MAIZE GENETICS

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STUDIES WITH BROKEN CHROMOSOMES

For several years, the behavior of chromosomes having a single broken end has been investigated. In all cases, the original break was induced at meiotic anaphases following crossing over between the homologues of chromosome 9, one of which is structurally modified. The primary purpose of this study was to determine the behavior of the broken end in subsequent nuclear generations, and this was fulfilled in establishing the chromatid type of breakage-fusion-bridge cycle in the gametophyte and endosperm tissues and the chromosomal type of breakage-fusion-bridge cycle in the sporophytic tissues. From these studies, material was obtained for other investigations, some of which were mentioned in last year's report, and which have been expanded during the year. Several incidental observations arising from them will be mentioned.

To continue these studies, a method of producing large numbers of functional gametes each containing a chromosome with a single, unsaturated broken end was desired. This was accomplished by partial elimination of the undesirable male gametes through differential pollen-tube growth which favored grains having a chromosome with a broken end. Plants that are heterozygous for a normal chromosome 9 and a chromosome 9 with a duplication of the short arm give rise to gametes having either a normal chromosome 9, a duplication chromosome 9, or a broken chromosome 9 (following a crossover involving the duplicated segment). Although cytological observations indicate that 18 per cent of the spores carry a broken chromosome, only 3.6 per cent are recovered from the pollen of these plants. Presumably because of chromosomal unbalance, pollen with the duplication does not compete well with pollen carrying a normal chromosome 9. Consequently, the pollen grain that mainly functions carries the normal chromosome 9. If, however, in such plants, the normal chromosome 9 is replaced by a chromosome 9 with a non-male-transmissible deficiency, only the pollen grains with a duplication or a recently broken chromosome can function. Competitive pollen-tube growth now favors those grains which carry a broken chromosome 9, because the chromosome 9 constitution in many of these grains is less unbalanced than in those carrying the duplication chromosome. If a large amount of pollen from such plants is placed on silks, pollen grains carrying broken chromosomes will function successfully in 75 to 90 per cent of the cases. This is a tremendous increase over the previously available 3.6 per cent. These figures are based on tests of nearly 30,000 pollen grains.

The chromatid type of breakage-fusion-bridge cycle occurring in the gametophyte divisions following a meiotic breakage of chromosome 9 can result in gametes carrying various degrees of deficiency and duplication of the short arm of chromosome 9. Kernels whose embryos have multiple duplications of the short arm can be identified on a genetic basis. Therefore, many individual kernels were isolated and the plants arising from them examined. Comparisons of plants that are monosomic, disomic, trisomic, tetrasomic, and pentasomic for the full short arm of chromosome 9 show no striking changes in growth or morphology that could be attributed to chromosomal unbalance.

An unusual type of chromosomal translocation involving chromosome 9, men-

tioned briefly in last year's report, has continued to appear. All such translocations were found in plants which had received a recently broken chromosome 9 from one parent. In all cases, only one translocated chromosome was present. It was composed of the long arm of chromosome 9 and a single arm of another chromosome of the complement, united at their centromere regions. The short arm of chromosome 9 and the complementary arm of the second chromosome were missing. The frequency and complete similarity in all cases of this unusual type of translocation suggest a particular type of action on the part of the chromosome 9 that is undergoing the breakage-fusion-bridge cycle. This cycle frequently produces a telocentric chromosome composed of the long arm of chromosome 9. The hypothesis is suggested that the newly produced terminal centromere fuses with a centromere of any one of the other chromosomes of the complement. This configuration eventually results in the elimination of one chromosome arm of the tripartite complex.

In last year's report, the identification of kernels that had received a chromosome with a single broken end from each gamete nucleus was described. Twenty such kernels were sown, and the seedlings arising from half of these showed that fusion had occurred between the two broken ends contributed by each gamete nucleus. In the remaining 10 plants, no evidence of such fusions was seen. To interpret more adequately the subsequent behavior of these two chromosomes, a larger sample was desired. Through the improved method of obtaining functional male gametes whose nuclei contain a chromosome 9 with a single broken end, several hundred kernels of the desired type were readily obtained. Among 138 such kernels selected for testing, 108 produced seedlings. The embryos in the remaining 30 were morphologically aberrant and were unable to grow. Among the 108 viable plants arising from these kernels, 72 gave evidence of fusion between the broken ends of the chromosomes 9 contributed by the two gametes. No evidence of such fusion was obtained from the remaining 36 plants. This does not mean that no fusions had occurred, for the subsequent behavior of the dicentric chromosome arising from such fusions could quickly nullify all evidence of fusion in the later nuclear divisions, which are the ones examined for this evidence. The behavior of the dicentric chromosome follows two main courses. During nuclear division, the two centromeres of each dicentric chromatid may pass to opposite poles in a spindle figure, producing two contiguous chromatin bridges stretched between the poles. When rupture of these two bridges occurs during late anaphase or early telophase, two newly broken ends enter each sister telophase nucleus. Fusion may then occur between these broken ends, re-establishing the dicentric chromosome condition and the chromosomal type of breakage-fusion-bridge cycle.

The second course that the dicentric chromosome may follow results in absence of fusion between the two broken ends in the nuclei that arise following the formation of such anaphase bridges. These two broken ends, which are observed in the nuclei of later generations, are permanently healed; for no subsequent fusions occur. Observational evidence strongly suggests that the healing process may be related to the nuclear cycle; that is, if a recently broken (unsaturated) end enters a telophase nucleus and has no other unsaturated end with which it may fuse, it will "heal" and become saturated or incapable of fusion during the period from telophase to the following prophase or during the reproductive cycle of the chromo-

some. The evidence leading to such an interpretation derives from the frequent observation of sister nuclei that are connected by a single chromatin bridge during late telophase and interphase. This would result if only one of the two contiguous bridges were ruptured during anaphase or early telophase. In that case, a single bridge would connect the two sister nuclei, and only one unsaturated broken end would have entered each nucleus. It is known that in sporophytic tissues such a single broken end will heal. If the single chromatin bridge connecting the two nuclei is not broken until the following prophase, the single unsaturated broken end within each nucleus may have healed. The second broken end, which will enter each nucleus following eventual rupture of the hitherto persistent bridge, may then have no unsaturated end with which it may fuse. It, in turn, will heal. The dicentric chromosome cycle is terminated and each nucleus has two broken ends, which, however, are saturated and incapable of further fusions. The nature of the healing process is not known; if, as this evidence suggests, it is related to the chromosome division cycle, experiments should be focused on this period.

Tests of the Amount of Crossing Over That May Occur within Small Segments of a Chromosome

Previous investigations have placed the locus of the mutant *yg-2* within the terminal chromomere of the short arm of chromosome 9. Rhoades had determined that the mutant *Dt* is located 7 crossover units beyond *yg-2*. This suggests that a relatively high percentage of crossing over must occur within a minute distal segment of the chromosome. To obtain some evidence on the amounts of crossing over that may occur within specific small regions, the following method was used. Plants were made heterozygous for terminal deficiencies of the short arm of chromosome 9. The extent of the deficiencies ranged from loss of the terminal chromomere to loss of four chromomeres. The normal chromosome 9 carried the recessive mutant *c*, and the deficient chromosome the allele *C* (*C*, colored aleurone; *c*, colorless aleurone). *C* is located within the fifth or sixth chromomere from the end of the short arm of chromosome 9. When pollen of such plants is placed on silks of plants homozygous for *c*, only the pollen grains carrying the normal chromosome 9 will function. Therefore, any *C* kernel that appears is the result of a crossover in the segment between the locus of *C* and the end of the deficient chromosome. The proportion of *C* to *c* kernels is thus a direct measure of the amount of crossing over that occurs within this segment. As the deficiency becomes shorter, the proportion of *C* to *c* kernels increases. The difference may be ascribed to the increasing length of the segment in which crossing over may occur. Since the increase of each segment is known, the amount of crossing over ascribable to this increment may be determined. The 11 deficiencies tested have been placed in five groups of descending order of length. Cytological observations of the exact position of a break that gives rise to a terminal deficiency are extremely difficult, because of the minute size of the chromomeres. Any one deficiency, placed in a particular size group, may be plus or minus a small part of a chromomere.

The table on page 151 shows that as the segment in which crossing over is measured becomes progressively longer, marked increases in crossing over occur. Toward the end of the series, an increase of half a chromomere may increase the crossover units by 10. Thus, if *yg-2* is lo-

cated toward the middle of the terminal chromomere, the location of *Dt* seven cross-

Deficiency	Per cent crossing over	No. of kernels examined
Deficient for 4 chromomeres:		
df 1297A-2	0.016	6130
df 1278A-4	0.052	1923
df 1501A	0.52	5748
df 1559B-2	0.72	1383
df 1463-2	0.94	9743
Deficient for 3 chromomeres:		
df 1265	1.25	5830
Deficient for 2 chromomeres:		
df 1533A	3.07	8791
Deficient for 1½ chromomeres:		
df 1507	8.33	4566
Deficient for 1 chromomere:		
df 1509	17.06	3826
df 1512D-2	21.1	1639

over units beyond *yg*-2 is not necessarily cytologically inconsistent. Since the normal amount of crossing over between *C* and *yg*-2 is only 19 per cent, it is highly probable that crossing over toward the tip of this arm is considerably more frequent per unit physical length than in other parts of the arm.

Deficiency Mutations: Progressive Deficiency as a Cause of Allelic Series

During the past year, major emphasis has been placed on expanding the studies of mutations associated with small terminal deficiencies of the short arm of chromosome 9. All such deficiencies originate from chromosomes that are broken during meiosis, as was explained earlier in this report. The short arm of the normal chromosome 9 terminates in a knob. A relatively thin chromatic thread connects this knob with the first distinct chromomere of the short arm. If a break occurs adjacent to the distal part of this first chromomere, a chromosome 9 deficient for the "stalk" of the knob results. Gametes having this deficiency are completely functional. Embryos homozygous for this deficiency are normal; but the seedlings, although normal in growth rate and morphology, are pale yellow and incapable of continued growth because of the defective chlorophyll condition. Because newly produced broken chromosomes 9 can be obtained in large numbers, this deficiency mutant has been produced repeatedly and independently in unrelated strains whenever the short arm of chromosome 9 is subjected to breakage, regardless of the method that produces this breakage. Seven unrelated and independently produced deficiency pale-yellow mutants have been selected for intensive study. When the stalk of the knob and approximately half of the terminal chromomere of the short arm of chromosome 9 is removed during breakage, the male and female gametes containing this deficient chromosome are functional. In the homozygous condition, this deficiency produces not pale-yellow but white seedlings. These seedlings are dwarfed, although their general morphological development appears to be normal. As with the pale-yellow mutants, the white-seedling mutants have occurred repeatedly in the progeny of independently produced broken chromosomes. Six of these mutants have been isolated for intensive study.

The allelic relations of all these mutants are being tested. Of the 21 possible combinations of the 7 pale-yellow mutants, 13 have been tested. Complete allelomorphism has been observed with all 13. Although tests of all individual combinations have not been completed, the types of combination that have been tested indicate complete allelism of all 7 mutants. These tests indicate that all pale-yellow deficiency mutants are similar in their character expression. Combinations to test the allelic relations of the white mutants have been made, but the

seedling tests have been completed on only 3 of the 15 possible combinations. In these 3 cases white seedlings appeared, indicating the allelic nature of the whites. The pale-yellow mutants have been combined with the white mutants. Of the 42 possible combinations, 14 have been tested. All 14 combinations gave rise to pale-yellow seedlings identical in appearance with the homozygous pale-yellow mutant type. The combinations so far tested have established this relationship for 5 of the 6 white mutants. It may be concluded, therefore, that the deficiency pale-yellow mutants are completely dominant to the deficiency white mutants.

Previous investigations had suggested that the well known and frequently used recessive mutant *yg*-2 (yellow-green plants) has its locus within the terminal chromomere of the short arm of chromosome 9. To determine the relation between *yg*-2 and these deficiency mutants, crosses have been made with all 13 deficiency mutants. Combinations of 6 of the 7 pale-yellow mutants with *yg*-2 have been tested. In all 6 cases, only normal green seedlings were produced. The deficiency pale-yellow mutant and *yg*-2 are not allelic. Combinations of the deficiency white mutants with *yg*-2 gave entirely different results. Although only 3 of the 6 combinations have been tested, all 3 combinations gave rise to *yg*-2 plants. This indicates the allelic relations of the deficiency white mutants and *yg*-2. Yellow-green-2 is dominant over deficiency white.

The combined results throw an interesting light on the nature of one form of allelism which would be puzzling to interpret if the cytology were not known. The cytological analysis allows a logical interpretation to be made. The allelic relationships may be represented by two series of descending order of dominance:

1. Normal green → pale-yellow → white
2. Normal green → *yg*-2 → white

Although the two series have a mutant in common, pale-yellow × *yg*-2 gives only normal green plants.

The interpretation of progressive deficiency will explain these results completely. Normal green plants have an unmodified chromosome 9, carrying *Yg*-2. This chromosome will cover any deficiency in a homologue and likewise the recessive mutant *yg*-2. The deficiency which produces pale-yellow is short and does not include the locus of *Yg*-2. Therefore, the chromosome carrying *yg*-2 covers the pale-yellow deficiency, whereas the deficient pale-yellow chromosome carries the dominant allele of *yg*-2. Thus only normal green plants result from this combination. It is cytologically obvious that the chromosomes 9 producing the white mutants have a longer deficiency than those producing the pale-yellow mutants. If it is assumed that the deficiency producing the white mutants includes the locus of *Yg*-2, the removal of this locus would allow *yg*-2 to be expressed when the *yg*-2 chromosome is combined with the deficient chromosomes 9 producing the white mutants. Only *yg*-2 will appear, for the chromosome carrying *yg*-2 will cover the deficiency present in the chromosome producing the white mutant. Progressive deficiency, therefore, will completely explain the allelic relations which these mutants show with each other and with *yg*-2.

Because newly broken chromosomes 9 give rise to these same mutants over and over again, studies are now in progress to determine the "mutation" rates.