MUTABLE LOCI IN MAIZE

BARBARA McCLINTOCK

Study of the origin and nature of instability of genic action at a number of different known loci in maize chromosomes has been continued during the past year. At any one locus of known genic action, different types of instability expression can appear. A hypothesis has been developed to account for the origin and subsequent behavior of these various types of unstable condition. Each type is considered as reflecting the operation of a particular chromosomal system that controls the action of the genic components at the locus. These controlling systems appear to be composed of distinct chromosomal units; but, unlike the genes, these units may move from one location to another within the complement.

Two well defined classes of controlling system have been dealt with in our study. They have been termed single-unit systems and two-unit systems. In the single-unit systems, only one controlling unit is recognized. When such a unit moves to a new location in the chromosomal complement, it imposes its specific mode of control of action of the genic components at this location. The time and place of genic action, as well as its type, will be an expression of the specificity of the particular controlling unit. Two-unit systems operate differently. When one of these units is inserted adjacent to a particular gene, it may immediately alter genic action, giving rise to a recognizable mutation. Changes in this unit may subsequently occur. These are reflected in altered expressions of the associated genic components. They occur only when the second unit of the two-unit system is also present in the nucleus. This second unit is independently located in the chromosomal complement. Instability of genic expression is a reflection, therefore, of the interaction of the two units. Changes in the gene-associated unit control the types of change in genic action, whereas the time and place of such changes are controlled by the second unit.

The main purpose of recent studies has been to examine the same system of instability expression at a number of different known loci, and to compare the action of various systems that may operate at the same locus.

The possibility of recognizing the opera-

tion of a particular system controlling instability of genic expression depends on its specificity. The *Ac* component of the two-unit *Ds-Ac* controlling system, described in previous reports, is highly specific in its action. Its operation is readily detected. In plants having *Ds* and *Ac,* mutability under the control of *Ac* has appeared at several known loci. The first recognized case was designated c^{m-1}. The origin of c^{m-1} by transposition of *Ds* to the locus of *C* has been described in previous reports. Subsequently, the *Ds-Ac* system of mutational control appeared at the *Bz* and *Wx* loci. Furthermore, several independent inceptions of this controlling system have occurred at each of these three loci. Other systems of mutational control have arisen, however, at the same loci. Since instability of genic action is considered to be an expression of the operation of a particular controlling system, which has been incorporated at the locus of the gene concerned, and not an expression of changes in the gene itself, it is to be expected that any one system can operate at any locus of known genic action. This expectation could be tested; and the *Ds-Ac* two-unit controlling system was chosen for this purpose. Instability at a selected locus can arise whenever the *Ds* component is transposed to the locus. By technically simple methods, it should be possible to detect the presence of this system within a few cell generations after incorporation of *Ds* at the locus. Two loci considered particularly appropriate for the initial tests were selected: that of A_1 and that of A_2. The results of these tests are summarized in the following section.

Origins of Instability at the A_1 and A_2 Loci

The genic components at the locus of A_1 in chromosome 3 and the locus of A_2 in chromosome 5 are associated with the development of anthocyanin pigment in the aleurone layer of the kernel and in the plant tissues. If both *Ds* and *Ac* are present in plants homozygous for these two dominant, stable factors, instability may arise at either locus if *Ds* is transposed to it. (By use of the term factor instead of gene, it is hoped that misconceptions regarding the nature of the change affecting the genic components at a locus may be avoided.) Transpositions of *Ds* usually occur late in the development of sporogenous tissues. Should one such event insert *Ds* at the locus of A_1 or A_2 in these plants, genic action at the affected locus might be altered. Such altered action could be detected in kernels on the ears of these plants if the progeny of the cell in which the transposition occurred produced a female gametophyte, and also if the sperm nucleus entering this female gametophyte carried the known recessive, a_1 or a_2, whose action is unaffected by *Ac*. Color development in the aleurone layer of the kernel so produced might be modified. Variegation might be exhibited if *Ac* was also present in this kernel.

The plants used as the female parents, in crosses to test this theory, had *Ds* located in the long arm of chromosome 5, closely linked to the factor *Pr*. One *Ac* factor was present, and all plants were homozygous for the stable, dominant factors A_1 and A_2. Pollen from plants homozygous for either a_1 or a_2 was placed on the silks of such plants. The resulting ears were examined for kernels showing variegation of aleurone color. Seventy-one ears were obtained from the first cross and 120 from the second. One kernel showing variegation for aleurone color was found on an ear when a_1 had been introduced by the male parent, and three were found on three different ears when a_2 had been introduced by the male parent. Plants were grown from all

four kernels, and tests initiated to determine the nature of the instability expressed.

In the plant derived from the single variegated kernel appearing in the first cross, mutability was being expressed at the locus of A_1. This new mutable condition, designated a_1^{m-4}, proved to be *Ac*-controlled. It arose at the normal A_1 locus in the *Ds-Ac*–carrying female parent. This could be determined from linkage studies. The female parent had been homozygous for A_1 and Sh_2, whereas the male parent had been homozygous for the recessive alleles. Sh_2 is very closely linked with A_1. Close linkage of a_1^{m-4} and Sh_2 was evident in backcross tests. Three other independent inceptions of instability at this A_1 locus have been examined. Two of them, a_1^{m-1} and a_1^{m-2}, are not *Ac*-controlled; but the third, a_1^{m-3}, is *Ac*-controlled and arose in a plant having the very same constitution as that which produced a_1^{m-4}.

Plants were grown from the three variegated kernels derived from crosses in which the pollen parent had been homozygous for a_2. In two of them, it could be shown that modifications had occurred at the locus of A_2 in the *Ds-Ac*–carrying parent. Both modifications resulted in instabilities of genic action at this locus, which were designated a_2^{m-3} and a_2^{m-4}. In the third plant, the chromosome 5 contributed by the female parent was not transmitted through either pollen or egg; therefore the nature of the alteration at A_2 could not be determined. Mutability at a_2^{m-4} is *Ac*-controlled. The nature of the controlling system associated with a_2^{m-3} has not yet been determined; it is not *Ac*-controlled, however. Initial tests were complicated by the presence of a defect in the chromosome 5 carrying a_2^{m-3}, which prevented pollen transmission. The locus of the defect is closely linked with *Pr*. Transmission of a_2^{m-3} through the pollen was limited. It occurred only when the grain carried a chromosome 5 without the defect. Such a chromosome is obtained by crossing over between a_2^{m-3} and the locus of the defect.

In considering the origins of a_2^{m-3} and a_2^{m-4}, it should be recalled that *Ds* was present in the same chromosome that carried A_2. In two of the three plants derived from the variegated kernels, the chromosome 5 with a modification at A_2 was also defective. It is known that events at *Ds* produce chromosomal alterations. Although some of them do not produce chromosomal aberrations that result in inviability, others do produce gross chromosomal defects, such as deficiency. It is not surprising, therefore, to find that some defect in chromosome 5, affecting viability, accompanied the inception of mutability in two of these three cases. This relationship affords additional, although indirect, evidence that altered genic expression originates through chromosome aberration.

Two earlier cases in our material of mutability produced by changes at the A_2 locus are designated a_2^{m-1} and a_2^{m-2}. Neither is *Ac*-controlled.

Instability of Sh_1 Action Induced by *Ds*

Two independent cases of insertion of *Ds* just to the left of the *Sh* locus in chromosome 9 have been studied. With regard to the hypothesis of origin of mutation through *Ds* events, these two cases have been exceptionally revealing. Early studies of *Ds* were focused on the phenomenon of its transposition from one location to another in the chromosome complement. For the sake of technical simplicity, cases were selected in which *Ds* had been transposed from one position in the short arm of chromosome 9 to another position within the same arm. Over twenty such cases

were examined in detail. In five of them, *Ds* had been inserted between *I* and *Sh*. These two factors lie close together in the chromosome, and approximately 4 per cent crossing over occurs between them. From linkage tests, it was determined that *Ds* had been inserted closer to *I* than to *Sh* in three of the five cases, and at approximately the same position in each. Crossing over between *I* and *Ds* was approximately one-fifth of that which occurs between *I* and *Sh*. In the other two cases, *Ds* was inserted just to the left of *Sh*. To determine crossover frequencies between *Ds* and *Sh* in these two cases, extensive tests were required. A number of kernels with exceptional phenotypes appeared in these tests. Studies were then initiated to determine the conditions associated with their appearance. They proved to be *Ds*-initiated mutations at the locus of *Sh*. A summary of the evidence may be given.

The initial tests were made in order to determine the crossover frequencies between *I* and *Ds* and between *Ds* and *Sh*. The tests were conducted as follows for both case 1 (*Ds* 4864A) and case 2 (*Ds* 5245): Plants having one or two *Ac* factors and carrying *I, Ds, Sh, Bz,* and *Wx* in one chromosome 9, and *C, ds, sh, bz,* and *wx* in the homologue, were crossed to plants homozygous for *C, ds, sh, bz,* and *wx* and having no *Ac* factor. From one such test with case 1, only a single kernel was found among a total of 4291 that could be considered to have a chromosome derived from crossing over between *Ds* and *Sh*. In phenotype, it was *I sh bx wx;* and *Ds* was present in the *I*-carrying chromosome. In 26 kernels, the phenotype was *I sh Bz;* and in 17 of them *Ds* was certainly present, located between *I* and *Bz*. (The presence of *Ds* may be detected only in those kernels that also have *Ac*.) In normal stocks, crossing over between *Sh* and *Bz* is approximately half that between *I* and *Sh*, or close to 2 per cent. If the kernels with the *I sh Bz* phenotype carried a chromosome derived from a double crossover in the two adjacent short segments, *I* to *Sh* and *Sh* to *Bz*, then kernels carrying the reciprocal crossover chromosome should also have been present. They should have had the *C Sh bz* phenotype. No kernels showing this phenotype were present. It would be even more difficult to explain in terms of crossing over the origin of the 17 kernels with *Ds* located between *I* and *Bz*. Double crossing over, involving the *Ds*-to-*Sh* and *Sh*-to-*Bz* segments, would have been required. This seems even less probable when it is recalled that only one kernel had a phenotype that could have appeared as a consequence of a single crossover between *Ds* and *Sh*. Obviously, the *I sh Bz* phenotype does not represent a product of crossing over. Another mechanism is responsible.

The results in similar tests of case 2 were much the same as those described for case 1. In one such test, 6683 kernels were obtained. Seven of them were *I sh bz wx* in phenotype, and *Ds* was present in the *I* chromosome. They could be interpreted as having originated through crossing over between *Ds* and *Sh*. Twenty-nine kernels were *I sh Bz;* and in 17 of them *Ds* was certainly present, located between *I* and *Bz*. No kernels having a *C Sh bz* phenotype appeared. The arguments given above for case 1, which excluded crossing over as a mechanism responsible for the appearance of *sh* in kernels having an *I sh Bz* phenotype, applied with equal force for case 2. On the other hand, this phenotype could be attributed to mutation at the locus of *Sh*. Since mutation to *sh* had not been observed to occur with such a high frequency in other genetic stocks of maize, or in those

cases in our material where *Ds* was inserted at other locations in the short arm of chromosome 9, it could be suspected that these mutations were produced by some event undergone by *Ds*. Tests were initiated to determine whether this was true.

Events at *Ds* will occur only if *Ac* is present in the nucleus. If the *sh* phenotype arises from a particular event at *Ds*, then no mutations to *sh* should occur if *Ac* is absent. Thus, had the experiment described above been conducted with plants having the same constitution with respect to markers in their chromosomes 9, but having no *Ac* factor, kernels with an *I sh Bz* phenotype would not have appeared on the ears, for no events at *Ds* would have occurred in any of the cells of these plants. Such a comparative test was conducted with case 2. All the plants in one culture had the same constitution as described above with respect to markers in chromosome 9, but differed with respect to *Ac*, which was present in some and absent from others. When those having *Ac* were crossed to plants homozygous for *C, sh, bz,* and *wx,* and carrying no *Ac* factor, some kernels with an *I sh Bz* phenotype appeared on the resulting ears, as in the experiment described earlier. When four of the sister plants that had no *Ac* were similarly crossed, no *I sh Bz* kernels appeared, among a total of 2553, on the resulting ears, and none of the kernels was variegated; for no *Ac* was present in any of them and therefore no breaks at *Ds* could occur. Pollen from these same four plants was placed on silks of plants homozygous for *C, sh, bz,* and *wx* and also carrying an *Ac* factor. *Ds* events could now occur, but only during the development of the kernels that had received *Ds* from the male parent and *Ac* from the female parent. Mutation to *sh* might originate in some of these kernels, which would then show segments of the *sh* phenotype but would not be totally *sh*. Among 1677 kernels produced from this cross, none having *I* and *Bz* was totally *sh* in phenotype. In the reciprocal cross, 1213 kernels were obtained. In such a cross, the *Bz* phenotype can be detected only in those kernels that also have *Ds* and *Ac*. No *I sh Bz* phenotypes were found among them. It should be emphasized that in these reciprocal crosses no evidence of crossing over between *Ds* and *Sh* appeared; that is, in none of the kernels with an *I sh bz wx* phenotype was *Ds* detected. They apparently carried a chromosome derived from crossing over between *I* and *Ds*. In both case 1 and case 2, the frequency of crossing over between *I* and *Ds* seemed to be the same as that between *I* and *Sh* in other stocks. Similarly, no change in crossover frequency between *Sh* and *Bz* was noted in either case.

The comparative tests outlined above support the hypothesis that the *sh* phenotype in the *I sh Bz* kernels originates through events at *Ds*, when the latter is located just to the left of *Sh;* for this phenotype appears only when *Ac* is also present.

A second test of this hypothesis was conducted with both case 1 and case 2. In plants that are homozygous for *I Ds Sh* and carry an *Ac* factor, mutations to *sh* should occur in a few cells late in the development of the sporogenous tissue, or in cells of the gametophyte. Kernels that are *I sh* in phenotype should appear on the ears from reciprocal crosses with plants homozygous for *C ds sh*. The frequency of appearance of such kernels in these reciprocal crosses is recorded in table 3. Many of the *sh* kernels certainly carried *Ds* in the *I* chromosome. Its location between *I* and *Bz* could be affirmed in the majority of cases because of the pattern of variegation produced by breaks at *Ds*.

If the described mutation results from inhibition of *Sh* action by *Ds* itself, then reversion to *Sh* might result from a subsequent event at *Ds* that removed this inhibitory action. In order to obtain more evidence about the nature of the events that are responsible for mutations at this locus, plants were grown from some of the *I sh Bz* kernels in which both *Ds* and *Ac* were present. Tests were conducted to determine the viability of the mutant *sh* when homozygous, the position of *Ds*, and whether or not reversion to *Sh* would occur.

TABLE 3

FREQUENCY OF MUTATION TO *sh* IN RECIPROCAL CROSSES

Cross	*I Ds Sh/ I Ds Sh;* 1 *Ac* ♀ by *C ds sh* ♂		*C ds sh* ♀ by *I Ds Sh/ I Ds Sh;* 1 *Ac* ♂	
Kernel type	*I Sh*	*I sh*	*I Sh*	*I sh*
Case 1 (*Ds* 4864A)	644	7	6349	128
Case 2 (*Ds* 5245)	9082	18	5004	34

For case 1, ten kernels were selected that had the *I sh Bz* phenotype and carried both *Ds* and *Ac*. Only six of them germinated. A mutant *sh* factor was present in four of the plants. It was not present in one plant, whose constitution was like that of the heterozygous parent: *I Ds Sh Bz Wx/ C ds sh bz wx;* 1 *Ac*. The sixth plant was triploid, with the following chromosome-9 constitutions: *I Ds Sh Bz Wx/I Ds sh Bz Wx/C ds sh bz wx*. Two *Ac* factors were present. A mutant *sh* factor was present in one of these *I*-carrying chromosomes.

An ear derived by self-pollination was obtained from each of the four diploid plants having a mutant *sh* factor. The ratio of *I* to *C* kernels indicated no reduction in viability of the mutant when homozygous. In crosses of these four plants to those homozygous for *C, ds,* and *bz,* a few kernels with an *Sh* phenotype appeared, but only on one of the test ears from crosses involving one of the four plants. It is impossible to be certain that they were not the result of contamination, but the associated phenotypes make it unlikely that they were. In three of the four plants, crossing over between *I* and *Ds* and *Ds* and *Bz* was the same as that which occurred in the heterozygous parent plants, indicating no decided shift in the position of *Ds* in the origin of the *sh* mutation. In the fourth plant, the frequency of crossing over between *I* and *Ds* was unchanged, but that between *Ds* and *Bz* appeared to be reduced.

In case 2, 21 kernels having an *I sh Bz* phenotype and also carrying *Ds* and *Ac* were selected for testing. Only 13 of them germinated. A mutant *sh* factor proved to be present in twelve plants. The constitution of the thirteenth plant was similar to that of the heterozygous parent: *I Ds Sh Bz Wx/C ds sh bz wx;* 1 *Ac*. The *I*-to-*C* ratio on ears derived from self-pollination of the twelve plants carrying a mutant *sh* factor indicated no reduction in viability of the kernels homozygous for this factor. Crossing over between *I* and *Ds* was unmodified in all twelve plants. That between *Ds* and *Bz* was unmodified in eight plants, but in four plants it appeared to be reduced. On the ears derived from crosses to plants homozygous for *C, sh, bz,* and *wx,* kernels having an *I Sh Bz* phenotype appeared in tests of three of the eight plants in which crossing over between *Ds* and *Bz* was unmodified. The rates of reversion were low in two plants, but markedly higher in the third plant (5933-1). This applied to rates of germinal mutation, that is, mutation occurring in the sporogenous or gametophytic cells. If mutation occurs during the development of the kernel, a sector with the *Sh* phenotype will be

formed. To be detected, such sectors must be large; in other words, the mutation must occur early in the development of the kernel. All late-occurring mutations will be overlooked. Some sectorial kernels developed on the ears derived from crosses of all three plants mentioned above, the frequency of such kernels being highest on the ears derived from crosses of plant 5933-1. It appeared that this plant carried a mutable *sh* locus; and tests conducted with the progeny of the plant during the winter of 1951–1952 fully confirmed its presence. Although *Ac* control of mutation is probable in this case, it cannot yet be demonstrated with certainty. Inability to detect all kernels showing mutation to *Sh* necessitates extended tests for this determination.

It is known from studies of *Ds*-initiated mutable loci involving factors associated with color development, which may be detected even in individual cells, that the frequency of reversion depends on the state of *Ds*. For some states, the rate is high; for others it is very low. Only three of the twenty examined Ds-initiated mutations to *sh* have provided certain evidence of subsequent reversion, although a fourth instance may possibly have occurred. The original *I sh Bz* kernels selected for testing did not show any detectable somatic reverson to *Sh*. They may represent, therefore, a selected class in which the state of *Ds* is one producing relatively few reversions. Tests of an unselected sample are now under way. They should give a better indication of the frequency of production of highly mutable *sh* loci through the medium of *Ds* events. Nevertheless, from the several tests outlined above, there appears to be little question that events at *Ds*, following its insertion just to the left of *Sh*, are capable of inducing mutation to *sh*. It is also certain that some of the mutants are capable of subsequent reversion to *Sh*. Since one of the selected *sh* mutants proved to be highly mutable, additional examples of high mutability may appear when appropriate methods for their selection are employed.

Summary

Instability of genic action, under the control of the two-unit system of which *Ac* is one of the components, has been examined at six different loci. (See table 4.) Four of these six loci are associated with anthocyanin pigment formation (*C, Bz, A*$_1$, and *A*$_2$), one with composition of the starch in pollen and endosperm (*Wx*), and the sixth with a morphological structure of the endosperm (*Sh*). It is clear that this two-unit system may operate at loci concerned in quite different types of phenotypic expression. It cannot be stated that the system could operate at any known locus; but it should be capable of operating at many. Mutational behavior under the control of *Ac* always follows a distinct pattern, regardless of the locus involved. No mutations occur when *Ac* is absent. They appear only when *Ac* is present, and the time and place of their occurrence are an expression of the particular state and dose of *Ac*. At five of the above-mentioned loci (column 3 of table 4), other systems controlling genic expression are known to operate.

Some aspects of the behavior of a_1^{m-2} are unique in our studies. Two distinct classes of mutation occur. One class produces phenotypes indistinguishable from that given by the normal *A*$_1$ factor: deep pigmentation in the aleurone layer and intense pigmentation in all parts of the plant. The other class produces a complex series of changes affecting anthocyanin development in plant and aleurone. Mutations in the latter class form a graded series with respect to the intensity of pigmentation produced. In the plant, however, pigmen-

tation is always restricted to the root, stalk, sheath, auricle, and glume; none appears to be present in the leaf, except in the midrib and along the edge. The effects of a particular mutation of this class on intensity of pigmentation may not correspond in plant and aleurone. For example, a mutation producing very slight pigmentation in the aleurone may give very intense pigmentation in the affected plant tissues. Also, some of the mutations observed produced totally colorless kernels and intensely pigmented plants. Another distinctive feature of a_1^{m-2} is related to the effect of crossing over in the immediate vicinity of this locus, that is, between a_1^{m-2} and Sh_2. The majority of crossovers within this short segment result in mutation that appears to belong to the second class mentioned above. In contrast, tests of crossing over between Sh_2 and a_1^{m-1}, a_1^{m-3}, or a_1^{m-4} have given no such clear indication of mutation accompanying crossing over. Still other aspects of behavior peculiar to a_1^{m-2} have been observed, but description will be postponed until further evidence is available.

TABLE 4

KNOWN LOCI (COLUMN 1) AT WHICH MUTABILITY, UNDER THE CONTROL OF *Ac*, HAS ARISEN (COLUMN 2), AND MUTABILITY AT THE SAME LOCI CONTROLLED BY OTHER SYSTEMS (COLUMN 3)

(The figures following the symbols represent the sequence of appearance in the Cold Spring Harbor cultures.)

Symbol for normal, dominant factor at locus	Instability controlled by *Ac*	Instability controlled by system other than *Ac*
C	c^{m-1}	c^{m-3}
	c^{m-2}	
	c^{m-4}	
Sh_1	See text for cases	
Bz	bz^{m-1}	bz^{m-3}
	bz^{m-2}	
Wx	wx^{m-1}	wx^{m-2}
	wx^{m-5}	wx^{m-3}
	wx^{m-6}	wx^{m-4}
A_1	a_1^{m-3}	a_1^{m-1}
	a_1^{m-4}	a_1^{m-2}
A_2	a_2^{m-4}	a_2^{m-1}
		a_2^{m-2}
		a_2^{m-3}

It is clear from our studies that different systems of control of genic expression can arise at any one locus in the chromosome complement, and that the same system may operate at different loci.

The organization of the chromosomes with respect to functional units, and the types of functional units that may be present, are not clearly understood. Our studies indicate that at least two classes of functional genetic unit are carried by the chromosomes: one of them potentially capable of determining a particular course of cellular reactions, the other associated with the control of this potential action. It has been possible to distinguish between these unit components at a locus and to describe, in terms of phenotypic expression, their modes of operation. Our studies also suggest that many mutations may be expressions of changes in controlling systems, the potential capacities of the gene units remaining unchanged. At present, there is no way to distinguish, on the basis of mutation alone, between alterations in potentialities of genic action and alterations in the controlling systems that leave the potential unchanged, except in those cases where the latter is clearly evident.