MUTATIONS IN MAIZE AND CHROMOSOMAL ABERRATIONS IN NEUROSPORA

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During the past year, studies of mutation-controlling systems in maize were continued at Cold Spring Harbor, and examinations of chromosomal aberrations in *Neurospora* were carried out at the California Institute of Technology.

MUTATIONS IN MAIZE

In previous Year Books, evidence has been presented concerning the nature of action of mutation-controlling systems in maize that are responsible for the behavior of mutable genes. Much of the detailed evidence was derived from study over a period of years of the system composed of the two chromosomal units, *Ds* and *Ac*. Extensive examination of other systems was postponed until the behavior of this system had been well explored. Knowledge of gene-controlling systems in maize is progressing rapidly through the efforts of investigators at other institutions who are examining some of the gene-controlling units that have appeared in their materials. One such unit, being investigated by Dr. R. A. Brink and his students at the University of Wisconsin, behaves much like *Ac*. My studies of the *Ds-Ac* system during the past year have been limited to obtaining some additional information about its action at one selected locus in the chromosome complement. This has involved further examination of the seven cases of change in action of *I* induced by *Ds* when the latter was located just to the left of Sh_1 in chromosome 9. The origins and general descriptions of these cases were outlined on pages 231–233 of Year Book No. 52. The newly acquired information provides a clearer understanding of the similarities and differences among them. Only a summary of this information need be presented here.

Among the seven cases in which the action of *I* (located approximately four crossover units to the left of *Ds* in the parent plants) was modified, *Ds* has been found to be present in the chromosome in each case and apparently not changed in location by the event that altered genic action in the segment of chromatin to the left of it. *Ds* remains near, and to the left of, Sh_1. The seven cases may be separated into two main classes: four that regularly produce viable homozygotes, and three that have given no viable homozygotes even though many attempts have been made this past year to obtain them. In all seven cases, the chromosome 9 having the altered region that includes the locus of *I* is transmitted normally through the female gametes; but transmission through the pollen grains is considerably reduced when these compete with others carrying normal chromosomes 9. The extent of this reduction is approximately the same for all the members of the first class and for two of the members of the second class, but is extreme for the remaining member of the second class. The seedlings derived from the homozygotes produced by members of the first class have all shown the same type of albescence phenomenon—disappearance of chlorophyll from the seedling leaves at about the three-leaf stage. Intercrossing has produced individuals carrying all possible combinations of the seven altered chromosomes 9, except combinations between those of class 2, which are apparently as inviable as are the homozygotes in each member of this class. Kernels with viable combinations show no pigment in the aleurone layer, resembling in this respect

the phenotypes of the homozygotes of class 1. The seedlings derived from them exhibit the same type of albescence phenomenon that characterizes the seedlings of the class-1 homozygotes. In none of the seven cases has there been any evidence of crossing over within the affected region in individuals having a normal and an altered chromosome 9. In each case, however, crossing over to the right of the affected region—between Sh_1 and Bz and between Bz and Wx—is normal.

The above-described relations among the seven independent cases of change in genic action within a segment of chromosome 9 located to the left of *Ds* are consistent with a hypothesis that they originated through removal of a similar segment of chromosome 9 in each case. The common segment removed would include the region beginning just to the left of the locus of Sh_1 and ending somewhere beyond that of *I*. The differences between members of the two classes and among members of the second class could then be explained by differences in the extent of loss beyond the locus of *I*—the members of class 2 having longer deficiencies than those of class 1, and one member of class 2 the longest. No direct evidence of deficiency in any one of these seven chromosomes was obtained, however, when each was compared with a normal chromosome 9 at the pachytene stage of meiosis. Also, it is known that deficiency need not be involved in such loss of genic action. A *Ds*-induced inhibition of action may be the cause, for this is known to account for some similar types of loss of genic action in the segment of chromosome 9 located to the right of *Ds*. The types and extents of such loss were described in Year Book No. 52.

System controlling genic expression of a_1^{m-1}. The occurrence of genic instability in many organisms, and its general resemblance to cases in maize, suggest that the gene-controlling units found in maize are not peculiar to that species. Rather, they reflect a type of nuclear organization and action that is probably present in many organisms. Thus it is necessary, before wider integrations can be made, to determine the kinds of gene-controlling systems that exist, how they differ from one another, and whether or not interactions occur among them. Attention is now being focused, therefore, on a system controlling genic expression which differs from the *Ds-Ac* system in some respects but resembles it in others. Although knowledge of the behavior of this second system is limited, it has been possible to formulate an interpretation from present information. This system operates to control genic expression of a_1^{m-1}, one of the mutable conditions that has arisen at the locus of A_1 in chromosome 3 in the Cold Spring Harbor cultures. (A_1 is associated with the appearance of anthocyanin pigmentation in plant tissues and in the aleurone layer of the kernel; a_1, known recessive allele, when homozygous, results in absence of anthocyanin pigmentation in both plant and aleurone.)

The system controlling genic expression at a_1^{m-1} is considered to be composed of two units, one inserted at the locus of A_1 in chromosome 3 at the time of origin of a_1^{m-1}, and one located elsewhere in the complement. The evidence suggests that this latter unit, tentatively designated as *Spm* for reasons that will be made evident shortly, may be altered in somatic cells and possibly may also be transposed from one location to another in the chromosome complement. In these respects, its behavior resembles that of *Ac*. Another apparent similarity is that *Spm* must be present in the nucleus if mutations are to occur at the locus of a_1^{m-1}. *Spm* and *Ac* differ in a significant way, however, and this is related to the contrasts in phenotype that are expressed in their presence and absence. The presence of *Ac* is detected by the alterations it induces at the locus of the gene whose mutations it controls (or at the locus of *Ds*), which lead to recognizable phenotypic modifications in somatic cells; its absence is indicated by the absence of any such modifications. With

regard to *Spm*, however, its presence or absence is recognized not only by the appearance or nonappearance of mutations at a_1^{m-1}, but also by another phenotypic difference that is sharply expressed in plants having certain states of a_1^{m-1}. When *Spm* is present in somatic nuclei, genic action at the locus of a_1^{m-1} is completely inhibited except in those cells that carry a mutation at this locus. Such mutations occur in some somatic cells during development. They permit the occurrence of some forms of action of the genic material at the locus of a_1^{m-1}, and this is recognized phenotypically in the tissues produced by the descendants of such cells. Therefore, both plants and kernels that have *Spm* are variegated in anthocyanin pigmentation. Spots or areas, each exhibiting a particular type and intensity of pigmentation—for different types of mutations are induced at a_1^{m-1} when *Spm* is present—appear on a colorless background. Removal of *Spm*, whether occurring somatically in some cells or produced as the consequence of meiotic segregations in plants heterozygous for it, results in partial release of inhibition of genic action, presumably at a_1^{m-1}, for the tissues of both the plant and the aleurone layer of the kernel are uniformly pigmented. With some of the states of a_1^{m-1}, the color is less intense than that which appears when the normal A_1 locus is present. The difference may be qualitative as well as quantitative, for the pigment does not seem to be the same as that produced by A_1. No spots or areas with altered pigmentation appear. In other words, no variegation is expressed in the absence of *Spm*. The symbol *Spm* has been used to designate this unit because it acts both as a suppressor and as a mutator.

The origin of a_1^{m-1} from a modification occurring at the locus of the normal A_1 gene was described in Year Book No. 50 (1950–1951). *Spm* was first discovered in a study of a particular state of a_1^{m-1}, derived from the original state. This derived state was selected early in the investigation of a_1^{m-1} because it was considered to be more suitable than the original state for an analysis of factors responsible for mutation at a_1^{m-1}. It was present in an individual kernel in the progeny of the plant in which a_1^{m-1} first appeared. Some aspects of the inheritance behavior of this state of a_1^{m-1} were outlined in Year Book No. 52. The evidence described there indicated the presence in variegated plants of a unit factor (or of several similar unit factors), not linked to the locus of a_1^{m-1}, that influenced the expression of a_1^{m-1}. It was then thought that removal of this factor from nuclei by meiotic segregations resulted in a particular type of mutation at the locus of a_1^{m-1}, and that in this respect the case resembled that of a_1^{m-2}, where removal of such a factor is known to lead to mutation at a_1^{m-2}. Results of subsequent investigation, however, carried out during the past year, suggest that removal of the unit factor, now designated *Spm*, does not lead to mutation at a_1^{m-1}, but rather removes an inhibitory action ascribable to *Spm*, as outlined above.

Evidence suggesting linkage of an *Spm* factor with *Y* or its allele *y* (*Y*, yellow starch in endosperm, dominant to *y*, white starch), located in chromosome 6 of the complement, was presented in Year Book No. 52 in table 3 of the report of this Department. An exploratory test involving only nine plants was made this year, partly in order to determine whether or not the apparent linkage could be validated. These plants were derived from the variegated kernels produced on the ear of plant 6046C-2, recorded in the above-mentioned table. Two or more independently located *Spm* units were assumed to be present in this plant, but one of them appeared to be linked to Y. It should be emphasized that, in backcross tests, segregation ratios typical of linked units would appear only on the ears of plants having a single *Spm* unit, and in such plants only when losses of *Spm* from many cells did not occur late in the development of the ear. Furthermore, transpositions of *Spm* to new locations would distort ratios or even eliminate

evidence of linkage if they occurred early in the development of an ear or if they were frequent during the later stages of development. An early-occurring loss of *Spm* from a cell whose descendants contribute to the formation of the ear is detected readily by the appearance of an area on the mature ear in which all the kernels carrying a_1^{m-1} show the characteristic pale aleurone color that develops in the absence of *Spm*. A few ears had such areas. Early-occurring transpositions of *Spm* can be detected by markedly altered linkage relations between the given units, by absence of such linkage, or by linkage of *Spm* to a factor carried in another chromosome. There was evidence of such changes in location of *Spm*, based on observed ratios produced by some of the ears.

The conditions, indicated above, that are required for the production of typical linkage ratios in backcross tests were present in eight ears obtained from six of the nine tested plants. The design of the test cross and the types of kernels that appeared on each of these eight ears are shown in table 17. From the data in this table it may be concluded that a single *Spm* unit was present in these six plants, linked to Y and located approximately 38 crossover units from Y. The presumed location in chromosome 6 of one of the *Spm* units in the parent plant, 6046C-2, has thus been confirmed.

Further description of the behavior of this gene-controlling system will be postponed until the evidence from tests now under way becomes available.

TABLE 17

PHENOTYPES OF KERNELS APPEARING ON EARS OF PLANTS WHOSE CONSTITUTIONS WERE a_1^{m-1} Sh_2/a_1 sh_2; Y/y WHEN THESE WERE CROSSED BY PLANTS HOMOZYGOUS FOR a_1, sh_2, AND y

PARENTAGE IN CROSS		PHENOTYPES OF KERNELS												Totals
		*Sh*₂ Color in aleurone						*sh*₂ Color in aleurone						
		Pale		Variegated		Colorless		Pale		Variegated		Colorless		
♀	♂	Y	y	Y	y	Y	y	Y	y	Y	y	Y	y	
6629A-1	1041-5...	36	58	66	35	0	0	0	0	0	0	85	89	369
6629A-1	1041-4...	33	54	51	36	0	1	0	0	1	1	101	98	376
6629A-3	1040-1...	34	52	43	37	0	0	0	1	1	0	85	83	336
6629A-4	1041-5...	23	65	56	36	0	0	0	0	0	0	90	84	354
6629A-6	1040-1...	34	67	78	37	0	0	0	0	1	0	86	113	416
6629A-7	1040-1...	29	59	58	36	4	1	0	0	0	0	105	100	392
6629A-9	1040-8...	39	41	49	38	0	0	1	0	0	0	79	90	337
6629A-9	1040-5...	41	58	50	34	0	1	0	1	0	0	79	115	379
Totals		269	454	451	289	4	3	1	2	3	1	710	772	2959

CHROMOSOME ABERRATIONS IN NEUROSPORA

During the winter of 1953–1954, examination of chromosome complements in asci of *Neurospora crassa* was undertaken at the California Institute of Technology, for the purpose of determining the nature of chromosomal aberrations known to be present in strains 4637 and 45502. All the stocks used in this study were obtained from Mary B. Mitchell, and all crosses were made by her. Her co-operation and interest were very much appreciated.

Previous investigations had shown that strain 4637 carries a reciprocal translocation between two nonhomologous chromosomes. Although the earlier studies were limited, they did indicate that chromosomes 1 and 6 are involved in the translocation. The purpose of this year's study was

to determine the position of break, in each of these two chromosomes, that gave rise to the translocation. Crosses were made between the wild-type strain 2522-1a and strain 4637R-1A. Nuclei in asci produced by this cross were examined at that stage of the first meiotic prophase when the chromosomes are maximally elongated. It was possible, by observing synaptic configurations of the chromosomes involved in this translocation and by comparing chromomere morphologies of paired elements within it, to determine the break position in each of the chromosomes.

The linear organization of chromosome 6, the next to the smallest chromosome of the complement, is unusually well defined, and this makes possible a ready identification of its component parts. That of chromosome 1, the longest chromosome of the complement, is less well defined; but two conspicuous chromomeres are present, which can serve as points of orientation in describing break positions. One of them is located near the middle of the chromosome, dividing it into two segments with relative lengths of 1 and 1.6. The other conspicuous chromomere is located near the end of the shorter of these two segments. The position of the break in chromosome 1 is in the longer of its two segments, approximately three-eighths of the distance from the free end of the segment. Chromosome 6 may be divided into several well marked segments. One terminal segment contains large, closely packed chromomeres. This is followed by a longer segment with smaller, less closely aligned chromomeres. A very conspicuous, deep-staining, dumbell-shaped chromomere separates the second segment from the remaining segment of the chromosome. This last segment, whose length is about one-quarter that of the total chromosome, contains a few small, rather widely separated chromomeres. The position of the break in chromosome 6 is in the second of the above-described segments, closer to the first segment than to the conspicuous dumbbell-shaped chromomere.

In crosses of strain 4637 to wild type, various types of synaptic configuration were produced by association of the two translocation chromosomes introduced by strain 4637 and the two corresponding normal chromosomes introduced by the wild-type strain. Homologous association of all components was frequent, resulting in the formation of a cross-shaped configuration having three short arms and one long arm. Failure of association of homologous components of one or more of the shorter arms of the cross was observed, however, in a number of nuclei. The remaining five pairs of chromosomes appeared to be normal, for no gross structural modifications were observed in any member of a pair.

In contrast to the relative ease with which the structural modification present in strain 4637 could be analyzed, that associated with strain 45502 proved to be very difficult. In crosses of this strain to wild type, a distinctive pattern of normal and abnormal spores appeared in many of the asci. Cytological examination indicated that there is no simple reciprocal translocation between two nonhomologous chromosomes in this strain to account for the distinctive patterns of spore types. The chromosome complements of four isolates of strain 45502 were examined: T45502-P1315-1a, T45502-1507-1A, T45502-1508-1a, and 70007TR-2A. The examinations suggested that in each of them the complement is composed of seven haploid chromosomes plus an extra chromosome whose length is approximately half that of the smallest chromosome of the complement, namely, chromosome 7. The origin of this extra chromosome could not be determined readily by inferences from synaptic associations, because of the many irregularities in chromosome associations that occurred when these strains were crossed to wild type and even when two of them, T45502-P1315-1a and 70007TR-2A, were intercrossed.

A brief description of some of the abnormal synaptic associations that were observed at the first meiotic prophase in asci

produced by crossing any one of the four strains of 45502 with wild type will indicate the nature of the complexities encountered in this analysis. One end of the fragment chromosome was often associated with an end of another chromosome. This did not necessarily reflect homology, because any one of the seven chromosomes of the haploid complement could enter into such an association. It occurred most frequently, however, with chromosome 7. When one end of the fragment chromosome was associated with an end of another chromosome, the homologue of this second chromosome sometimes formed a terminal association with a member of still another pair of chromosomes. Although most of the aberrant associations occurred between ends of chromosomes, a few nuclei were observed in which longer segments of two nonhomologous chromosomes were synaptically associated.

Because of these irregularities in synaptic behavior, determination of chromosome composition and organization in the ascus nuclei was difficult. In all nuclei, pairing occurred between some of the homologues. The same pairs, however, were not present in each nucleus. Nevertheless, by noting in each nucleus which chromosomes were paired and by comparing the members of each pair with regard to their internal structure, it was possible to determine whether or not any structural rearrangement was present in any member of a given pair. For two of the four examined isolates of 45502, this method produced no evidence of gross structural modifications in any of the seven regular chromosomes of the complement. In the other two isolates, the composition of chromosome 7 was not determined with certainty, but the other six chromosomes appeared to be normal.

As was stated earlier, the origin of the small extra chromosome present in each of the four isolates of 45502 was not determined. The high frequency of association of this fragment with a member of the chromosome 7 pair suggests a possible derivation from chromosome 7. Some support for this inference is given by the similar high frequencies of such associations that appeared in many of the nuclei produced by crossing 45502-P1315-1a with 70007TR-2A. Each parent presumably contributed the seven haploid chromosomes plus the fragment. Thus it was to be expected that nuclei with eight homologously associated pairs of chromosomes would be numerous. Instead, they were relatively rare. More often, one or both of the fragment chromosomes were synaptically associated with another chromosome of the complement. Sometimes there appeared very complex configurations, involving members of several different chromosomes of the complement. Chromosome 7 was a component of most of these aberrant configurations.

The aberrant synaptic associations appearing at the first meiotic prophase in all crosses involving these four isolates of 45502 were reflected at the late diakinesis and metaphase stages. Associations between nonhomologous chromosomes were noted. Also, univalents were present in a number of nuclei. No concerted effort was made, however, to analyze these stages, or still later ones in the meiotic process. Therefore it is not known whether or not many abnormal disjunctions of chromosomes occurred at anaphase I as a consequence of abnormal pairing or lack of pairing. Neither is it known to what extent such disjunctions could contribute to abnormal spore formation.

The difficulties encountered in the analysis of the chromosome constitution of 45502 were intensified in the first period of observation by the presence of another chromosomal abnormality, which was later found to have been introduced by the wild-type parent. The initial observations were made in asci produced by the cross of 45502-P1315-1a to wild-type strain 2292-2A. In addition to the fragment chromosome, a structural abnormality was noted in one of the two chromosomes 5. This chromosome was considerably longer than its normal homologue, its length being com-

parable to that of chromosome 3. A segment of uncertain origin, added to or inserted into one arm of the chromosome, was responsible for the increased length. Synaptic associations were very frequent between this abnormal segment and the fragment chromosome. In addition, other complex types of synaptic configurations were formed, incorporating not only the fragment chromosome and the abnormal chromosome 5, but also one or more other chromosomes of the complement. Frequently, homologues of chromosome 7 or of both chromosome 7 and chromosome 2 were components of these configurations. Only after an analysis had been made of the chromosomes in asci derived from other crosses was it realized that the abnormal chromosome 5 had been introduced by the wild-type parent 2292-2A.

Confusion in interpreting the chromosome constitution of 45502—arising from this initial lack of knowledge of the constitution of the wild-type parent, 2292-2A, used in some of the crosses—seriously hampered progress toward a solution of the main problem, which was to determine the chromosome abnormality responsible for the distinctive spore pattern and for the accompanying false linkages of certain genetic markers. Nevertheless, the knowledge gained from this experience is of some general significance. It indicates the necessity for determining the chromosome constitution of wild-type and tester stocks before they are used in crosses requiring cytogenetic analysis. It also suggests that some of the discordant results derived from genetic analyses may be due to undetected structural modifications of chromosomes in strains presumed to be normal in their chromosome constitutions.

Although the study of chromosome constitutions and behavior in strains derived from 45502 had to be terminated before the cytogenetic relations had been clarified, the increased knowledge of chromosome organization and behavior in *Neurospora* which it provided will serve as a guide in future studies of this type.

BIBLIOGRAPHY

BERNSTEIN, M. H. Deoxyribonucleoproteins of cell nuclei: Sensitivity to X-rays. Nature, vol. 174, p. 463 (1954).

BERRIE, A. M. M. The effects of temperature on ultraviolet-induced mutability in *Escherichia coli*. Proc. Nat. Acad. Sci., vol. 39, pp. 1125–1133 (1953).

DEMEREC, M. (editor). Advances in Genetics, vol. 6. 488 pp. New York, Academic Press (1954).

——— What makes genes mutate? Proc. Amer. Philos. Soc., vol. 98, pp. 318–322 (1954).

HERSHEY, A. D. Functional differentiation within particles of bacteriophage T2. Cold Spring Harbor Symp. Quant. Biol., vol. 18, pp. 135–139 (1953).

——— Nucleic acid economy in bacteria infected with bacteriophage T2. II. Phage precursor nucleic acid. Jour. Gen. Physiol., vol. 37, pp. 1–23 (1953).

——— Some central problems of viral growth. *In* The dynamics of virus and rickettsial infections, pp. 13–15. New York, Blakiston Co. (1954).

KAUFMANN, B. N. See PAIGEN, K.

KAUFMANN, B. P. Chromosome aberrations induced in animal cells by ionizing radiations. *In* Radiation biology, ed. A. Hollaender, pp. 627–711. New York, McGraw-Hill Book Co. (1954).

——— See MCDONALD, M. R.

KUNITZ, M., and M. R. MCDONALD. Ribonuclease. *In* Biochemical preparations, vol. 3, ed. E. E. Snell, pp. 9–19. New York, John Wiley & Sons (1953).

LABRUM, E. L. The effect of generation time on the delayed appearance of induced mutants in *Escherichia coli*. Proc. Nat. Acad. Sci., vol. 39, pp. 1221–1227 (1953).

LEVINTHAL, C., and N. VISCONTI. Growth and recombination in bacterial viruses. Genetics, vol. 38, pp. 500–511 (1953).

MCCLINTOCK, B. Induction of instability at selected loci in maize. Genetics, vol. 38, pp. 579–599 (1953).

MCDONALD, M. R. The effects of X rays on dilute solutions of crystalline trypsin: Continued inactivation after termination of irradiation. Brit. Jour. Radiol., vol. 27, pp. 62–63 (1954).

——— The inactivation of dilute solutions of crystalline trypsin by X-radiation. I. Kinetics and characteristics. Jour. Gen. Physiol., vol. 38, pp. 93–103 (1954).

——— and B. P. KAUFMANN. The degradation by ribonuclease of substrates other than ribonucleic acid. Jour. Histochem. and Cytochem., vol. 2, pp. 387–394 (1954).

——— See KUNITZ, M.

PAIGEN, K. The occurrence of several biochemically distinct types of mitochondria in rat liver. Jour. Biol. Chem., vol. 206, pp. 945–957 (1954).

——— and B. N. KAUFMANN. Effects of X-irradiation on amount and composition of nucleic acids in liver. Jour. Cell. and Comp. Physiol., vol. 42, pp. 163–178 (1953).

VISCONTI, N. See LEVINTHAL, C.

VON BORSTEL, R. C. See WHITING, A. R.

WHITING, A. R., and R. C. VON BORSTEL. Dominant lethal and inactivation effects of nitrogen mustard on *Habrobracon* sperm. Genetics, vol. 39, pp. 317–325 (1954).