MODE OF OPERATION OF A SYSTEM OF CONTROLLING ELEMENTS IN MaIZE

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Mode of Operation of a System of Controlling Elements in Maize.

General descriptions of controlling elements in maize and of their modes of operation have been given in a number of reports appearing in recent years (for literature citations, see McClintock, 1956b). It is the purpose of this report to consider some of the experimental methods that have been employed to discover the mode of operation of the system of controlling elements which has been referred to in other publications as the Spm (Suppressor-mutator)-a<sub>m-l</sub> system. Controlling elements may be defined as those unit components, carried in the chromosomes, that serve to control gene action both with regard to type and degree and to the tissues or parts of a tissue in which this will occur. The different controlling elements are recognized by means of their distinctive modes of control of gene action, regardless of the primary type of action of the gene substance itself. They exhibit Mendelian inheritance patterns and their locations in the chromosome complement may be determined by use of ordinary genetic techniques. However, they may undergo change in location within the chromosome complement, appearing at new locations and disappearing from previously determined locations, without losing their distinctive properties in the process. This process has been termed transposition and
the several methods that have been used to detect such transposition were presented previously (McClintock, 1956b).

For many years, efforts of the author were concentrated on analysis of the system composed of the two controlling elements designated Ac (Activator) and Ds (Dissociation). This system was chosen for extended studies because both elements of this system could be identified readily and regardless of the location in the chromosome complement that each might occupy. Thus, change in location of these elements, and the effects produced when one of them is inserted at the locus of a known gene, could be detected and subsequently analysed. Altogether, the operation of this system at eight known gene loci has been examined. Also, two or more independent insertions of one of these elements at four of these eight gene loci were detected and the consequence of this examined in each case. It was concluded from these studies that this system should be able to operate at any gene locus provided that the effects of its operation at a particular gene locus is not lethal. Most important, however, is the realization that the mode of control of gene action may be predicted in advance, for it will follow the rules that apply to the operation of this system. Knowledge of the mode of operation of the Ac-Ds system has been useful in guiding experiments aimed at revealing the mode of operation of other control systems. This applies particularly to methods for identifying controlling elements.
and for detecting their transpositions. It has also provided a model for recognizing and subsequently appraising the changes in state of the affected gene locus. Such changes must be recognized if confusion in designing experiments and interpreting results is to be avoided.

Some years ago, a large number of different variegates appeared in the progeny of individual plants with a history of having been subjected to the breakage-fusion-bridge cycle in their early development (McClintock, 1951). At the time these variegates appeared, it was realized that it would not be possible to examine each of them. Therefore, only a few among the many that appeared were selected for subsequent study. The Ac-Ds system was discovered among one of these selected cases; and as the mode of operation of this system became apparent, attention was focused mainly upon it.

Study of the other selected cases either was discontinued or it was sharply curtailed until adequate time could be found for a detailed examination. The system responsible for control of gene action in one of these latter cases is now sufficiently understood to allow conclusions to be drawn regarding types of controlling elements that are involved in it and their modes of operation. This is the system responsible for control of gene action at both $a_1^{m-1}$ and $a_2^{m-1}$, mentioned in previous reports. In this report, attention will be given mainly to $a_1^{m-1}$. 
The history of origin of \( a_1^{m-1} \) from a modification that occurred at a standard \( A_1 \) locus is as important for an appreciation of the controlling elements involved in this system and their modes of operation as were the histories of origin of modified gene action that appeared in the Ac-Ds carrying cultures for an appreciation of the presence and mode of operation of the elements of that system. The modified \( A_1 \) locus, designated \( a_1^{m-1} \), was the third recognized case of change in gene action in a sequence which commenced with an alteration at a previously unidentified gene locus concerned with chlorophyll production. The plants having this first member of the sequence exhibited variegation for chlorophyll pigmentation. This variegation was one of those originally selected among the many that appeared in the original cultures, as described above. In the early stages of examination of this variegate, a number of plants in one culture were self-pollinated. On the ear produced by one of these plants, some kernels appeared that exhibited variegation for anthocyanin pigmentation. Spots of deep pigmentation appeared in a colorless background. The plants derived from them also exhibited variegation for anthocyanin pigmentation. Subsequent tests of these plants and their progeny indicated that an alteration had occurred at the standard \( A_2 \) locus in one chromosome 5 of the parent plant. This altered locus was given the designation \( a_2^{m-1} \). Tests were then undertaken to examine the changes in expression of gene action at \( a_2^{m-1} \) and to
determine the factors involved in control of this. In the course of this study, a number of plants in a culture in which the system responsible for control of gene action at a \textsuperscript{2} \textit{m-l} was present, were used as pistillate parents in crosses with plants that were homozygous for the standard recessive, \textit{a}_1, in chromosome 3, and for the standard \textit{A}_2 locus in chromosome 5. On one of the ears this cross produced, a single kernel was found that exhibited pigment spots of anthocyanin in a non-pigmented background. A plant was grown from this kernel and this plant, in turn, exhibited variegation for anthocyanin pigmentation. As expected, tests crosses utilizing both pollen and ear of this plant indicated the presence in it of an altered \textit{A}_1 locus, and was thereafter designated \textit{a}_1 \textsuperscript{m-l}. It was evident that the alteration had occurred at the standard \textit{A}_1 locus in one chromosome 3 in the parent plant, and late in development of one of the ears of this plant, for only one kernel on this ear exhibited altered \textit{A}_1 action. Studies aimed at determining the components of the system responsible for control of gene action at both \textit{a}_1 \textsuperscript{m-l} and \textit{a}_2 \textsuperscript{m-l} were continued but initially only on a limited scale. Only recently has time been found to examine this more completely. It is now known that the same system of control of gene action operates at both \textit{a}_1 \textsuperscript{m-l} and \textit{a}_2 \textsuperscript{m-l}. Very likely this same system also operated to control action of the gene that was associated with chlorophyll production, the initial variegated in the sequence. This postulate is based upon the patterns of variegation that were exhibited and upon inheritance behavior for study of this case was discontinued some years ago.
and therefore direct tests to determine identity of control systems cannot now be made.

In order to learn of the system that is responsible for control of gene action at $a_1^{m-1}$ and $a_2^{m-1}$, a large number of different types of tests were conducted. The results obtained from each are consistent with one another on the basis of the eventually determined modes of operation of the components of this system. In order to comply with space requirements, only a selected set of tests will be given here. These are chosen in order to illustrate the salient features of the mode of operation of this system and they will be confined to studies conducted with $a_1^{m-1}$. Those conducted with $a_2^{m-1}$ will be given in a separate report.

From examination of the Ac-Ds system, it was learned that insertion of the Ds element at the locus of a gene initiated the primary modification that brought this gene under the control of the Ds-Ac system. In many cases, the action of the gene was noticeably altered by this event and detection of the insertion of Ds at the locus could be made shortly after it occurred. Subsequent change at the locus results from the effects Ac exerts on the Ds element. The consequence of this is either removal of Ds from the locus of the gene or a modification at the locus, induced by Ds, that effects a change in its organization,---a change in state of the locus.
Both types of events can give rise to recognizable changes in action of the gene substance. With regard to $a_{m-1}^1$, insertion of a particular controlling element at the standard $A_1$ locus is considered to have occurred and to have been responsible for the initial change in gene action. Like $Ds$, it is this element that directly controls the type of gene action that will occur at the $A_1$ locus and the types of change in this action that may occur subsequently. It appears to be the same element that is present at $a_{m-1}^2$. This conclusion is based on the response of both $a_{m-1}^1$ and $a_{m-1}^2$ to the presence of an independently located element designated Suppressor-mutator (Spm). All action of the gene substance at both $a_{m-1}^1$ and $a_{m-1}^2$ is suppressed when Spm is present in the nuclei of a plant except in certain cells. The particular type of expression of the gene that these modifications effect is thereafter maintained either in the presence or the absence of the Spm element. In this regard, the Spm element is complementary to the element located at $a_{m-1}^1$ and $a_{m-1}^2$. It serves to activate it and as a consequence of this, stable mutations are produced. In this respect, it resembles $Ac$ in the $Ds-Ac$ system.
is not present in the nucleus. When it is removed, either through meiotic segregation or by means of somatic transposition, anthocyanin pigment may appear in the kernels and plants having either $a_1^{m-1}$ or $a_2^{m-1}$ and its distribution is uniform. In other words, there is no variegation. The type and intensity of pigmentation is an expression of the particular state of either the $a_1^{m-1}$ or $a_2^{m-1}$ locus that is present. These states and their origins will be considered shortly. When Spm is returned to the nuclei by appropriate crosses, gene action at $a_1^{m-1}$ and $a_2^{m-1}$ is suppressed except in those cells where mutation-producing events occur. Thus, Spm serves not only to activate the complementary element residing at $a_1^{m-1}$ and $a_2^{m-1}$ and the eby conditions stable mutations at those two loci, but it also must act upon this element in yet another manner for its presence results in suppression of known potentials for gene action, except in those cells where mutation-producing events occur. These two seemingly different aspects of the mode of control of the Spm element on the element residing at $a_1^{m-1}$ and $a_2^{m-1}$ are responsible for its being designated Suppressor-mutator. However, this seemingly dual action may be the expression of only one process rather than of two unrelated processes, as will be indicated later.

The states of $a_1^{m-1}$

All examinations of the effect of a controlling element at a gene locus
must be conducted with the affected locus. Since, in addition to stable mutations, the controlling element may also initiate structural or organizational modifications of the locus that alter its subsequent expression, that is, change the state of the locus, it is necessary to consider states of the affected locus and their origins before detailed results of experiments can be presented. With $a_1^{m-1}$, a changed state is readily recognized by the appearance of an individual kernel or plant that exhibits an altered response of the locus to the presence and absence of Spm. For example, when the controlling element first entered the locus of $A_1$, it effected a particular type of modification in the structure or organization of the locus. In the absence of Spm, some gene action occurred. Kernels were lightly but uniformly pigmented as were the plants derived from them. In the presence of Spm, however, all gene action was suppressed except in some cells where mutation-producing events occurred at the locus of $a_1^{m-1}$ that allowed the gene substance to be active in the descendants of this cell. Each such event did not result in the same degree or type of gene action but many of them restored the full or near full $A_1$ type activity. Many of these events occurred relatively early in plant and kernel development. Unlike $a_1$ variations in dose of Spm had no effect on altering the time of occurrence of these and in this respect the action of Spm differs from that of $A_1$. 

events. A number of mutation-producing events occurred in germinal cells and this made it possible to examine the nature of the mutation in the next generation. Another type of change at $a_1^{m-1}$ also occurred in a few of the germinal cells. These resulted in altered expressions of the locus both in the presence and absence of Spm. Some of them were detected initially in individual kernels in the progeny of the original $a_1^{m-1}$ carrying plant. When this plant was crossed to plants that were homozygous for the standard recessive, $a_1$, which does not respond to Spm but responds, instead to Dt, the locus in the majority of kernels that received it was unmodified. Among the kernels receiving the unmodified $a_1^{m-1}$ locus and also Spm, the variegation pattern was much the same. There were many pigmented areas, indicating early occurring mutation-producing events. Many of these exhibited the full $A_1$ type of pigmentation. However, an occasional kernel appeared that exhibited a quite different pattern of pigmented areas. (Two kernels) were found among several thousand that had only small pigmented areas. These were uniformly distributed and the intensity of pigment in them was either quite light or very dark. Another kernel appeared that had a number of large pigmented areas as well as some small areas but the intensity of the
pigment in all of them was relatively light. It ranged from very faint in some areas to intense intensity in others.

Plants were grown from the three described kernels. Tests conducted with them and their progeny indicated that the pattern of variegation exhibited in the presence of Spm was heritable. The altered $a_1^{m-1}$ locus, the changed state of the locus, in each case responded in its own particular way to the presence of Spm and also to its absence. In the absence of Spm, the state of $a_1^{m-1}$ in the first described kernel produces deep pigmented kernels and plants. That in the second described kernel gives rise to very lightly pigmented kernels but rather darkly pigmented plants. The state of $a_1^{m-1}$ present in the third described kernel produces no pigment in either plant or kernel in the absence of Spm. Subsequently, other states of $a_1^{m-1}$ have been isolated. Each is distinguishable from the other by the types of mutation and the time and frequency of their occurrence in the presence of Spm and as well as the type of expression of the gene substance that appears when Spm is present. No relationship was noted among the different states between the control of time of occurrence of mutation in the presence of Spm and that of type of gene action that occurs in its absence. Figure 1 illustrates the distinctiveness of several of the states of $a_1^{m-1}$ and Table 1 records the range of differences that is apparent among the presently isolated states of $a_1^{m-1}$.
The integrity of a state of \( a_l^{m-1} \) is maintained in heterozygous plants and this applies to plants that carry a different state in each of their chromosomes 3. In such plants, each state responds to Spm in its own predictable way and the variegation patterns each produces will be expressed in the plant or kernel tissues. Figure 2 illustrates this. The kernels in the photographs carry the state shown in — of figure 1 in one chromosome 3 and the state shown in — of figure 1 in the homologue. The pattern of mutation produced by each state as readily recognized in these kernels. In the plants, normal segregation of the two states occurs at meiosis and each may be recovered in the expected proportion from the gametes that these plants produce.

States of \( a_l^{m-1} \) are maintained unchanged in the absence of Spm. In its presence, however, new states may arise and the frequency of occurrence of this in germinal cells is related to the time of occurrence of mutation-producing events that a particular state exhibits. If the state produces some early occurring mutations, then new states of \( a_l^{m-1} \) may appear in the germ cells, and the frequency of this is proportional to the frequency of occurrence of these early mutations. If, on the other hand, the state is one in which mutations occur only late in development of the plant or kernel, then few or no altered states may be recovered in the gametes of
these plants. The mutation-producing events at \( a^{m-1}_1 \) and regardless of state, give rise to stability of expression of the locus. The particular type of gene action the mutational event produces continues to be expressed in subsequent generations both in the presence and in the absence of Spm. This suggests that the change responsible for these mutations may have removed the controlling element from the locus or it may have resulted in its inactivation with regard to Spm. Since the changes in state arise at the very same developmental period as the mutation-inducing events occur, it is concluded that they represent a modification at the locus induced by the controlling element residing there that did not result either in its removal or inactivation. In other words, the element responded to the presence of Spm at the appointed time and in the appointed cell but the consequence of this was not the usual one, i.e., the mutation-producing event. Instead, the responding element itself was either modified or it induced some reorganization at the locus that modified its capacity to respond to Spm with regard to time and also with regard to the types of mutations it can induce in the absence of Spm. It is as evident in this case as it is with Ds that a change in state is one of the consequences of the response of the
of the controlling element residing at a gene locus to the independently located element of the system, Spm in this case and Ac in the case of Ds.

Detection of the Spm element and its mode of operation

In the early examination of $a_{1}^{m-l}$, no evidence of the presence of the Spm element was detected. This was because the original $a_{1}^{m-l}$ carrying plant had a number of Spm elements located at various positions in the chromosome complement. All of the gametes it produced had Spm elements in them. Since the dose of Spm has no appreciable effect on the pattern of mutation produced by $a_{1}^{m-l}$, differences in number of Spm elements in a kernel or plant is not made directly evident by this means as it is with Ac.

It was only after several generations of crossing of $a_{1}^{m-l}$ plants to a tester stocks which did not have Spm, that definite ratios of variegated to uniformly colored kernels appeared on the test cross ears. These ratios indicated the presence in the $a_{1}^{m-l}$ carrying plants of an independently located element that is associated with control of $a_{1}^{m-l}$ expression. They also indicated that the number and the location of this element was not the same in all tested plants. In the meantime, several different states of $a_{1}^{m-l}$ had been isolated on the basis of the altered variegation patterns that appeared in individual kernels on several of the first testcross ears, as described earlier. In successive generations of crossing of
plants carrying these different states of $a_{m-1}$ to the $a_1$ tester stocks, the same kind of ratios of variegated to non-variegated kernels also began to appear. It was then assumed that the uniformly pigmented (non-variegated) kernels and plants carried $a_{m-1}$ but not the independently located element. On the other hand, this element was assumed to be present in those kernels and plants that showed pigmented areas in a non-pigmented background. On this interpretation, the independently located element was exerting a suppressor-mutator type of control of gene action at $a_{m-1}$. Since the dose of this element obviously did not affect the pattern of mutational events, control of this must reside at the $a_{m-1}$ locus itself, the type depending upon the state of the locus. It was evident that the phenotypes of the nonvariegated kernels and plants also reflected the state of the $a_{m-1}$ locus that was present in them.

The hypothesis stated above was subject to test. If it were correct, evidence in support of the following four statements should be obtained:

1. All variegated kernels and plants carry at least one Spm element.

2. No Spm element is present in the non-variegated class of kernels and plants. (Germinal mutations, described earlier, are excluded from this class.)

3. The $a_{m-1}$ locus in the non-variegated kernels and plants is capable
of responding to Spm if this element is subsequently introduced into a
nucleus.

(4) The type of response to Spm and the phenotypes produced in its
absence is a function of the state of the $a_{m-1}$ locus.

A large body of evidence in support of these statements is now available
and it has been obtained by various types of test, only a few of which
need be outlined here.

In order to facilitate identification of the presence or absence of
Spm in a particular plant, so-called Spm tester stocks were developed.

These stocks have either one or the other of the two states of $a_{m-1}$ shown
in $-$ and $-$ of figure 1, and these states were selected for the following
reasons. In the first place, when Spm is present, very few germinal
mutations or changes in state occur. Therefore, nearly all of the
gametes produced by plants carrying these states of $a_{m-1}$ and also Spm
have an unmodified $a_{m-1}$ locus in them. Secondly, the pattern of variega-
tion each produces in the presence of Spm is distinctive and non-obscuring.

Thirdly, in the absence of Spm, one of these states, figure 1, gives rise
to darkly pigmented kernels and this is a useful character in some tests.

The tester stocks were made homozygous for one or the other of these two
states. They were also made homozygous for either $Sh_2$ or $sh_2$, located
very close of \( a_{1}^{m-l} \) (about one-quarter of a percent crossing over occurs between them).

An example of one series of tests will illustrate some of the methods employed to determine Spm constitutions of individual plants. The silks of two ears of a variegated plant carrying \( a_{1}^{m-l} \) and \( Sh_{2} \) in one chromosome 3 and \( a_{1} \) and \( sh_{2} \) in the homologue and also \( Y \) in one chromosome 6 and \( y \) in its homologue, received pollen from a plant that was homozygous for \( a_{1} \), \( sh_{2} \) and \( Y \). The state of \( a_{1}^{m-l} \) in the pistillate plant was that shown in figure 1. From this cross, the two ears produced a total of 745 kernels. There were 181 \( Sh_{2} \) kernels in which the aleurone layer was uniformly and rather darkly pigmented; 69 of these were \( Y \) and 112 were \( y \). The aleurone layer in another 188 \( Sh_{2} \) kernels exhibited a number of spots of the full \( A_{1} \) type pigment in a colorless background and 117 of these were \( Y \) and 71 were \( y \). The aleurone layer in the remaining \( Sh_{2} \) kernels was completely colorless and the starch in its endosperm was \( y \). Among the 375 \( sh_{2} \) kernels on these two ears, the aleurone layer in 373 of them was totally colorless; 186 of these kernels were \( Y \) and 187 were \( y \). The remaining 2 \( sh_{2} \) kernels exhibited spots of full \( A_{1} \) type pigment in a colorless background. The phenotype of the starch in one of them was \( Y \) and that in the other was \( y \). Since
\( a_1^{m-1} \) is closely linked to \( Sh_2 \), nearly all of the \( Sh_2 \) class of kernels on these two ears should carry an \( a_1^{m-1} \) locus and nearly all of the \( sh_2 \) kernels should be homozygous for the standard recessive, \( a_1 \). The close linkage of \( a_1^{m-1} \) to \( Sh_2 \) is obvious for only 1 \( Sh_2 \) kernel in the total of 370 was completely colorless and only 2 \( sh_2 \) kernels in a total of 375 had pigment in the aleurone layer.

On the basis of the interpretation given above, the uniformly pigmented kernels should have no \( Spm \) in them whereas those exhibiting spots of the full \( A_2 \) type pigment in a colorless background should have this element. From the ratio of these two classes among the \( Sh_2 \) kernels (161 to 187) it could be concluded that the variegated pistillate parent plant had one \( Spm \). The ratio of \( Y \) to \( y \) in each of these two classes indicated that this \( Spm \) element was carried in the \( Y \) bearing chromosome.

It was then necessary to determine whether or not these conclusions were valid. For this purpose, 104 plants were grown from various types of kernels on these two ears and tests were conducted with them. The phenotypes of the selected kernels were as follows: 11 uniformly pigmented \( Sh_2 Y \), 13 uniformly pigmented \( Sh_2 y \), 17 variegated \( Sh_2 Y \), 8 variegated \( Sh_2 y \), 1 variegated \( sh_2 Y \), 30 colorless \( sh_2 Y \) and 24 colorless \( sh_2 y \).

All 24 plants derived from the uniformly pigmented kernels were themselves
uniformly pigmented. All of the 26 plants derived from the variegated kernels showed small streaks of the $A_1$ type pigment in a non-pigmented background. And, as expected, all 54 plants derived from the colorless $sh_2$ class of kernels lacked anthocyanin pigment. Each plant was then tested for presence or absence of Spm by crossing it with a plant in an Spm tester stock.

To illustrate how the tester stocks can serve to reveal the presence or absence of Spm, those tests conducted with the 54 plants derived from the colorless, $sh_2$ class of kernels will be considered first. This is a completely objective test since the presence or absence in any one of them of Spm could not be assumed on the basis of phenotypic expressions. One or more ears of each of these plants received pollen from plants that were homogeneous for either state -- or -- of figure 1, for $Sh_2$ and for $y$; the pollen parents were uniformly pigmented indicating the absence of Spm in them according to the stated hypothesis. If the plant being tested has no Spm, then all of the kernels on the resulting ear will be uniformly colored. If, however, the plant being tested carries Spm, then it should be present in some of its gametes. Following introduction of the $a_{m-1}$ locus from the male parent, the presence of Spm in those kernels that received it from the female parent should be revealed by the appearance in them of small,
deeply pigmented spots in a colorless background due to activation of the \( a_{m-1} \) locus by the Spm element. In those kernels that did not receive Spm, the aleurone layer should be uniformly pigmented. Among the 30 plants derived from the colorless, \( sh_2 \), \( Y \) class of kernels, it could be determined on this basis that 15 had a single Spm element and 15 had no Spm. In 13 of the 15 plants that had Spm, linkage of it with \( Y \) was evident (A, table 2) but in the 2 remaining plants, no linkage of Spm with \( Y \) was evident (B, table 2). The reason for the absence of Spm with \( Y \) in these 2 plants will be considered in the next section. It need only be mentioned here that this is not unexpected.) Among the 24 plants derived from the colorless, \( sh_2 \), \( y \) class of kernels, 6 had a single Spm element (C, table 2), and 18 had no Spm.

Each of the 24 plants derived from the uniformly pigmented kernels was uniformly pigmented. Each was tested for presence or absence of Spm in the described manner and in none of them was Spm found to be present. All of the plants derived from the variegated kernels showed streaks of the \( A_1 \) type pigment in a non-pigmented background and the test crosses indicated the presence of Spm in each of them. In 16 of the 17 plants derived from the variegated \( Sh_2 \) \( Y \) class of kernels, one Spm element was present and in 15 of these plants, it was linked with \( Y \) (D, table 2). In one of these
plant, however, no evidence of linkage of the single Spm element with Y was noted (E, table 2). In the remaining plant in this group, 2 Spm elements were present, neither of which was linked with Y (F, table 2). More than one test cross ear was obtained from 11 of these 17 plants and the number and location of the Spm element was the same in the cells producing all ears except for one plant. In this plant, one Spm element was present in the cells producing the main ear and it was linked with Y (G-1, table 2). In the cells that produced the tiller ear, however, a single Spm element was present but it showed no linkage with Y (G-2, table 2).

In all 8 plants derived from the variegated Sh2 y class of kernels, one Spm was present (H, table 2). One Spm element was also present in the plant derived from the variegated sh2 Y kernel. This plant was used as a pollen parent in crosses with plants having different constitutions: homozygous also for a1 and sh2, and having no Spm, homozygous for several different states of a1 m-1 but having no Spm, and to plants heterozygous for different states of a1 m-1 Sh2/a1 sh2, but y/y, no Spm. In this third group, plants with different states of a1 m-1 were represented. All tests indicated the presence in the pollen parent of a1 m-1 in one sh2 carrying chromosome and of Spm in the Y carrying chromosome, and that this Spm element was activating the various different states of a1 m-1.
The tests described above were conducted with the progeny of a single plant in a culture. There were 19 variegated plants in this culture. Each was derived from a variegated kernel that appeared on an ear of a variegated plant that was $a_1^{m-1} Sh_2 / a_1 Sh_2$, $Y/y$, $wx/wx$ (chromosome 9), $pr/pr$ (chromosome 5) in constitution when pollen of a plant homozygous for $a_1$, $sh_2$, $y$, $Pr$, and $Wx$ and having no Spm had been placed on the silks of this ear. All kernels appearing on this ear were $Sh_2$ and the distribution of phenotypes among them were as follows: 28 uniformly dark pale $Y$, 7 uniformly dark pale $y$, 55 variegated (spots of deep pigmentation in a colorless background) $Y$, 86 variegated $y$, 75 colorless $Y$, and 103 colorless $y$. Among the kernels showing anthocyanin pigment, the ratio of uniformly dark pale colored kernels to variegated kernels indicated the presence of at least 2 and possibly 3 Spm elements in the pistillate parent plant and one of these appeared to be linked with $Y$. The silks of ears of 9 plants derived from the variegated $Y$ class of kernels on this ear and of 10 plants derived from the variegated $y$ class received pollen from plants that were homozygous for $a_1$, $sh_2$, and $y$, and had no Spm. The ratio of kernel types appearing on the resulting ears produced by each of these 9 plants is entered in table 3. In this table, the 9 plants are placed in four groups, A to D, according to the assumed constitution of Spm in each that the ratio of
kernel types suggested. The 6 plants in A of this table were assumed to have a single Spm element located in the Y bearing chromosome. Progeny from 4 of these 6 plants were grown and again tested for Spm. Those derived from plant 6629A-1, line 1 of A of table 3, were considered separate above. The total number of progeny plants in this group that were tested and their origins are entered in the last line of A of table 3. These tests verified the presence of Spm in the variegated kernels and plants and its absence in the uniformly pigmented kernels and plants. They also verified that assumed Spm constitution and location in the pistillate parent plant. Only those tests conducted with the 116 plants derived from the colorless sh2 class of kernels will be summarized here. Among the 56 plants derived from the al sh2 Y class of kernels, 32 carried Spm and 24 had no Spm. In 30 of the 32 plants having Spm, linkage of it with Y was expressed, I, table 2. In two plants, the Spm element was not linked with Y, as mentioned earlier (B, table 3). Among the 60 plants derived from the al sh2 Y class of kernels, 17 had a single Spm element (J, table 2) and 43 had no Spm. In order to verify the absence or linkage of Spm with Y, an ear of one of these plants had been self-pollinated
and another ear of this plant had been used in the cross with an \( a_{m-1}^m \) \( Sh_2 \) y, no Spm tester plant. The progeny from both of these ears were again tested for Spm constitution and location. The silks of ears of 6 plants derived from \( a_1 \) \( sh_2 \) kernels in the \( Y \) class on the self-pollinated ear received pollen from the \( a_1^m \) \( Sh_2 \) y, no Spm tester plants. From the kernel types on the resulting ears it could be concluded that 2 of 6 plants were \( Y/Y \) in constitution and that one of them had no Spm whereas the other had 1 Spm (199 pale colored kernels : 169 variegated kernels on the test cross ear). The remaining 6 plants were \( Y/y \). One Spm was present in 4 of them but it was not linked with \( Y \) (K, table 2). An Spm element appeared to be carried at allelic positions in a pair of chromosomes in the remaining 2 plants (I, table 2).

Seventeen plants derived from the variegated \( Sh_2 \) \( Y \) class of kernels on testcross ear of this same parent plant were crossed by plants in the \( Spm \) tester stocks. In 16 of these 17 plants, one Spm was present and on none of the ears produced by 15 of them was there any evidence of linkage of Spm with \( Y \) (K, table 2). However, the ratio of kernel types appearing on the test cross ear of one of them suggested such linkage (N, table 2). The remaining plant had 2 Spm elements, neither of which was linked with \( Y \) (O, table 2).
Tests of Spm constitution in 9 plants derived from the variegated Sh₂
Y kernels on the ear produced by the test cross with the other plant
entered in B of table 2, suggested that the Spm element in this plant had
been carried in the Y bearing chromosome but at a new location that was
farther removed from Y. Seven of the 9 progeny plants had a single Spm
element and in all of the test cross ears, loose linkage of \( \frac{Spm}{Y} \) with \( Y \) was
expressed (P, table 2). One plant of the 9 had 2 Spm elements (Q, table 2)
and in the remaining plant had 3 Spm elements (R, table 2).

Further examples of the progeny test method of determining Spm
constitution and location will not be given here. It should be mentioned,
however, that such tests were conducted with the indicated progeny of
the plants entered in B, C, and D of table 3, and also with the progeny of
the 10 other plants of this same culture. Also, many additional
were conducted with the progeny of plants having other states of \( a₃₋¹ \)
or combinations of states, i.e., carrying different states of \( a₃₋¹ \) in
each chromosome 3. The tests clearly support the 4 statements given on
page , and particularly statements (1) and (2). More direct support for
statements (3) and (4) was obtained by other tests to be described shortly.
Various different locations of Spm, either in different chromosomes of the
complement or in different locations within the same chromosome, were
discovered in these tests and each determined position of it was
subsequently verified by means of progeny tests. However, it is clear
from the tests so far described that Spm does not remain at one location
within the chromosome complement but disappears from a known location and
appears at a new location and this will be considered in a separate
section. Before this is described, it is necessary to show that the
Spm element, regardless of location, is capable of acting upon any one of
the states of $a_1^{m-1}$. 
Responses of different states of $a_{1}^{m-1}$ to the same Spm element

Evidence for statement (3), page xxv, appeared in all tests in which either one of the two states of $a_{1}^{m-1}$, present in the tester stocks, had been used in crosses with plants carrying Spm. Each state responded to Spm in its characteristic manner. In order to determine whether the Spm element present in a particular plant would be capable of activating other states of $a_{1}^{m-1}$, several additional types of test were performed. One of them utilized different ears produced by a single plant. In one such test, these plants were homozygous for $a_{1}$. Spm could be either present or absent in any one of them. Pollen from a plant of the tester stock carrying either state - or state - was placed on the silks of an ear of one such plant. The silks of another ear of the same plant received pollen from another plant that was homozygous for a different state of $a_{1}^{m-1}$ and in which no Spm was present. If the pistillate plant had no Spm, then all kernels on both ears were uniformly pigmented, and the intensity of this reflected the state of $a_{1}^{m-1}$ introduced by the pollen parent (excluding state -, figure 1, which gives colorless kernels in the absence of Spm). If, however, the pistillate parent carried Spm, then both variegated and non-variegated kernels appeared on each ear, but the phenotypes of the two classes of kernels on each ear, reflected the state of
\( a_{m-1} \) introduced by the pollen parent. Again, if the activating element showed linkage with a genetic factor among the kernels on one ear, the same linkage was usually expected. Usually, the same linkage on the other ear, and an example of this is given in 5 of table 2. In this test, the main ear of an \( a_{1}/a_{1}, Y/y \) plant received pollen from one of the Spm tester stocks. The kernel types on the ear this cross produced, 5-1, table 2, indicated the presence in the pistillate parent of an Spm element carried in the Y chromosome.

The silks of a tiller ear of this same plant received pollen from a plant that was homozygous for state 1, figure 1, and also for y. The pollen parent was uniformly pigmented indicating the absence of Spm in it. The kernel types on the ear this cross produced are entered in 5-2 of table 2. The ratio of variegated to non-variegated kernels in the Y and y classes was much the same on both ears. Thus, it could be concluded that the Spm element, carried in the Y chromosome of the pistillate plant, was capable of activating either state of \( a_{m-1} \). Pollen from the same collection that was used in the latter cross was also placed on the silks of a plant homozygous for \( a_{1} \) and for y, but in which Spm was known to be absent. All of the 29 kernels on the ear this cross produced were uniformly lightly pigmented and all were y. This test confirmed the absence of Spm in the pollen parent.
Another type of test that was employed to indicate the response of different states of \( a_1 \text{m-1} \) to the same Spm element, utilized the pollen of plants that were homozygous for \( a_1 \) and in which \( \bar{a}_1 \) Spm element was present. The types of test conducted with two such plants, number 6861-1 and 6861-7, are illustrated in table 4. Both of these plants were homozygous for \( a_1 \) and \( sh_2 \), and a single Spm element was present in each as shown in the ratio appearing in the table. The silks of an ear of each plant received pollen from the \( a_1 \text{m-1} \) tester stock that carries state 5718. The types of kernels on the resulting ear are entered in A of table 4. These ratios indicate the presence of \( \frac{1}{2} \) Spm in each plant. Both plants were used as pollen parents in crosses with plants that were homozygous for state 5719A-1 but in which no Spm was present. The types of kernels on the ears resulting from these crosses are entered in B of table 4. Again, a 1 : 1 ratio of variegated (Spm) to non-variegated (no Spm) kernels appeared on these ears, indicating the presence of \( 1 \) Spm in each of the two pollen parents. These same two plants were also used as pollen parents in crosses with plants that were \( a_1 \text{m-1} \) \( Sh_2/ a_1 sh_2 \) in constitution and had no Spm. The state of \( a_1 \text{m-1} \) in these plants was that which gives no anthocyanin pigmentation in the kernel and plant in the absence of Spm (state 5720, figure 1) but gives many mutations to the lower alleles of \( A_1 \) in its presence.
Other ears of these same plants received pollen from plants that were homozygous for $a_1$ and $sh_2$ but had no Spm. The types of kernels appearing on the ears resulting from each of these two types of cross are entered in C of table 4. Again, it is evident that plants 6861-1 and -7 each have one Spm element that is capable of acting on this state of $a_1^{m-l}$. The results obtained from the described tests are those to be expected if the Spm element in plants 6861-1 and -7 is capable of acting on different states of $a_1^{m-l}$. The same type of test as that just described was conducted with $a_1^{m-l} sh_2/a_1 sh_2$ plants having more than one Spm element and the ratio of kernel types on the test cross ears was that expected if each of the Spm elements present in the $a_1^{m-l} sh_2/a_1 sh_2$ plant was capable of acting on each of the states of $a_1^{m-l}$.

Still other types of test were conducted to determine the capacity of a Spm element to act on different states of $a_1^{m-l}$. One of them utilized the pollen of a plant that was $a_1^{m-l} sh_2/a_1 sh_2$ in which a single Spm element was present at a known location in the chromosome complement. When such a plant was used as a pollen parent in crosses to plants that were $a_1^{m-l} Sh_2/a_1 sh_2$ and having no Spm but among which different states of $a_1^{m-l}$ were represented, the types of kernels on the resulting ears clearly indicated the capacity of the Spm element in the male parent to act not only upon the state
of the \( a_{1}^{m-l} \) locus delivered by the male parent, but also upon the state of the \( a_{1}^{m-l} \) locus delivered by the female parent. Again, when plants carrying Spm that were \( a_{1}^{m-l} sh_{2}/ a_{1} sh_{2} \), among which different states of \( a_{1}^{m-l} \) were represented, were used as pistillate parents in crosses with a plant that was homozygous for one state of \( a_{1}^{m-l} \) and also for \( sh_{2} \) but in which no Spm was present, activation of the \( a_{1}^{m-l} \) state delivered by the male parent by the Spm element delivered by the female parent was indicated in all tests of this type.

Detailed consideration of the various types of test mentioned above cannot be given here. However, all of them clearly established the similarity of the Spm element carried in the many different tested plants, and regardless of its number or its location in the chromosome complement of a given plant. They also established the ability of the Spm element to act upon any of the selected states of \( a_{1}^{m-l} \) and they indicated that control of type of gene action in the absence of Spm and control of the as well as its type in the presence of Spm is solely time and frequency of occurrence of mutation a function of the state of \( a_{1}^{m-l} \).