if the location of the elements has a chromosomal continuity with the possible new site of the transposed element.

The question of the maintenance of integrity of the pattern type during the transposition event has not yet been answered. A complete elucidation of this problem will necessitate the study of many cases of instability originating from a wide variety of initial pattern types. Attempts to explore this problem are in progress.

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