MUTABLE LOCI IN MAIZE

BARBARA McCLINTOCK

Previous reports have stated that a number of unstable loci have recently arisen in the maize cultures. In a particular cell of a plant, a normal "wild-type" locus becomes altered; the normal, dominant expression of this locus changes and gives rise to a recessive expression (or, in several cases, a recessive locus becomes unstable and mutates toward a dominant expression). This expression of the locus need not be permanent. In some descendent cells, a second change may occur within the locus that results in the restoring of the capacity of the locus to express the dominant phenotype or brings about an intermediate expression between full recessive and full dominant. In the latter case, a third alteration may occur in some descendent cells that steps up the phenotypic expression toward the full dominant or reduces it toward the full recessive.

When a locus changes from a stable to an unstable state, recognition of the occurrence depends upon several factors. A clear-cut phenotypic expression of the changed locus is essential. This limits ease of recognition to those gene loci associated with the production of some obvious plant constituent, such as the chlorophyll pigments or other plant pigments, or to contrasting morphological characters striking enough to allow the contrasts to be de-

tected in single small sectors of some part of the plant. The presence of a changed locus that gives a recessive expression often may remain hidden in a heterozygous state until a self-pollination or some special cross is made that allows its presence to be uncovered. No attempt has been made to detect the presence of these hidden changed loci, and thus the frequency of occurrence of such changes is not known. The unstable loci that have been discovered have all appeared in the progeny of crosses designed for other purposes. It is suspected that the number of mutable loci still undetected may be quite great, with numerous independent occurrences of unstabilization of the same locus. The detected unstable loci include many previously unknown to maize geneticists. Some well known loci in maize have also become unstable in these cultures. These include two independently arising unstable *yg* loci (yellow-green chlorophyll), four independently arising unstable *c* loci (*c*, colorless aleurone; *C*, colored aleurone), three independently arising unstable *wx* (waxy) loci (*wx*, starch staining red with iodine; *Wx*, starch staining blue with iodine), and one unstable a_2 locus (anthocyanin in aleurone and plant). With the exception of the two *yg* loci, all these unstable loci have originated by a change in a normal, previously quite stable locus showing dominant expression. In the case of unstable *yg*, the recessive *yg* locus mutates to form chlorophyll of a much darker color than the normal *yg* locus expresses.

These mutable loci fall into two distinct classes: (1) those that require the presence of a second locus, the activator locus (*Ac*), for instability to be expressed and maintained, and (2) those that do not require such a second locus for instability to be expressed. The *Ac* locus itself is unstable and resembles in this respect the second class of unstable gene loci.

Two recognizable subdivisions of the mutation process are shown by all the unstable loci: (1) control of the time and the apparent frequency of mutations in a tissue by a factor capable of changing during a mitosis so that altered rates of mutation will be expressed in descendent cells following such a change; and (2) the subsequent change at the locus that occurs in a particular cell and gives rise immediately to recognizable altered phenotypic expression in this cell and its descendent cells. The term "state of a locus" has been used to distinguish the differing potentialities of a mutable locus for expressing visible mutations in descendent cells. The mutations giving rise to changes in state of a locus are readily recognized by the altered rates of the second type of mutation that follow from such an event. In the second class of mutable loci—those controlled by *Ac*—the time and apparent frequency of mutations of the affected locus are controlled by the states of both loci: the locus controlled by *Ac* and the *Ac* locus itself.

During the past year, attention has been directed mainly to those loci that require the *Ac* locus for continued expression of instability. All these loci that are known are in the short arm of chromosome 9, possibly because the genetic methods being used allow certain mutable loci in this arm to be readily detected. Those receiving particular study include the *Ds* (Dissociation) locus, mentioned in previous reports, two independently arising mutable *c* loci, and two independently arising *wx* loci. In each case, the mutable recessive state of the previously stable dominant locus arose in a particular cell of a plant that also possessed an *Ac* locus. The origin of this potent *Ac* locus is quite unknown. It has been traced to a culture

grown in the summer of 1942, but its presence in still earlier cultures cannot be traced. It cannot be stated that *Ac* induces the initial state of instability of a locus, although a causal connection may be suspected.

None of these *Ac*-controlled mutable loci will show any type of instability if the *Ac* locus is absent from the nucleus. As soon as *Ac* is introduced into a nucleus having such a susceptible locus, instability is resumed. Mutations at the susceptible locus sometimes may occur in the initial nucleus following the introduction of *Ac*. To illustrate the control of mutability of a locus by *Ac*, the mutable c^{m-1} locus may be used. This locus has been given the symbol c^{m-1} because it was the first of the several mutable *c* loci isolated; the normal *c* locus, used for many years in genetic experiments, is designated c^s because it is a stable locus not mutating under the influence of *Ac*. The behavior of this unstable recessive *c* locus in the designated constitutions is indicated below:

c^{m-1} *ac*: no mutations; aleurone layer colorless

c^s *ac*: no mutations; aleurone layer colorless

c^s *Ac*: no mutations; aleurone layer colorless

c^{m-1} *Ac*: mutations to *C* occur; aleurone layer variegated for color

It should be emphasized that, with similar constitutions, the same type of responses will be registered by any of the other loci that are activated by *Ac*.

Nature of the *Ac* Action

The Ds locus. The *Ds* locus was discovered before any of the other *Ac*-controlled unstable loci. *Ds* is located at the position demarking the proximal third of the short arm of chromosome 9, between one and two crossover units to the right (toward the centromere) of the *Wx* locus. The normal, nonmutating locus has been designated *ds*. An observable *Ds* mutation arises as the consequence of breakage of two sister chromatids within the *Ds* locus in each case. This breakage is followed by fusions of broken ends to give rise to a U-shaped acentric fragment composed of the two sister segments of the distal two-thirds of the short arm, and a U-shaped dicentric chromosome composed of the proximal third of the short arm, the centromere, and all of the long arm of each of the two sister chromatids. Nearly all the known loci of this chromosome are carried in the distal two-thirds of the short arm of chromosome 9 and are thus included in the acentric fragment after a *Ds* mutation. The genetic methods of observing the time and frequency of the *Ds* mutations in various parts of the plant have been explained in previous reports (Year Books Nos. 45 [1945–1946] and 46 [1946–1947]).

The *Ac* locus acts upon *Ds*, or any other locus susceptible to it, usually quite late in the development of any of the sporophytic tissues. In contrast, action of *Ac* on susceptible loci may be apparent at all stages in the development of the endosperm tissues. In this respect, the endosperm behaves like an extension of the sporophytic tissues.

As stated above, the detectable *Ds* mutations are associated with breaks at this locus that are followed by 2-by-2 fusions of those broken ends of sister chromatids that lie adjacent to one another. This type of fusion following breakage is not the only one that could occur. Restitutions could take place, re-establishing the previous organization of the chromatids; or crisscross fusions of broken ends could occur, simulating crossing over but involving sister chromatids in somatic nuclei. It is not known whether or not the latter

types of fusion do occur with expected frequencies at the *Ds* locus. The evidence suggests that they may occur, at least occasionally. It is known that breaks at the *Ds* locus may be followed directly or indirectly by fusions of broken ends other than those giving rise to the U-shaped dicentric chromatids. This is shown by the fusions that occur after an occasional coincidental break in another part of the chromosome. It is possible then for a broken end associated with a *Ds* mutation to fuse with one of these other newly broken ends. This gives rise to a gross chromosomal aberration that can be analyzed. Such chromosomal aberrations have been observed in individual cells or clusters of cells in the examined sporocytes of the *Ds Ac* plants. Several of these translocations have been found in individual plants, and have received further study. These aberrations have been useful in interpreting occasional inconsistencies in the behavior and the location of the *Ds* and the *Ac* loci. It is now known that the *Ds* locus may change its position in the chromosome after such coincidental breaks have occurred. One very clear case has been analyzed, and, through appropriate selection of crossover chromatids, strains having morphologically normal chromosomes 9 have been obtained. As a consequence of this aberration, the *Ds* locus in these strains has been shifted from a position a few units to the right of *Wx* to a position between *I* and *Sh*. This is a very favorable position for showing the nature of the *Ds* mutation process. When the usual type of *Ds* mutation occurs at the locus in this new position, a U-shaped dicentric chromosome is formed. At the succeeding anaphase a chromatin bridge is produced, which subsequently breaks. The breakage-fusion-bridge cycle is thus initiated and can be genetically expressed in succeeding nuclear divisions. This is because the loci of *Sh, Bz,* and *Wx* now lie between *Ds* and the centromere. With appropriate genic constitutions of chromosome 9, detection of the breakage-fusion-bridge cycle is certain after a *Ds* mutation has occurred in this new location. These observations have been made, and the genetic analysis completely confirms the cytological analysis of the nature of the *Ds* mutation process.

The inheritance of Ac as a separate locus. Ac behaves in inheritance as a single independent locus. Tests for the presence of *Ac* in F_2 progenies of *ds ds Ac ac* plants have given ratios of 1 *Ac Ac* to 2 *Ac ac* to 1 *ac ac;* backcrosses of *Ac ac* plants by *ac ac* plants have given ratios of 1 *Ac ac* to 1 *ac ac* plant. Crosses of *Ac Ac* plants by *ac ac* plants have given progenies all of which were found to be *Ac ac*. In the many crosses that have been made with plants that were heterozygous for both *Ds* and *Ac,* independent inheritance of the *Ac* and *Ds* loci was clearly established, except in three independently arising cases. In these three cases, *Ac* was obviously linked with *Ds* and could be located 6 to 20 crossover units to the right of it. Such linkages were maintained in later tests of the progeny of one of these plants, when both the crossovers and the noncrossover chromatids were tested. Similar tests are now under way for the other two cases. Cytological examination of heterozygous plants in each of the three cases showed no observable chromosomal abnormalities. Chromomere matching between synapsed homologues was perfect for all of chromosome 9.

Either of two possibilities may explain this change in genetic location of *Ac*. First, *Ac* may be located toward the end of the long arm of chromosome 9 and normally show no genetic linkage with *Ds* because the crossover distance is too great. In the case of *Ds-Ac* linkage, a newly

arising crossover modifier, not associated with a gross chromosomal abnormality, would need to be invoked to explain the observations. This modifier would need to be closely linked with *Ac*, as it has not been removed in the crossover tests so far made. Second, the *Ac* locus may have been removed from its former position and inserted into a new position in chromosome 9 in a manner similar to that observed for the transposition of the *Ds* locus, described above. Because *Ac* induces breaks at specific loci and gives evidence of undergoing a specific breakage process itself, this latter explanation is not improbable. Tests are now under way to determine whether or not either of these alternatives can apply.

The effects of dosage of Ac. Studies of the effects of various dosages of *Ac* have shown that the time and apparent frequency of *Ac*-controlled mutations is in large measure a function of dosage of *Ac*. This applies both to the sporophytic tissues and to the endosperm tissues. The endosperm tissue is 3*n*. Here, one, two, or three doses of *Ac* can be obtained and observations made of the effects of each of these dosages on any of the *Ac*-controlled mutable loci. All *Ac*-controlled mutable loci respond alike to the various *Ac* dosages. The description given below of responses of the *Ds* locus in endosperm tissues can be applied equally well to the responses of any of the other *Ac*-controlled mutable loci.

In *Ds ds ds* kernels, the mutation rates of the single *Ds* locus have been compared in *Ac ac ac*, *Ac Ac ac*, and *Ac Ac Ac* endosperm constitutions. One dose of *Ac* allows mutations to occur relatively early; considerable irregularity in both time and frequency of mutations is apparent, for the endosperm is often divided into various larger or smaller sectors each showing its own special rate of mutation. This irregularity is only apparent, as subsequent evidence will show. Two doses of *Ac* delay the timing of *Ds* mutations so that they occur relatively late in the development of the endosperm. The frequency may be very high, however. When three doses of *Ac* are present, only relatively few, very late-occurring *Ds* mutations are recognized in these kernels. Three doses of *Ac* appear to decrease the frequency of *Ds* mutations, but this appearance is probably deceptive. It is more likely that the increased dosage of *Ac* so delays the timing of *Ds* mutations that only the very earliest-occurring of these can possibly show in the endosperm, and that if the endosperm cells had continued to divide, very large numbers of *Ds* mutations would have appeared. In other words, the tissues mature before these potential *Ds* mutations can be expressed. The validity of this interpretation will be apparent when the various states of the *Ac* locus are defined and their dosage responses compared. It may be concluded, then, that the absolute dosage of *Ac* is one main factor controlling the time and apparent frequency of mutations: the higher the *Ac* dosage, the later the occurrence of *Ds* mutations.

The frequency of *Ds* mutations is complicated to analyze because of three factors: (1) the maturing of tissues with high dosages of *Ac* before *Ds* mutations can be expressed; (2) changes in state of a single *Ac* locus, resulting in changes in response of the *Ds* locus that resemble either an increase or a decrease in the dosage of *Ac;* and (3) changes in state of the *Ds* locus, resulting in changes in response to *Ac* dosages. Factor (1) has been briefly considered above. Factor (2) will be considered next, and factor (3) will receive brief consideration later.

Changes in state of the Ac locus. As mentioned above, in the endosperms of all

kernels having the constitution *Ds ds ds* and *Ac ac ac* there is great variability in the time and frequency of *Ds* mutations within a single kernel. Various numbers of relatively large or small sectors may be present, each showing a special time and frequency of *Ds* mutations, or showing no *Ds* mutations at all. In most of these kernels, a few relatively early *Ds* mutations are observed. Several male parents of the constitution *Ds ds Ac Ac,* when crossed to *ds ds ac ac* female plants, produced kernels with unusually favorable sectors of the above-mentioned types. In over 90 per cent of the *Ds*-carrying kernels, sectoring occurred early in the development of the endosperm. Each sector showed a very distinct type of mutation pattern. The sectors in two of the most common classes of kernel will be described for the purpose of illustration. In one class, the kernel was composed of four very distinct sectors. In one sector, no *Ds* mutations or only a few very late mutations occurred; in the second sector, the *Ds* mutations occurred late but relatively frequently and were uniformly distributed within the sector; in the third sector, the *Ds* mutations occurred at a time midway in development of the endosperm and at a medium rate; there were fewer mutations per area than in the second sector, and they occurred earlier. The fourth sector arose from a nucleus that had undergone a *Ds* mutation; no further *Ds* mutations could be registered in this sector. The second most frequent type of kernel was composed of only three sectors, similar to the first, second, and fourth sectors of the above-mentioned type. It had no sector of medium-timed mutations. Nearly all the kernels resulting from these crosses could be classified on the basis of the presence of combinations of two, three, or all four of these distinct types of sectors. The origin of a sector, in each case, could be traced to a nucleus arising from the earliest divisions in the endosperm. The ratio of kernels with specific combinations of sectors was surprisingly constant. Some combinations occurred with high frequencies, others with relatively low frequencies; and some combinations were not found at all.

It is obvious that something occurs in the early divisions of the endosperm that results in nuclei with differing potentialities for expressing the time and frequency of *Ds* mutations. Immediately upon examination of these kernels, one is impressed with the resemblance of the mutation patterns in the various sectors to the patterns that have been obtained by combining various dosages of *Ac*. Here, however, only one *Ac* locus was introduced into the primary endosperm nucleus. Obvious questions present themselves: Do these sectors arise as the consequence of some abnormality that results in segregations of various dosages of *Ac* in these early nuclei? If so, what abnormalities occur and what is the segregation mechanism? Neither nondisjunction of chromatids containing *Ac,* nor transposition of the *Ac* locus to another position in the chromosome set followed by mitotic segregation, satisfactorily explains the observed ratios of the various types of kernels. A satisfactory fit is obtained if it is assumed that (1) the *Ac* locus is composed of a number of identical and probably linearly arranged units, and (2) changes in the number of units can take place at the locus during or after chromosome reduplication such that one chromatid gains units that the sister chromatid loses. If the *Ac*-carrying chromosome is double at the time of entrance into the primary endosperm nucleus, such an event involving one of the two chromatids during the subsequent division could result in four nuclei, two with unaltered units within the *Ac* locus

and two with altered units within the *Ac* locus (one of these having an increased number of units and the other a decreased number). The four resulting nuclei would not be alike with respect to dosage of units within the single *Ac* locus. If the number of units within the *Ac* locus controls the time and frequency of *Ds* mutations in a manner comparable to known dosages of whole *Ac* loci, the time and frequency of *Ds* mutations in the various sectors could be explained. If such a change in units at an *Ac* locus is accompanied by a *Ds* mutation and involves one of the four chromatids carrying *Ds*, the kernels that have one sector produced as the consequence of a *Ds* mutation could be accounted for. This interpretation is subject to several kinds of test. It should be possible to predict the *Ac* type of control in those sectors that have undergone an early *Ds* mutation. Special genetic methods for detecting the *Ac* action in these sectors have been devised. Preliminary results from one test indicate a good fit with expectation; the most favorable tests should be available in the summer's harvest.

Other evidence supports the assumption that the *Ac* locus is composed of a number of identical units and that these units can increase or decrease as the consequence of a somatic mutation within the *Ac* locus. This considers what has been called the "state of the locus." The various states of the *Ac* locus are recognizable by the time and frequency of the mutations it produces when combined with one particular *Ds* locus. This particular *Ds* locus must have a constant state. A *Ds* locus can be maintained in a constant state by keeping it isolated in an *ac ac* plant; for without *Ac* the *Ds* locus is quite stable and remains unchanged. *Ac* loci have been isolated that produce in *Ac ac ac* constitutions relatively late *Ds* mutations. When such late mutations result from a single dose of *Ac*, the *Ac* locus is considered to be in a high state. Other *Ac* loci have been isolated that in single doses induce many early mutations. These are called low-state *Ac* loci. In an *Ac Ac ac* constitution (two doses) a low-state *Ac* locus may give a mutation pattern resembling that obtained from a high-state *Ac ac ac* (one dose) constitution. Again, the *Ac Ac Ac* (three doses) constitution of a low-state *Ac* locus may give mutation patterns resembling those produced by a high-state *Ac Ac ac* (two doses) constitution. The effects produced by three doses of a high-state *Ac* locus are most instructive, because the delay in timing of mutations with this particular state and dosage of *Ac* is so great as to result in no visible mutations, or only an occasional one. The tissues have ceased dividing before the potential mutations can occur. The variously exhibited states of individually selected *Ac* loci and the effects produced by each in one, two, and three doses, respectively, give added support to the interpretation that the state of any one *Ac* locus is an expression of the number of reduplicate units present within this locus. It is obvious that the *Ac* locus itself is mutable, for *Ac* loci with different states can be recognized and isolated in the progeny of a cross of *Ac ac* by *ac ac*. It is suspected that what can be seen in the sectorial kernels described above are mutations of the *Ac* locus.

The Mutable *c* Loci

Mutable c^{m-1}. One of the two mutable *c* loci being studied originated from the cross of a female plant homozygous for *c sh ds ac* by a male plant homozygous for *C Sh Ds* and heterozygous for *Ac* (*Ac ac*). The same male plant was used in making a number of similar crosses. On one of the

resulting ears, a single aberrant kernel was observed. Instead of showing either a complete *C* color (*C Ds/c ds/c ds, ac ac ac* constitution) or a variegation pattern composed of colored aleurone with colorless sectors, owing to *Ds* mutations (*C Ds/c ds/c ds, Ac ac ac* constitution), this kernel was obviously composed of colorless aleurone in which colored areas were present. In pattern of variegation, it was the reverse of expectation: a *c* locus appeared to be mutating to *C*. This kernel was removed, a plant was grown from it, and various crosses were made to determine the nature of the altered expression of aleurone color. Appropriate genetic analysis indicated that a mutable *c* locus was present which had arisen from the normal *C* locus present in the male parent. This *C* locus had changed to a mutable *c* locus capable of mutating back to the original *C*. This mutable *c* locus is *Ac*-controlled and responds in precisely the same manner as the *Ds* locus to various doses of *Ac* and to various states and observable changes in state of the *Ac* locus. With respect to *Ac*, the responses of the two loci are amazingly alike. *Ds* mutations, however, are known to be the result of some mechanism leading to chromosomal breakage and fusion, whereas the mutations at the *c* locus appear to involve quite a different mechanism, leading to changes in expression of the locus from recessive to dominant.

Similarities in response of *Ds* and c^{m-1} to *Ac*, together with the known breakage mechanism at the *Ds* locus and also the obvious changes in state of the *Ac* locus that can be explained on the basis of a chromosomal breakage mechanism, lead one to suspect that some kind of chromosomal breakage and fusion mechanism may likewise be responsible for these reverse mutations.

Because c^{m-1} and c^{m-2} are very different in their phenotypic expressions, however similar they are in other respects, it should be stressed that visible mutations at the c^{m-1} locus always result in the expression of a full dominant phenotype. Varying expressions of color intensity to either side of that expected from a single dose of normal *C* do not appear.

Mutable c^{m-2}. The second mutable *c* locus, c^{m-2}, arose in a somatic cell of a plant of the constitution *I Sh ds/C Sh ds, Ac Ac*. The plant was sectorial for this mutable *c* locus. With respect to dosage of *Ac*, the production of sections, etc., the c^{m-2} locus responds exactly as do the *Ds* and c^{m-1} loci. The phenotypic expression of mutations at the c^{m-1} and at the c^{m-2} locus is distinctly different. The mutations of c^{m-2} give rise to sectors showing great variation in color intensity, from a very faint to an intensely deep color—often much deeper than that produced by a single, double, or even triple dose of *C*. Any one intensity may appear as a mutant sector in any part of the aleurone layer on any one kernel; also, any one kernel may show a number of different mutations each having its own particular color intensity.

The very great differences in phenotypic expression of the two independently arising mutable *c* loci suggest that the normal *C* locus may be composed of at least two blocks, each having its own particular organization and function but both necessary for producing the *C* phenotype. They are not strictly complementary, for combinations of c^{m-1} and c^{m-2} in the same endosperm do not result in the production of *C* color. The mutable c^{m-1} locus is assumed to have arisen from a normal *C* locus after alterations had occurred in only one block. On the other hand, the mutable c^{m-2} arose from a normal *C* locus after alterations that involved only the second block. The c^{m-1} locus undergoes muta-

tional changes only in the one affected block, whereas the mutations of c^{m-2} involve changes only in the second block. A possible alternative to this interpretation will be considered in the concluding paragraphs of this report.

The Mutable *wx* Loci

Two mutable *wx* loci are now under investigation. Each was found as a single aberrant kernel on an ear resulting from the cross of a plant homozygous for *wx ds* and *ac* by a plant that was homozygous for *Wx* and also for *Ac*. A different male parent was involved in each cross. The first case found, wx^{m-1}, was recognized because the aberrant kernel showed a *wx* phenotype with *Wx* sectors and spots instead of the expected full *Wx* phenotype. Because other well marked genetic characters were involved in this cross, it could be determined that no contamination had occurred to give rise to this kernel and that the mutable *wx* condition had arisen at the *Wx* locus in the chromosome 9 contributed by the male parent. No known mutable *wx* loci were present in any of the stocks at the time of discovery of the original kernels of either wx^{m-1} or wx^{m-2}.

The second case, wx^{m-2}, was first observed as an aberrant kernel because it showed a recessive *wx* phenotype, instead of the expected *Wx*. No mutability was recognized in the original kernel. The mutability of the locus was recognized later, when the pollen of the plant arising from this kernel was examined.

A third case of a mutable *wx* locus arising from a normal dominant *Wx* locus was observed in the cross of a female *Wx Wx, Ac Ac* plant by a male *wx wx, ac ac* plant. The kernel was almost completely *wx*, with only small specks of *Wx*. The mutations to *Wx* occurred late in the development of the endosperm. This type of mutation pattern suggested that the mutability was *Ac*-controlled; for two doses of *Ac* were present in the endosperm, and two such doses are known to give this pattern with all *Ac*-controlled mutable loci. The embryo in the kernel did not germinate, and so this case of an independently arising mutable *wx* locus has been lost.

The two recovered mutable *wx* loci have received only preliminary study because of their recent origin. The wx^{m-1} locus is *Ac*-controlled and responds to *Ac* in all its various aspects as do the *Ds*, c^{m-1}, and c^{m-2} loci. It is not known whether wx^{m-2} is controlled by *Ac*.

All *Ac*-controlled mutable loci show mutations only late in the development of the sporophytic tissues. In the sporogenous cells, such mutations may be delayed until the premeiotic or meiotic nuclei are formed. The starch in the pollen of plants homozygous for a normal *wx* locus stains a clear red-brown color with dilute solutions of iodine and potassium iodide. The starch grains in the pollen of plants homozygous for a normal *Wx* locus stain a dark blue color with this same solution. In heterozygous plants, as expected, two types of pollen grains are present in equal proportions, those staining red-brown (the *wx*-carrying grains) and those staining deep blue (the *Wx*-carrying grains). In the two original plants carrying these mutable *wx* loci, a normal nonmutating *wx* locus was present in one chromosome 9 and the mutable *wx* locus in the homologous chromosome 9. Examination of the pollen of these two plants gave the first indication that the primary mechanism responsible for mutations of the *wx* loci could be associated, at least in some cases, with chromosome breakage and fusion. In both plants, most of the pollen grains, when stained with iodine, were visually *wx* (red-brown) in phenotype.

In the plant carrying wx^{m-1}, however, about 2 to 3 per cent of the grains were clearly different in staining reaction. These stained blue with iodine, the intensities of color ranging from light gray-blue to the very deep blue characteristic of pollen grains carrying a normal *Wx* locus. A high percentage of these blue-staining grains, often more than 50 per cent, were small and only partially filled with starch granules. This contrasted greatly with the proportion of such defective grains (only 2 to 3 per cent) in the *wx*-staining class. The pollen of the plant carrying wx^{m-2} differed from that of the plant carrying wx^{m-1} in that the recognizable blue-staining grains were confined mainly to the defective class. Less than 1 per cent of the pollen grains in this plant were defective, and in approximately half of these the starch granules stained blue. Here, also, the intensities of color ranged from a light gray-blue to a very deep blue. Only a very few of the normal-appearing pollen grains showed any trace of blue color, and it was always very faint. This contrasted greatly with the pollen of the wx^{m-1} plant, where many of the deeply blue-staining grains were normal in appearance. The questions arise: (1) Why is the proportion of defective grains so high in the mutated class in both plants? (2) What is the reason for the various intensities of the staining reaction? (3) Why are the normally developed grains with mutated loci so different in staining reaction in these two plants?

An interpretation, subject to further testing, has been formulated. It assumes that the normal *Wx* locus is composed of a number of identical, reduplicated units, each unit contributing its share in the ultimate conversion of precursor starch to the amylose type characteristically produced by the *Wx* locus. The more units are present at the locus, the greater is the conversion and the stronger the iodine staining reaction. Published quantitative chemical studies (Sprague, Brimhall, and Hixon, *Jour. Amer. Soc. Agric.*, vol. 35, pp. 817–822, 1943; Brimhall, Sprague, and Sass, *Jour. Amer. Soc. Agric.*, vol. 37, pp. 937–944, 1945) of the nature of the starch in endosperms having various genic constitutions of *wx*, *Wx*, and a low allele of *Wx* known as wx^a have given a sound basis for assuming a quantitative reaction of the *Wx* locus. The starch in *wx wx wx* endosperm is composed of amylopectin. With one dose of *Wx* (*Wx wx wx* constitution), about 18 per cent of the starch is synthesized to amylose, the remainder being amylopectin. With two and three doses of *Wx*, more starch is synthesized to amylose, but not in proportion to dosage (20 and 22 per cent, respectively). Tests of wx^a, however, have shown a proportionality with dosage. Here, one dose of wx^a (wx^a *wx wx* constitution) gives 0.65 per cent amylose, and two and three doses give 1.20 and 2.40 per cent, respectively. These published results suggest that the number of reduplicated units in the normal *Wx* locus may be high enough to approach, in a single dose, an effective utilization of the available substrate to be converted. This is apparently not so for a single dose of the wx^a allele. Here, too few units may be present and much of the available substrate may be unutilized. This available substrate could be utilized if more gene units were present. Added doses of the wx^a allele could accomplish this. On the assumption that different numbers of gene units are present in the *Wx* and wx^a alleles, it is possible to explain the different dosage responses of these two alleles.

According to the hypothesis that the normal *Wx* locus is composed of a number of reduplicated units, it is assumed that the mutable *wx* loci arose from a

normal *Wx* locus by loss of units from the locus. The remaining units are too few to produce a visual change in the color of the starch when stained with iodine. If the mutation process results in an increase of units within the locus, enough amylose may be produced to give a visible color reaction with iodine. Since the wx^a locus gives a light orchid stain in the endosperm and a lighter stain than full *Wx* in the pollen grain, the number of residual units in the two mutable *wx* loci should be even lower than that present in wx^a. Because individual mutations occurring at the wx^{m-1} and wx^{m-2} loci are not alike, but result in each case in the production of one particular intensity of color reaction, it must be assumed that the increase in the number of units at any one mutation is not constant; some mutations result in only small increases, others in larger increases in number of units, and so on. The various grades of intensity of staining reflect these variable increases in the numbers of units: the more units are present, the more amylose is produced, and consequently the more intense is the staining reaction.

It is believed that the defective grains in the mutated class could arise as the consequence of a breakage-fusion mechanism that results in U-shaped dicentric chromatids and an acentric U-shaped fragment, as described for the *Ds* mutations. If this break occurred within the *wx* locus or to the left of it, the dicentric chromatid could have more amylose-producing units in it than either one of the original chromatids: increased or doubled in the first case, and doubled in the second case. Breakage of this bridge in the succeeding anaphase could occur nonmedially and give rise to one broken chromatid having all these increased numbers of units and a sister chromatid having none. Successive breakage-fusion-bridge cycles could double, triple, quadruple, etc., the number of units. Only a few mitoses would be required for building up large increments of such units. Are the defective *Wx*-staining grains the result of this mechanism? Are they defective because they have lost segments of the short arm of chromosome 9 that always accompany the formation of such dicentric chromatids? Will a deficiency of one-half to two-thirds of the short arm of chromosome 9 give rise to a detectable pollen grain, and will it be of this particular defective type?

An affirmative answer to this last question has been obtained by examining the pollen of *Wx Ds/Wx Ds, Ac Ac* plants. This pollen should all be *Wx*-staining except for the grains produced after a premeiotic or meiotic *Ds* mutation; these should be deficient for at least two-thirds of the short arm of chromosome 9. If, in these grains, some starch granules are formed, the starch should give the *wx* staining reaction, because a *Ds* mutation would place the *Wx* locus in the acentric fragment. This fragment is usually lost to the nucleus in the division following the *Ds* mutation. The dicentric chromosomes in the nuclei formed after a *Ds* mutation should have no *Wx* locus at all. It has previously been determined, through other types of experiments, that a deficiency of the *Wx* locus will give rise to starch of the *wx* staining type. By examination of the pollen of *Wx Ds/Wx Ds, Ac Ac* or *Ac ac* plants, it has now been determined that such small, partially filled, *wx*-staining grains are present in the relatively high numbers expected. Only rarely does one find such grains in the pollen of *Wx Wx ac ac* plants, and those found may arise from occasional adjacent fusions of broken ends after crossover breakage at meiosis. There is no obstacle,

then, to considering that the defective *Wx*-staining grains in the pollen of the wx^{m-1} and wx^{m-2} plants have arisen as the consequence of dicentric chromatid formation followed by the breakage-fusion-bridge cycle. The *Wx* locus is to the left of *Ds*, and the segment of chromosome 9 absent from these grains is even smaller than that found after a *Ds* mutation. The various intensities of the *Wx* staining reaction in the defective class of pollen grains may merely represent the various dosages of *Wx* units present in these grains; for various dosages could be anticipated.

The breakage-fusion-bridge cycle alone cannot explain many of the mutations from *wx* to or toward a *Wx* phenotype. Normal, functional *Wx*-staining pollen grains are present in the wx^{m-1} plant. It is possible that these are the reciprocal products of a breakage mechanism that sometimes gives rise to dicentric chromatids. A crisscross type of fusion may occur between broken ends after breakage at one locus in two sister chromatids. If the breakage occurs within the compound locus at unequal positions in the two chromatids, one resulting chromatid might gain enough units at a single breakage and fusion to produce a quantity of amylose starch that would give a detectable staining reaction. Several successive mutations could step up the unit dosage within the locus considerably. A graded series of staining intensities could be expected to follow such events. Examination of the mutation process in the endosperm tissues carrying c^{m-2} and wx^{m-1} suggests that such successive mutations may occur.

Examination of the kernels resulting from crosses involving the wx^{m-1} locus has been illuminating. It has shown: (1) that mutations of wx^{m-1} occur, as expected, in the endosperm tissues; (2) that these mutations differ in phenotype, some giving rise to sectors showing only very faintly staining starch, others to sectors showing intensely blue-staining starch, with all others falling between these extremes; (3) that at least five grades of intensity can be recognized among the mutations within a single kernel; and (4) that twin sectors frequently occur, one sector showing a deeper blue stain than the sister sector. Examination of these kernels also showed that a single mutation may be followed in successive divisions by further mutations, and that these latter result in starch with either an increased or a decreased staining intensity. In other words, the mutation process may result in changes in either direction: toward full dominant or toward full recessive. A few sectors arising as the consequence of the breakage-fusion-bridge cycle were present in these kernels. Large areas within these sectors gave the *wx* staining reaction, but there were subsectors showing the *Wx* staining reaction. Within these subsectors, a wide variation in intensity of staining reaction was observed. The spatial relations of the cells showing these varied intensities of staining reaction were instructive; for adjacent areas probably arising from sister cells and showing inverse relations of color intensities were most obvious. The differences in intensities of staining reaction in these twin areas ranged from slightly detectable to pronounced, with extremes showing a deep blue adjacent to a red-brown (*wx*). This latter observation strongly supports the interpretation of the relation of dosage to the intensity of the staining reaction. Dosage changes appear to be responsible for the changes in staining reaction within these sectors having dicentric chromatids; the greater the dose, the more intense the staining reaction, and vice versa. It is realized that increments or decrements of units must fall within the range that can

give visually distinguishable changes in intensities of color.

It is not mere speculation, therefore, to consider that the quantitative grades of mutation occurring at the mutable *wx* loci may follow increases in the number of identical units within a depleted locus that is normally composed of a relatively large number of such reduplicated units. The question arises, What is the primary mechanism responsible for such increases and decreases, and do all mutable loci reflect this same general mechanism?

Conclusions

It may be premature to consider in detail the question asked in the concluding sentence above. Because so many mutable loci have recently appeared in the maize cultures, because in many respects they all behave in very much the same way, and because this behavior is similar also to that described for other mutable loci both in maize and in other organisms, it may be profitable to review briefly the pertinent facts about the cases described in this report, in order to ascertain the similarities and dissimilarities among these cases. They involve the mutable locus *Ac* and the mutable loci it controls—*Ds*, c^{m-1}, c^{m-2}, and wx^{m-1}.

Ds stands alone in that the chromosomal consequences of a mutation at this locus are known. The detectable *Ds* mutations unquestionably arise as a consequence of some mechanism that either brings about breakage and fusion between sister chromatids at the *Ds* locus or simulates this mechanism in its consequences; for dicentric chromatids are produced after a *Ds* mutation. In the pattern of mutations, c^{m-1} and *Ds* are strictly comparable. Variegation patterns produced by *Ds* mutations in *C ds/C ds/I Ds, Ac ac ac* constitutions can be so similar to variegation patterns produced by *c* to *C* mutations in c^s c^s c^{m-1}, *Ac ac ac* constitutions that they may be indistinguishable by mere observation of the kernels. Yet the formation of dicentric chromatids alone cannot explain most of the observed c^{m-1} mutations. This likewise applies to the c^{m-2} mutations and to many of the mutations of wx^{m-1}. Some form of chromosome breakage and fusion, however, may be involved.

As stated earlier, mutations of c^{m-1} and c^{m-2} differ greatly in phenotypic expression. Visible mutations of c^{m-1} give rise each time to the full dominant expression expected from a single dose of *C*, whereas those of c^{m-2} are quantitatively expressed, with color intensities varying from faint to extremely deep. The normal *C* locus is known to give dosage effects: the more whole *C* loci present, the greater the depth of color. By means of duplications of the short arm of chromosome 9, it has been possible to observe effects of doses up to and including six *C* loci. With the highest dose, the color of the aleurone is unusually deep. Some of the mutations of c^{m-2} result in intensities greater than that shown with three doses of the normal *C* locus, whereas others are so faint that they obviously have produced much less pigment than is produced by a single normal *C* locus. The various consequences of mutations of the c^{m-2} locus are in complete agreement with the hypothesis that the mutations result from graded increases in the number of units within a depleted locus and that they express themselves phenotypically by graded increases in the substance or substances responsible for the phenotypic character. The observed mutations of c^{m-1} do not lead to this hypothesis, for they show no quantitative subdivisions. Similarity of the c^{m-1} mutations to *Ds* mutations in basic response to *Ac*, and their conformity to the general pattern of the *Ac*-controlled

mutations that do give quantitative effects, has led to formulation of a subsidiary hypothesis rather than abandonment of the general hypothesis. The validity of this subsidiary hypothesis is subject to tests that are now being conducted. Assuming the fundamental mutation process to be similar for all *Ac*-controlled mutable loci, the genes in the mutating c^{m-1} block of the *C* locus could be related to some chemical process that requires a threshold number of units for expression to be fulfilled; or it may be that the mechanism bringing about a change in units at this mutable *c* locus assures a specific increase in number of units.

The mutations occurring at the c^{m-2} and wx^{m-1} loci are amazingly similar in every respect. The mutations fall into a graded quantitative series in both cases. Also, both give twin or adjacent sectors showing the same grades of contrasting intensities of expression of the dominant character. There can be little doubt that the same mechanism is responsible for the mutations occurring at these two different loci. With regard to the wx^{m-1} locus, there is evidence for believing that the unit number within the locus may be responsible for the expression of unit increases of the dominant phenotypic expression. Considering the obvious similarities between the two cases, it would be difficult to avoid concluding that the same conditions apply to mutations at the c^{m-2} locus. In this connection it may be recalled that mutations at the *Ac* locus likewise suggest a mutation mechanism involving changes in the numbers of units at the locus.

Because many of the mutations occurring at the two mutable *c* loci, the wx^{m-1} locus, and the mutable *Ac* locus do not result in dicentric chromatids, as do *Ds* mutations, and do not lead to detectable gross chromosomal aberrations, the mechanism, if it is a breakage phenomenon, must restore the normal chromosome morphology. Unequal breakage within the locus, followed by the crisscross type of fusion mechanism, could accomplish this end.

Any mechanism that gives rise to an increase in numbers of units at a locus might also give rise to the reverse condition; that is, to chromatids with decreased numbers of units. If so, chromosomes with loci having various unit numbers should appear as isolates in these cultures. Some of these isolates should show more visible mutations than others under given conditions. The frequency of visible mutations would depend upon the initial number of units present in the locus before mutation occurred. The more units were present, the greater would be the chance that the increase in the units during any one mutation would be sufficient to produce a visible effect; and the converse would also be true. Isolates from the different mutable loci, showing just these expected variations in the rates of visible mutations, have been obtained. The term "state of the locus" applies as well to the mutable loci controlled by *Ac* as to the *Ac* locus itself. What has been termed a high-state locus gives high rates of mutation, and a low-state locus gives low rates of mutation. It is possible, therefore, that the number of units present at a mutable locus is correlated with the state of the locus as well as with the expression of visible effects. It is a matter of degree. If the initial number is high, but not high enough to produce a visible effect, the state of the locus may be considered high. Conversely, if only a few units remain in the locus, the state of the locus may be considered low.

The above conclusions are supported by the many observations that have been made especially of the chlorophyll-producing types of mutable loci. As mentioned in

previous reports, the motivation for this study of mutable loci was the observation of twin sectors, apparently arising from sister nuclei, that showed inverse rates of visible mutations. The most extreme of these twin sectors showed a mutation to dominant in one sector, and in the sister sector either a complete recessive or a very much reduced rate of mutation as compared with that in the surrounding tissue. In these observed cases of twin sectoring, it is obvious that the factor or factors controlling the rate of mutation and the visible mutations themselves are of the same general nature, if not actually different resultants of the same mechanism. The factor responsible for twin sectoring acts at a mitosis, and the apparent result is that one chromatid gains something that the sister chromatid loses. In the interpretation given, this gain and loss are considered to be an increase and a decrease, respectively, of identical units at the locus, which, in turn, controls not only the appearance of visible mutations but also the state of the locus as reflected in the time and frequency of occurrence of future visible mutations.

The above interpretations are being used as a working hypothesis in continuing studies of mutable loci. The evidence at present points toward the presence of reduplicated units within a locus, these units often expressing themselves in a quantitative manner through unitary action on substrates responsible for phenotypic characters. The hypothesis that the phenotypic expression of a locus depends upon the number of such units present in any one chromosome, and that some mechanism or mechanisms can alter this number and give rise to visible mutations, is both sufficiently simple and sufficiently integrative to afford precise direction in some types of experimentation. It is believed that this approach to one phase of the over-all mutation problem may be productive, even though it is realized that the details will need clarification and may be subject to degrees of modification. With so many mutable loci behaving in very much the same manner, it is unlikely that many different, unrelated mechanisms are involved. It is more likely that one general type of condition exists in all these mutable loci and that this condition may be altered in any of these loci by one kind of mechanism.

In this report, it has not been possible to include a discussion of the many other observations and conclusions that are relevant to the subject, such as the restabilization of a mutable locus, the behavior of *Ac*-controlled mutable loci when two or more are present in the same nucleus, or the changes at the *Ac* locus that often appear to accompany an *Ac*-induced mutation. Nor has it been possible to describe the accumulated evidence on some of the non-*Ac*-controlled mutable loci, or to mention the many new mutations that are constantly arising in these cultures. These studies are being continued and will be reported later.