GENETIC AND CYTOLOGICAL STUDIES OF MAIZE

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Further Studies of the Spm System

The manner in which the *Spm* (Suppressor-mutator) element in maize controls gene action and mutation at the modified A_2 locus in chromosome 5, designated a_2^{m-1}, was outlined in Year Book 57. The alternating cycles of activity undergone by *Spm,* and their relation to the patterns of anthocyanin distribution in plant and in kernel, were described. It was reported that the time of change from one phase of activity to the other during development appears to be under genetic control. Some isolates of *Spm* remain active during all stages of development, or become inactive in only a few cells very late in the development of plant or kernel; other isolates undergo early change in phase; and still others may be completely inactive during all stages of development, or return only very occasionally to the active phase. Studies aimed at revealing some of the conditions responsible for controlling changes in phase of *Spm* activity were continued this year. It is now evident that the readily distinguishable differences between plants or within different parts of the same plant with regard to changes in phase are reflections of different types of modification of the *Spm* element itself and also that such modifications occur at the time of change in phase.

Investigation of cyclical changes in phase of *Spm* requires examination of its activity in all parts of individual plants, including the kernels produced by different ears of a single plant. The type of *Spm* modification occurring in an individual cell of a plant during development is reflected in the progeny of that cell. The phenotype of the tissues derived from it indicates not only that a reversal of phase of *Spm* activity has occurred but also that the modification of *Spm* responsible for the reversal is likewise responsible for controlling the time and frequency of occurrence of subsequent reversals.

Much of the evidence was derived from a study of sequential changes in phase of one particular isolate of *Spm* whose behavior was followed through four generations of plants. It was possible to select kernels from ears of plants in which *Spm* was either unaltered or had been modified in an individual cell during plant development; and plants grown from such selected kernels exhibited either the parental type of *Spm* behavior or some modification of it. Each progeny plant was then examined in the same manner as the parent plant. Again, kernels selected from some of the ears were sown, and the plants derived from them were investigated. This method of examining the behavior of *Spm* has made it possible to draw the inferences mentioned above, namely, that each change in phase of *Spm* is associated with an event in some cell that alters the *Spm* element itself, and that each such event effects a particular type of modification of *Spm*. The type of modification is made evident in the descendent cells by the time and frequency of occurrence of subsequent reversals of phase; and the different modifications of *Spm* produce a wide range of effects in this regard.

An unanticipated aspect of *Spm* behavior was revealed during the year. It had been observed previously that, when an *Spm* element in its active phase is incorporated into a nucleus carrying an inactive *Spm* that rarely reverts to its active phase, the inactive *Spm* is apparently activated. The pattern of anthocyanin distribution that develops in plant and kernels is not the one that is expected when a single active *Spm* element is present in a plant. Instead, it resembles the one that appears when two initially active *Spm* elements are introduced into the zygote, or when three are present in the primary endosperm nucleus and each of them independently undergoes inactivation during development.

It has now been learned, however, that the inactive *Spm* element suffers no permanent alteration by reason of its association with the active *Spm*. By means of meiotic segregation in plants having both these elements, their association within a nucleus can be terminated, and the subsequent action of each can be observed in progeny plants where it is present alone. To identify each of these two elements in the progeny, the relative locations in the chromosome complement of the *Spm* elements in the parent plant must be known. Therefore, progeny tests were conducted with two sets of plants. In one set, the inactive *Spm* element was located in chromosome 9 and was closely linked with *wx*, whereas the active *Spm* element was independently located. In the other, the location of the two elements was reversed: the active *Spm* was linked with *wx* and the inactive *Spm* was independently located. In the individual progeny of either set it was possible to determine whether or not *Spm* was present, whether both elements or only one had been received from the parent, and, if only one, which of the two it was.

The progeny tests showed that, as long as the inactive *Spm* element is carried in a nucleus having an active *Spm*, it behaves like an active *Spm* element. When its association with the active element is terminated, however, it behaves much as it would have behaved if it had never been so associated. In other words, its initial constitution is not altered.

Another test, similar to those just described, was conducted with plants having two initially active *Spm* elements, one located in chromosome 9 and closely linked with *wx,* and the other independently located, in order to see whether each would exhibit its particular cycle of phase reversal after they were separated by meiotic segregation. Because one element was closely linked with *wx* and the other segregated independently of it, it was possible in the progeny (except in a few individuals that carried a transposed *Spm* element) to identify each *Spm* element, and to compare the behavior of each in those individuals that received one or the other. By this means it was learned that the association of the elements did not affect their subsequent control of phase reversal: each retained its own type of control.

It may be pointed out at this time that there appears to be more than a superficial resemblance between the *Spm* system of control of gene expression in maize and the system that controls the expression of flagellar antigens in the bacterium *Salmonella,* described by Lederberg and Iino. The resemblance is evident in the mode of control of gene action, the changes in phase of activity, the duration of a phase and the changes in duration that may occur, and the "states" of the genes whose action is being controlled.

Chromosome Constitutions of Some South American Races of Maize

Studies at Cold Spring Harbor were interrupted during the winter of 1958–1959 while I spent several months in Colombia examining the chromosome constitutions of plants belonging to different races of maize from Ecuador, Bolivia, Chile, and Venezuela. I was invited to make such examinations by the Committee on Preservation of Indigenous Strains of Maize, of the National Academy of Sciences–National Research Council in Washington, several of whose members have taken part in identifying and classifying these races.

The collection and propagation of indigenous races of maize of the above-mentioned countries have been the responsibility of some of the members of the Rockefeller Foundation who are associated with its agricultural program in Colombia, namely, Dr. Lewis M. Roberts, Director; Dr. David H. Timothy, in charge of maize improvement in Colombia; Dr. William H. Hatheway, whose knowledge of maize races is extensive; and Ing. Ricardo Ramírez, who also has a broad comprehension of the subject. Each of these men has contributed his talents to the project of collection, identification, and propagation. I am very grateful for their highly effective assistance, as well as for the many courtesies shown by each of them during my study of chromosome constitutions of some of the races.

All chromosome examinations were made at the Universidad Nacional, Facultad de Agronomía e Instituto Forestal, located in Madellín, Colombia. Facilities of the Institute were generously and courteously extended to me by the Director, Dr. Garcés, and by Dr. Sánchez, in whose department the examinations were made. I am particularly indebted to Señorita Rocío Díez P., who worked with me daily in order to learn to identify maize chromosomes and whose progress was so rapid that she was able to make a contribution to the study.

The sporocytes of plants whose chromosomes were to be examined had been collected and stored in a deep-freeze unit. Races of Ecuador, Bolivia, and Chile were selected by Dr. Roberts and Ing. Ramírez to be explored in the limited time available. Pachytene stages of the microsporocytes of plants belonging to these races were examined for the presence of knobs in any of the chromosomes; when knobs were found, their location, size, and morphological characteristics were determined. If B-type chromosomes were present, the number was ascertained. Note was taken also of any readily identifiable structural

modification affecting chromosome organization.

There are more than twenty known locations in the 10 chromosomes of maize at which knobs may be present. At any one location, knob morphology may vary with respect to size or shape, or both. Previous studies by maize cytologists had shown that a particular type of knob at any one location is heritable in that it passes to successive plant generations without change. Their work had also revealed that number and distribution of knobs differ among different strains of maize: some strains have no knobs; others have a few, at specific locations; and still others have many, whose types and locations depend on the strain.

As the data accumulated, it became increasingly evident that detailed knowledge of knob constitution would be a useful adjunct to the observation of morphological and physiological properties as criteria for characterizing races. Its usefulness for considerations of modes of origin of races was also evidenced, particularly during the examination of races indigenous to high-altitude regions in the three countries. At the completion of this examination it was found that plants of all but 2 of the 32 highland races studied were amazingly similar with regard to knob constitution. In these 30 races, a small knob was present at one particular location in the long arm of each chromosome 7. In some but not all of the plants of these races, a very tiny knob was present in the long arm of chromosome 6. When present, it was always at the same location —an important fact because there are three knob locations in the long arm of this chromosome at each of which the knobs may differ in size or shape. No other knobs were found in the chromosome complements of the 30 races, except in 2 plants among the 125 examined. These 2 were derived from different collections made in Ecuador. Each of them carried one additional knob, which in both cases appeared in only one homologue of the chromosome in which it was located. In one plant, the extra knob was in the long arm of one of the two chromosomes 2; in the other, it was located in the long arm of one chromosome 8. These 2 exceptional plants probably resulted from an earlier contamination with a race having other knob constitutions.

The strikingly uniform pattern of knob constitution described above characterized 9 of the 10 highland races of Ecuador that were examined, 11 among 12 highland races of Bolivia, and all 10 of the highland races of Chile included in the study. In addition, B-type chromosomes were found in some plants of many of these races, the number per plant ranging from 1 to 6. In contrast to the highland races, many of the lowland races of these countries differed greatly from one another with regard to number, location, and size of knobs and also with regard to degree of heterozygosity of a knob at any one location. Within many of these races, however, there was a consistent pattern of distribution of knobs at particular locations in the chromosome complement as well as consistency in the type of knob found at any one location.

The similarity of chromosome constitution among most of the Andean races of Ecuador, Bolivia, and Chile was so impressive that we wanted to learn whether or not it extended to the highland races of Venezuela. Sporocyte collections had been made from plants of only 4 such races, but chromosomes from all the available collections were examined. Knob constitutions in the 4 races proved to be very different from those of the Andean races previously examined. In 2 of them, knobs were present in all the chromosomes except chromosome 10, and some chromosomes had two or three knobs; some of the knobs, moreover, were exceedingly large. In addition, a high degree of homozygosity was exhibited; that is, in each homologous pair of chromosomes the same type of knob was present at the same location in each

member of the pair. Plants of the other 2 races examined had some of the same knobs, but often a particular knob appeared on only one of the two homologues of a chromosome.

At the time the chromosome examinations were being made, I did not know the exact location from which a particular collection had come but only the country and the elevation. After the examinations had been terminated, the collected data were taken to Bogotá, where, with the invaluable cooperation of Dr. Timothy and Dr. Hatheway, the relations of chromosome constitutions to exact geographical locations were plotted. It then became more fully apparent that there are significant relations between knob constitution and geographical location, and that it would be possible to utilize a knowledge of knob constitutions in attempting to trace the origins and migrations of maize races. It is also possible to draw inferences about the probable contributions of hybridization to the development of some of the races.

The impression gained from these preliminary studies is that present-day maize may have derived from several different centers. Migration from such centers in the past was followed by hybridization. Examination of the chromosome knobs of plants from various geographical locations may help to identify some of these centers.

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