

THE SUPPRESSOR-MUTATOR SYSTEM OF CONTROL OF GENE ACTION IN MAIZE

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Part I

The manner by which controlling elements in maize affect the action of genes and induce specific changes in this, has been examined by a number of investigators in recent years. Several ^{independent} systems, each composed of one or more controlling elements, have been identified. It is ^{considered} ~~recognized~~ that controlling elements are ^{chromosomal} ~~unit components~~ apart from the genes even though they are carried by the chromosomes and reside at specific sites in them. It was possible to ~~determine~~ this when it was learned that these elements may undergo transposition from one site to another in the chromosome complement. It was further learned that the mode of control of gene action was a reflection of the specificity of operation of the individual components of a system and ^{the} interactions that occur between ^{them}. Furthermore, it was learned that one such system would control the action of different genes, in like manner.

For induction of operation of a particular system at a known gene locus, it is only necessary that one of its elements be inserted at a site within or close to the locus of the gene. This is shown in the two known cases of control of gene action by the element Ac (Activator) at the P locus in chromosome 1 and the Bz₁ locus in chromosome 9

(Brink et al,

McClintock, 1956 a, b, c).*

*
Footnote:

Brink and his associates have used the term Modulator (Mp) instead of Activator (Ac). This term was given to the controlling element at the P locus before it was recognized to^{be} the same Ac element that operates in the Ds-Ac system, first reported in 1947.)

A system composed of the above mentioned Ac element and a second element, designated Dissoaiation (Ds), also has shown that the mode of control of gene action resides in the ~~two~~ controlling elements. In this system, it is the Ds element that becomes inserted at or close to the locus of the gene whose action is being controlled. The operation of this system at a number of different gene loci ~~has been extensively~~^{was} examined by the author (McClintock, 1947 to 1956) and ~~an independently~~ arising case of its ~~action~~^{operation} at the Bz₂ locus in chromosome 1 ~~has been~~^{was} investigated by Nuffer (195). This system also has been used for specific purposes by a number of other persons (Brink et al.,

Fabage, Schwartz, Oliver, Notani, Peterson,
Dollinger, Sprague).

The Suppressor-mutator (Spm) system is another ~~one~~ in which the mode of control of gene action by its componenet elements could be examined at more than one gene locus. ~~In this report, a detailed account of this~~

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Its ~~mode of~~ ^{independent control of gene expression} operation at ~~the~~ two ~~gene~~ loci A_1 in chromosome 3 and ^{at that of} A_2 in chromosome 5, ~~has~~ been compared. Both A_1 and A_2 are

associated with the production of anthocyanin pigmentation in plant and kernel. ^{or} When a plant or kernel is homozygous for ^{the standard} a recessive ^{pigment} allele, either a_1 or a_2 , no anthocyanin appears in plant or kernel.

When ^{both} ~~A_1 and A_2~~ are present, ~~both~~ the plant and the kernel are deeply pigment. ^{on this study} ~~Of the two cases~~, the Spm system of control ^{of gene action appeared} ~~arose~~ first at the locus of A_2 and in the course of ^{examination} ~~study~~ of instability of expression of a gene associated with chlorophyll development. In this ^{examination} ~~study~~, a number of plants of a culture were self-pollinated and on the ear produced by one plant, kernels appeared that exhibited variegation for anthocyanin pigmentation. Each showed ^{some} areas of deep pigmentation, ^{and in some kernels} other areas of pale pigmentation ^{in a background that was} ~~and still other areas that were~~ totally colorless.

^{The attempt to learn} ~~Examination~~ of the system responsible for the newly appearing ^{anthocyanin} variegation commenced with plants grown from kernels on the above mentioned ear. It was soon realized that the variegation, ^{instability of gene expression, responsible for the} was initiated ^{that had occurred to} by some modification ^{of} the standard A_2 locus in chromosome 5, ~~and that~~ ~~this was associated with the instability of its expression.~~ This ^{varieties of gene express in different parts of} ~~instability~~ could be exhibited in plant or kernel when the modified locus

was either homozygous or was heterozygous with the standard recessive, a_2 . The modified A_2 locus was then given the designation a_2^{m-1} and study of it was continued.

best
 No clear impression of the mode of operation of the system

concluded from observations and tests
 responsible for variegation ~~was gained from the~~ early study of a_2^{m-1}

numerous types of different patterns of variegation were noted in this

~~In the course of this, however,~~ the silks of an ear of a plant carrying *during this period,*

the system responsible for control of gene action at a_2^{m-1} received

pollen from a plant that was homozygous for the standard recessive, a_1 ,

carried in chromosome 3, and for the standard A_2 , carried in chromosome 5.

In this cross, all of the kernels on the ear were expected to be fully

pigmented. This was found for all kernels except one. The exceptional

kernel exhibited variegation for anthocyanin pigmentation. It had

spots of deep pigmentation in a colorless background. The plant grown

from it also exhibited variegation for anthocyanin pigmentation and tests

conducted with it indicated that the variegation was associated with ~~some~~

modification of the standard A_1 locus that had occurred in a cell whose

descendants produced the female gametophyte in the ovule that gave rise

to the original variegated kernel. The ~~new~~ ^{ad} ~~modification~~ ^{locus} was then

given the designation a_1^{m-1} and study of it was continued.

Early examination of a_1^{m-1} behavior suggested that a relatively simple system was associated with control of gene action but as the study progressed, it became evident that the system was far from being a simple one. Nevertheless, some appreciation of its mode of operation was gained during this period. At the time, study of a_2^{m-1} ~~was~~ *had been* discontinued as no ~~clues~~ ^{clue} had been grasped of the mode of operation of the system associated with it. After the basic mechanism responsible for control of gene expression at a_1^{m-1} was recognized, study of a_2^{m-1} was recommenced, with the expectation that some similarity ^{ps} might be found in the control systems associated with each. It was ~~soon~~ ^{subsequently} realized that the mode of control of gene expression was essentially ~~the same~~ ^{similar} for both a_1^{m-1} and a_2^{m-1} . With this in mind, further tests were conducted and these, in turn, proved that gene action at both of these loci was controlled by the very same system of elements. By means of these concurrent studies of a_1^{m-1} and a_2^{m-1} , confounding aspects of behavior given by each, when examined alone, were resolved. It then became apparent that lack of recognition of the cyclically occurring changes in action undergone by the dominant element of the system, Suppressor-mutator (Spm), was ^{one of} the main ~~reason for confused impressions of the mode of operation of the system associated with control of gene action at a_2^{m-1} involved gained in the early part of this study.~~ *cause of confusion in attempts to find an interpretation of* ^{associated with control of gene action at a_2^{m-1}} *These cycles of*

control

of change in phase of activity of Spm are responsible for many different types of phenotypic expression that appear within individual plants or kernels and also for highly discrepant ratios of particular phenotypes that appear in progeny derived from either self-pollination or from test crosses of some plants.

2. Mode of operation of the Spm System a) *Basic elements of the system.*
- An outline of the mode of operation of the Spm system will be given here in order that the following sections, each dealing with some particular aspect of this, may be read with ^{an} appreciation of the operation of the system as a whole. The Spm system is considered to be composed, ^{assumed to be} ~~primarily~~ ^{basically}, of two ~~basic~~ controlling elements. One of these is located at or close to the gene whose action is being controlled, and in the cases under discussion, at the locus of A_1 in chromosome 3 and of A_2 in chromosome 5. The other controlling element, Spm, is ^{known to be} located elsewhere in the chromosome complement, ^{and it may} ~~This latter element~~ undergo ~~transposition~~ transposition from one location to another, ~~in the complement~~. On different occasions, ~~the~~ transposition has resulted in insertion of it at a site sufficiently close to a known gene marker to allow ready detection of the new location by means of linkage studies with the marker; and its insertion at and transposition from ~~specific~~ sites in chromosomes 3, 5, 6, and 9 have been examined.

It has not yet been proved that a transposable element resides at the locus of the gene whose action is being controlled by the Spm system. That this is to be expected is indicated by evidence of the ~~presence~~ presence of such an element at ^a ~~the~~ gene locus ^{exhibiting instability of expression} obtained from study of another two element control system, Ds- Ac. It could be shown that the Ds element of this system is inserted at or near the locus of the gene whose action is being controlled by the system, and that Ac resides elsewhere in the complement. ^{Both Ac and} Ds ^{are} ~~is known to be~~ a transposable element.

Again, the fact that a_1^{m-1} arose in a plant in which the a_2^{m-1} -Spm system was operating, and that the behavior of both a_1^{m-1} and a_2^{m-1} are impressively alike, strongly supports the assumption that ~~the same~~ ^{and that it is the same element in both cases} a transposable element resides at each of these two loci, ~~and that each~~ ^{at may be considered, then, that each} arose from insertion of this element, first from an unknown location in the chromosome complement to the locus of A_2 and subsequently to the locus of A_1 .

b) *Mode of action and the behavior of the Spm element.*

In each of the two cases under discussion, modification of gene action probably occurred as an immediate consequence of insertion of ~~the~~ ^{the} ~~controlling~~ ^{above mentioned} element at the locus. In both cases, ~~this~~ ^{it} appears to have effected a reduction in capacity of the gene to contribute to

anthocyanin pigment formation. This reduced capacity is exhibited when the Spm element is absent or inactive either in a plant or a kernel ^{or when it is inactive in some part of a plant or kernel.} ~~or when it is inactive in some part of a plant or kernel.~~

← In plants and kernels that ~~are~~ ^{have} A_1 and A_2 , anthocyanin pigment intensity is deep either when Spm is present or when it is absent.

In contrast to this, pigment intensity is reduced in those kernels and plants that are homozygous for the original state of either a_1^{m-1} or a_2^{m-1} or are heterozygous ^{with} for one of these and the corresponding recessive allele ^{is present but is} whenever, Spm ~~is~~ absent or inactive. When, however, ^{Spm} ~~it~~ is present

* ~~Footnote.~~ The constitutions of these plants would be:
 for a_1^{m-1} : A_2A_2 or A_2/a_2 ; a_1^{m-1}/a_1^{m-1} or a_1^{m-1}/a_1 and
 for a_2^{m-1} : A_1A_1 or A_1/a_1 ; a_2^{m-1}/a_2^{m-1} or a_2^{m-1}/a_2

and active ⁱⁿ both plants and kernels ^{having one of} these ~~same~~ constitutions, anthocyanin pigment formation ^{appears but only} ~~is suppressed~~ except in well defined areas, ~~of either plant or kernel.~~ Within these areas, pigment appears. Three distinctly different types of events are responsible for the appearance of these pigmented areas. The first of them is associated with some ^{i.e., at} ~~mutation-type~~ event occurring at the locus of the gene concerned, either a_1^{m-1} or a_2^{m-1} as the case may be. ^{Such an event can} This results in reestablishment of

the full or near full capacity of the locus to contribute to anthocyanin production formation. The ^{re mutation-type} events occur in individual cells whose progeny cells

may then exhibit intense pigmentation. When ^{one such} ~~this~~ occurs in a cell of

the germ line of a plant, gametes may be formed that carry the locus with restored ^{gene} activity, and ^{Cases of this} ~~its presence~~ may be detected ^{among individuals of} the progeny.

Tests of such ^{individuals} ~~progeny~~ indicate that this ^{mutation-type event} ~~modification~~ results in stability for no further changes in its activity occur ^{with} the of gene expression ~~both~~ in the presence and/absence of Spm.

^{Although} The second and third ~~types~~ of event ^{may result in the appearance of} ~~give rise to quite similar~~ ^{phenotypes}, the nature of the ^{newly} event ^{is part the same} appearing areas ~~but~~, the event responsible for each ^{is} different and the consequences of the ~~event~~ ^{each} are ~~also~~ distinctly different. The first of

these is associated with a ~~somatically occurring~~ trans position of Spm ^{from one location to another in the chromatin complex}

^{a somatic nucleus} which, through ^{subsequent} segregation of chromatids at ^{the following} a mitotic anaphase, ~~removed~~ ^{one of the two sister} from ~~the~~ nucleus. The progeny cells arising from one in which

Spm has been ^{so} removed ^{show} exhibit the ^{same} reduced grade of pigment intensity

that characterizes the appearance ^{of} whole plants or kernels in which Spm is ^{totally} absent, ^{and the area is uniformly pigmented; no variation appears within it.} ~~The phenotypic expression within the area or progeny~~ ^{ears or from pollen produced within} kernels and plants derived from such an area, is quite stable. ~~Since~~

^{in case other} ~~Spm is absent~~, no further change occurs ~~at the locus of the gene involved,~~ unless Spm is again introduced in some subsequent cross.

The third type of event gives rise to pigmented areas exhibiting the very same phenotype as that just described. However, the cause for ^{it}

this is not removal of Spm from the nucleus but rather its complete inactivation without change in ^{to} location. The phenotypic expression ^{resulting}

^{from} this inactivation ~~produces~~ is stable as long as Spm remains in ~~its~~ ^{the} inactive phase. Return to the active phase ~~in~~ some subsequent cell again will initiate not only suppression of gene action in the immediate progeny cells, but also it will initiate ^{one more} ~~again~~ the same round of events just described. Consequently, within some of the large lightly pigmented areas of a plant or kernel, smaller, non-pigmented areas may be formed and within them, in turn, both deeply pigmented and lightly pigmented areas may appear. Whether or not a variegated pattern of the type just described will appear following a particular inactivation of Spm, and ^y ~~and~~ should it appear, just what type of pattern of variegation will be exhibited, depends upon the duration of the inactive phase of Spm. Following some inactivations, the inactive phase may persist through many cell or even plant generations. Following others, the duration of the inactive phase may be short, with frequently occurring returns to the active phase in some cells that ^{maybe} ~~are~~ only a few cell generations removed from that in which inactivation occurred. And, a large range of difference in duration of either ^{an} ~~the~~ inactive or the ~~inactive~~ phase of Spm has been noted.

On the basis of the above description of events occurring at the locus of either a_1^{m-1} or a_2^{m-1} and ^{also} to the Spm element ^{itself}, it is evident that ~~the~~ variegated patterns in plants ^a may be simple and readily interpreted if ~~the~~ Spm ^{element in it is one with} with a long duration ^{an} of its active phase ^{of long duration,} ~~is present~~ and if transposition of it is infrequent or does not occur during ~~during~~ developmental stages.

In contrast, the pattern may be very complex if the active phase ^{and is followed by inactive phase of various duration,} of Spm is of short duration or if transpositions of it occur in a number of cells ¹ rather early in development.

in a plant kernel

When two or more active Spm elements are present, the frequency of occurrence of the mutation-inducing events at either a_1^{m-1} or a_2^{m-1} remains the same. In other words, the pattern of variegation ^{associated} produced ~~with the mutation-producing events~~ ^{increased} by ~~such events~~ ^{unaffected} is ~~unaltered~~ by/dose of the Spm element. In contrast, the pattern of variegation induced by transposition or ^{by} change in phase of Spm is much altered by increased dose of Spm. The number of pigmented areas attributable to these events is decreased as the Spm number increases. For this reason, the number of Spm elements in a plant or kernel ~~is able to~~ ^{effects} in a marked way the over-all pattern of variegation. ~~observed~~ However, in any one plant or kernel ~~that~~ ^{exhibits} only that pattern of variegation attributable to mutation at the gene locus, it is not possible to know without test whether this pattern is

produced because ^{of the presence of} two or more ^{active} Spm elements ~~are present~~ or because of the ^{whether it is produced}

presence of only one Spm element which has a very long duration of its active phase, ^{also} and ^{involves} few or no transpositions during early + mid-developmental stages.

The time of change in phase of activity of Spm during development of plant or kernel also ^{affects the} ~~contributed to modification~~ of patterns of variegation. With the original state of both a_1^{m-1} and a_2^{m-1} , mutations ^{in some cells} to a full or near full expression of gene action ~~may~~ occur early in plant or kernel development if a fully active Spm element ~~was~~ ^{is} present w_1

at the beginning of development. Such mutations give rise to large, deeply-pigmented areas in ~~both~~ ^{either} plant ^{or} and kernel. However, if an

inactive Spm is present ^{initially} that returns to the active phase in some cells ~~but~~ only late in development, ^{that} only small areas ^{may be present} are ~~produced~~ in which

it is active. These areas are characterized by the ^{appearance} ~~presence~~ of small spots of deep pigmentation ~~appearing~~ in a non-pigmented background.

Since mutation to full capacity of gene action ~~can~~ occurs only ^{in some cells} when Spm is active, delayed ⁱⁿ ~~change in phase of activity~~ ^{from the inactive to its active phase} of Spm ^{would} give rise ^{under these conditions} only to small spots of deep pigmentation, within

^{all} the restricted areas in which it is active. ^{from the above discussion,} It is evident, ^{then,} that the type of variegation exhibited by a plant or kernel may be very ^{complex} ~~complicated~~

if the Spm element in it is undergoing change in phase of activity at

that occurs in cells

at different times during development. It is largely because of this that the early study of a_2^{m-1} lead to no clear understanding of the system involved in its control, although other conditions, which will be mentioned ^{later} ~~shortly~~, likewise contributed to this. In contrast, appreciation of the system controlling a_1^{m-1} behavior was more readily attained. The reason for this is now ^{clear} ~~evident~~. In the early studies of it, the Spm element present in the plants ^{investigated} had a very long duration of its active phase. So few changes to the inactive phase occurred during development that these did not obscure the generally consistent

And, it was this consistent ^{of the same manner of operation of the} pattern of behavior that allowed interpretations to be drawn ^{and tests of} ~~and tests of~~ ^{variation and inherent behavior that was observed} ~~them to be made.~~

c) ~~The~~ States of a_1^{m-1} and a_2^{m-1}

One of the most ^{striking manifestations} ~~consistent~~ aspects of the ^{presence} ~~behavior~~ of a controlling element at a gene locus is the modification of ~~it or~~ induced by it that ^{results} ~~will give rise~~ ^m ~~to~~ ^{in expression of mutation,} ~~subsequently to~~ change either in frequency of ^{of this,} ~~visible~~ mutation ^{or} ~~at the locus of the gene,~~ ^{the} ~~in~~ type of mutation, or both, in) ^{the pattern of variation resulting from this, is in marked contrast} ~~type and frequency of mutation, in comparison to that which was appearing~~

^{expressed} ~~before this~~ modification occurred. ¹ Since each such modification is ^{with other conditions being equal, each will} ~~with other conditions being equal, each will~~ ^{typical and} ~~heritable in that it~~ continues to produce its own particular pattern ^{Therefore, each such modification must be} ~~and type of mutation in subsequent generations, it is called a change in~~

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and with respect to a_1^{m-1} and a_2^{m-1} , these are termed changes in state of a_1^{m-1} or a_2^{m-1} .

Study of several different control systems indicates that all manifestations of change in action of the gene ^{mutate} ~~of a~~ heritable ~~type~~, _^ whether it be a stable mutation or an altered state, are expressions of modification induced by the controlling element of the system ~~associated~~ ^{with} resides at the locus of the gene. In a two element system such as Ac-Ds, Dt-a₁, En-palegreen, etc., ^{each} the modification ^{represents} ~~arises as a consequence~~ of ^a ~~the~~ response of the ~~element~~ at the gene locus to the presence of the independently located element of the system, which is Spm in the cases under consideration. Therefore, a change in state of a_1^{m-1} or a_2^{m-1} will occur only when this element is present and active and only at those times during development when mutation may also occur. A change in state is, then, only one of several possible consequences of a single response of the element associated with the gene to the presence of the complementary element of the system.

^{to new state} ^{mixed}
From ~~consideration of~~ [^] origins of new states, ~~given above~~, it is [^] ~~clear~~ ^{evident} that isolation of a newly altered state for subsequent examination requires that it arise in some cell of the germ line and be present in a gamete. Therefore, the chance ^{of} isolation of a new state depends

largely upon the time during development of a plant when the controlling element associated with the gene locus is responsive to the independently located element of the system and, indeed, ^(within the same system) the time of this is one of the distinctive expressions of state. ^{and} If, in a plant, the state is ^{one in which} such that it responds relatively early in development, and if such responses occur in sporogenous cells, a number of gametes may be produced, each of which carries either a mutation or an altered state. Both types of change may be detected in the progeny of this plant, and each may thereby be isolated for subsequent study. If, on the other hand, the state is one in which the element associated with the gene locus responds to the independently located element only very late in development of the plant, few or no gametes may be produced that carry either a mutation or an

altered state. ^{From} ~~With~~ the former state, ^{new} ~~changes to other~~ states ^{be detected} would arise with appreciable frequencies, whereas ~~with~~ the later state they would ^{arise} arise

be detected

either very rarely or not at all. ^{from} ~~Considering~~ the ^{described conditions relating to} ~~factors~~ associated

in the process

^{detection} ~~with their~~ origin and isolation, ^{a new state makes it} ~~it is~~ evident that no conclusions ~~are~~

regarding relative stabilities of the two states are justified from ^{frequency of appearance of new states in process tests} comparisons of ~~this type~~. With the latter state, as many changes of state ^{be produced} may occur as with the former but there is no way of detecting and isolating ^{many of those that do occur} those produced by the latter. Therefore, in this study, ~~with~~ each new

state was derived from one in which response of the ^{gene-associated} element ~~associated~~
~~with the gene locus~~ to an active Spm occurred relatively early in
 plant development.

As stated above, mutation to a stable expression of gene action ^{or} ~~and~~ to
 change in state ^{is} ~~are~~ the consequence of ^a response of the gene-associated
 element to Spm. Methods are available for determining the relative
 frequency of occurrence of different types of mutation to a stable gene
 expression but it is difficult, if not impossible, to estimate accurately
 the relative frequency of occurrence of modifications effecting change in
 state. This is because ~~some of them give a type of expression that~~ ^{the} ^{expression of them}
~~differs only slightly from that/which it originated, and it is often~~
~~difficult to detected~~ ^{such} ^{in individual kinds,} cases of this. [↑] Those that effect a marked change
 are, ^{in expression} (On the other hand,) readily recognized, and ^{it is} ^{evident} with both
 a_1^{m-1} and a_2^{m-1} that the ^{appearance} ~~frequency~~ of such ^{markedly altered} ^{strikingly} changed states is considerably
^{frequent} ~~less than the frequency of appearance of modifications effecting a stable~~
 gene expression.

From the original state of a_1^{m-1} , whose gene-associated controlling element may respond to an active Spm very early in development, 18 independently arising changes of state were selected. Each was recognized in an individual kernel appearing on ~~an~~ ^{those} ears that developed when pollen of the original plant carrying a_1^{m-1} was placed on silks ~~of~~ ^{is quite stable in the presence of Spm.} ears of plants that were homozygous for the standard recessive, a_1 .

Observations were made of the variegated pattern in the plant derived from each, and each, in turn, was crossed to plants homozygous for a_1 in order to examine the expression of the modified a_1^{m-1} locus in its ~~the~~ progeny. ~~Subsequent~~ ^{of the altered state} tests were then conducted with the plants derived from selected kernels in the progeny in order to establish the mode of expression in subsequent generations, ^{both in the presence and absence of Spm.}

With regard to ~~expression~~ ^{patterns} of variegation, the 18 selected cases of change in state of a_1^{m-1} , ~~the 18 selected cases~~ ^{is added into} could be placed within five main groups. The ~~2~~ ^{are} in the first group ~~were~~ characterized by some early occurring mutations to full or near full A_1 expression and very ^{and an active Spm is required for this} many late occurring mutations of this type. In contrast, the original state gives rise to many early occurring mutations ^{and} to various ^{different} levels of A_1 expression, ^{but there are} and ~~to~~ fewer later occurring ones. In the absence

In the absence of Spm, the expression of the ~~two~~^{two} altered states in the first group differed. With each, pigment developed slowly in the plant until it is quite intense but in the kernels, one of the ~~so~~ two is characterized by the appearance only of faint pigmentation whereas the other ~~gives rise to deeply pigmented kernels.~~ In this regard, the original state expresses a medium grade of pigment intensity in the kernels in the absence of Spm.

However, the other are much more intensely pigmented
~~There were~~ ^{Fur} ~~altered states~~ ^{could be placed in a} ~~in the~~ second group. These ~~were~~^{are} characterized by production, in the presence of Spm, of late and very frequently occurring mutations in both plant and kernel, which usually gave the full or near full expression of pigment intensity. Again, subdivisions of them could be made because of differences in degree of pigment intensity that were expressed in kernels in the absence of Spm.

A Four of the 18 selected altered states fall within a third group.

These ~~were~~^{are} characterized by the appearance, when Spm was present, of ~~mutant areas that are small because the mutation-inducing events~~
~~mutations that occurred~~ late in development of both plant and kernel, ~~but~~

However, The frequency of ~~this was~~^{such events} ~~to~~^{was} much less than that ~~given~~^{exhibited} by members of the group just described. Here, also, they differed among themselves with regard to intensity of pigment appearing in ~~the~~ kernels when Spm was absent.

are
There ~~were~~ two altered states in the fourth group. Very few

^{Spm} mutations to an A_1 ~~type expression occurred~~ ^{phenotype are expressed} in either plant or kernels
for the reasons such a phenotype are always small.
and all of them arose late in development, ^{selected} The remaining 5/cases fall

within a fifth group. In the presence of Spm, nearly all of the mutations

~~xxxxxx~~ expressed ^d only low levels of pigment intensity and the type of

pigment appears to differ ^{With two of these states,} from that produced by A_1 . ~~in some of them,~~

an occasionally mutant area ^s appeared ^s that exhibited the full A_1 type

but the frequency of appearance of such areas is low and the areas always is small.
pigmentation, ~~There are differences among them~~ The states within this

group differ from one another ^{not only} both in the time of occurrence of mutation,

in the presence of Spm,

and also in the degree of pigment production in ⁱⁿ the absence, ~~of Spm.~~

^{anthocyanin}
With one, no/pigment is produced in either plant or kernel in the absence

of Spm. In this respect, the phenotype is quite similar to that

appearing in plants and kernels that are homozygous for the standard a_1

recessive.

From the total of 18 examined ~~cases of altered state~~, 6 have been
widely used over a period of years in study of the Spm system.

Photographs of kernels, figures , illustrate the the phenotype each

produced in both the presence and ^{the} absence of Spm.

Detection and analysis of altered states of a_1^{m-1} was uncomplicated because, as mentioned earlier, the Spm element in the original plant, and in the progeny derived from it, had an active phase of long duration.

Had ~~they~~ ^{it} undergone frequent change in phase of activity, variegation patterns and inheritance behavior would have been so complex that ^{most likely} no certain conclusions ~~would~~ have been drawn from them of the precise ^{nature of}

~~the~~ state of a_1^{m-1} that might be present in a particular plant or kernel.

Such, ^{indeed, conditions} were the ~~circumstances~~ encountered in early study of a_2^{m-1} .

Variegation patterns and inheritance behavior were so complex that even though different states were recognized, a precise characterization of each could not be formulated ^{then} ~~as~~ ^{because} the ~~contribution~~ ^{relation} of cyclical change

in activity phase of Spm to altered expression of variegation and ~~to~~ ^{irregular nature of phenotypes in progeny tests} ~~patterns of inheritance~~ had not yet been ^{recognized} discovered. It was not until

these states were examined in the presence of an Spm element ~~with~~ ~~axiang~~ an active phase of long duration that it was possible to characterize them precisely. It was then found that their types were similar to those that had appeared in the a_1^{m-1} cultures. There was one, however, that was quite unlike any of the selected states of a_1^{m-1} . Initially, its behavior was an additional cause of confused impressions of the mode of

operation of the system responsible for control of gene action at a_2^{m-1} that were generated early in the study. Subsequently, it proved an invaluable tool in analysis of this system. In the absence of Spm, or when it is inactive, plants and kernels having this state are intensely pigmented and the degree of this is nearly equivalent to that produced ~~when~~ ^{by} the standard A_2 , ~~is present~~. When an Active Spm is present, all pigment formation is suppressed in the kernels, except in those cells in which Spm has become inactive or has been removed by transposition. No mutations to give a stable A_1 expression have yet been identified. Thus, the variegation patterns appearing when this state is present are reflections solely of modifications occurring to the Spm element itself, either transposition of it or change in phase of its activity, and the ~~use~~ ^{usefulness} of this state ^{for investigating} ~~in analysis of~~ cycles of activity of Spm will be described in one of the sections of this report.

Some definite conclusions may be drawn regarding the significance of state in control ^{of} ~~of~~ variegation expression. For this purpose, all examined states of either a_1^{m-1} or a_2^{m-1} that give rise to stable mutations, ~~which are expressed by type~~ ^{in both and intensity} of anthocyanin pigment ~~formation and~~ ^{activity} ~~by intensity of this~~, may be grouped together, and this includes all of them except the one just mentioned above. It ~~is~~ ^{maybe} concluded that in the

presence of an active Spm element, both the time of occurrence of mutation during development of plant or kernel and the type of this that ^{and intensity of any one type} ~~may occur~~, are expressions of the state of the a_1^{m-1} or the a_2^{m-1} that may be present in a particular plant or kernel, ^{this expression will be constant regardless of} ~~for increased doses~~ of Spm, ~~do not alter this expression~~. Again, the type and intensity of anthocyanin pigment formation in kernel and plant in the absence of Spm or when it is present but inactive, is also an expression of state.

ff The different states ^{$a_1^{m-1} + a_2^{m-1}$} ~~have been~~ useful in this study. ^{at times it was} ~~It has been~~ necessary to select ^a particular states ~~for~~ ~~analysis~~ specific purposes of analysis, and the reasons for this will be made apparent in later ^{requirements for solution of a particular problem} sections when the ~~purposes~~ are discussed.

ff In the absence of Spm, all states are quite stable and ^{some} ~~many~~ of them have been carried through many plant generations without ~~giving~~ any indication of change. Those states that give rise to early occurring mutation in the presence of Spm ^{an active also} give rise in its presence to some ^{newly} ~~nearly~~ altered states ^{and} that may be recognized in individual progeny ~~derived~~ ^{sub} from plants, ~~having one such state~~. In contrast, those states that are characterized by very late occurring mutations do not give rise to or must do so very rarely altered states in the presence of Spm. ^{also at times} In studies ~~of~~ ^{conducted with} two of them, and carried through 9 generations of plants, not one case of altered state has been encountered.

d). Types of change in action of Spm other than those related to its cycles of activity.

In addition to the activity cycles undergone by Spm, mentioned earlier, another type of modification occurs to it that effects a marked alteration in pattern of mutation produced by different states of a_1^{m-1} or a_2^{m-1} . These modifications appear to arise in individual cells in which a fully active Spm element had been present. The descendent cells then express the presence of the modified Spm element in them by exhibiting markedly altered patterns of mutation. Evidence now available does not suggest that the responsible event is associated with change in location of Spm in the chromosome complement, for it has been determined in several cases that an Spm element whose location is known may undergo such a modification without coincident ^{detectable} transposition.

The ^{type of} modifications of Spm under consideration, effect its capacity to suppress gene action at either a_1^{m-1} or a_2^{m-1} and to induce mutation ^{of either of them} to stable alleles. When one such Spm element is present, anthocyanin pigment, resembling that appearing in the absence of an Spm element, is produced in plants but it develops at a very slow rate. No pigment may appear in young plants but it develops gradually as the plant ^{progresses towards} ~~reaches~~ maturity. In kernels, the capacity of such an Spm element to suppress gene action likewise is

reduced but the degree of this is less marked than in the plant. The difference may be related to the more limited time that is available for accumulation of pigment in the kernel. Its rapid maturity stops the process. Whatever may be the cause, very little or sometimes no pigment of the type produced in the absence of Spm appears in kernels having one such modified Spm element. However, spots of pigment derived from mutation at either a_1^{m-1} or a_2^{m-1} , as the case may be, do appear but the number of them is much lower than that produced with a fully active Spm element. For each state, the reduction is expressed as a proportion of that given when a fully active Spm is present. Both the suppressive and mutative capacity of Spm appears to be weakened, and for this reason, such modified Spm elements are designated Spm-w in contrast to the so-called standard Spm element that gives full suppression of gene action of a_1^{m-1} or a_2^{m-1} and a constant and predictable pattern of mutation with any one state of a_1^{m-1} and with all but one exceptional state of a_2^{m-1} .

In subsequent discussions that deal with Spm-w, it will be necessary to distinguish it from the standard Spm element. Therefore, in these discussions, the standard Spm element will be ~~designated~~ symbolized as Spm-s.

