MUTATION IN MAIZE

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The origin of instability at a number of loci of known genic action in maize chromosomes has been described in previous Year Books, and the nature of the unstable expression discussed. In some of these cases, instability appeared when Ds, a transposable chromosomal unit, was inserted next to the locus. The original and the subsequent mutations were shown to be expressions of changes induced at the locus by Ds. Ds is known to produce chromatin alterations. It was suspected, therefore, that the mutations it induces may be accompanied by some form of chromatin alteration or reorganization. If so, no two mutation-inducing events need produce exactly the same type of change at the locus, even though the resulting modification effects the same change in expression of the known genetic factor. It was decided, therefore, to examine a number of Ds-induced mutations in order to determine whether or not differences could be detected among them. For this purpose, a number of independently occurring mutations at a selected locus were required. The two cases, described in Year Book No. 51 (1951–1952), in which Ds was inserted into the short arm of chromosome 9 just to the left of Sh_1 were selected because each has provided a large number of newly induced mutations to sh_1 (shrunken endosperm).

ORIGIN OF THE MUTANTS

All the mutants used in this study appeared in the progeny derived from crosses of plants homozygous for C, ds, sh_1, and bz, and carrying no Ac factor, to plants having Ac and one of the following constitutions: (1) $I\ Ds\ Sh_1\ Bz/I\ Ds\ Sh_1\ Bz$, (2) $I\ Ds\ Sh_1\ Bz/I\ Ds\ Sh_1\ bz$, or (3) $I\ Ds\ Sh_1\ Bz/C\ ds\ sh_1\ bz$. I (inhibitor of aleurone color, dominant in action to C, which produces aleurone color) is located approximately four crossover units to the left of

Sh_1. Bz (allele of bz, recessive mutant which alters the color of the aleurone layer and the plant tissues to a bronze shade) is located approximately two crossover units to the right of Sh_1.

The frequency of occurrence of germinal mutations to sh_1 among the kernels on the ears resulting from the above-described crosses was recorded in Year Book No. 51. On the ears produced by the first two of these crosses, a few completely colored, Sh_1 kernels were present. Such kernels were expected for the following reasons. In the two cases under consideration, most of the alterations produced by Ds result in a dicentric chromatid and a reciprocal acentric fragment. The position of break in the short arm of chromosome 9 is at the locus of Ds, just to the left of Sh_1. The acentric fragment, carrying I, is subsequently lost during a mitotic cycle. If such an event occurs in a premeiotic, meiotic, or gametophytic nucleus, gametes may be formed that are deficient for a segment of chromosome 9 extending from the locus of Ds to the end of the arm. Previous studies had shown that female gametophytes having such deficiencies are viable and functional, and that kernels in which I is absent can be produced from them. In the crosses described here, such kernels should be completely colored. Plants derived from them should have a long terminal deficiency of the short arm of one of their chromosomes 9. As will be shown later, this proved true of only some of these plants. Morphologically normal chromosomes 9 were present in the others, but the action of I carried by the chromosome received from the female parent was inhibited.

CHANGE IN ACTION OF GENES LOCATED TO THE RIGHT OF Ds

An analysis of plants derived from some of the kernels carrying newly induced sh_1

mutants that appeared on the ears from cross 3 listed above was given in Year Book No. 51. The mutants appearing on the ears from crosses 1 and 2, however, are of particular importance, because neither crossing over in a heterozygote nor contamination could account for the mutant phenotype. Forty-nine plants carrying newly induced alterations at the locus of Sh_1 have been examined. All were derived from kernels that were I sh_1 in phenotype. Sixteen were selected from the ears produced by cross 1. Ten of these kernels were completely colorless, indicating the absence of either Ds or Ac. In four others, areas exhibiting either the C Bz or the C bz phenotype were present, indicating the presence of both Ds and Ac. In the remaining two kernels, areas showing only the C bz phenotype appeared, indicating the absence or inhibition of Bz. Nine I sh kernels were selected from the ears produced by cross 2. Five of them were completely colorless, and four had areas of both the C Bz and the C bz phenotypes. From the ears produced by cross 3, nineteen I sh_1 kernels were selected that showed areas of the C Bz and the C bz phenotypes, and five additional kernels were selected that showed areas only of the C bz phenotype.

Subsequent study showed that in thirty-seven of the forty-nine plants derived from the selected I sh_1 kernels, Bz was present in the I chromosome and unmodified in action. The remaining twelve plants were all bronze (bz) in phenotype. As will be shown later, this phenotype appeared in some of these plants because the locus of Bz had been altered by the same event that had altered the Sh_1 locus.

COMPARISONS BETWEEN sh_1 MUTANTS

Modification affecting only Sh_1. Similarities and differences among the thirty-

seven cases of mutation to sh_1 in which the action of Bz was unaffected by the mutation-inducing event will be considered first. In all cases, the chromosome carrying the new sh_1 mutation was transmitted normally through the male and female gametophytes, and the homozygotes were viable. Ds was present in each, and its position was not detectably altered by the event that produced the mutation to sh_1. Reversions to Sh_1 occurred in nine of the thirty-seven cases. In two of them the frequency of reversion was high, and in both cases it was Ac-controlled. The reversions to Sh_1 were not associated with loss or change in location of Ds. The behavior of the sh_1 mutants differed not only with regard to the occurrence of reversions, and to frequencies of reversions when they did occur, but also with regard to frequency of crossing over between the locus of sh_1 and the loci of its nearest known neighbors, I and Bz. In one case consistent results have been obtained in tests extending over three successive generations. When this sh_1 is present, crossing over between I and sh_1 is increased about 50 per cent, in comparison with standard frequencies, and crossing over between sh_1 and Bz is increased approximately 300 per cent. In several other cases, the mutation to sh_1 resulted in marked reductions in crossing over. In a backcross test involving one such case, no crossing over between sh_1 and Bz was observed in a total of 3156 tested gametes; crossing over between I and sh_1 was slightly reduced, but that between Bz and a marker, Wx, located to the right of Bz, was unaltered. In all cases where a modification in crossing over was expressed, it was confined to the vicinity of the sh_1 locus.

It may be concluded that the described dissimilarities among these sh_1 mutants reflected dissimilarities in the primary mu-tation-inducing events, even though all were initiated by Ds.

Simultaneous modification of the action of Sh_1 and its neighboring gene, Bz. As was stated above, twelve of the forty-nine plants derived from I sh_1 kernels produced from crosses 1 to 3 exhibited the recessive bronze phenotype. Four of the kernels from which these plants arose had appeared on ears produced by cross 1, three came from ears produced by cross 2, and five from ears produced by cross 3. (These last five were selected because of the appearance of C bz areas in the aleurone layer and the complete absence of any detectable Bz action, and also because in four of them the endosperm was noticeably defective.) Only in the four cases derived from cross 1 was it certain that the Sh_1 and Bz loci had been altered by the same event. The female parent in this cross was homozygous for both Sh_1 and $Bz;$ whereas one of the chromosomes 9 in the female parents used in the other two crosses carried the known recessive, bz. In the remaining eight of the twelve plants, the presence of this recessive in the I sh_1–carrying chromosome could account for the bronze phenotypic expression, the mutation-inducing event having altered only the Sh_1 locus. Evidence obtained from subsequent study of one of the three cases derived from cross 2 and four of the five cases derived from cross 3 suggests, however, that a longer segment within the chromosome had been altered, and that it extended from the locus of Ds to or beyond that of Bz or its allele bz. This evidence will appear in the following discussion.

One of the four bronze plants obtained from the I sh_1 kernels of cross 1 was completely male- and female-sterile, and therefore the modification responsible for the bronze phenotype could not be examined further. Cytological examination of the

other three plants revealed no structural alteration in their chromosomes 9. Marked differences were noted, however, in genetic behavior. In one of them, the I-carrying chromosome behaved as though the known recessives, sh_1 and bz, were present, in that it was normally transmitted through the male and female gametophytes, and the kernels homozygous for it were normal in appearance. Also, no reversions to Sh_1 or Bz were observed. The behavior of the I-carrying chromosome in the other two plants was decidedly aberrant. It suggested that a segment of the chromosome, including the Sh_1 and Bz loci, was either absent or greatly modified. In one of these cases, transmission of the chromosome was nearly normal through the female gametophyte but markedly reduced through the pollen grain. Kernels homozygous for the modified segment were not produced, and most of the kernels heterozygous for it were abnormal in appearance—smaller than normal and showing few to many wrinkled regions. In the second of these two cases, the chromosome was transmitted at a reduced rate through the female gametophyte and was not transmitted through the pollen. Most of the kernels heterozygous for this segment were defective, and in many of them the embryo appeared to be dead. In both cases, Ds was present in the chromosome having the modified segment. Although its exact position has not yet been determined, in both cases it was either adjacent to or a component of the genetically modified segment. No reversions to Sh_1 or to Bz were found in either case.

The genetic behavior of the I-carrying chromosome in two of the three bronze plants grown from the I sh_1 kernels from cross 2 was normal. It resembled that of a chromosome carrying the known recessives sh_1 and bz. Ds was present in the chromosome in each of these plants, and

not noticeably altered in location. The behavior of the I chromosome in the third plant was similar, except that somatic reversions to Bz occurred and they were Ac-controlled. The frequency of reversion was low, and no germinal mutations appeared in tests of several thousand gametes. No reversions to Sh_1 were observed. In the event that they did occur, with as low a frequency as the Bz reversions, detection would be difficult. Only germinal reversions to Sh_1, or those occurring in very early development of the kernel, can be recognized with certainty. Ds was present in this chromosome, and again its location had not been altered noticeably by the event that produced the changed phenotypic expressions of Sh_1 and Bz. It was close to sh_1 and to the left of the mutable bz locus.

In one of the five bronze plants derived from I sh_1 kernels produced by cross 3, no aberrant behavior of the I-carrying chromosome was observed. Normal transmission occurred through the male and female gametophytes, and the kernels heterozygous and homozygous for the chromosome were normal in appearance. Ds was present in this chromosome. No reversions to either Sh_1 or Bz were seen. In the four remaining plants, the I chromosome behaved as if it had a modified segment, the modification being similar to that in the two cases described above. Here, again, there was a reduction of gametophytic transmission of the chromosome carrying the modified segment. In one plant, transmission through the female gametophyte was normal, and that through the pollen grain only slightly reduced. The heterozygous kernels were normal in appearance, but no homozygotes were obtained. Another plant showed nearly normal transmission through the female gametophyte, but markedly reduced transmission through the pollen grain. No homozy-

gous kernels were obtained, but the heterozygotes were normal in appearance. The behavior in the remaining two plants was similar to that in the plant just described, except that most of the kernels heterozygous for the modified segment were defective—small kernels, with few to many wrinkled areas. Cytological examination did not reveal a detectable structural modification in the short arm of chromosome 9 in any of the plants that were heterozygous for these modified segments. Again, Ds was present in all the chromosomes with modified segments, and it appeared to be located adjacent to or to be a component of the modified segment. No somatic reversion to either Sh_1 or Bz was observed.

Although it is clear from the descriptions above that alterations affecting both the locus of Sh_1 and that of Bz had occurred in at least nine of these twelve cases, and that homozygotes exhibiting the recessive expression could be obtained from two of them, it is not yet possible to state whether or not deficiency was responsible for the recessive expression, except in the one case where reversion to Bz occurred. In that case, deficiency of Bz is excluded. Marked deleterious effects are produced by some of these alterations, even in the heterozygote; and this situation has not previously been encountered in studies of heterozygous deficiencies in maize. Although deficiency cannot be excluded as the cause of the recessive phenotypic expression in every case, there is at present no direct evidence of it in any one case.

Change in Action of Genes Located to the Left of Ds

The previous sections have considered changes in expression of known genes located to the right of Ds, namely, Sh_1 and Bz. Changes in action of I, located four crossover units to the left of Ds, may also

occur. Evidence of this has been obtained from plants derived from the colored kernels appearing on the ears of crosses 1 and 2. As was explained earlier, the appearance of such colored kernels on these ears was anticipated; and plants derived from them were expected to have a chromosome 9 with a long terminal deficiency of the short arm, extending from the locus of Ds to the end of the arm. It was considered necessary to test this expectation. Consequently, some of the colored kernels were selected and plants were grown from them. Eighteen plants were obtained from the colored kernels of cross 1 and two from those of cross 2. Owing to the pressure of other work, time was not found to examine the chromosomes of these plants. In order to obtain seed for later examination, each plant was self-pollinated and also crossed by plants homozygous for C, sh_1, and bz. The types of kernels appearing on the ears of three of the plants derived from colored kernels produced by cross 1 and one of the two plants derived from such kernels from cross 2 were quite unexpected. They indicated that the appearance of color in the original kernel from which each plant arose was not due to a terminal deficiency, but rather to some more localized modification within chromosome 9 that had affected the action of I. Since the kernel types and the frequencies of types were the same on the ears of all four plants, they can be considered collectively. On the self-pollinated ears, a few completely colorless, Sh_1 kernels appeared. There were 87 of them among a total of 1224 kernels, and the percentages on the four ears were 4.6, 7.7, 7.7, and 9.0. The remaining kernels were all colored: 604 were Sh_1, and nearly all of these were very lightly colored; 533 were sh_1, and these had the depth of color that is produced by three doses of C. On the ears resulting from the backcross, no colorless kernels were

present. The Sh_1 and sh_1 classes of kernels appeared in equal frequencies. Nearly all the Sh_1 kernels were lightly colored, and nearly all the sh_1 kernels showed the color associated with three doses of C. It was obvious that either a pollen-transmissible deficiency of the I locus was present in these plants, or the action of I had been modified in such a way that it was no longer able to inhibit color development in the presence of C.

Plants were grown from the different classes of kernels on the self-pollinated ear and the backcross ear of the four exceptional plants. The seedlings arising from the completely colorless, Sh_1 class of kernels from the self-pollinated ears of all four plants were normal in morphology. The chlorophyll coloring in the leaves, on first emergence, appeared to be only slightly lighter than normal. As the seedlings aged, the color faded until the leaves were a very light yellow, after which the seedlings died. Seedlings arising from the other classes of kernels were normal in all respects. Cytological examinations were made of the chromosomes 9 in some of the plants arising from the light-colored, Sh_1 kernels derived from the self-pollinated and the backcross ears, in order to determine if any detectable structural alteration was present in the short arm of one of them. No evidence of structural change was noted in any case.

It may be concluded that the completely colorless, Sh_1 kernels on the self-pollinated ears—those that gave rise to seedlings with modified chlorophyll expression—represented the homozygotes in all four cases. I normally inhibits color expression in the aleurone layer of the kernel when C is also present in the nucleus; and in kernels homozygous for I no color develops. In the cases described above, complete suppression of color did not occur in the presence of C, although kernels homozygous

for the modified I were completely colorless. In these cases, not only the action of I had been altered, but also that of some previously unknown factor associated with chlorophyll development or stability; and this alteration was similarly expressed in the homozygous seedlings in all four cases.

It is evident from the foregoing discussion that genetic material located to the left of Ds had been modified. Ds was known to be present in the chromosome showing modified action of I in three of the four cases, although its position has not yet been determined. Tests of its presence and location in all these cases are now under way.

The progeny obtained from three others of the plants derived from colored kernels produced by cross 1 resembled that of the four plants just described, with the exception that no colorless, Sh_1 kernels appeared on the self-pollinated ears. In other words, no homozygotes were produced on these ears. Cytological examination of the plants derived from the light-colored, Sh_1 kernels showed no detectable alteration in the short arm of chromosome 9. It remains to be determined in what way the alteration affecting the I locus in these three cases differs from that in the four cases described above. Tests for this purpose are now being carried out.

In the remaining thirteen plants derived from colored kernels, the expected deficiency of a segment of the short arm of chromosome 9 was found. It included approximately the distal one-third of the arm, from the locus of Ds to the end of the arm. Cytological examination revealed that in ten of these cases the deficiency had been produced by a Ds event that resulted in a dicentric chromosome 9 and an acentric fragment, the latter including the locus of I. In the remaining three cases, a translocation had occurred. A segment from another chromosome had been translocated

to the short arm of chromosome 9 at the locus of Ds. A segment of the short arm, from Ds to the end of the arm, had subsequently been lost to the nucleus, with the result that I was deleted.

From the cases described in this and previous sections, it is evident that Ds can initiate changes affecting the action of genes on either side of it, and that the effect may be localized, close to Ds, or may spread along the chromosome. As was mentioned earlier, it is difficult to determine the maximum extent of this effect, because the more extended alterations include genetic materials whose absence or modified action adversely affects gametic transmission and viability, even in the heterozygote.

MEIOTIC SEGREGATION AND MUTATION

It has been demonstrated, with regard to the Ac-controlled mutable loci, that the time of occurrence of mutation during the development of a tissue is a reflection of the dose of Ac present in the nucleus: the higher the dose, the later the time of occurrence of mutation. Somatic segregations of Ac occur, and result in the formation of sister cells whose nuclei differ with respect to Ac—its presence or absence, and the dose. The progenies of the two sister cells reflect these segregations by showing either no mutations or altered times of mutation. In other words, control of mutation at these loci is an expression of the constitution of the nucleus with regard to Ac, and this constitution can be altered as a consequence of somatic segregation. In genetic studies, somatic segregations have not been fully examined or appreciated. Meiotic segregations, on the other hand, have provided the key information for an interpretation of genetic mechanisms in general. When plants are heterozygous for one or more genetic factors, meiotic segregations

of chromosomes accomplish abrupt changes in nuclear constitution. The resulting spores or gametes differ with regard to these factors. The orderliness of meiotic segregation gives rise to definite ratios of particular genetic constitutions among spores and gametes. It is this orderliness that allows us to make deductions about the constitution of the parent.

Mutation at a mutable locus may occur whenever a particular genetic constitution is present in the nucleus. Such constitutions may arise from somatic segregation or from meiotic segregation. If mutation occurs in all cells having a particular constitution, then meiotic segregation in heterozygous individuals may produce spores or gametes among which a definite fraction will undergo mutation at the mutable locus. When the heterozygous individual is crossed to one that can serve to test the gametic constitutions, definite ratios of mutant to nonmutant individuals should appear in the progeny.

Two cases of instability, designated a_1^{m-1} and a_1^{m-2}, which arose at the A_1 locus in chromosome 2 have given evidence of mutation that occurs in spores or gametophytes receiving particular genetic constitutions as a consequence of meiotic segregation. Neither a_1^{m-1} nor a_1^{m-2} is Ac-controlled. Both undergo a number of different types of mutation in the somatic tissues. Two main classes of mutation occurring at a_1^{m-2} are recognized in the somatic tissues, but mutations that occur in the spore or gametophyte, as a result of particular genetic constitutions arising from meiotic segregations, belong to only one of these classes. Similarly, somatic mutations occurring at a_1^{m-1} are diverse in type, but those occurring in the spore or gametophyte as a result of particular genetic constitutions of the nucleus created by meiotic segregations are of only one type. Although more extensive data indicating the relation be-

tween meiotic segregation and mutation are available for a_1^{m-2} than for a_1^{m-1}, the latter will be used to illustrate the nature of the evidence. This is because a factor has been found, located in chromosome 6, that actuates mutation at a_1^{m-1} in the spores receiving it. The location of the factor influencing mutation at a_1^{m-2} in spores has not yet been determined.

The original state of a_1^{m-1} allowed many mutations to occur early in the development of the plant and endosperm tissues. Each resulted in production of anthocyanin pigmentation; and many grades of expression were recognized, each resulting from a particular mutation. When many mutations occur early in the development of a plant, it is not easy to recognize the nature of control of mutation at the mutable locus, or to describe accurately its inheritance pattern. Therefore, in crosses of the original plant carrying a_1^{m-1} to plants homozygous for the stable recessive, a_1, a search was made for kernels exhibiting changes in state of a_1^{m-1}, in the hope of finding a state of a_1^{m-1} in which mutation would occur late in the endosperm tissues. Delay of mutation beyond the meiotic stages would allow a more accurate analysis to be made of the inheritance pattern of the mutable locus and of its mutation-controlling system. Several kernels did exhibit such a change in state of the mutable locus. In them, the mutations were expressed by dots of the full A_1 color and by small areas that were pale in color. Five such kernels were removed from four different ears, and plants were grown from them in the greenhouse during the winter of 1950–1951. Each plant was self-pollinated and crossed to plants homozygous for the stable recessive, a_1. In tests of all five plants, the inheritance behavior was the same. The ratio of kernel types on the ears suggested autonomous control of mutation at a_1^{m-1}. One case will be considered below.

On the self-pollinated ear of this greenhouse plant, there were 243 kernels. Among them, 7 carried a germinal mutation that produced pale aleurone color; 181 were variegated, with dots of A_1 and small pale areas, like the kernel from which the plant arose; and 55 were completely colorless. Since this plant was a_1^{m-1}/a_1 in constitution, the observed ratio of kernel types was that to be expected if mutation at a_1^{m-1} is autonomously controlled. This plant was used as the pollen parent in a cross to a plant homozygous for a_1, and again the expected ratio of kernel types appeared: 9 kernels with germinal mutations to pale aleurone, 181 kernels with dots of full A_1 and small pale areas, and 165 completely colorless kernels. The plant carrying a_1^{m-1} was heterozygous for Y (Y, yellow endosperm, dominant to y, white endosperm), and the plant to which it had been crossed was homozygous for the recessive, y. On the self-pollinated ear, all 7 of the kernels carrying germinal mutations to pale aleurone were Y, and on the backcross ear 8 of them were Y. No significance was attached to this relationship when the ears were first examined, as the total number of kernels with germinal mutations was small.

Kernels were selected from both the self-pollinated ear and the backcross ear, and plants were grown from them in the summer of 1951. Tests of the plants arising from the kernels having germinal mutations indicated that the mutation was completely stable in subsequent generations. Plants were obtained from variegated kernels of both the Y class and the y class that had appeared on the ears derived from self-pollination and backcrossing. These, in turn, were self-pollinated and crossed to plants homozygous for a_1. It was desired to introduce the recessive sh_2 (shrunken endosperm) into plants carrying a_1^{m-1}, because sh_2 is known to be very closely

linked to the A_1 locus (they are only a quarter of a crossover unit apart). Therefore, some of the plants derived from the variegated kernels, all of which were homozygous for Sh_2, were crossed either to or by plants homozygous for a_1 and sh_2 and also for y. Because of the pressure of other work, time was not taken to examine the ratio of kernel types on the resulting ears. Variegated kernels that were nonyellow (yy) were selected from two of these ears, however, and plants were grown from them in the summer of 1952. These were again crossed reciprocally to plants homozygous for a_1 and sh_2, and all the kernels on the resulting ears were examined. The examination revealed an extraordinary situation with regard to the relative number of kernels carrying germinal mutations. On most of these ears, such mutations appeared in approximately 50 per cent of all kernels in the Sh_2 class—that is, the class in which the a_1^{m-1} locus was represented.

In the first culture, consisting of eight plants, six plants showed this phenomenon. The sixteen ears produced by these six plants yielded 3793 kernels, which could be separated into the following classes: full A_1 color expression, Sh_2—3 kernels; pale color, Sh_2—1043 kernels; variegated (dots of full A_1 color and small areas of pale color), Sh_2—906; colorless, Sh_2—10; pale color, sh_2—1; variegated, sh_2—1; colorless, sh_2—1829. Three ears were obtained from each of four plants, and two apiece were obtained from the remaining two plants. On each ear, the ratio of pale-colored to variegated kernels in the Sh_2 class was close to 1:1. The numbers were as follows: plant 1, 83 to 79, 73 to 66, and 27 to 24; plant 2, 127 to 107, 124 to 97, and 22 to 22; plant 3, 54 to 56, 44 to 45, and 59 to 50; plant 4, 15 to 11, 115 to 76, and 91 to 80; plant 5, 102 to 92, and 42 to 51; plant 6, 45 to 31, and 20 to 19. A single ear was obtained from each of the remaining two

plants in this culture. On one of them, there were 104 pale, Sh_2 kernels and 211 variegated, Sh_2 kernels—an approximate 1:2 ratio. On the other there were 5 pale, Sh_2 kernels and 136 variegated, Sh_2 kernels; no segregation-like ratio was exhibited on this ear.

In the second culture, nine plants were crossed by plants homozygous for a_1 and sh_2. On thirteen ears produced by seven of these nine plants, the very same type of 1:1 ratio in the Sh_2 class of kernels was obtained. Among a total of 5157 kernels, the following types appeared: pale, Sh_2—1368; variegated, Sh_2—1216; colorless, Sh_2—10; pale, sh_2—1; variegated, sh_2—3; colorless, sh_2—2559. On the ear of one of the remaining two plants in this culture there were 72 pale, Sh_2 kernels and 157 variegated, Sh_2 kernels, or a 1:2 ratio of kernel types in the Sh_2 class. Three ears were obtained from the ninth plant; and, in sharp contrast with the situation described above, the ratios were not the same on all three ears. There were 48 pale, Sh_2 to 78 variegated, Sh_2 on one ear; 48 pale, Sh_2 to 160 variegated, Sh_2 on the second ear; and 18 pale, Sh_2 to 85 variegated, Sh_2 on the third ear.

When the plants in the two cultures were used as pollen parents in the reciprocal cross, a majority of the resulting ears likewise exhibited unit ratios of Sh_2 kernels having germinal mutations to those having none. Of the total of seventeen plants in the two cultures, seven were used as pollen parents. Five of them, which had given a 1:1 ratio when used as female parents, also produced this ratio when used as male parents. The numbers of the different types of kernels among a total of 2527 were: pale, Sh_2—672; variegated, Sh_2—591; colorless, Sh_2—15; pale, sh_2—1; colorless, sh_2—1248. One of the two remaining plants had shown a 1:2 ratio when used as a female parent. This same ratio appeared

when it was used as a male parent, with the following distribution of kernel types: pale, Sh_2—55; variegated, Sh_2—97; colorless, Sh_2—2; variegated, sh_2—1; colorless, sh_2—161. The seventh plant, when used as a female parent, had shown no segregation-like ratio (5 pale, Sh_2 to 136 variegated, Sh_2). When it was used as a male parent, however, a 1:3 ratio appeared on the resulting ear: full A_1, Sh_2—1; pale,

examined in order to determine whether or not they presented similar segregation-like ratios. Some of the plants were Y/y as well as a_1^{m-1}/a_1 in constitution, and five of them had been backcrossed to plants homozygous for a_1, sh_2, and y. The ears produced by four of these five plants showed a striking linkage of phenotypes. On the ears of three plants, germinal mutations to pale appeared with a high frequency

TABLE 3

PHENOTYPES OF KERNELS APPEARING ON EARS PRODUCED BY THE CROSS OF PLANTS HOMOZYGOUS FOR a_1, sh_2, AND y BY PLANTS WHOSE CONSTITUTIONS WERE $a_1^{m-1}\ Sh_2/a_1\ Sh_2$; Y/y (PART I), OR $a_1^{m-1}\ Sh_2/a_1\ Sh_2$; y/y (PART II)

PLANT NUMBER	PARENTAGE IN CROSS	Color in aleurone					Colorless aleurone		
		Pale		Variegated		Total			
		Y	y	Y	y		Y	y	Total
Part I:									
6046B-1..........	♂	107	20	12	89	228	119	113	232
6046B-3..........	♀	32	1	58	95	186	93	88	181
6046C-2..........	♀	28	7	55	86	176	75	103	178
6047A-1..........	♀	30	31	173	162	396	209	228	437
6047A-3..........	♀	48	82	76	47	253	115	146	261
Part II:									
6047C-1..........	♀	...	20	...	174	194	...	163	...
6047C-3..........	♀	...	92	...	97	189	...	203	...
6047C-4..........	♀	...	58	...	184	242	...	251	...
6047B...........	♀	...	4	...	398	402	...	391	...
6047B...........	♂	...	5	...	482	487	...	483	...

Sh_2—57; variegated, Sh_2—168; colorless, Sh_2—3; colorless, sh_2—216.

The 1:1 ratio of kernel types in the Sh_2 class on the backcross ears obtained during the summer of 1952 suggested that the a_1^{m-1}-carrying plants were heterozygous for a factor that induces mutation to pale in the haploid spore receiving it. As was stated earlier, the kernels from which these plants arose had been produced on ears of plants grown the previous summer, whose kernel types had not yet been examined. The kernel types of these summer-1951 plants, and those of their sibs, were now

among kernels of the Y class and with a low frequency among kernels of the y class. The ear produced by the fourth plant showed the reciprocal linkage relationship. Part I of table 3 illustrates this. The plants in culture 6046 of table 3 came from variegated kernels with yellow endosperms, from the self-pollinated ear of a plant grown in the greenhouse the previous winter. Those in culture 6047 were derived from variegated kernels appearing on the ear resulting from a cross of this plant to one homozygous for a_1 and y. The A plants of this culture came from kernels

with yellow endosperms, whereas the B and C plants came from kernels with white endosperms. The B plant was derived from a variegated kernel that exhibited a newly altered state of a_1^{m-1}. Instead of dots of full A_1 color in the endosperm and sizable streaks in the plant, this state produces only specks of deep A_1 color in the endosperm and small streaks in the plant.

A 1:1 ratio of pale to variegated in the colored class appeared in crosses of only three of the plants listed in table 3, two cases in part I and one in part II. The linkage of pale kernels to Y and of variegated kernels to y in the cross involving plant 6046B-1, and the reciprocal linkage relationship exhibited in the cross involving plant 6047A-3, are both very definite. These linkages suggest that a heritable factor, located in chromosome 6, controls the occurrence of mutation at the a_1^{m-1} locus in those spores receiving it as a consequence of meiotic segregation. It is also evident, as the data from the crosses involving plants 6046B-3 and 6046C-2 indicate, that mutation need not occur in all the spores receiving this factor. Until further information is available, it may not be profitable to consider why this should be so. It can be mentioned, however, that the study of segregation-like ratios involving the a_1^{m-2} locus has provided information that may apply to a_1^{m-1}. On the ears of field-grown plants, sharp segregation ratios appeared. On the ears of plants grown in the greenhouse, segregation ratios were usually absent and germinal mutations were infrequent; in some cases, none were seen. The greenhouse plants came from kernels of the very same ears that provided

kernels for the field-grown plants. This would suggest that certain physiological conditions within the plant, in addition to the genetic constitution of the nucleus, influence the occurrence of mutation, and that these conditions are in some way environmentally or nutritionally controlled.

It should be stated that the two cultures grown in the summer of 1952 which produced so many ears showing an approximate 1:1 ratio of Sh_2 kernel types were derived from kernels appearing on the ears of two of the crosses recorded in table 3. The plants in one of these cultures were grown from variegated kernels with white endosperms, chosen from an ear produced by the cross involving plant 6047A-3. Plants in the other culture came from the variegated kernels on an ear produced by plant 6047C-4. With regard to these ratios, one further point is of interest. It may be recalled that many of the ears showed a slight excess of kernels with germinal mutations. Results of a study of a_1^{m-2} have helped to make this understandable, also. They suggest that the excess represents mutations that occurred in a few cells prior to meiotic segregation. Spores derived from such cells would carry a pale mutation regardless of whether or not they had received the mutation-inducing factor as a consequence of meiotic segregation.

It is now known that particular changes in genetic constitution of nuclei, whether arising as a consequence of meiotic segregation, as described above, or through somatic segregation, as previously mentioned, influence the occurrence of mutations at a number of mutable loci.