[7] Istrati, Gh., *Compt. Rend. Soc. Biol.*, **129**, 1010–1013 (1938).
[8] Wagner-Jauregg, Th., *Centralbl. f. Bakt.*, **144**, I. Orig., 31–32 (1939).
[9] Okell, C. C., and Blake, A. V., *Jour. Path. & Bact.*, **33**, 57–63 (1930).
[10] Shwartzman, G., "Phenomenon of Local Tissue Reactivity," N. Y., 461 pp. (1937).
[11] Ajo, C., *Proc. Soc. Exper. Biol. & Med.*, **47**, 500–501 (1941).
[12] Sterges, A. J., *Jour. Bact.*, **43**, 317–327 (1942); *Ibid.*, **44**, 138 (1942).
[13] Mueller, J. H., and Miller, P. A., *Ibid.*, **41**, 301–303 (1941).

THE FUSION OF BROKEN ENDS OF CHROMOSOMES FOLLOWING NUCLEAR FUSION

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Communicated September 22, 1942

When, through radiation or other causes, chromosomes are broken within a single nucleus, 2-by-2 fusions may occur between the broken ends. These fusions may lead to rearrangements of parts of the chromatin complement, giving rise to various chromosomal aberrations which are detected as reciprocal translocations, inversions, deficiencies, etc. Since, in the well-investigated cases, the breakages occurred within a single nucleus, the conditions that lead to fusions of broken ends could not easily be ascertained. The following questions have been asked: (1) Must two or more chromosomes be in intimate contact at the time of breakage in order that fusions may occur? (2) If no intimate contact is necessary at the time of breakage, are the broken ends "unsaturated," that is, capable of fusion with any other unsaturated broken end? (3) If question (2) can be answered in the affirmative, what forces are involved which lead to the contact and subsequent fusions of the two unsaturated broken ends? Likewise, (4) how long will these broken ends remain unsaturated, i.e., capable of fusion?

Questions (1) and (2) could be answered if the following conditions were present: Assume that fusion occurs between two nuclei each of which possesses one chromosome, one end of which has been broken. Each nucleus will then have a single broken end. When these nuclei fuse and their chromosomes intermix within a single nucleus, the chromosome with a broken end contributed by one nucleus could fuse with the chromosome with a broken end contributed by the second nucleus. The chromosome fusion should occur between these two broken ends. This experiment may easily be conducted in maize. The two nuclei that fuse can be the male and the female gametes, respectively. The method of obtaining

gametes having a chromosome with a single "unsaturated" broken end has been reported previously.[1] This method may be briefly summarized. Plants were obtained which possessed one normal chromosome 9 and one chromosome 9 with a duplication of the short arm. This duplicated arm extended beyond the normal short arm, the serial order of parts within the duplicated segment being the reverse of that of the normal short arm. When the duplicated segment is involved in crossing-over, a dicentric chromatid may be produced which is the equivalent of two chromosomes 9 attached at the ends of their short arms. Breakage of this dicentric chromatid during a meiotic anaphase results in the entry into a spore nucleus of a chromatid with a single broken end. Fusion then occurs at the position of breakage between the two sister halves of this broken chromatid, forming a new dicentric chromatid. This, in turn, produces a bridge configuration in the first gametophytic division, as the two centromeres of the dicentric chromatid pass to opposite poles in the anaphase figure. Again, a broken chromatid enters each telophase nucleus. In each nucleus, fusion again occurs between the two sister halves of this broken chromatid at the position of this latter breakage. This *chromatid* type of breakage-fusion-bridge cycle continues in successive gametophytic mitoses. Therefore, all the nuclei of the fully developed male or female gametophyte will possess one chromosome with a single broken end. Following fertilization, two nuclei from the female gametophyte and one from the male gametophyte fuse to form the primary endosperm nucleus. If the nuclei of one of the gametophytes possesses such a chromosome carrying dominant genes in the arm with the broken end, and if the other gametophyte possesses a normal, non-broken chromosome carrying the recessive alleles, variegation for these genes will be apparent in the fully developed endosperm. This is because the chromosomes with the broken ends continue the breakage-fusion-bridge cycle in successive nuclear divisions during the development of the endosperm. Following various non-median breakages of the bridge configurations, the dominant genes may be deleted from some nuclei and duplicated in the sister nuclei. Continued repetition of this type of breakage during the development of the endosperm produces a conspicuous variegation pattern. The behavior of the chromosome with the broken end in the sporophytic tissues of these kernels is entirely different. The chromatid type of breakage-fusion-bridge cycle ceases when the zygote is formed. The newly broken end "heals." Following this healing, no further fusions occur. The broken end is as stable in its subsequent behavior as any normal chromosome end.

There is no reason to suspect that the condition of those chromosomes in the gamete nuclei that participate in endosperm fusions differ from those participating in zygotic fusions. However, in one case, the broken end remains unsaturated, and in the other case the broken end heals. It is

reasonable to believe, therefore, that the healing process occurs subsequent to zygotic fusions and not before. One may tentatively assume that a chromosome end, broken in the pre-gametic division, is unsaturated at the time of zygotic fusion. If each gamete contributes a chromosome with an unsaturated broken end, one could expect fusion to occur between these two broken ends. The reasoning behind this expectation is based on the behavior of ring-shaped chromosomes in sporophytic tissues.[2] Ring-shaped chromosomes are frequently broken during mitotic anaphases. Following such breakage, the telophase nuclei receive a chromosome both ends of which are broken. Fusion may then occur between these unsaturated broken ends, reëstablishing the ring-shape. This is a chromosome fusion, not a chromatid fusion. This behavior has suggested that fusion of unsaturated broken ends in sporophytic tissues may be chromosomal in contrast to gametophytic and endosperm tissues where chromatid fusions may occur. The experiment to be described furnishes proof of the correctness of these assumptions.

To test whether the broken ends are unsaturated in the gamete nuclei, two such ends were introduced into the zygote nucleus, one contributed by the male gamete and one by the female gamete. Detection of kernels whose zygote nuclei had received such chromosomes was accomplished by introducing contrasting endosperm markers carried by the chromosomes with the broken ends (*i*, aleurone color, and *wx*, waxy starch, located in the short arm of one parental chromosome 9 and the alleles *I*, inhibitor of aleurone color, and *Wx*, normal starch, carried by the other parental chromosome 9). The endosperms of those kernels that receive a broken chromosome 9 from each parent should show a very distinctive type of variegation. All three broken chromosomes 9 would undergo the break-fusion-bridge cycle. This would lead to variegation for *I-i* and *Wx-wx*, variegation for depth of color in the *i* regions due to multiplication of the number of *i* genes, and scarred and pitted regions in both the *I* and *i* sectors due to the presence of cells which are homozygous deficient for segments of the short arm of chromosome 9.[3]

Plants heterozygous for the duplication chromosome 9 and homozygous for *i wx* were crossed by plants heterozygous for the duplication and homozygous for *I* and *Wx*. Three types of kernels, with respect to endosperm characters, should be produced; (1) *I Wx*, non-variegated kernels following fusion of a nucleus carrying a non-broken *I Wx* chromosome with nuclei carrying either a non-broken or a broken *i wx* chromosome; (2) kernels variegated for *I-i* and *Wx-wx* following fusion of a nucleus carrying a broken *I Wx* chromosome with nuclei carrying a non-broken *i wx* chromosome; (3) kernels resulting from the fusion of a nucleus carrying a broken *I Wx* chromosome with nuclei carrying a broken *i wx* chromosome. As stated above, the endosperm character of this latter type of kernel could be

anticipated. When observations were made of the kernels resulting from this cross, these latter kernels were very conspicuous. From a total of 18,243 kernels examined, 20 possessing an embryo were of this latter type. More of this type were present but they were germless. These 20 kernels were germinated to determine what had happened to the two broken chromosomes 9 which had entered the zygote. If both broken ends had healed without fusion, normal-appearing plants would be expected to arise from these kernels. If the two broken ends had fused, a dicentric chromosome would have been produced. It would be composed of the chromosome 9 contributed by the male gamete and the chromosome 9 contributed by the female gamete, with their short arms fused end-to-end. When this dicentric chromosome divided and when the two centromeres of each chromatid passed to opposite poles at a mitotic anaphase, two contiguous bridges should be formed. Following breakage of these two bridges, two chromosomes, each with a freshly broken end, should enter each sister telophase nucleus. As stated previously, one could expect fusions to occur between the broken ends of these two chromosomes in each sister telophase nucleus. This would reëstablish the dicentric condition, for again the two chromosomes 9 would be joined to form one chromosome with two centromeres. Repeated anaphase bridge configurations should be expected in subsequent divisions following this *chromosomal* type of breakage-fusion-bridge cycle. The cells of a plant possessing such a dicentric chromosome undergoing this behavior should be composed of various types of homozygous and heterozygous duplications and deficiencies of the short arm of chromosome 9, following repeated non-median breaks in the anaphase bridges. Consequently, these plants should be conspicuously modified in appearance, because of the variation in degree of duplication or deficiency in the many nuclei of the plant.

In the seedling stage, 10 of the 20 plants arising from the kernels classified as having received a broken chromosome 9 from each parent were obviously of the type expected if a dicentric chromosome were present. The presence of the dicentric chromosome was confirmed by examination of the division figures in the young roots, where nearly half of the observed anaphase figures showed two contiguous bridges derived from a dicentric chromosome. Nine of the remaining plants were normal in appearance, and one plant was normal in morphological growth but pale yellow[4] in color and died in the seedling stage. No bridges were observed in the roots of these latter 10 plants.

Due to aberrant growth and death of many cells, 5 of the plants with a dicentric chromosome died in the seedling stage. The remaining 5 plants continued to grow. In all 5 plants, as growth continued, sectors of tissue developed which showed no aberrant growth patterns. These sectors were quite normal in appearance. Gradually, these recovered sectors gained the

ascendency in growth until most of the plant was normal in appearance. In one plant, 3 normal side shoots developed from the base of a very aberrant main shoot which was obviously dying. Root tips were taken at various times from all of the 5 plants that had survived the seedling stage. In all cases, vigorous growth of some side branches of the root system was noted. Examination of division figures in these roots no longer showed any dicentric bridges. The examined cells possessed the normal chromosome number of 20, instead of the 18 monocentric chromosome plus 1 dicentric chromosome previously observed in the younger roots. Sporocytes for examination of the chromosomes at pachytene were obtained from two of the three recovered shoots of one plant and from the recovered main shoots of the four other plants. In all cases, 10 bivalent chromosomes were present, one of which was a bivalent chromosome 9. The two chromosomes 9 were not fused at the ends of their short arms. In most cases, the composition of the short arm of each member of the bivalent was greatly modified, although in each tassel sample the two chromosomes 9 maintained their respective morphologies in all examined cells. Of the 12 chromosomes 9 examined from these six samples, no two were alike. The composition of the short arms of the chromosomes 9 in the two recovered branches from one plant originally possessing a dicentric chromosome was entirely different. In each case, it was apparent that the cells of the examined part of the tassel had originated from one individual cell whose cell ancestors had previously been undergoing the chromosomal type of breakage-fusion-bridge cycle involving the original dicentric chromosome 9. This could be determined in each case by the comparative morphologies of the short arms of the two members of the bivalent. In several cases it was possible to determine the minimum number of fusions, bridges and breaks that must have occurred before "healing" of the two broken ends had taken place in the nucleus of the particular cell that gave rise to the recovered sector. The factors involved in the process of healing of two such broken ends within a single nucleus are still undetermined.

The pachytene chromosomes were examined in the surviving 9 of the 10 plants which were normal in morphological growth from the earliest seedling stage and which showed no bridges in the earliest roots. This examination showed that 4 of these plants had received a broken chromosome 9 from each parent. However, morphological analysis of the short arms of the two chromosomes 9 in each case gave no indication that fusions had ever occurred between their broken ends. In one plant, one parent had contributed a broken chromosome 9, but it was not possible to determine whether the other parent had contributed a broken chromosome 9. In the remaining 4 plants, each parent had contributed a broken chromosome 9. However, the broken end of one chromosome 9 had fused with a broken end of a chromosome other than that of the chromosome 9 introduced by the

second gamete. In each case, the broken end of the second chromosome 9 had no unsaturated end with which it could unite. As expected, this single broken end thereafter healed.

Conclusions.—The experiments outlined allow some specific answers to be given to the questions presented in the first paragraph of this paper. Question (1) may be answered in the negative. Two chromosomes do not have to be in contact at the time of breakage in order that fusions may occur between their broken ends. This was shown by the fusion that occurred in the zygote or in an early embryonic nucleus between a broken end of chromosome 9 contributed by the male gamete and a broken end of the chromosome 9 contributed by the female gamete. Question (2) may be answered in the affirmative. This was shown by the fusion of these two chromosomes, which produced a dicentric chromosome, and the subsequent behavior of this dicentric chromosome which, for some time, followed the chromosomal type of breakage-fusion-bridge cycle. Question (3) cannot be answered directly from the present observations. Nevertheless, the observations imply that some force exists which accounts for the fusion of unsaturated broken ends of chromosomes. Question (4) likewise cannot be answered directly. Nevertheless, it is certain that the unsaturated state does not persist indefinitely. An unsaturated broken end will become saturated or healed and incapable of fusions when only one such broken end is present in sporophytic tissues;[1] or, as shown in this report, two such broken ends may heal without fusions even when these two ends are present in the same nucleus.

[1] McClintock, B., *Genetics*, **26**, 234–282 (1941).
[2] McClintock, B., *Ibid.*, **23**, 315–376 (1938).
[3] McClintock, B. (unpublished).
[4] This mutant type appears when a plant is homozygous deficient for a small terminal segment of the short arm of chromosome 9.

NON-RANDOM UNCOILING IN HETEROBRACHIAL CHROMOSOMES

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Communicated October 2, 1942

In theories regarding the mechanics of chromosome spiralization much depends upon whether there is a definite (inherent?) pattern of coiling or whether the direction of coiling is purely a matter of chance, determined independently in the two arms of the chromosome. Reversals of direction