MUTABLE LOCI IN MAIZE

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During the past year, the study of instability of action of the genetic factors at various loci in maize chromosomes was continued. This study, extending over a six-year period, has shown that many different genetic factors may exhibit such instability. By appropriate methods, it is possible to detect the instability of expression of the factors at a particular locus shortly after the event at the locus leading to this behavior has occurred. Different origins of instability at any one locus may thus be discerned. Comparisons of such cases have shown that the genetically active components at any one locus may vary in type and in range of expression, and that differences may exist in their mode of control. From this evidence interpretations have been derived regarding the responsible mechanisms. These have been developed in an article now in press (*Cold Spring Harbor Symposium on Quantitative Biology,* vol. 16, 1951) and therefore need not be stated here. This report will be limited to brief reviews of newly acquired information concerning the origin and behavior of mutable conditions at various loci.

Two new cases of instability have appeared involving the bronze locus in the short arm of chromosome 9 (*Bz,* dark red or purple color in plant and aleurone; *bz,* recessive allele, plant and aleurone color a bronze shade). Each appeared in a single tested gamete of a plant that carried *Ds* and *Ac,* and they are much alike in behavior. Both are *Ac*-controlled; and they appear to have originated by transposition of *Ds* to the bronze locus, for *Ds*-type breaks in the chromosome occur at this locus, as well as mutations from *bz* to the apparently full *Bz* phenotypic expression. Their behavior resembles in many respects that of c^{m-1}, described in previous reports. They have been designated bz^{m-1} and bz^{m-2}.

The *Wx* locus has been concerned in several other studies. The factors at this locus are associated with production of amylose starch in the pollen and in the endosperm of the kernel. When only the recessive, *wx,* is present, the starch consists of amylopectin, and this stains reddish brown with iodine–potassium iodide solutions. The normal dominant, *Wx,* in single, double, or triple doses, produces amylose starch, in quantities amounting to approximately 22, 25, or 28 per cent, respectively, of the total starch content of the endosperms. This starch, in each of these doses of the dominant, stains a dark blue with I-KI solutions.

In Year Book No. 48 (1948–1949) a series of alleles produced by mutations of the *Ac*-controlled wx^{m-1} locus was described. Many of these alleles may be distinguished by the physical appearance of the starch in the endosperm, but a more accurate criterion is the intensity of the blue stain when a solution of I and KI is applied. The intensity of the staining reaction is an indication of the amount of amylose starch produced by a particular allele; and a progressive increase in intensity of stain is exhibited by kernels carrying single to triple doses of the particular allele. (One and two doses are obtained by combinations with the recessive, *wx*.) The alleles arising from mutations at wx^{m-1} produce amounts of amylose ranging from only a fraction of 1 per cent of that produced by the normal allele, *Wx,* to approximately the same amount as *Wx*. Mutations at wx^{m-1} occur only when *Ac* is present in the nucleus, and the alleles so produced may again mutate if *Ac* is present. When *Ac* is absent, the alleles appear to be stable. A number of different germinal mutations of wx^{m-1} have occurred in plants carrying wx^{m-1} and one *Ac* factor. Those carried in gametes in which *Ac* was not present served as the source of stable strains of particular alleles. The action of these selected alleles in single, double, and triple doses was examined visually by means of the iodine-solution test. Chemical analyses to determine the amylose content produced by some of the alleles in single to triple doses were conducted by Dr. B. Brimhall and Dr. G. F. Sprague, of Iowa State College. Table 1 reproduces the data supplied by these investigators.

TABLE 1

Allele designation	Percentage amylose starch produced by allele in single to triple doses		
	Single dose	Double dose	Triple dose
4744A-3	2.4	4.0	5.8
5220A	3.5	4.6	7.3
4744E-1	3.5	4.6	7.2
4744E-2	3.7	5.8	*
2d sample	....	5.3	....
4744G-2	4.3	6.5	*
5013D	4.6	5.0	9.3
4742B-2	6.5	7.3	13.2
4721F	16.8	22.2	25.0
4744C	18.5	20.0	25.5
5750B	25.0	27.0	28.0
5016A	23.0	27.0	*

* Sample having triple dose of allele not available at time of analysis.

Selection of the alleles whose action is shown in table 1 was not random; they included some of the lower and some of the higher ones. Other alleles with still lower activity, as estimated by the iodine staining reaction, have been isolated. Some others that may fall between isolates 4742B-2 and 4721F of the table were not available in graded doses to be included in the analyses. The samples analyzed, together with the others mentioned, suggest that mutations at wx^{m-1} may give rise to a series of alleles distinguished by small differences in the amount of amylose starch

they produce. Thus, a relatively large number of quantitative alleles may be anticipated.

Several other cases of independently originating instability at the *Wx* locus have been investigated. One of them, designated wx^{m-3}, was detected by differences in starch constitution in regions of an individual kernel on an ear coming from the cross of a female plant carrying a previously normally behaving *Wx* locus in each chromosome 9, by a male plant homozygous for the stable recessive, *wx*. The modification producing this instability occurred at the *Wx* locus in one of the chromosomes 9 of the female parent. Studies of the gametes produced by the plant arising from this kernel, and tests of its progeny, revealed the type of instability expression. It appears that wx^{m-3} is autonomous; no separate activator factor is required for the occurrence of mutations. The types of mutation differ markedly from those produced at wx^{m-1}. No alleles showing intermediate expressions of the action of the factors at this locus have been isolated, and only very rarely does a kernel exhibit a region that indicates mutation to intermediate action. The mutations are nearly always from recessive to apparent full-dominant expression. The dominant so produced may mutate again to the full recessive.

Two additional mutable conditions at the *Wx* locus have recently been found. Both arose from modifications at a previously normally behaving *Wx* locus. Each was first recognized in an individual kernel, which had regions showing visible changes in appearance of the starch as well as the associated less intense blue staining with iodine solutions. A plant was obtained from each kernel, and the study was continued. Neither of these cases appears to require a separate activator factor, but the studies are too incomplete to permit adequate description of behavior. In one of them, however, the rate of production of germinal mutations is high. Selections were made of some of these, each showing a particular rate of reaction, and the plants arising from them were crossed to tester stocks this past summer to determine the subsequent stability of the selected mutants.

Variegation in starch composition resembling instability of action of factors at the *Wx* locus commonly occurs in kernels that have *Ac* located a few crossover units to the left of this locus. Areas showing various grades of intensity of blue stain with iodine solutions may appear in kernels that have received from their female parent a chromosome 9 carrying the stable recessive *wx*, and no *Ac*, and from their male parent a chromosome 9 carrying *Ac* at this location and also a normal *Wx*. Attempts are now being made to determine whether these altered phenotypic expressions arise from modifications at the *Wx* locus and as a consequence of the spatial relation between *Ac* and *Wx*.

Unstable behavior of the factors located at a number of other positions in the chromosome complement has also been examined. Because the number of such cases is continually expanding, it has not been possible to examine each with the required degree of thoroughness. For many of them, only a cursory examination was attempted, with a view to gaining some information, even if limited, about the nature of instability in each case. The presence of these mutable conditions is detected by variegation in a plant or endosperm character. Ten of those examined are associated with production of pigment in the aleurone layer of the kernel, or in both kernel and plant tissues. The loci of six of them are known. Three are at the A_1 locus in chromosome 3, one is at the *I* locus in chromosome 9, and two are at the *R* locus in chro-

mosome 10. One involves a previously unidentified locus whose factors resemble in action those of the *R* locus. One suggests alterations at *Pr* (purple anthocyanin pigment; *pr,* recessive allele, red pigment; located in chromosome 5). Its mutations are from the recessive, which results in red pigmentation, to various grades of intensity of the dominant expression, which is purple pigmentation. Tests for the locations of the remaining two have not been completed, but the mutation types do not suggest that the factors at any of the previously known loci are involved.

Study of instability of expression of factors associated with anthocyanin pigmentation in the plant tissues only has been confined to determining the patterns of this expression; no attempt has been made to determine the loci involved. One such case suggests alterations at the *B* (booster of plant color) locus in chromosome 2; another suggests alterations at the *Pl* (purple plant color) locus in chromosome 6; and a third suggests instability at the *R* locus in chromosome 10 that alters the anthocyanin pigmentation in the plant tissue only.

In another case, the factors at the locus involved are associated with pigmentation of the pericarp layer of the kernel. Although this mutable condition has received only cursory examination, its action will be mentioned because of an interesting relation between mutations in the pericarp and intensity of pigment formation in the cells of the aleurone layer underlying the mutant areas. The pericarp tissue forms the outer layer of the kernel. It is maternal in origin, whereas the underlying aleurone layer is derived from the endosperm. In this case, the pericarp is colorless until a mutation occurs. The mutations result in pigment formation in the pericarp. The type of pigmentation produced by mutation resembles that associated with the cherry allele at the *R* locus. Some diffusible substance is produced as a consequence of these mutations, and this substance is used by the cells of the underlying aleurone to intensify their own pigmentation. This is evident because the aleurone cells located under the regions of colored pericarp are more intensely pigmented than those underlying the pericarp areas in which no mutations have occurred. If the pericarp layer is peeled off, its pattern of mutations is still revealed in the aleurone by regions of more intense pigmentation.

The alteration at the *I* locus is of some interest because of the nature of the action of *I,* which had been analyzed earlier. *I* acts to produce an inhibitor of pigment formation in the aleurone layer when its allele *C* (aleurone color) is also present. Kernels homozygous for *I,* however, are completely colorless. In kernels with *I I C* constitutions, aleurone color does not develop. In *I C C* constitutions a faint blush of color may appear at the base of some of the kernels. The dosage action of the *C* allele has been described in previous reports; the higher the dose, the more intense the pigmentation. The expression of both *I* and *C* is quantitative. *I* appears to be concerned with the production of some substance or substances that compete for reactive sites with the substance or substances associated with *C* action. As the dose combinations given indicate, *I* appears to be more effective than *C* in this respect. If more *C* factors were present, however, the increment might result in the development of considerable amounts of pigment in the aleurone layer. This possibility was examined by using a chromosome 9 with a duplication of the short arm, each member of which carried a *C* factor. When two such chromosomes were contributed by the female parent and a single *I* factor

by the male parent, the dose ratio in the endosperm was four *C* factors to one *I* factor. The aleurone layer in such kernels was uniformly pigmented. The intensity of this pigmentation was slightly less than that produced by a single dose of *C* (*C c c* constitutions). The alteration at the *I* locus effected a change that lowered the capacity of *I* to inhibit *C* activity.

The above-mentioned alteration occurred at a previously normally behaving *I* locus. It was detected in an aberrant kernel on an ear resulting from the cross of a female plant homozygous for *C* by a male plant carrying *I*. Much pigmentation appeared in the aleurone layer of this kernel. Variegation was exhibited by small areas that were either lighter or darker in color. The plant arising from this kernel was self-pollinated, crossed reciprocally with plants carrying *C*, and also crossed to plants carrying *c*. Examination of the resulting ears indicated the nature of the action attributable to the alteration at the *I* locus. Kernels homozygous for this modified *I* were completely colorless, as were those carrying this *I* and also *c*. The reduced capacity for functioning was exhibited in combinations with *C*. A single dose of modified *I* with a double dose of *C* produced kernels that were darkly pigmented. Those having a double dose of *I* and a single dose of *C* were more lightly pigmented. In some of the kernels with *I C C* constitutions, variegation was exhibited; regions that were colorless or more deeply colored than the surrounding areas were present.

During the year, much effort was focused on examination of alterations affecting the action of factors at the A_1 locus in chromosome 3 (A_1, aleurone and plant anthocyanin color; a_1, recessive allele, colorless aleurone and altered plant color). Three independent cases of inception of instability at this locus have been isolated, and they will be described shortly. Attention will first be given, however, to summarizing the results of efforts to produce mutations of the known stable recessive allele, a_1.

Experiments aimed at the production of mutations of a_1 were described in Year Book No. 49 (1949–1950). During the past year, these experiments were considerably expanded. Because the methods employed have been described, only a summary of the additional results need be included here. Thirteen plants homozygous for a_1 and having the desired constitutions with respect to chromosome 9 (one carrying a long terminal deficiency of the short arm, the other having a duplication of this arm in the reverse order) were used in crosses to plants homozygous for a_1 and having normal chromosome constitutions. No *Dt* (Dotted) factor was present in any of these plants. As was mentioned in the previous report, the purpose of the experiment was to determine whether or not mutations to A_1 would occur in the absence of *Dt* and, if so, whether these mutations would be of the type produced by *Dt*. At the present writing, the kernels on 315 ears derived from these crosses have been individually examined; and on 86 of the ears one, or occasionally two or several, kernels showing one or more spots of the A_1 phenotype were found. Among the 93,078 kernels examined, 117 had such mutant areas; and all these areas were small, resembling those produced by the *Dt* factor. In 93 of them, only one A_1 spot was present. The other 24 were distributed as follows: 9 with two A_1 dots, 4 with three, 6 with four, 1 with five, 2 with seven, 1 with seventeen, and 1 with eighty-four. Except in the one kernel having eighty-four spots, the A_1 spots were confined to one area of the kernel. This would be expected if the event leading to mutation depended upon an earlier event that evolved a *Dt*-like factor.

Mutations at a_1, of the *Dt* type, would appear only after such an event had occurred, and only in that area of the kernel derived from the cell in which it had taken place. In the kernel having eighty-four of them, the A_1 dots were distributed throughout the aleurone layer. In the plant grown from this kernel no mutations to the A_1 phenotype appeared; and no such mutations occurred when this plant was crossed to and by plants homozygous for a_1. The alteration responsible for the mutations to the A_1 phenotype in the kernel was probably present in only one of the two sperms that functioned in the origin of this kernel—the one that entered the endosperm nucleus.

A control experiment, accompanying the one described above, was made by intercrossing plants homozygous for a_1 but having normal chromosome constitutions. Only one kernel among a total of 21,464 examined had a single small spot of the A_1 phenotype.

The origins of the three independent inceptions of instability at the locus of A_1, mentioned above, may be reviewed briefly. The first case, designated a_1^{m-1}, was detected in a single aberrant kernel on an ear resulting from a cross in which the male parent was homozygous for a_1 and the female parent heterozygous (A_1/a_1). The aleurone layer of this kernel was variegated; both colored and colorless areas were present. The types of variegation and color expression suggested that mutations were occurring from the recessive to higher alleles of A_1. The plant derived from this kernel was likewise variegated for anthocyanin pigmentation. Tests of its progeny confirmed the presence of an unstable a_1 locus and showed that this instability had arisen in one of the chromosomes 3 contributed by the female parent. This a_1^{m-1} is autonomous, in that no separate activator factor is required for expression of instability. A number of different types of mutation occur. They affect the type and amount of pigment that will be formed in the aleurone and plant tissues, and the time and place of its formation.

The second inception of instability was detected in much the same way as was a_1^{m-1}. It was present in a single aberrant kernel on an ear from a cross in which the male plant had been homozygous for a_1 and the female plant heterozygous (A_1/a_1). In this kernel the variegation was expressed by dots of the A_1 phenotype. The plant derived from it also showed variegation for anthocyanin pigmentation. Subsequent tests indicated that this mutable condition, designated a_1^{m-2}, arose in one of the chromosomes 3 contributed by the female parent.

The third mutable condition, a_1^{m-3}, was detected by variegation for anthocyanin color in the aleurone layer of some kernels on the self-pollinated ear of an individual plant and on the ears coming from reciprocal crosses with plants carrying a_1. Both parents of this plant were homozygous for A_1, and no instability affecting its behavior had been detected in these parents or their antecedents. Six sister plants were also self-pollinated and crossed reciprocally with plants homozygous for a_1, and no instability of phenotypic expression of A_1 was detected.

Both a_1^{m-2} and a_1^{m-3} arose in plants that carried *Ds* and *Ac*. Preliminary evidence suggests that the mutations are controlled in each case by an activator. This may prove to be *Ac*. Until the possible relation with *Ac* has been clarified, a description of the mutational types given by these two mutable loci will be postponed.

Some other loci carrying factors associated with the development of kernel characters have exhibited mutability. Changes at one such locus affected the production of yellow pigment in the endo-

sperm cells. They were first detected in some kernels on an ear of a plant that was homozygous for *y* (*Y*, yellow pigment formation; *y*, recessive allele, no yellow pigment formed). In these kernels, areas were present showing various grades of intensity of yellow pigment. Study of this case was confined to determining that the mutable condition was heritable. Instability of the action of factors at other loci has resulted in altered development of either the aleurone layer or the underlying endosperm cells. These types have proved to be heritable, but no attempt has been made to study their behavior in detail.

A number of cases of instability have appeared that were originally revealed by somatic changes affecting chlorophyll development. Except in three cases that appeared early in the study, no attempt has been made to investigate in detail the nature of the mutation processes concerned. The study was confined to determining whether or not the mutable condition was heritable.

The above description of different mutable loci and their origins has been given in order to indicate the range of genetic expression of this kind that can occur in a single organism and the large number of known loci as well as previously unknown loci that may be concerned. On the basis of the types of mutation and their modes of control, it has been possible to subdivide the mutable conditions into classes. It is now known that mutable conditions belonging to different classes may arise at any one locus. No theory of gene action or mutation that has been seriously considered by the majority of geneticists is adequate to account for these various types of expression of the factors at a locus and their modes of control. It has been necessary, therefore, as was pointed out early in this report, to reconsider former concepts and to develop others, where required, in order to incorporate the new evidence.

All the types of mutation described in this and previous reports have been heritable. Some cases have been found, however, in which the phenotypic expression of instability did not prove to be heritable. Several of them have been given some attention. The general pattern of such instability may be illustrated by the following case. A plant homozygous for the recessive factors *c* and *wx* in chromosome 9 and *pr* in chromosome 5 was crossed by one carrying the dominant alleles. All the kernels on the resulting ear showed bizarre types of variegation. Every kernel had several, and some many, regions showing unexpected phenotypes. Some regions showed the *c* phenotype in the aleurone layer. The underlying cells might be all *Wx*, some *Wx* and others *wx*, or all *wx*. Other regions obviously were produced because dicentric chromatid formations, involving the chromosome 9 carrying the dominant factors, had occurred in the ancestor cell that produced the region. Chromosome 5 also was involved in chromosome aberrations, for many areas of the *pr* phenotype appeared. Of considerable significance was the appearance of twin areas, in large numbers. These are adjacent regions of similar size, which suggest origin from sister cells. Several classes of such twin areas appeared: (1) one area of colorless aleurone, the other having a deeper color than that of the surrounding aleurone cells; (2) one area showing the red (*pr*) phenotype and the other a deeper purple (*Pr*) than the surrounding aleurone cells; and (3) two regions showing reciprocal quantitative differences in color intensity—that is, one lighter and one darker than the surrounding areas. That some if not all of this variegation resulted from chromosome aberrations was suggested by the concomitant losses of *C* and *Wx* in some

areas, indicating deletion, and the occurrence of breakage-fusion-bridge cycles in others, indicating dicentric chromatid formations. Plants were obtained from some of these kernels and crosses were made to test for recurrence of the expression in the following generation. None appeared on the many test ears examined, and the experiment was discontinued at this stage.

A second case, very similar to the one just described, was also examined. It likewise proved to be nonheritable, in that the abnormal phenotypes did not reappear in the following generations. No evidence has appeared to suggest what factors are responsible for these nonheritable types of variegation.