class rather than the pale class. Because of the very sharp expression of variegation among the Spm carrying kernels and also because of the faint pigment produced in its absence, this state of a\(^{m-1}\) has been useful in a number of studies of the Spm system.

Subsequent tests conducted with plants having state 5718 a\(^{m-1}\) and Spm have indicated that the appearance of kernels having the A\(_1\) phenotype in its progeny is rare. The number with this phenotype derived from tests of plant 6046B-4 entered in table 15, is unusually high. In this case, the same collection of pollen was used for both the self-pollination and the test cross entered in this table. It is suspected that the tassel of this plant from which pollen was collected had a small sector and that the pollen grains derived from anthers in this sector were unusually high responsible for the appearance of the large number of kernels exhibiting the A\(_1\) phenotype.

A number of plants were grown from one ears produced by crosses conducted with plant 5720. With rare exceptions, the state of a\(^{m-1}\) in this plant and in its progeny gives rise in plant and kernel to mutant areas that exhibit only low levels of pigment intensity. As mutation inducing events may occur very early in development of plant sporogenous cells, or in gametophytic cells, and of the kernel, a number of
gametes are formed by plants having this state that carry a mutant of $a_1$$m^{-1}$. Therefore, many kernels appear on test cross ears that express this.

One grade of pigment intensity is expressed in each but the range of this among the mutant carrying kernels is from very faint to medium dark.

When a plant having this state 5720 of $a_1$$m^{-1}$ and also Spm is used as a pollen parent in the cross to plants homozygous for $a_1$, only one of the three chromosomes 3 in the endosperms of kernels on the resulting ear carries this $a_1$$m^{-1}$. Among the variegated class of kernels with this constitution, mutant areas appear. Each expresses one particular grade of pigment intensity within it. When, however, the reciprocal cross is made, among the variegated kernels on the resulting ear, mutant areas appear within which there are smaller areas exhibiting deeper shades of pigment intensity.

This is expected as these kernels have the state of $a_1$$m^{-1}$ in two of its three chromosomes 3, and mutation-inducing events occur to each independently of one another. Types of kernels appearing one ears produced from crosses of plants that were $a_1$$m^{-1}$ (state 5720)/$a_1$ to plants homozygous for $a_1$ are given in table 16. In this table it will be seen that the number of uniformly pigmented kernels is high as well as the number of colorless among the variegated pigmented kernels, not one but many different shades of pigment kernels. It was subsequently learned that with state 5720, no anthocyanin
pigment is produced in either plant or kernel when Spm is absent. The high rate of germinal mutation produced by this 5720 state of a<sup>m-l</sup> resembled that produced by the original state of a<sup>m-l</sup> from which it was derived. However, with this original state, the majority of germinal mutations that express high levels of pigment intensity resembled or mutations give rise to the full or near-full type expression. The proportion of germinal mutations that express lesser levels of pigment intensity were produced with considerably lower frequencies. Because of the very high rate of germinal mutation given by the original state of a<sup>m-l</sup>, it was not considered to be a useful one for analysis of the mode of operation of the Spm system and tests of it were not continued beyond the 1951 season. In contrast, state 5720, which is similar to the original state of a<sup>m-l</sup> in terms of frequency of mutation but was derived from it, has been quite useful.
5. Individuality of expression of each state of a_{m-1} when two different ones are present in a plant or kernel.

During the summer of 1951, crosses were made between plants carrying different states of a_{m-1} and the type of expression of each state in the kernels on the resulting ear was examined. The combinations that would bring together in a single kernel the following states of a_{m-1}: state 5719A-1 with state 5720, and with the original state, state 5718 with the original state, state 5720 with the original state, and state 5719A-2 with state 5720, not previously discussed. In later years, other combinations of states were made, however, no advantages were found.

The most instructive cross that illustrated the individuality of each state of a_{m-1} when combined in a kernel on a plant was that between plants carrying state 5719A-1 and another carrying state 5720, and the types of kernels appearing on the ear produced by one such cross will be given. Pollen from plant 6042-9, that carried state 5720, was placed on the silks of a tiller ear of plant 6046C-1 in order to make clear the results obtained from this cross. It will be necessary to compare other ears produced with each of these two plants which was homozygous for state 5719A-1. The first ear of the main stalk of plant 6046C-1 was self-pollinated and on the resulting ear there were 4 uniformly pigmented kernels exhibiting type pigments, 31 that were uniformly pale colored, and 377 that had small spots of the type pigment in a colorless background, the typical pattern produced by state 5719A-1. Pollen of this plant was placed on the silks of a plant homozygous for a_{1} and on the resulting ear there was 1 kernel uniformly pigmented kernels which was homozygous of the type
212 kernels that were uniformly pale colored, and 465 that expressed the variegation pattern typically produced by state 5719A-1. Plant 6042-9 variegated had been derived from a kernel on the self-pollinated ear of plant 5720. Pollen from a plant homozygous for $a_1$ had been placed on the silks of a tiller ear of this plant. The kernel types that appeared on the resulting ear were as follows: 32 uniformly pigmented kernels, each present with a low level of intensity of pigmentation; all of them this level was low, 102 variegated kernels with both large and small areas exhibiting low levels of pigment intensity in a colorless background, and in addition, 239 kernels in which no pigment was observed. When pollen of plant 6042-9, with an ear of a tiller state 5720, was placed on silks of a tiller ear of plant 6046C-1, it was then learned that the pollen parent was homozygous for state 5720. Thus, the cross was made between a plant homozygous for state 5719A-1 and one homozygous for state 5720. There were 216 kernels on the ear this cross produced. Four of them were uniformly pale colored. In 18 others, spots with being $A_1$-type pigment were present in a uniformly lightly pigmented background and the pattern of the $A_1$ spots was that characteristically produced by state 5719A-1. The intensity of the background pigment was uniform over the aleurone layer for each kernel but the level of this differed among these kernels. And, these levels were the same as that on...
appearing among the uniformly pigmented kernels on ears produced by the cross of plants having state 5720 to plants homozygous for \( a_1 \). In addition, there were 194 variegated kernels. These kernels had the pattern of \( a_1 \) spots produced by state 5719A-1 and also the pattern of lightly pigmented areas characteristically produced by state 5720 (see photos). The over-all pattern was that which could be expected from superposition of the pattern produced by one state over that produced by the other. Each state expressed its own type of response to the presence of Spm.

It was assumed that in the 18 kernels showing the variegation pattern produced by state 5719A-1 on a uniformly pigmented background, a mutant stable in the presence of Spm, had been contributed by the parent carrying state 5720. The correctness of this interpretation was made evident in tests of the plants derived from kernels of this type that were conducted in a subsequent year. The plant derived from each carried a mutant whose expression was stable either in the presence or the absence of Spm and the state 5719A-1 of a \( m^{-1} \) in the homologue, which continued to express its individual variegation pattern in subsequent generations.

A description of the types of test that established this will be postponed until a description may be given of the means by which such tests are made possible.
As described earlier, plant 5700A was derived from a very pale colored kernel on an ear produced by cross of the original \( a_1 \) carrying plant to one that was homozygous for \( a_1 \). Pollen of plant 5700A was placed on silks of an ear of a plant homozygous for \( a_1 \). Two types of kernels appeared on this ear, those that were very pale in color and those that were colorless. An accurate classification of the two types was not attempted as the intensity of pigment in the pale class was so low that it was feared that some in the class would be placed with the colorless class. Some kernels that certainly had pigment in them were selected from this ear, and the plants grown from them in the summer of 1951 were given culture number 6078. At least one ear of each plant in this culture received pollen from a plant homozygous for \( a_1 \) and \( sh_2 \). On all ears so produced, there were only the two classes of kernels: very pale colored and colorless, and accurate classifications were also difficult to make in some of these ears. As an example, the first ear on the main stalk of plant 6078-5 received pollen from a plant homozygous for \( a_1 \) and \( sh_2 \). On the resulting ear, 256 kernels certainly had pigment in them, but the remaining 314 appeared to be colorless. A tiller ear of this plant was self-pollinated and similar difficulties were encountered in making accurate classifications of the two classes of kernels. Among the 424 kernels
this ear produced, 254 had faint pigment in them but the remaining 170 appeared to be colorless. The silks of an ear on another tiller of the plant received pollen from plant 608OC-7 whose constitution was \( a_1m^{-1} \) (state 5719A-2). The kernel types appearing on this ear were surprising. Before describing (see description of this state, see pages ) the first ear on the main stalk of plant 608OC-7 had been self-pollinated and among the 234 kernels produced, one was fully \( A_1 \) in phenotype, none were pale, 172 were variegated for dots of \( A_1 \) in a colorless background, and 61 were colorless. The second ear on the main stalk received pollen from a plant homozygous for \( a_1 \). On the ear this cross produced there were 11 pale colored kernels, 97 showing dots of \( A_1 \) in a colorless background, and 127 that were colorless. Pollen collected from a tiller of the plant 608OC-7 was placed on the silks of an ear produced by a second tiller of plant 6078-5. The types of kernels appearing on this ear were surprising. There were 2 kernels exhibiting the full \( A_1 \)-type pigmentation, 75 kernels that were uniformly pale colored, and 132 that appeared to be colorless.

In addition, there were 259 variegated kernels. Eighty-three of them had dots of \( A_1 \) in a colorless background—the pattern of variegation associated with state 5719A-2. The remaining 176 variegated kernels exhibited few large areas of full \( A_1 \)-type pigment and also very many small spots of this that were situated quite close to one another. In some of these
kernels there were a few areas and in other a few areas that appeared

to be colorless. It was suspected immediately that the plants in culture
in the absence of Spm, this state

6078 had a modified state of $a_1^{m-1}$ but no Spm. Introduction of Spm

through a male gamete of plant 6080C-7 revealed the pattern of variegation

this state would produce in its presence. Subsequent tests substantiated

examining some aspectes of the Spm system of operation. Plants derived

from the newly appearing variegated class of kernels on the ear produced

by the cross of 6078-5 by 6080C-7 were also examined. Some of them were

$a_1^{m-1}$(state 5700A)/$a_1$ and others were $a_1^{m-1}$(state 5700A)/$a_1^{m-1}$ (state

5719A-2). Both states appeared in the progeny of plants having this

latter constitution, each having retained its own properties side by side in the nuclei of one plant. Further discussion of the manner in which

these tests were made will be postponed until the methods adopted for them

may be considered.
Besides the intercrosses of plants carrying the distinctive states of \( a_1 \), described above, crosses were made between plants having the original state of \( a_1 \) in one chromosome 3 and \( a_1 \) in the homologue, with plants having one of the following derived states: state 5718, state 5719A-1, and state 5720. The types of kernels appearing on the ears of these crosses produced will not be considered here in detail. As stated earlier, the \( a_1 \) state of \( a_1 \) gives rise to many germinal mutations. The majority of kernels and plants that have one such germinal mutation in them exhibit the full \( A_1 \) phenotype. However, other germinal mutations give anthocyanin and thus a wide range in pigment intensity. Many kernels and plants carrying the cross conducted with plants carrying state 5718 or state 5719A-1 to plants carrying the original state of \( a_1 \). Before these phenotypes are discussed, those that appear on ears produced by cross of plants considering this, attention will be given to the behavior of the original \( a_1 \) state of \( a_1 \) in plants that carry this state in one chromosome 3 and \( a_1 \) in the homologue.

In the summer of 1951, 48 plants were grown from variegated kernels appearing on ears produced by cross of the original \( a_1 \) carrying plant to plants that were homozygous for \( a_1 \). These kernels exhibited a pattern of variegation that was common to most of the variegated kernels produced by
test crosses conducted with the original \( a_{m-1} \) carrying plant (photo ).

The plants derived from these kernels likewise exhibited the same bizarre types of variegation pattern that characterized the appearance of the kernels. It was obvious that change of \( a_{m-1} \) was occurring in a number of cells of the plant and many of these occurred early in plant development.

The plants were composites of distinct sectors in each of which a particular phenotype was expressed. Many of them exhibited the \( A_1 \) phenotype. In others, the pigment was uniformly distributed within the sector but its intensity was low. Still other sectors were distinguished by distinctive types of variegation patterns within them. "Blood" streaks of the \( A_1 \) type pigment in a non-pigmented background, and the number and size of these streaks was not the same in all sectors exhibiting distinctive variegated patterns. In a few plants, the type of pattern of variegation exhibited by one tiller differed greatly from that of the main stalk and of other tillers of the same plant.

Some of the variegated plants that were \( a_{m-1}/a_1 \) in constitution were used as female parents in crosses with plants homozygous for \( a_1 \) and \( sh_2 \).

The types of kernels appearing on the resulting ears are given in A of table 17. Pollen of two plants were used on silks of plants carrying
a modified state of \( a_{m-1} \) (state 5718 or 5719A-1). To serve as a control
to such crosses, part of the pollen collected from each plant was placed on
silks of ears of plants that were homozygous for \( a_1 \). The kernel types on
produce \( \times \) test of two plants
these ears are shown in B of table 17. In this table, the kernels that
appeared to be fully \( A_1 \) in phenotype are placed under the heading "\( A_1 \)".

Under the heading of "pale" are placed those kernels that were uniformly
pigmented but in which the intensity of this was lower than that produced
among the kernels. Within this class, there was a range in intensity from very faintly
pigmented in some kernels to medium dark for others. All kernels
exhibiting variegation for anthocyanin pigmentation are entered under the
heading "variegated". Most of these kernels exhibited large and small
areas with the \( A_1 \) phenotype and pale areas. In the last column of this

table are entered the number of kernels expressing a markedly altered
pattern of variegation, similar in types to those that gave rise to the
altered states that have been considered in previous sections. It may be
seen that the number of them is low. However, other changed states,
less easy to detect, were being produced. One of them was detected
because it was present in the cell whose descendant cells gave rise to an
ear that had been used in a cross with a plant homozygous for \( a_1 \) and sh2.
The pattern expressed by the variegated kernels on the resulting ear was of one type and it differed from that given by the original state of $a_l^{m-1}$. This similarity in pattern made it possible to know that an altered state was present in the cells that gave rise to this ear. In the presence of Spm, this altered state produces many small $A_l$ dots, located very close to each other, and also some larger areas exhibiting the $A_l$ phenotype (Photo.). To the naked eye, some kernels having this pattern of variegation may appear to be uniformly pigmented. However, microscopic examination of them reveals the pattern of $A_l$ spots that is responsible for this deception. The dots of $A_l$ are so close together that there distinctiveness can not be seen except when viewed under magnification. (In all studies of variegation, kernel types on ears are examined under magnification in order that details of importance in them would not be undetected.) In the absence of Spm, kernels in which this state of $a_l^{m-1}$ is present have pigment in them, but this is so light in intensity that detection of it sometimes is difficult. The types of kernels on the ear of the original plant having this state 5996-4 are given in C of table 17. In plant 5996-4, one Spm was present and it was carried in one of its two chromosomes 6. (See footnote to table 17.)
On the ears produced by crosses of plants that were \( a_1^{m-1} \) (original state) to plants that carried either state 5718 or 5719A-1/a1, the original state of many of the kernels that received \( a_1^{m-1} \), or a mutant derived from it, could be recognized. Likewise, those that received \( a_1 \) from the plant having the original state of \( a_1^{m-1} \) and the derived state of \( a_1^{m-1} \) from the other parent also could be recognized. This may be seen from the photograph of kernel types appearing on one such ear (photo. ). However, kernels with a distinctly new phenotype appeared on these ears. They had the pattern of \( A_1 \) dots given by the derived state of \( a_1^{m-1} \) but pale these appeared on a pigmented background instead of a colorless background. This suggested that the plant carrying the original state of \( a_1^{m-1} \) had contributed a mutant of \( a_1^{m-1} \) giving a reduced grade of pigment intensity and that this mutant was stable in the presence of Spm. Tests of plants derived from such kernels indicated the correctness of this interpretation. In other words, the original state of \( a_1^{m-1} \) was giving rise to mutants that were stable in the presence of Spm. Some of the pale mutants produced by the original state of \( a_1^{m-1} \) that are stable in the presence of Spm very much resemble in their phenotypic expression that given by some of the derived states in the absence of Spm. This is strikingly illustrated by one type that resembles that produced by state
In the absence of Spm, kernels having state 5719A-1 are deeply pigmented whenever Pr is present in them. However, when the kernels are homzygous for the recessive allele, pr, the kernels are only lightly pigmented. Some of the stable mutants derived from the original state of \( a_1^{m-1} \) give these same phenotypes with the alleles of Pr. It would appear that the production of either a stable mutant or a new state by the original state of \( a_1^{m-1} \) is associated with the fate of the controlling element at the \( a_1^{m-1} \) locus. Its removal or total inactivation could slight give rise to the stable mutants whereas a/shift in its location, without inactivation, may be responsible for the origin of some of the altered states. It is apparent, nevertheless, that the production of stable mutants by the original state of \( a_1^{m-1} \) is far more frequent than the production of obviously altered states of the types that have been described in this report.

In the cross of \( a_1^{m-1} \) (original state) \( \times a_1 \) by \( a_1^{m-1} \) (state 5718 or 5719A-1) / \( \bar{a}_1 \), the number of Spm xixixixixixixixixixix was known only in the plant carrying the derived state. The Spm number in plants having the original state could not be determined readily from the types of kernels appearing on ears produced by testcrosses conducted with it. The frequency of occurrence of germinal mutation was too high. Nevertheless,
the types of kernels appearing on the ears produced by stress of plants carrying the original state to plants carrying the derived states, whose constitutions were given above, is instructive, and the types of kernels appearing on six ears produced by this type of cross will be given.

There was a total of 2175 kernels with the following phenotypes:

- the 277 with $A_1$ phenotype, 71 that were uniformly pale colored and had a derivative of the $m\ldots m$; some of these obviously $m\ldots m$ introduced by the parent moving it. Eighteen other kernels were uniformly pale colored but superimposed on this were dots or spots of $A_1$ with a pattern given by the derived state of $a_1^{m-1}$ used in the cross.

There were 1207 variegated kernels that fell into two distinct classes:

- 602 exhibited the pattern that is characteristically produced by the original state of $a_1^{m-1}$ and in some of these, the presence of the derived state was suspected, and 605 that exhibited the dots or characteristically produced by the derived state and spots of $A_1$ in what appeared to be a colorless background. In addition, there were 602 colorless kernels. Tests of plants derived from kernels exhibiting the variegated pattern of the derived state on a pale pigmented background, and those derived from ones in which both the original state and the derived state were suspected to be present, confirmed the suspected constitutions of the kernels from which each was derived. From the former,
the presence of a stable mutant in one chromosome 3 and the derived state either 5718 or 5719A-1, as the case may be, in the other chromosome 3. Plants derived from the latter type of kernel had the original state of \( a_1 \) in one chromosome 3 and the derived state in the other.

Tests of \( a_1 \) behavior that have been discussed in detail in previous sections of this report were all conducted during the early period of study of \( a_1 \). From them was developed an interpretation of the primary mode of operation of the system responsible for control of gene expression at \( a_1 \). The origin and behavior of different states of \( a_1 \) were considered, and the discovery of an independently located element, \( Spm \), whose mode of action was outlined, was likewise treated. Nevertheless, up to this time in the study of \( a_1 \), no revise tests of this interpretation had been conducted.

The interpretation that had been developed implied the following conditions. \( Spm \) is an independently located element in the \( a_1 \) system, and it is subject to transposition from one location to another in the chromosome complement. In its presence, change occurs at the \( a_1 \) locus and this may lead to one of two main consequences. One of them results in a stable mutant expression; each mutant expresses one mode of gene action, recognized by the degree and
anthocyanin and kind of pigment that it produces in plant and kernel. The second
results in an altered state of $a_{m-1}$, each state expression thereafter one
particular type of presence to Spm. This is related to control by the
altered $a_{m-1}$ itself of the time during development when subsequent change
will occur to it, the types of mutants that these latter changes will
induce, and the number of cells in which such events will occur. The
number of Spm elements present in the nuclei of a plant or kernel does not
alter these qualities of a state of $a_{m-1}$. In the absence of Spm, a
states of $a_{m-1}$ function in the production of anthocyanin pigment in both
plant and kernel, and again, the type and level of its production
is a quality of state of $a_{m-1}$. This function is suppressed
in plant and kernel when Spm is present in the nuclei. Removal of Spm from
of a somatic cell will allow the functional activity of the state of $a_{m-1}$
that is present to be expressed in its descendant cells.

The above statements incorporate the primary conditions imposed by the
interpretation. More evidence was required in order to test the general
validity of this interpretation. Therefore, such tests were conducted
during the summer of 1954. There were many such tests and they will be the
subject of the section of this report that follows.
The interpretation of mode of control by Spm of gene expression at \( a_1^{-m-1} \), outlined in previous sections, required precise verification, and tests devised for this purpose were conducted during the summer of 1954.

The interpretation considers that no Spm is present in the uniformly pale colored kernels on ears derived from crosses entered in tables 2 to 8 and 10 to 15, but that it is present in all kernels exhibiting pigmented areas in a colorless background. It also considers that each state of \( a_1^{-m-1} \) will respond to any isolate of Spm in a manner that is characteristic of the state. In other words, it will be the state of \( a_1^{-m-1} \) that will govern the pattern of variegation when any one Spm is present. To illustrate, if pollen of a plant homozygous for \( a_1 \) and carrying 1 Spm is placed on the silks of a number of plants, each derived from a pale colored kernel and in each of which a different state of \( a_1^{-m-1} \) is present, then on the ear produced by each plant, half of the \( a_1^{-m-1} \) carrying kernels should be pale colored and half should be variegated. On each ear, the pattern of variegation among the variegated kernels should express that which is characteristic of the state of \( a_1^{-m-1} \) present in the ear bearing plant.

If the pollen parent should have 2 non-linked Spm elements,
then on each of the resulting ears there should be a ratio of one pale
colored kernel to 3 variegated kernels among the \( a_{m-l} \) carrying kernels
and again, the pattern exhibited by them should be that fixed by the
state of \( a_{m-l} \) that is present.

Several types of test-cross were devised that made it possible
to learn whether or not \( Spm \) were present in any one plant, and to demonstrate
with certainty the control of phenotype by state of \( a_{m-l} \) both in the
presence and absence of \( Spm \). These were conducted during the summer of
1954. The plants to be tested were derived from selected kernels on ears
produced by crosses made in previous growing seasons. The types of
kernels selected, the number of plants derived from each type, and the
origin of the ear from which each selection was made, is given in table
18. All kernels derived from one ear were grown under one culture
number, that entered in the last column of this table. However, the
derived from one ear \( \ldots \) were separated from one another, and
grown under the same culture number but to which was added a distinctive
letter of the alphabet. If other genetic markers also were segregating
among the kernels on the ear, all those \( \ldots \) phenotype were
sown \( \ldots \) separately from those \( \ldots \) each given the same culture
number but appropriately distinguished by a letter of the alphabet following
the culture number.
The most critical types of test-cross were conducted with plants belonging to three different categories. The plants belonging to the first of these were uniformly pigmented. They were derived from pale colored kernels on the self-pollinated ears of plants having one particular state of \(a_1^{m-1}\). Each of the tester plants in this first category was homozygous for the particular state of \(a_1^{m-1}\) that was present in the parent plant. They were also homozygous for \(Sh_2\). Within this category, four different states were represented, and these are entered in part I of table 19. The culture number of each from which their origin may be traced through table 18, as well as the constitutions with regard to genetic markers carried in chromosomes 5, 6, and 9, are also given in this table.

If the plants in part I of table 19 are used in crosses of the following types, only pale colored, \(Sh_2\) kernels should appear on the resulting ears:

1. Crossed with plants of the standard \(a_1/a_1\) tester stock.

2. Self-pollinated or sib-crossed

3. Intercrossed with plants in the same category, bringing together two different states of \(a_1^{m-1}\).

4. Crossed to plants derived from the pale class of kernels in table 18.
(5) Crossed to some of the plants derived from the colorless, \( sh_2 \) cultures entered in kernels in table 18.

If the plants in part I of table 19 are used in crosses of the following type, both pale colored, \( sh_2 \) kernels and variegated kernels should appear on the resulting ears:

(1) Crossed to some of the plants derived from the colorless kernels in cultures entered in table 18.

(2) Crossed to plants derived from the variegated class of kernels in cultures of table 18 in which the constitution of the plants are \( a_{l}^{m-1} Sh_2/a_{l} Sh_2 \).

From cross (1) immediately above, all the variegated kernels on the resulting ear should exhibit that expected to be produced by the state of \( a_{l}^{m-1} \) present in the tester plant. Also, if one ear of the \( a_{l}/a_{l} \) plant received pollen from a tester plant homozygous for one state of \( a_{l} \) and another ear of the same plant received pollen from a tester plant that was homozygous for another state of \( a_{l}^{m-1} \), then among the variegated kernels on each ear, a distinctive pattern of variegation should be expressed, one pattern on one ear and another pattern on the other ear. The pattern expressed would reflect the individual response of the state of \( a_{l}^{m-1} \) contributed by the tester stock to the presence of the same Spm element.
From type-cross (2) immediately above, two class of variegated kernels should appear on the resulting ear. One should exhibit the variegated pattern produced by one state of $a_{1}^{m-1}$ superimposed on that produced by the other. These would be the kernels receiving $a_{1}^{m-1}$ from each parent. The second class should exhibit only the pattern that is produced by the state of $a_{1}^{m-1}$ delivered to it by the tester plant, and these kernels would be those receiving $a_{1}$ from the plant being tested, and $a_{1}^{m-1}$ from the other plant.

On the ears produced by the type-crosses described above, it should be possible to determine the Sm number in that part of the plant that produced the ear used in the cross. This could be deduced from the ratio of pale to variegated kernels that appear on the ear. Also, if the plant being tested were heterozygous for one or more of the genetic markers in chromosomes 5, 6, and 9, given in table 19, then the location of Sm with respect to those markers could be determined, provided that the tester plant used in the cross, these markers were homozygous recessive.

More than 500 test-crosses were conducted with tester plants in the first category, those entered in part I of table 19. From the kernel types on the resulting ears it was possible to detect not only the presence or absence of Sm in many plants, whose phenotypes were known, and to conclude this with the phenotype of the plant, either variegated under question.
but also, when present, the number of Spm elements that were present in any one tested part of a plant. In many plants it was also possible to determine the location of Spm with reference to the three given genetic markers, Pr in chromosome 5, Y in chromosome 6 and Wx in chromosome 9. These tests also served to confirm the individuality of states of the different markers in the control of pattern of variegation in the presence of Spm and type of gene expression given in its absence. Before evidence of this is presented, the usefulness of tester plants in each of the two remaining categories will be described.

For some types of test, it would have been desirable to use plants that were homozygous for both $a_{1}^{m-1}$ and $sh_{2}$. At the time, no plants of this constitution had been constructed. Therefore, kernels that were $a_{1}^{m-1} sh_{2} / a_{1} sh_{2}$ appearing on ears produced by the crosses entered in table 5 and 8 were selected and plants grown from them. All of them had the 5719A-1 state of $a_{1}^{m-1}$. Four plants were derived from pale colored, $sh_{2}$ kernels, and 6 plants were derived from variegated, $sh_{2}$ kernels. The ears from which each of these were selected is indicated in table 18. The phenotypes of these plants with respect to Spm and the alleles of Pr, Y, and Wx are entered in parts II and III of table 19.
The plants in Part II of Table 19 were all uniformly pigmented.

When pollen from any one of them was placed on silks of plants in the standard \( a_1 \) tester stocks, one-half of the kernels on the resulting ears were uniformly pale colored, \( \text{sh}_2 \), and the other half were colorless, \( \text{sh}^2_2 \). When the same pollen was placed on silks of ears produced by plants derived from the pale, \( \text{Sh}_2 \) class of kernels which had the constitution \( a_{m-1} \text{Sh}_2 / a_1 \text{sh}^2_2 \), the ratio of kernel types on the resulting ears were as follows: 2 pale colored, \( \text{Sh}_2 \) : 1 pale colored, \( \text{sh}_2^2 \) : 1 colorless, \( \text{sh}^2_2 \).

However, when the pollen was placed on silks of ears of sister plants, derived from the variegated kernels, pale and variegated kernels appeared on the resulting ear, not only in the \( \text{Sh}_2 \) class but also in the \( \text{sh}^2_2 \) class. This indicated that the variegated plant carried an \( \text{Spm} \) to which the \( a_{m-1} \) in the /\( \text{sh}^2_2 \) chromosome derived from the tester stock could respond. It was important to compare the ratio of pale to variegated in the \( \text{Sh}_2 \) class with that in the \( a_{m-1} \) carrying \( \text{sh}_2^2 \) class in order to learn whether or not \( \text{Spm} \) was located in the same chromosome 3 that had \( a_{m-1} \) and \( \text{Sh}_2 \). If so, the ratio of variegated to pale kernels in the \( \text{Sh}_2 \) class would be greater than in the \( \text{sh}_2^2 \) class. Tests conducted during the summer of 1954 did not reveal a case of this but similar types of test conducted in later years did reveal the presence of an \( \text{Spm} \) element in chromosome 3 in some plants.