STABILITY OF THE SUPPRESSOR ELEMENT IN TWO MUTATOR SYSTEMS AT THE A_1 LOCUS IN MAIZE[1]

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THE widely accepted hypothesis for the mechanism of induction of mutability at specific loci by the two-element mutator systems found in corn is that proposed by McCLINTOCK (1950) and adopted by BRINK (1958, 1964) and PETERSON (1960). The hypothesis provides that a controlling element called an "operator" (McCLINTOCK 1961) moves adjacent to the gene site (A_1, for example) under the influence of a second element (*Ac* or *Spm*), called a "mutator" or "regulator." The operator suppresses the action of the adjoining structural gene, resulting in a recessive phenotype (a). This phenotype continues until the operator loses its ability to suppress gene action, through the mutator causing the operator either to "turn off" or to move away from the site. In either case something like normal gene function is restored, producing lineages of cells which express various degrees of A phenotype depending on the system involved, hence the mutable gene designation (a^m).

In the absence of the mutator, the operator element is viewed as permanently in position at the gene site in the "on" state. In this state it suppresses the gene, causing a stable recessive phenotype, which remains unchanged as long as the mutator is absent. When by appropriate crosses the mutator is returned, the operator is again subject to being "turned off" or is removed and the gene thus is restored to some degree of normal function. During all of these changes and through many replications the structural gene apparently remains the same. This hypothesis is a useful one; it fits most of the known facts about two unit-systems, but it is difficult to test.

Similar reasoning has developed in the formulation of the episome concept (JACOB and WOLLMAN 1959; JACOB and MONOD 1961), where more firm biochemical evidences are available to support the hypothesis of the addition of an element. For lack of these kinds of evidence for the systems in maize, other tests more reasonably applied to this organism are desirable.

If the suppressor element hypothesis is correct, that is, if a^m is actually A with a separable controlling element suppressing its action, one would expect that the element so easily placed or removed by its mutator could be dislodged, inactivated, or destroyed in the absence of the mutator. Such an event would result in the restoration of gene action, an event which ought not to be reversible within the strain since the culture would be free of the mutator factor.

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Another prediction that might be made if a^m is a structural gene plus a controlling element is that, since both must be reproduced at each cell division, errors in replication would be expected to occur such that one or both would be missing from some cell lineages. The loss of the controlling element alone would permit the freed structural gene to function.

To test whether the operator might be dislodged, three destructive or mutagenic agents (X rays, ultraviolet light, and ethyl methanesulfonate) may be applied to cultures carrying known mutable loci but lacking their respective mutators. All three of these agents are effective in producing chromosome breaks and gross aberrations in corn. Ultraviolet light and ethyl methanesulfonate (EMS) were chosen because they also cause discrete changes on the genic level (NUFFER 1957; NEUFFER and FICSOR 1963). X rays, which yield only gross changes in corn, were included for comparison.

A test for spontaneous errors in replication may be accomplished by producing seeds that are homozygous a^m *dt*. These colorless seeds may then be examined for colored dots which represent reversions to *A*.

MATERIALS AND METHODS

The mutator systems used in this investigation were studied through their activities at the A_1 (anthocyanin) locus on chromosome 3. The a^m *Dt* system used in experiment 1 consisted of a highly mutable recessive allele a_1^m (no anthocyanin) and the mutator factor *Dt* (dotted) located on chromosome 9. Dotted causes a_1^m to change to A_1 producing sectors of purple or red pigment in the aleurone layer and in those plant tissues when anthocyanin pigments normally develop. The mutable allele for this system is designated simply as a^m and the system as the *Dt* system. The a^m-*Spm* system, designated *Spm* by MCCLINTOCK (1961), used in experiment 2 consisted of a mutable allele (a^{m-1}) and its mutator factor *Spm* (suppressor of mutability). When *Spm* is present, the phenotype of a^{m-1} kernels is colorless with full colored dots. When it is absent the expression is dilute color with no dots. A recessive allele (a^s) which is stable in the presence of either *Dt* of *Spm* was used in experiment 2. Also the marker sh_2 (shrunken endosperm) which is 0.25 map units distal to A_1 was used to distinguish between the alleles where progeny test confirmations were required. In experiment 3 the stock used was made heterozygous for alleles representing the two systems (a^m and a^{m-1}) so as to test both simultaneously and for yg_2 (yellow green seedling) for the purpose of determining frequencies of chromosome breakage. The yg_2 locus is near the end of the short arm of chromosome 9.

The treatments used were as follows: In experiments 1 and 2 mature pollen of the indicated genotype was subjected to either 1000r of unfiltered X rays (150 kv 9 ma) or to a 30 second bilateral exposure to two Westinghouse (WL 782H) sterilamp tubes at a distance of 4 inches (NUFFER 1957) and crossed immediately on the proper ear stock. Pollen for control pollinations was handled in the same manner but not subjected to treatment. In experiment 3 seeds with embryos of the genotype $a^m\, a^{m-1}$, *spm spm*, *dt dt*, $Yg_2\, yg_2$ were treated with ethyl methanesulfonate by soaking for 8 hours in a 0.075M aqueous solution, washing with water and then planting in soil. Distilled water was used for soaking of seed for the X-ray treatment (1000r applied following soaking) and for the control. No treatment was used in experiment 4.

RESULTS

The first experiment was designed to test the *Dt* system which is similar to the *Ds-Ac* system as pointed out by MCCLINTOCK (1951a) but differs in a number of respects (NUFFER 1961). Treated and untreated mature pollen from homo-

FIGURE 1.—Several colorless seeds from the cross $a^m\ dt \times a^m\ dt$, (Experiment 1) including one with several sectors of dotted tissue. The pollen was treated with ultraviolet light. The streaks of white are due to a diseased condition of the ear.

zygous $a^m\ dt$ plants was crossed on an $a^m\ dt$ ear stock. The resulting ears produced all colorless seeds which were examined for large colored sectors and for dots. Seeds from the control and X-ray treatment and all but three from the ultraviolet light treatment were completely devoid of typical anthocyanin pigment. Several seeds from both groups had a flush of red color, but these proved to be germless, repeating a well known phenomenon in maize in which germlessness is associated with nongenetic coloration of otherwise colorless seeds. The three truly exceptional seeds included one with a single tiny dot, one with several dots, and one with several sectors of dotted tissue which covered a large portion of the seed surface (Figure 1). None of the three (Table 1), except possibly the one-dot

TABLE 1

Frequency of reversion to A *(colored sectors) and of induction of mutator (groups of colored dots, either on a colorless seed of* a^m dt *or in a colorless sector on a dilute seed of* a^{m-1} spm) *resulting from treatment of* a^m *pollen in the absence of the mutator element*

Genotype	Treatment	Seeds examined	Reversion to *A*	Mutator induced	Losses of a^m
Experiment 1					
a^m, *dt*	X ray	7694	0	0	. .
	UV	2868	0	3	. .
	control	2128	0	0	. .
Experiment 2					
a^{m-1}, *spm*	X ray	5528	0	1	69
	UV	1958	0	2	153
	control	3024	0	0	11

case, should be considered as simple reversions to *A* but instead as induction of new *Dt* factors which then cause reversion. If the treatment destroyed the suppressor element in one of the chromatids of the pro-endosperm nucleus of the pollen grain, a half-seed sector should result (on the average). This sector would be solid rather than composed of dots. If, on the other hand, a new *Dt* factor were induced, a sector would arise having continuing reversions to *A* which would appear as dots. A seed with one dot could be either an extreme deviation from a half-seed sector involving a newly arisen *A* or more likely, a single dot in a larger half-seed sector involving a newly induced *Dt*. The material in the experiment with a^{m-1} *spm* is better suited for distinguishing between the above possibilities, as the borders of the induced mutator sector are clearly defined. The possibility of delayed events resulting from either a multistranded treated chromosome or from subsequent chromosome breakage is not ruled out but is considered of little consequence in interpreting these results.

The second experiment was designed to test the mutator systems for which the suppressor element hypothesis was developed. The *Spm* system was used because it is especially suited for distinguishing between loss of the structural gene, loss of the suppressor, and induction of a new mutator. The mutable allele in this system (a^{m-1}) gives a colorless seed with colored dots when *Spm* is present and dilute colored seed with no dots when it is absent. The same treatments used in the first experiment were applied to pollen from plants of the genotype a^{m-1}-Sh_2/a^s-sh_2, *spm spm*, *dt dt*. This pollen was crossed on the same a^m-*Sh*, *dt* ear stock producing ears with nonshrunken seed that are expected to be half dilute ($a^{m-1}/a^m\ a^m$) and half colorless ($a^s/a^m\ a^m$) but all free of mutator-induced dots.

The seeds were examined for various sectorial changes. Sectors observed here provide valid data because the chromosomes of mature pollen grains are already effectively doubled for the next division. A single strand may be affected by discrete treatment to produce a half-seed sector; the dilute seeds could show colorless sectors (loss of the structural gene), full colored sectors (loss of the suppressor), or colorless sectors with colored dots (induction of a new *Spm*). The colorless seeds could express colored or dotted sectors. Colored sectors on colorless seeds are not expected since the a^s allele present in the pollen parent is stable in all known situations. Sectors of dots might occur if a new *Dt* were induced that would then activate the responding a^m allele of the ear parent.

The initial plan to examine the dilute seeds for colored sectors, colorless sectors without dots, and colorless sectors with dots was accomplished, but the planned examination of colorless seeds for sectors of dots was impossible because of the high frequency of apparent colorless seeds with dilute "recovery" spots, a common phenomenon (STADLER 1939) presumably resulting from abortive deletion of the a^{m-1} bearing chromosome segment. An extreme case is seen in the left hand seed of Figure 2.

The data from this experiment are summarized in the bottom half of Table 1. They show negative results for reversion to A but three presumptive cases of induction of *Spm*. The most striking of these, from X-ray treatment, is shown

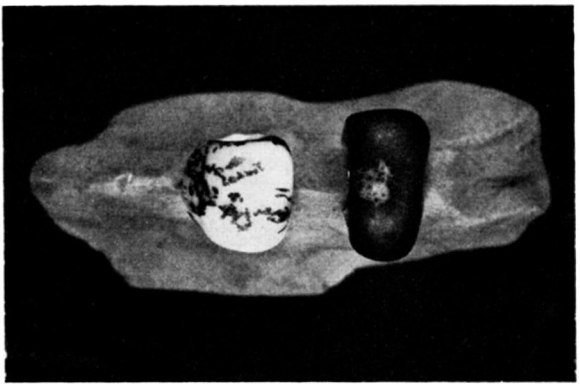

FIGURE 2.—Two exceptional seeds from the cross a^m-*sh*/a^m-*sh*, *dt dt spm spm* × a^{m-1}-*Sh*/a^s-*sh dt dt*, *spm spm*, (Experiment 2) showing (left) a colorless seed with many dilute recovery spots and (right) a dilute colored seed with a colorless sector within which are included many *Spm*-type dots. The pollen was treated with X rays.

in Figure 2, right hand seed. This kernel shows the dilute-colored background with a colorless sector bearing typical a^{m-1} *Spm* dots. Table 1 also includes a typical number of fractional losses of the a^{m-1} bearing segment (colorless sectors with no dots).

The third experiment was devised to test the action of the chemical mutagen ethyl methanesulfonate. Seeds with embryos of the genotype a^m/a^{m-1}, *spm spm*, *dt dt*, Yg_2 yg_2 were treated with EMS or X rays for comparison and planted. The material was made heterozygous for yellow green to provide an indicator of the frequency of loss of *Yg* or of mutation to *yg*. The treated and control seedlings were observed for purple sectors through maturity. The supporting genotype (A_2 *B Pl* R^r) with a^{m-1} provides a weakly colored plant on which purple sectors of A_1 tissue would easily be seen. The frequency of yellow green sectors (loss of *Yg*) was noted for comparison, and a final count was made at flowering. The data are summarized in Table 2. No purple sectors were seen on the 168 plants examined, but many yellow green sectors were seen on the treated plants, demonstrating that the mutagen treatments were effective.

It should be pointed out that not all yellow green sectors produced by EMS

TABLE 2

Comparison of the frequency of reversion to A (colored leaf and sheath sectors) and losses of Yg_2 *(yellow-green leaf and sheath sectors) in plants grown from seeds of* a^{m-1}/a^m, dt dt, spm spm, Yg/yg *treated with ethyl methanesulfonate and X rays*

Treatment	Number of plants	Colored sectors	Yellow green sectors extending from leaf tip to base	Yellow green sectors not extending length of leaf
Experiment 3				
EMS 8 hr, 0.075 M	70	0	434	9800
X rays, 1000r	42	0	151	2940
Control	56	0	14	280

treatment represent chromosome breakage and loss or gene mutation but that a considerable number may be due to irreversible changes in the chloroplasts brought about by EMS. This is deduced from the observation (unpublished) that EMS treated seeds that are homozygous for *Yg* produce seedlings with more yellow green sectors than expected when calculated on the basis that the frequency of expressed losses of *Yg* in the homozygote should be equal to the square of the frequency of expressed losses in the heterozygote since only simultaneous losses of both dominant alleles in the homozygote should produce a genetically yellow green sector. However, even if chloroplast changes account for as many as half of the sectors, the data in Table 2 represent a sizable frequency of segmental losses from the treated chromosome and provide an adequate indication of treatment effectiveness.

The fourth experiment designed to determine the frequency of spontaneous copying errors in the replication of the suppressor element was accomplished by examining 5600 colorless seeds of a^m a^m a^m, *dt dt dt* endosperm genotype for colored dots four cells in size or larger (the safe limit of visibility to the unaided eye). Since each seed had an average of 60,000 observable cells on the surface examined, each with three susceptible a^m alleles, and since dots four cells or larger in size are clearly discernible, each seed represents $60{,}000 \times 3 \div 4$ or 45,000 chances for the occurrence of a replication error that would cause a visible colored dot to occur. Examination of 5600 seeds represents observation of 252,000,000 chances for loss of the *Dt*-responding suppressor element. No dots were found on the seeds examined.

DISCUSSION

Failure to obtain reversion of the suppressed structural gene in either of the two a^m alleles by various kinds of treatment demonstrates a remarkable stability. This stability is further supported by the absence of spontaneous reversions in large numbers of aleurone cells. Errors in copying and transmission of information are a common occurrence in biological material, hence their absence, when predicted as in this case suggests an interesting relationship between the gene and its suppressor element. The association appears to be so close that the types of agents used to induce separation are ineffective. This is difficult to visualize in light of what is now known about the chemical structure of the genetic substance. The specificity of the structural gene presumably resides in one or more DNA molecules. The suppressor element is viewed as being near but externally associated with the DNA. Both ultraviolet light and ethyl methanesulfonate are known to produce discontinuities in the DNA molecule as well as to break whole chromosomes; the case for X-ray action in corn is not clear as only gross effects are known to occur (NUFFER 1957; NEUFFER and FICSOR 1963). Therefore the absence of observable disassociation of gene and suppressor with these agents suggests special relationships that require interpretation. One explanation may be that any type of separation of gene and element other than that brought about by the legitimate mutator causes inactivation of the whole complex. This could

occur by distortion of the structural gene so that activity is lost and a recessive mutant is produced, or by loss of the gene or a small segment of the chromosome with the same result, or by chromosome breakage with loss of one or more genes and with possible associated lethality. The first two possibilities would produce recessive mutants at the A_1 locus which would not be distinguishable in these experiments from a^m without its mutator. The third possibility would produce losses due to chromosome breakage that could not be distinguished from radiation and chemically induced breaks. Separation of gene and suppressor also might occur through normal crossing over. In the systems studied here this has not been found (NEUFFER 1965), but in some other related systems (MCCLINTOCK 1965) the suppressor has been found to separate by crossing over.

Although the occurrence of separation by crossing over is evidence for an element being present that is spatially removed from the structural gene it is conceivable that any given mutator system may involve a closely linked two-unit system, one supporting unit of which may be removable by crossing over leaving the second unit present but inactive. Consequently the occurrence of separation by crossing over (or, if it occurred, by mutagenic agents) cannot be considered proof of the presence of a suppressor element. Absence of separation on the other hand, is contrary to prediction for the controlling element hypothesis.

It is possible to devise other schemes (NUFFER 1962) for explaining the results obtained. The most attractive of these involves a structure with a missing part instead of an added element. When a configuration is inactivated by removal of a part, the only way to restore activity is to replace the part. This is a very specific kind of step which requires specific nonrandom action. To apply this idea to the mutator system, it may be assumed that each gene has a key configuration which is essential for its function but is not a part of its code. If the mutator is capable of removing that key (perhaps to a new site), the gene then becomes nonfunctional but is still able to replicate properly even in the absence of the mutator. Function is restored only when the mutator restores the original key or replaces it with a similar one from another site. If the key is drawn from another site, this action may produce a new site of mutability that is also responsive to the action of the mutator. The restored functional gene may have a different efficiency of action because the key material substituted was different from the original. The specificity of the mutator resides in its being limited to certain kinds of keys or to certain ways of removal. Hence *Dt* cannot replace the key removed by *Spm* and vice versa.

The postulation of a key configuration satisfies many of the known facts about mutator systems but requires that there be in the nucleus a supply of key material; otherwise, once it is removed and lost by segregation, the key material would be impossible for the mutator to replace. The source may be other genes, a constant source like heterochromatin, or the mutator itself.

The data presented here bear on another important question; namely, the origin of the mutator factor. In these experiments, three cases of new *Dt* expression were induced by ultraviolet light, and three cases of *Spm* were induced, two by ultraviolet light and one by X rays. Since the well authenticated cases of

mutator induction previously known were brought about by chromosome breakage caused by the breakage-fusion-bridge cycle (McCLINTOCK 1950, 1951a, b), and since chromosome breakage is a common result of both ultraviolet light and X-ray treatment, the facts reinforce the conclusion that chromosome breakage is the mechanism by which new mutators arise.

There are other significant indications from these results. For example, the relatively low frequency of *Dt* and *Spm* cases as compared to the high number of breaks in a single chromosome arm (last two columns, Table 1) indicates that breakage in a few very specific areas is required for induction of new mutator elements, rather than breakage in various locations or in large areas such as those occupied by heterochromatin. Similarly, the fact that ultraviolet light, which is known to be more discrete in its action and less efficient than X rays in producing chromosome breaks (NUFFER 1957), produced significantly more cases of mutator induction than X rays, which are gross in action, suggests that the sites for induction of mutator action are physically small and probably limited in number.

SUMMARY

Cultures carrying both *Dt*- and *Spm*-responding mutable A_1 alleles, but lacking the mutators, were treated with ultraviolet light, X rays, and ethyl methanesulfonate in an effort to induce reversion of the mutable alleles to *A*. The results were negative, but several cases of induction of *Dt* and *Spm* were found. These appear to result from chromosome breakage at a limited number of sites.—Similar cultures were examined for spontaneous reversion to *A*, which would indicate copying errors in the suppressor element. No cases were found in 252×10^6 chances.—The remarkable stability observed in this material poses some questions about the suppressor element hypothesis. The induction of new *Dt* and *Spm* activity reinforces the conclusion that chromosome breakage is the mechanism for their origin and the greater efficiency of ultraviolet in this induction suggests that the sites of origin of *Dt* and *Spm* are physically small and probably limited in number.

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