CYTOGENETIC STUDIES OF MAIZE AND NEUROSPORA

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THE MUTABLE Ds LOCUS IN MAIZE

General considerations. In last year's report a summary account was given of several newly arising unstable gene loci. The instability of all but one of these loci was phenotypically expressed by the appearance in an otherwise recessive plant of sharply defined sectors of dominant tissue or of tissue showing an intermediate condition between recessive and dominant. Each of these sectors arose following a mutation in the unstable locus occurring in an individual cell during the development of the tissue. When an unstable locus is present, the tissues of the plant show a pattern of variegation which is related to the time and frequency of mutations occurring in particular cells during the development of the tissue. Observations of the behavior of the unstable loci have suggested that a common underlying phenomenon is associated with the expression of instability in all the cases examined. Several generalizations may be formulated concerning this phenomenon. Two separable factors are known to be associated with the expression of instability. The first factor is concerned with the particular state of the unstable locus in the cells of a developing tissue. The state of a locus is reflected by the time of occurrence of phenotypically visible mutations and by the frequency and distribution of these mutations. The second factor is concerned with the mutation at the unstable locus that gives rise to the phenotypically recognizable altered expression of the locus. During the development of a tissue, the state of a locus may remain unchanged. This results in a tissue showing one particular and readily recognizable type of variegation pattern. Changes in the state of a locus may, however, occur. These changes arise abruptly and appear to be associated with an event that occurs during a mitotic cycle. Following such a change, the variegation pattern is altered in the descendants of this cell. There may be fewer or, conversely, more mutations in the descendent cells than would have occurred had the event that gives rise to a change in state not taken place. The evidence suggests that the change in state may be related to the reproductive cycle of the chromosome, for it has frequently been observed that when a change occurs the state of the mutable locus in each sister chromatid may become altered. The state of the mutable locus may be quite different in the two chromatids, and the state in each chromatid, in turn, different from

that which existed in the immediate mother cell. In brief, it is the state of the locus at a particular stage in development that determines the time and rate of future mutations, and this state may be altered by an event occurring at a mitosis often considerably in advance of the genotypic mutation itself.

During the past year attention has been concentrated on one of the mutable loci, because its action and its location are particularly favorable for an analysis of the factors associated with mutability. Furthermore, the type of action at this locus is unique in its cytogenetic aspects and of considerable general interest in this respect alone. In this one case, mutability is expressed not by a visible phenotypic change in the action of a gene, but rather by dissociation of the bonds that normally would maintain a linear cohesiveness of this locus with an adjacent locus in the chromosome. As an ultimate consequence of the mutation, the chromosome is dissociated into two completely detached segments. Because one of these segments is acentric, it is not capable of directed movement in the spindle figure and subsequently is lost to the nuclei of descendent cells. This mutable locus has been designated *Ds* because the most readily recognizable consequence of its action is this dissociation. By both cytological and genetic methods, the *Ds* locus has been placed in chromosome 9 at approximately the position that demarcates the proximal third of the short arm. The acentric segment that is produced as a consequence of a dissociation mutation is composed, then, of the distal two-thirds of the short arm. This segment contains the loci of most of the known mutants of chromosome 9. Collectively, these mutants affect characters of the pollen, the endosperm of the kernel, the seedling, and the mature plant. Consequently, dissociation mutations at the *Ds* locus may be traced by genetic analyses in all stages of the life cycle when a plant carries dominant alleles and *Ds* in one chromosome 9 and recessive alleles and a normal *ds* locus in the homologous chromosome 9. Whenever a dissociation mutation occurs in a cell during the development of a tissue of such a plant, an acentric segment carrying the dominant factors is produced. This acentric segment is subsequently lost from the nucleus during a mitotic cycle. The result is a nucleus having only the recessive alleles that are present in the homologous segment of the *ds*-carrying chromosome 9. All the cells arising from this cell will be recessive in genotype and also in phenotype, if the expression of the particular recessive factor is cell-specific and if this phenotypic expression is not subject to changes that may be caused by diffusible gene products from the adjacent dominant cells. The presence of a recessive sector in a mature tissue indicates that a dissociation mutation occurred in the ancestor cell that gave rise to this sector. In plants of the given constitution, therefore, the mature tissues can be expected to show variegation for recessive sectors. From the size, frequency, and distribution of these recessive sectors in any one tissue the state of the locus in this particular tissue or sector of tissue can be recognized.

Control of Ds activity by Ac. Accumulating evidence indicates that the *Ds* locus will undergo dissociation mutations only when a particular dominant factor is present. This factor is designated *Ac* because it activates *Ds*. *Ac* is probably located in the long arm of chromosome 9, but its exact position has not been determined. By the end of this growing season, the analysis of the action of *Ac* on the *Ds* locus should be well advanced. At the present time the evidence suggests the following relations between *Ac* and *Ds*. If *Ac* is not present, the *Ds* locus is com-

pletely normal in behavior and indistinguishable from *ds*. If, by appropriate crosses, however, *Ac* is introduced into the primary endosperm nucleus, the *Ds* locus again becomes mutable and dissociations may begin to occur shortly after the introduction of *Ac*. *Ac* will not affect a normal *ds* locus, however; in the presence of *Ac, ds* remains stable.

Cytological aspects of the action of the Ds locus. Genetic evidence indicated that the dissociation mutations take place relatively late in the development of the sporophytic tissues. This was confirmed by cytological observations of the sporocytes of *Ds Ac* plants. In the sporogenous cells of the anthers of the majority of plants examined, the dissociation mutations—recognized by the constitution of the chromosome 9 bivalent—most frequently occurred in a late-premeiotic nucleus or sometimes in the meiotic nucleus itself. Genetic and cytological evidence also indicates that dissociation mutations may be delayed throughout the period of meiosis and only begin to take place in the following gametophytic nuclei. In some plants, however, dissociation mutations were observed to have occurred in relatively young premeiotic nuclei. The relation of this variable timing of dissociation mutations to the particular state of the *Ds* locus will be discussed later.

In making preparations of the sporocytes with the usual aceto-carmine staining techniques, considerable difficulty was encountered in obtaining an adequate number of well spread and sharply stained meiotic prophase figures. Consequently, it was necessary to attempt an improvement in the techniques. Methods that had been developed in the fall of 1946 for the study of meiotic prophase chromosomes of the fungus *Neurospora* were tried, and found to be likewise superior for similar stages in maize. These methods introduce the use of lactic acid, either in the fixing fluid or in the staining solution. Young anthers were fixed for 12 to 24 hours in a fresh mixture of four parts of 95 per cent alcohol to one part of lactic acid. The sporocytes in the meiotic prophase states were forced out of the anther in a drop of aceto-orcein; a cover slip was placed over the drop, and the slide gently heated. An unusually sharp differential stain resulted. The cytoplasm was only slightly stained; the chromosomes, in contrast, were brilliantly stained, and the centromeres were sharply delimited in each chromosome. Considerable stretching of the chromosomes sometimes occurred, however, during the flattening of the sporocytes. When equal parts of lactic acid and acetic acid were used in the fixative in place of lactic acid alone, the chromosomes stained sharply with aceto-orcein but were less subject to stretching during the flattening of the sporocytes. A third method involved the restaining of aceto-carmine preparations with an orcein stain consisting of 1 per cent orcein in a mixture of equal parts of lactic acid, acetic acid, and water. Brilliant contrast in staining resulted. Initial use of this stain on the sporocytes did not give satisfactory results.

Some of the major aspects of chromosome 9 behavior that are associated with the presence of the *Ds* locus were reviewed in Year Book No. 45. It is now suspected that the dissociation mutation process is not a simple breakage of bonds at the *Ds* locus, although this is usually the eventual consequence. In making observations of the chromosomes, it was necessary to be able to identify accurately the *Ds*-carrying chromosome in the sporocytes of a *Ds ds* plant. Crosses were made, therefore, between *Ds*-carrying plants with morphologically normal chromosomes 9 and *ds ds* plants having a chromosome 9 with a small terminal knob at the end of the

short arm and a short duplication of chromatin extending beyond the knob. The heteromorphic end of the short arm of the chromosome 9 bivalent in the meiotic prophases of the resulting plants allowed the *Ds*- and *ds*-carrying chromosomes to be readily identified. In these plants, a number of sporocytes were observed in which the *Ds*-carrying chromosome was deficient for the terminal two-thirds of the short arm as a consequence of a previous dissociation mutation in an ancestor cell. In all cases, without exception, it could be determined that only the *Ds*-carrying chromosome had been affected by this action. Some sporocytes were observed, however, in which the *Ds*-carrying chromosome had not simply lost two-thirds of its short arm, but had been subjected instead to some other modification, whose history is not understood at present. Among the aberrant types, those showing the complete loss of the *Ds*-carrying chromosome in a sector of sporocytes in the anther were the most frequent. In the recognized cases, the losses occurred earlier than the more frequently observed dissociations at the *Ds* locus. In other cases, a small sector of sporocytes was present in which the *Ds*-carrying chromosome was missing; in these cases, however, a small ring-shaped chromosome was present in the cells of these particular sectors. This ring chromosome probably is composed of a segment of the *Ds*-carrying chromosome 9, although positive identification could not be made. A few other types of aberrant configurations also were observed. In all these relatively rare types of aberrant behavior, only the *Ds*-carrying and never the *ds*-carrying chromosome was involved. It is obvious that the presence of the *Ds* and *Ac* loci is in some way responsible for these aberrant types of chromosome 9 behavior. It is hoped that a more complete analysis of these relatively rare types of sporocytes will yield some insight into the nature of the action that occurs as a consequence of the combined presence of the *Ds* and *Ac* loci. It will be necessary to observe many thousands of sporocytes before a sufficient number showing aberrant types of chromosome 9 behavior can be found. With the improved techniques recently developed, it is hoped that this may be more readily accomplished. Until this needed information has been obtained, it would be premature to attempt to project a sequence of events that result directly in a dissociation or in one of the rarer types of alterations involving the *Ds*-carrying chromosome 9.

The stability of the state of the Ds locus. As mentioned previously, abrupt changes may occur in the expressed pattern of dissociation mutations in a tissue or sector of tissue. The general pattern, in each case, is the product of the time, the frequency, and the distribution of dissociation mutations that have occurred in individual cells during development. At present it is not known to what extent these changes in the expression of dissociation mutations are controlled by altered conditions at either the *Ds* or the *Ac* locus, or at both, or by other genetic conditions not yet identified. Until further evidence has accumulated, the observed patterns will be considered a reflection of the state of the *Ds* locus even though this restricted definition may later require modification. The patterns of dissociation mutations are visible in tissues of plants that have a *ds*-carrying chromosome 9 with recessive factors in its short arm and a *Ds*-carrying chromosome 9 with their dominant alleles. The recessive factors *wd*, *pyd*, and *yg* (*wd*, white leaf tissue; *pyd*, pale-yellow leaf tissue; *yg*, yellow-green leaf tissue), located at or close to the end of the short arm, have been used to determine the pattern of dissociation mutations in leaf tissues of seedlings

or mature plants. The series of alleles *c* (colorless aleurone), *C* (colored aleurone), and *I* (inhibitor of *C* color)—located approximately at the position demarking the distal third of the short arm—and the recessive factors *sh* (shrunken endosperm), *bz* (bronze, modifier of *C* color), and *wx* (waxy starch in endosperm and pollen)—located, in the order given, between *C* and *Ds*—have been used to examine dissociation mutations in the endosperm of the kernel. The alleles *Wx* and *wx* have been used to estimate the number of pollen grains in individual anthers of *Wx Ds/wx ds* or *wx Ds/Wx ds* plants that are deficient for the terminal two-thirds of the short arm of chromosome 9 because of previous dissociation mutations in ancestral nuclei.

When silks of plants that were homozygous for *ac* and for *C, sh, bz, wx,* and *ds* received pollen from plants carrying *Ac* and a chromosome 9 with the dominant alleles *I, Sh, Bz, Wx,* and *Ds,* a number of kernels on the resulting F_1 ear were variegated because of the presence of sectors of cells with the phenotypic constitution *C sh bz wx*. With a few exceptions, each sector composed of multiple-recessive cells arose following a dissociation mutation that had occurred in the ancestor cell of the sector. Subsequent elimination, during a mitosis, of the acentric segment of the chromosome 9 carrying *I Sh Bz Wx* resulted in the absence of these dominant factors from the descendent nuclei. In crosses involving any one male parent carrying a particular *Ds* and a particular *Ac* locus, the majority of variegated kernels fell into one main class with respect to the type of variegation pattern they exhibited. Great differences in pattern types exist. For example, the majority of variegated kernels on the F_1 ears may show a speckled appearance because of the presence of a number of small patches of cells that are *C sh bz wx* in constitution. The size of a speckle here depends on the time of occurrence of the dissociation mutation. If it occurs very late, the colored speck may be composed of only one, two, or a few aleurone cells, but if it occurs somewhat earlier, the colored speck is composed of more aleurone cells. Crosses involving a different male parent may give rise to F_1 ears in which the majority of variegated kernels show early dissociation mutations. These kernels are characterized by large areas of recessive tissue and, in extreme cases, by only small patches of dominant tissue—residual areas where dissociation mutations have not occurred.

In a number of variegated kernels, there were relatively large, sharply defined sectors in which the pattern of dissociation mutations within the sector contrasted greatly with the pattern exhibited by other parts of the kernel. These sectors indicated that a change of state had occurred in the ancestor cell that gave rise to the sector. Often these changes in state occur at a relatively early period in the development of the endosperm. The most instructive cases were exhibited by those kernels in which a change of state could be traced to the first or second mitotic division in the endosperm. A change in state in the first division may give rise to a kernel one-half of which shows one pattern of dissociation mutations and the other half a contrasting pattern of dissociation mutations. Or a kernel may be divided into three or four sectors, each with its own particular pattern, following changes of state that occurred in the first and second mitotic divisions of the endosperm. Because of the free nuclear division that takes place in the early development of the endosperm, this tissue is not ideal for a study of early cell lineages. Nevertheless, early changes in state can be recognized in many kernels. The prospects of de-

termining the contrasting nature of the altered states of two sister chromatids, following a mitotic cycle that introduces a change in state in both chromatids, are considerably better in this tissue than in the sporophytic tissues.

In the sporophytic tissues, dissociation mutations occur late in the life of any one tissue. This is in contrast with the endosperm tissue, where dissociation mutations may occur at any time during development. Changes in state, however, may occur at any time during the development of the sporophytic tissue. In this respect, the two tissues are comparable. Barring heterofertilization, the *Ds* locus in the first endosperm nucleus and that in the zygote nucleus are carried by sister chromatids. If no change of state had occurred in the division that gave rise to these two chromatids, the conditions that govern the states of both *Ds* loci should be alike. Several lines of evidence have indicated that this is probably true. Kernels showing early dissociation mutations in the endosperm give rise, in general, to plants having relatively early dissociation mutations in the sporophytic tissues. Conversely, kernels with late dissociation mutations in the endosperm tissues give rise to plants showing relatively late dissociation mutations in the sporophytic tissues. Present evidence indicates that the state of a particular *Ds* locus may remain relatively unchanged in most of the cells of a plant. Plants arising from kernels that showed early dissociation mutations gave rise in the next generation to variegated kernels the majority of which showed early dissociation mutations. Conversely, plants arising from kernels that showed late dissociation mutations gave rise in the following generation to variegated kernels the majority of which showed late dissociation mutations. Even though a *Ds* locus may remain in one state throughout a number of consecutive mitoses, changes in state nevertheless are not infrequent. At present, no critical evidence is available concerning possible genetic or environmental factors that may influence the state of a locus or its expression as reflected in dissociation mutations. Although changes in state of a locus and the subsequent changes in the frequency and distribution of dissociation mutations are interrelated, the alteration that is associated with a change in state and the alteration that results in a dissociation mutation are distinct and separable.

Continuation of Studies of the Chromosomes of Neurospora crassa

Study of the mutable loci in maize was interrupted during the fall and early winter of 1946 in order to continue the investigations of the chromosomes of *Neurospora crassa* begun several years earlier. The earlier work was preliminary and exploratory, and no time was spent in obtaining the necessary illustrations of chromosome morphology and behavior during ascosporogenesis. In addition, the preliminary study indicated a need for improvements in techniques in order that consistently good preparations could be obtained of the chromosomes and nuclei of the ascus. Efforts were concentrated, therefore, on these two objectives. The investigations were conducted at the California Institute of Technology, with the collaboration of Mr. Jesse R. Singleton. Approximately one hundred photomicrographs were taken, illustrating chromosome and nuclear behavior from the prefusion stages in the crosier to the binucleated stage in the ascospore. New or modified techniques were devised, which greatly improved the quality of the preparations. This applied significantly to the meiotic prophase stages,

where the chromosomes are greatly extended. In the previous investigation, the minute morphology of the extended chromosomes could rarely be observed, but with the present techniques this morphology is sharply defined in many figures. Mr. Singleton has succeeded in mapping the chromomere organization of each of the seven chromosomes. Each chromosome has an individually recognizable chromomere organization, including deep-staining regions which, because of their positions in the chromosomes, probably represent the heterochromatic regions known to be adjacent to the centromeres. It can now be stated with certainty that no heteromorphic pair of chromosomes is present in *Neurospora crassa*. These techniques have also made it possible to observe more critically the mutual relations of the two homologues of a bivalent during the mid-meiotic prophase stages, that is, before diplotene. Many bivalents were observed in which the two homologous chromosomes were lying side by side but not in direct contact at any point along their length. These favorably oriented bivalents showed no relational coiling of the two homologues about each other, and it was obvious that the distance between them was quite constant—amounting to approximately half a micron—so that in their spatial relation they resembled railroad tracks. Technical methods were devised by Mr. Singleton for softening the ascus wall in order to flatten the asci, and also for achieving a sharp differentiation of the spindle figures and the centriole. From preparations kindly donated by Mr. Singleton, photographs were taken to illustrate the peripheral position of the chromosomes in the spindle figures and the rather bizarre organization and behavior of the centriole in the third division in the ascus.

Though the primary objectives of this interim study of the chromosomes of *Neurospora* were fulfilled, a supplementary factor of possibly greater value was the progressive realization of the possibilities for utilizing this material in attacking a number of cytological and cytogenetic problems, both old and new.