THE PRODUCTION OF HOMOZYGOUS DEFICIENT TISSUES WITH MUTANT CHARACTERISTICS BY MEANS OF THE ABERRANT MITOTIC BEHAVIOR OF RING-SHAPED CHROMOSOMES*

BARBARA McCLINTOCK

Cornell University, Ithaca, New York and University of Missouri, Columbia, Missouri[1]

Received February 25, 1938

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I. INTRODUCTION

IT IS the purpose of this paper to describe the method by which viable tissues, homozygous deficient for a known region of a chromosome, may be produced in maize. The chromosomal region involved includes the locus of the gene *Bm 1* in chromosome V (allele of *bm 1*, brown midrib, producing a brown color in the lignified cell walls). The lignified cell walls of the homozygous deficient tissue exhibit the features characteristic of the known recessive gene *bm 1* although the locus of this gene is absent.

The method of obtaining the homozygous deficient tissue is related to the unique behavior of ring-shaped chromosomes during somatic mitosis. This behavior has been briefly mentioned in previous publications (McCLINTOCK 1932; RHOADES and McCLINTOCK 1935). Ring-shaped chromosomes do not always maintain themselves unaltered through successive nuclear cycles in the maize plant. They may (1) increase in size through duplication and reduplication of segments of the original ring, (2) decrease in size by deletions of segments from the ring, (3) be totally lost from the nuclei or (4) be present in increased numbers in the different nuclei. Whatever the method by which a change in size occurs, only ring chromosomes are produced from ring chromosomes.

In maize it has been found that deficiencies in certain regions of the

* The cost of the half-tone illustrations in this paper is met by the Galton and Mendel Memorial Fund.

[1] Contribution from the Department of Botany in coöperation with the Department of Field Crops, Genetics Research Project, Missouri Agricultural Experiment Station, Journal Series No. 570.

chromosomes may be transmitted successfully through the egg but not through the pollen (BURNHAM 1932; STADLER 1935). Pollen possessing a deficient chromosome plus a ring-shaped fragment chromosome should be functional if the ring-shaped fragment completely compensates for the deficiency. By utilizing a deficiency transmissible through the eggs and rendered non-lethal in the pollen by the inclusion of a ring fragment covering the deficiency, a zygote with two deficient chromosomes plus a ring chromosome can be produced. This zygote is heterozygous for the

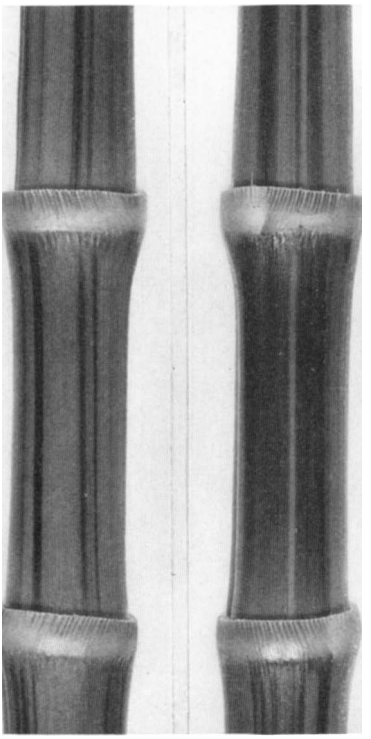

FIGURE 1.—Two sides of a stalk of a variegated plant. The leaves at the two nodes have been removed. The dark bands are *bm1*, the light bands, *Bm1*.

deficiency. This heterozygosity in the resulting individual would be maintained as long as an unaltered ring chromosome was present. Should the ring chromosome be lost in subsequent nuclear divisions, or should it change in size through loss of a segment within it, the tissues arising after such loss or alteration would be homozygous deficient for the entire deficiency in the first case or for regions within the limits of the deficiency in the second case.

Two cases of deficient rod chromosomes with complementary ring chromosomes were available for this study. The two cases arose in the

progeny of X-rayed pollen containing a normal haploid complement with the dominant gene *Bm 1*. This pollen, when placed upon silks of *bm 1* plants with a normal chromosome complement, gave rise, among a progeny of 466, to two individuals which were variegated for *Bm 1* and *bm 1* (figure 1). Aberrant behavior of a ring chromosome produced by the X-ray treatment and carrying the gene *Bm 1* was suspected to be the cause of the variegation.

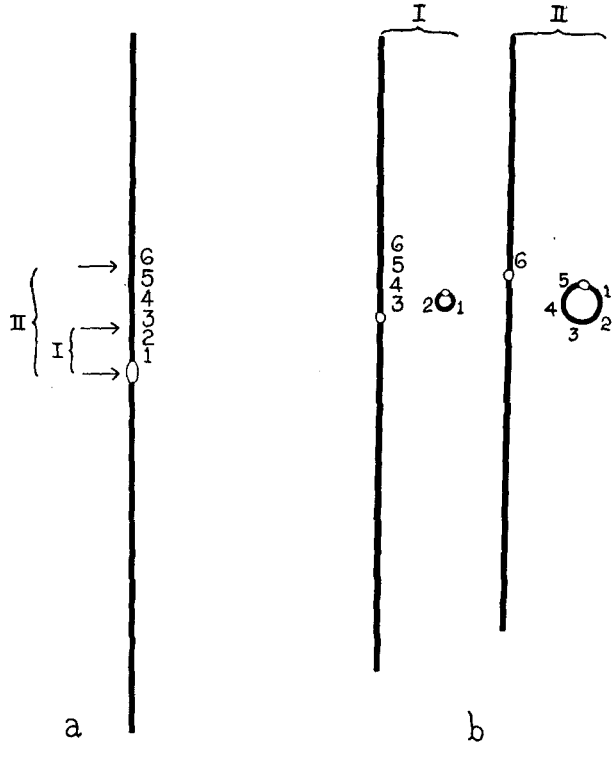

FIGURE 2.—a. Diagram of a normal chromosome V. The slightly bulging section represents the spindle fiber attachment region. The sets of arrows, I and II, point to the positions of breaks which gave rise to the two deficient rod chromosomes and their compensating ring chromosomes illustrated in I and II of b. The deficient rod and compensating ring chromosomes of I are referred to in the text as Def 1 and R 1 respectively, those of II as Def 2 and R 2 respectively

Examination of synaptic configurations in sporocytes revealed not only the presence of a small ring-shaped chromosome in each plant but also a deficiency in one chromosome V. In each case, the size of the ring-shaped chromosome and the extent of the deficiency in the rod-chromosome were comparable. The deficiency in both cases involved a section of the short arm immediately adjacent to the spindle fiber attachment region. Since the ring fragment in both cases possessed a small but definite spindle fiber attachment region, these regions being clearly visible in meiotic prophases,

it was assumed that in each case the deficient rod and its compensating ring chromosome arose as the result of two breaks in the normal chromosome V, one break passing through the spindle fiber attachment region, the other breaking the chromosome at a distance from the spindle fiber attachment region equal to approximately 1/20 (Case I) and 1/7 (Case II) of the total length of chromosome V (figure 2). Fusions two by two of the broken ends resulted in a deficient rod and a compensating ring chromosome each with a section of the original spindle fiber attachment region. Since both the deficient rod and the ring chromosome possessed a section of the spindle fiber attachment region, both could be maintained through nuclear cycles.

Proof that the ring chromosome represented the region for which the rod chromosome was deficient was furnished by the synaptic configurations produced by homologous associations of the three chromosomes: the normal chromosome V contributed by the female parent, the deficient rod chromosome V and the small ring chromosome contributed by the male parent (figures 25 and 26 and photographs of the same, 17 and 18, Plate II). Cytological examination of different portions of the tassel disclosed the loss of the ring chromosome in several branches. Similarly, within a single anther, groups of cells were found lacking the ring chromosome. It was suspected, therefore, that the ring chromosome carried the locus of *Bm 1*, its loss during somatic mitoses being responsible for the presence of the *bm 1* (brown) streaks in these plants. Conclusive proof for this was derived from the progeny of these two plants when crossed to normal *bm 1* plants. The progeny included variegated (*Bm 1* and *bm 1*) and *bm 1* plants. Of the variegated plants, microsporocytes of 148 individuals were examined for the presence of the ring chromosome. The ring fragment was found in 146 of these individuals although in many plants several branches of the tassel lacked the ring fragment. In two plants no ring chromosome was found in the several branches of the tassel which were collected. Of the totally *bm 1* plants, 47 were examined. In no case was a ring chromosome found. In a *bm 1* tiller of a variegated plant, a considerably reduced ring chromosome was found. It is probable in this case that the *Bm 1* locus had been deleted from the ring chromosome through somatic alterations to be described in the next section. Individual collections were made on the two sides of plants which were approximately half *bm 1* and half variegated. In these cases, the presence of the ring chromosome could be established only on the variegated side.

II. THE MITOTIC BEHAVIOR OF RING-SHAPED CHROMOSOMES

The interpretation of the variegation and of the production of homozygous deficient tissues has been based on a knowledge of the behavior of

ring-shaped chromosomes in somatic nuclear cycles. A description of what has been observed regarding the appearance and behavior of the ring chromosomes in meristematic regions is therefore necessary before the individual cases can be considered. Although the primary cause of irregularities in the nuclear cycles is undoubtedly the same for large and small ring-shaped chromosomes, the subsequent behavior and the genetical consequences vary in these two extremes. The behavior of large ring-shaped chromosomes will be considered first; this will be followed by an account of the small ring-shaped chromosomes; finally, correlations and conclusions will be drawn regarding ring-shaped chromosomes in general.

Mitotic behavior of large ring-shaped chromosomes

Since the two ring-shaped chromosomes of cases I and II, figure 2, are both small, a large ring-shaped chromosome originally representing most of chromosome II has been examined (McCLINTOCK 1932). The observations were made on longitudinal sections of actively growing root tips. Observations at meiotic prophase in this plant had clearly indicated that changes in size and hence chromatin content of the ring chromosome were occurring in the premeiotic nuclei. Groups of related cells usually had similar ring chromosomes but the differences in unrelated cells were very great. In a few cells the altered ring chromosome was larger than the normal chromosome II. In some cells it had been reduced to only a few chromomeres. All gradations between these two extremes were found in different sporocytes of this same plant. The smallest ring chromosome has obviously undergone a great loss of chromatin. The original ring chromosome possessed a single knob. Evidence for duplication of segments other than the obvious increase in size of the ring chromosome was clearly registered in some cells by the increase in the number of knobs. Rings with two, three and four knobs were found.

It was suspected that the alteration in chromatin content of the ring was related to the division cycle of the chromosome. Observations of mitoses in root tip meristems suggested the manner in which the alterations occur without, however, revealing the primary cause. If one assumes that during the splitting process or after the split has occurred, a crossover took place between the two sister chromatids, a double-sized, continuous ring with two spindle fiber attachment regions would be produced. A second crossover between the two sister chromatids could result in an interlocking of the sister ring chromosomes provided the second crossover did not counteract the first. The presence of double-sized rings with two spindle fiber attachment regions at late anaphase and early telophase was clearly evident in a number of cells (figures 5, 7, 15, 16; photographs 4, 5, 8, Plate I). Unfortunately the presence of interlocking rings could not

be determined directly since the chromosomes of maize in somatic cells are relatively small. Many anaphase figures were suggestive but none could be definitely distinguished from double-sized rings with a twist at the mid-region. From the point of view of the origin of such configurations it would be important to know the relative percentage of each type. From actual counts it is certain that the double-sized rings are present in at least one-third of the aberrant figures. The actual number of late anaphase and early telophase figures with chromatin bridges produced by double-sized or interlocked rings amounted to approximately 8 percent of a total of 1145 figures recorded in roots whose ring chromosome had not materially reduced in size in most of the cells (D, table 1).

TABLE 1

The frequency of normal and aberrant somatic anaphase and early telophase configurations in plants with different ring chromosomes.

	RING CHROMOSOME	NORMAL	ABERRANT	% ABERRANT
A	R1	605	1	0.16
B	R2	1195	14	1.1
C	R2 plus enlarged R2	1169	76	6.1
D	Large ring chromosome II	1053	92	8.1

Since the fate of the double-sized or interlocked rings is not the same in all late anaphase and telophase figures, a number of types of behavior from anaphase to late telophase have been diagrammed in figure 3. Representative drawings from different cells are given in figures 5 to 24 and photographs of Plate I. In the diagrams, the behavior of double-sized rings has been emphasized since this type could be clearly recognized in many cells. They are either clearly open or show a twist at the mid-region. Some of the interlocked rings should produce figures resembling those shown in the diagram and would not be easily distinguished from them.

In most of the mitotic cycles the ring chromosome splits along a single plane, separation of the two halves proceeding normally at anaphase, figure 4. In the late anaphase figures the double-sized rings produce a double bridge the chromatin of which is pulled taut (figure 5, photograph 1, Plate I). It is suspected that breakage of the chromatin bridges sometimes occurs during this period (photograph 10, Plate I). Since such figures were not included in the counts mentioned above, the 8 percent of anaphase and telophase figures with bridges represent the minimum number of cells in which double-sized or interlocked rings occurred. Some of the telophase figures suggest an early breakage of one or both strands of the double bridge (figures 8 and 9 and photographs 5 and 6, Plate I).

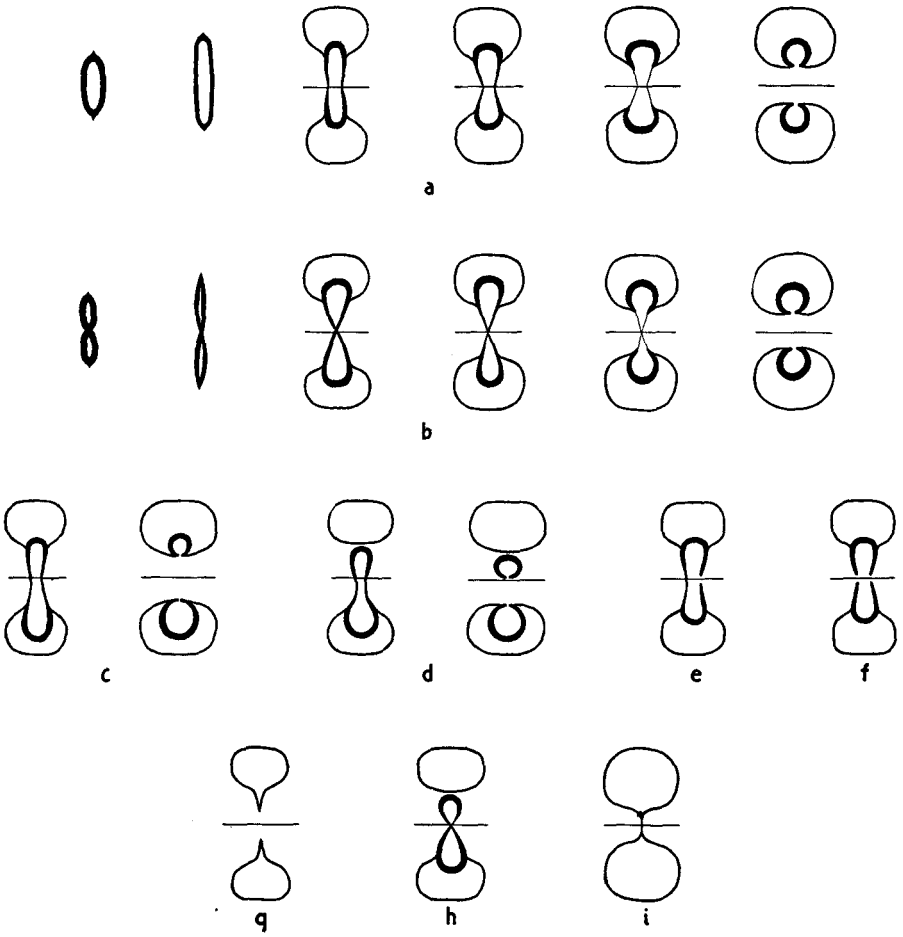

FIGURE 3.—Diagrams illustrating the behavior in somatic mitosis of double-sized ring chromosomes with two spindle fiber attachment regions produced from the two split halves of a single ring chromosome.

a. Successive stages from mid-anaphase to mid-telophase of a medianly placed double-sized ring chromosome. The cell plate determines the positions at which breaks will occur in the two chromatin bridges.

b. Similar to a except that a twist is present in the bridge strands of the double-sized ring chromosome.

c. Appearance in early and mid-telophase of a double-sized ring which was non-medianly placed in the spindle figure. The components entering each daughter nucleus vary in chromosome length and constitution.

d. Similar to c except that the upper portion of the double-sized ring chromosome is not included in the reorganizing telophase nucleus. Such behavior results in the loss of a component of the ring chromosome from one of the daughter nuclei.

e. Appearance at mid-telophase of a double-sized ring chromosome with one broken bridge strand.

f. Appearance at mid-telophase of a double-sized ring chromosome with both strands broken.

g. Appearance at very early telophase suggesting an early breakage of bridge strands of a double-sized ring chromosome (or two interlocked sister ring chromatids).

h. Comparable situation as illustrated in d except that the strands of the bridges are twisted at the cell-plate region.

i. Fine bridge of chromatin between two resting nuclei suggesting that a breaking of the strands had not occurred at telophase.

In many cases, breakage of the strands composing the bridge does not occur at anaphase; compare photographs 9 and 10, Plate I. The moving apart of the spindle fiber attachment regions in the double-sized rings is retarded by the tension of the chromatin bridges. The subsequent behavior is conditioned by the position in the spindle figure of this retarded ring or of two retarded interlocked rings. As the telophase sets in there is an

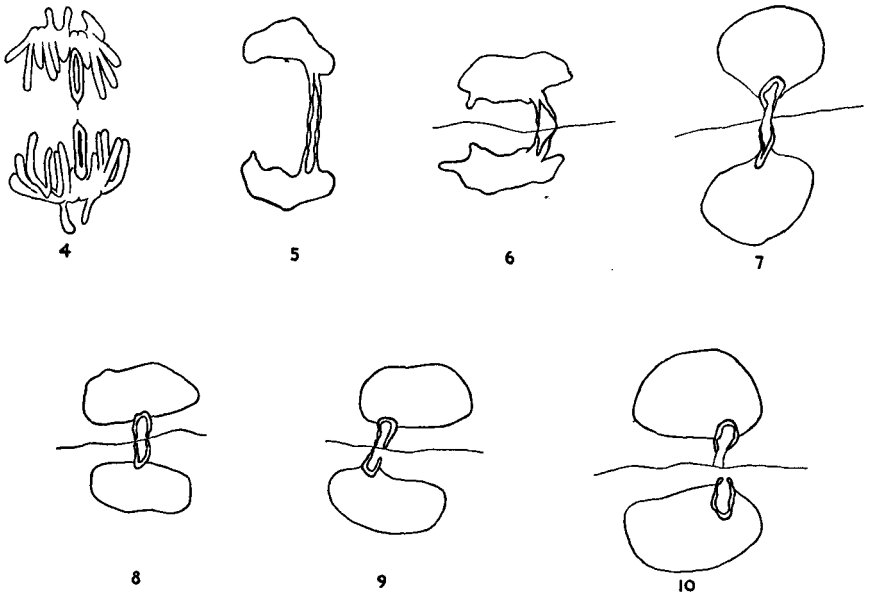

FIGURE 4.—Normal separation of a ring chromosome in somatic anaphase.
FIGURE 5.—A double-sized ring chromosome in early telophase.
FIGURE 6.—A double-sized ring chromosome at a slightly later stage than that shown in figure 5.
FIGURE 7.—Double-sized ring chromosome at mid-telophase. The bridge strands close to the cell-plate have become very thin. The shape of the chromosome within the nuclei has become discernible.
FIGURE 8.—Mid-telophase. Early breakage at the cell-plate region of two bridge strands of the double-sized ring. See comparable figure, photograph 6, Plate I.
FIGURE 9.—Mid-telophase. Early breakage at the cell-plate region of one bridge strand of a double-sized ring. See photograph 5, Plate I.
FIGURE 10.—Late telophase. Breakage of bridge strands of a double-sized ring chromosome at the cell-plate region and withdrawal of the chromatin into the nucleus at the lower part of the figure.

immediate release of tension on the chromatin bridges produced through the swelling of the forming nuclei (photograph 2, Plate I). As the nuclei continue to swell and approach the cell-plate, the chromatin of the ring *within* the nuclei is relaxed, allowing the form of the ring chromosome to be clearly defined (figures 8, 9, 11, 16; photographs 3, 4, 5 and 8, Plate I). At this stage the tension on the chromatin threads from the nuclear mem-

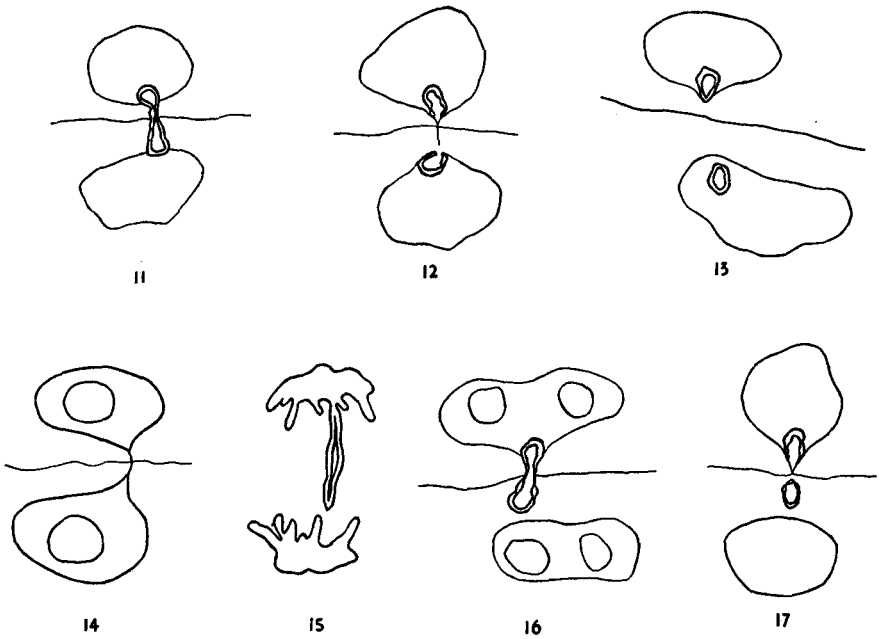

FIGURE 11.—Double-sized ring at mid-telophase. See photograph 3, Plate I.
FIGURE 12.—Similar stage to that shown in figure 10 resulting from a previous double-sized ring with a twist at the mid-region or from two sister ring chromatids which were interlocked.
FIGURE 13.—Sister nuclei at late telophase. The positions of the ring chromosomes suggest a previous bridge formation which has broken.
FIGURE 14.—Resting stage. Sister nuclei with a fine connecting chromatin bridge.
FIGURE 15.—Non-median position of a double-sized ring chromsome at late anaphase. See photograph 7, Plate I. In the photograph there is a twist in the ring chromosome.
FIGURE 16.—Mid-telophase. The result of a non-median placement of a double-sized ring chromosome at anaphase. See photograph of the same, 8, Plate I.
FIGURE 17.—Mid-telophase. The result of a non-median placement of a double-sized ring chromosome with a twist, or of two interlocked sister ring chromatids.

brane to the cell-plate again increases. The threads become thin and taut as if being pulled into the nuclei (figures 7, 16, 17; photograph 8, Plate I). In a few cases, these fine chromatin threads are seen in relatively late telophase nuclei (figure 14). Since they usually do not persist into late stages, breakage must usually occur during the earlier telophase period. There were many sister telophase nuclei observed in which the ring chromosome in each nucleus was close to the region of the nuclear membrane lying nearest the cell-plate (figures 12 and 13). Such figures probably represent the last stage in the progress of the previously double-sized or interlocked rings. It should be emphasized that fusions of broken ends must occur after such breakage, since only ring chromosomes have been found to arise from ring chromosomes although rod chromosomes might be expected.

It sometimes happens that the passage of one spindle fiber attachment

region of a double-sized ring proceeds toward its pole in advance of the opposing spindle fiber region. Consequently, the double-sized ring is not medially placed in the spindle figure. The cell-plate then intercepts the chromatin bridges in a non-median position (figure 15, photograph 7, Plate I). As a result, the components of the double-sized ring entering sister telophase nuclei will be unequal in size and chromatin constitution. One segment of the double-sized ring is sometimes not included in the telophase nucleus on its side of the cell-plate (figures 16 and 17, photograph 8, Plate I).

If the chromosome is not split at anaphase, fusions of broken ends could give rise in the next division to normally disjoining sister ring chromosomes, or if twists are present in the chromonema before fusion, to a continuous double-sized ring or interlocked sister ring chromosomes when

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EXPLANATION OF PLATE I

All magnifications are approximately ×1100.

Plate I.—Individual cells from longitudinal sections of the growing points of roots. Photographs 1 to 10 are of the large chromosome II ring. Photographs 11 to 14 show an enlarged R2 chromosome. Photographs 15 and 16 are of the normal R2 chromosome.

Photograph 1. Late anaphase. Bridge produced by separation of the split halves of a ring-shaped chromosome which is in the form of a double-sized continuous ring. There is a twist of the strands at the mid-region.

Photograph 2. Early telophase. Beginning of relaxation of tension on the strands of the double bridge.

Photograph 3. Mid-telophase. Complete relaxation of tension on strands of bridge.

Photograph 4. Mid-telophase. Double-sized ring chromosome.

Photograph 5. Mid-telophase. A double-sized ring chromosome. The strand to the right appears to be broken.

Photograph 6. Mid-telophase. A double-sized ring chromsome. Both strands appear to be broken at the cell-plate region.

Photograph 7. Late anaphase. Non-median placement in the spindle figure of a double-sized ring chromosome.

Photograph 8. Mid-telophase. The result of a non-median placement in the spindle figure of a double-sized ring chromosome. The strands adjacent to the cell-plate have become attenuated. The lower segment of the ring chromosome was excluded from the forming nucleus.

Photograph 9. Very early telophase. Chromatin bridge produced by a double-sized ring chromosome with twisted strands, or possibly two interlocked sister ring chromatids.

Photograph 10. Very early telophase. Figures such as this suggest an early breakage of the strands of a double-sized ring chromosome or of interlocked sister ring chromatids.

Photograph 11. Typical late anaphase position of a small ring-shaped chromosome which will be excluded from the reforming telophase nuclei.

Photograph 12. Early telophase. Excluded ring chromosome which was previously non-medianly placed in the spindle figure. The cell-plate has passed below it.

Photograph 13. Late anaphase. Stage in the process of exclusion of two closely associated ring chromosomes.

Photograph 14. Similar to photograph 13.

Photograph 15. Typical late anaphase position of a small ring-shaped chromosome which will be excluded from the telophase nuclei.

Photograph 16. Mid-telophase. The result of a previously excluded ring-shaped chromosome. The cell-plate has passed below the ring.

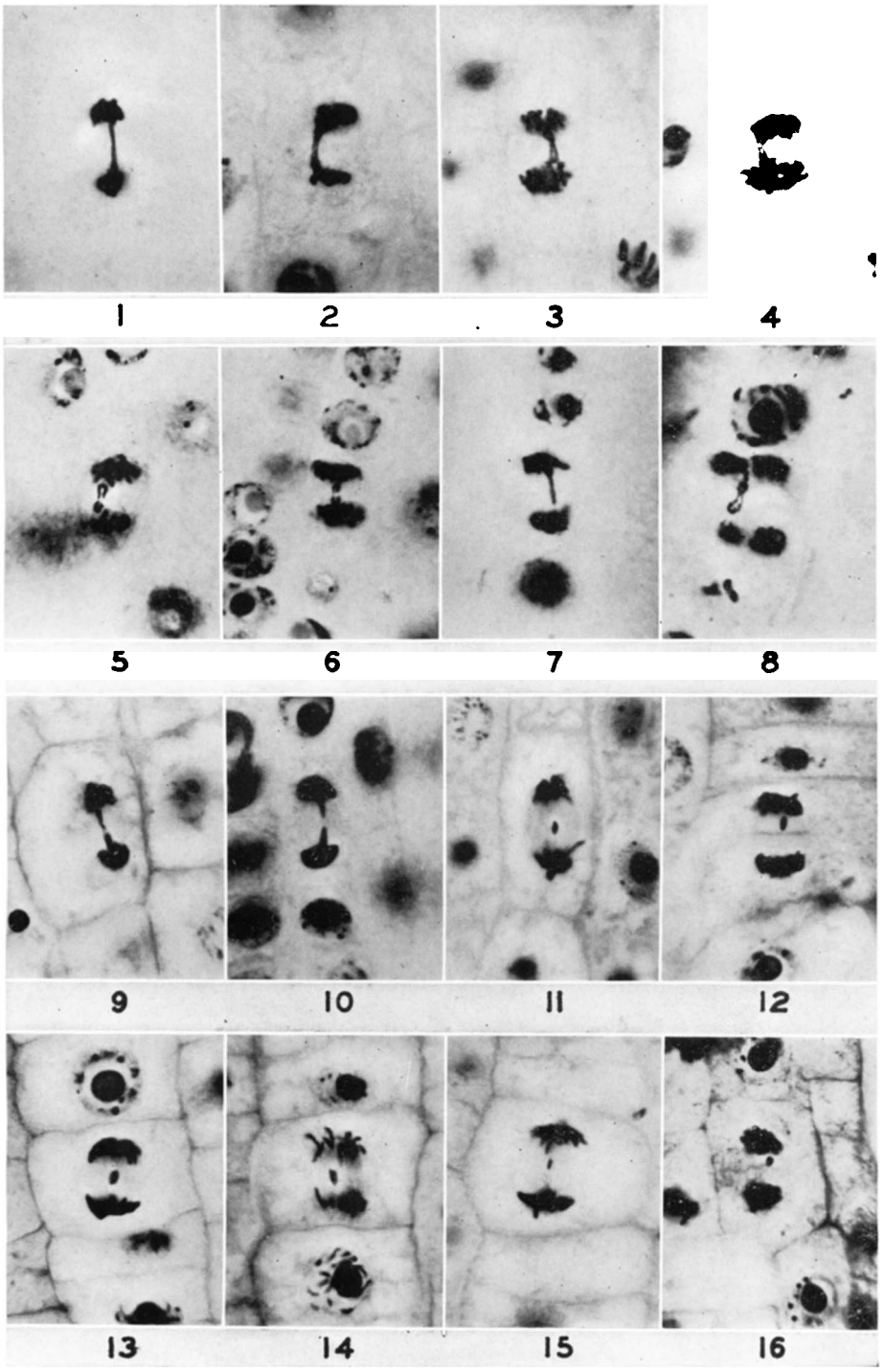

PLATE I

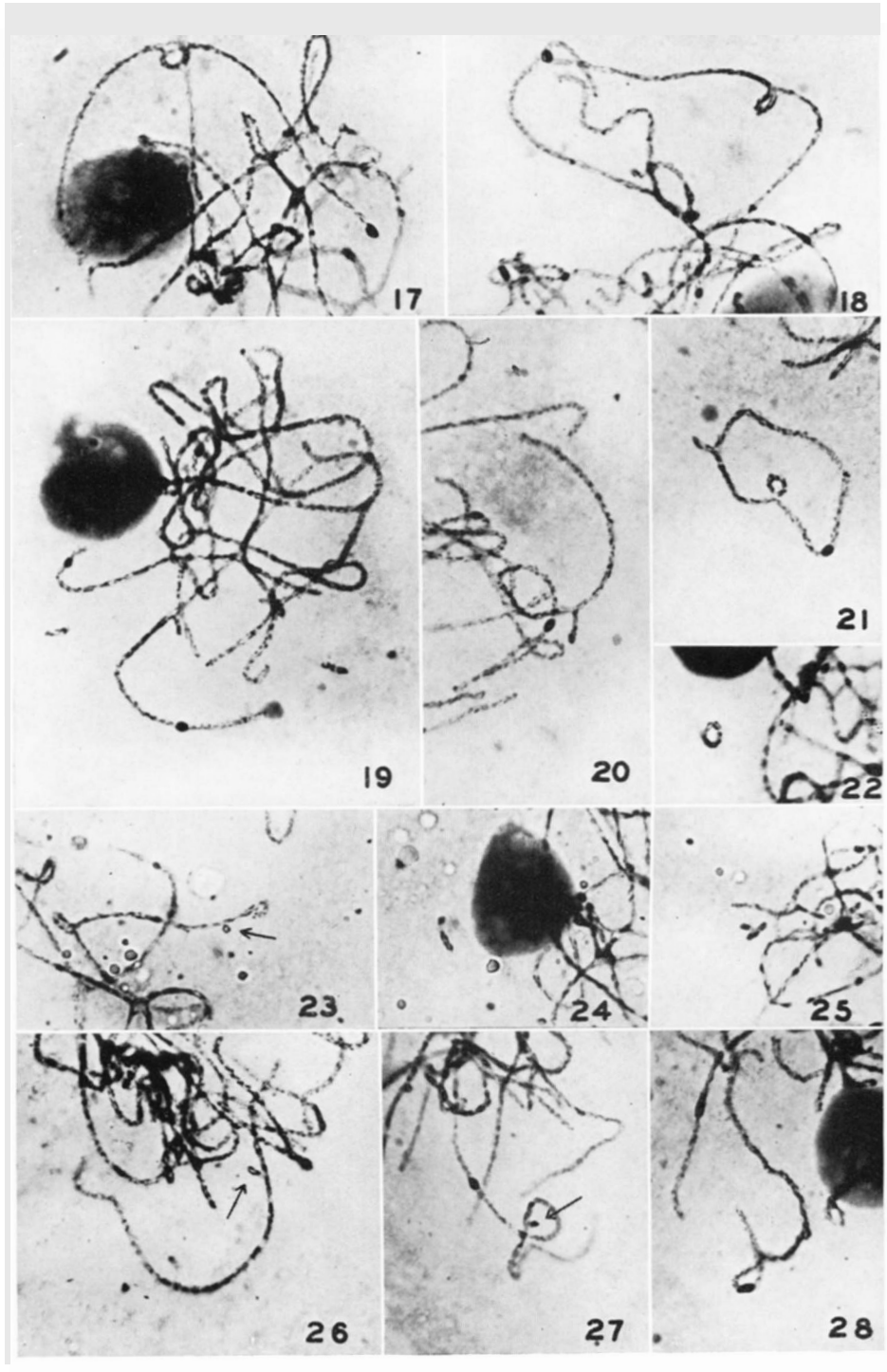

PLATE II

simple assumptions are made regarding the method of splitting or reduplication of a chromonema along a single plane. If the chromosomes are split at anaphase, two by two fusions of the two adjacent broken ends of sister chromatids could result immediately in a continuous double-sized ring. If fusions two by two took place between the non-adjacent broken ends, continuous double-sized rings or interlocked sister ring chromatids could result. If the single (no anaphase split) threads were very much twisted or the double (split present at anaphase) threads coiled about one another, complex configurations would appear in the next anaphase. Only rarely was a figure found suggesting any complexity. If such behavior were the secondary cause of double-sized or interlocked ring chromatids (it cannot be the primary cause, see discussion), adjacent cells in the longitudinal rows could be expected to show chromatin bridges in an appreciable percent of the cases. They were present in a number of longitudinally adjacent cells. However, a very large number of such figures would be necessary to allow a satisfactory statistical study to be made. Although a large number of anaphase and telophase figures with aberrant configurations have been observed, the numbers of these in adjacent cells were insufficient for such a study.

The mitotic behavior of small ring-shaped chromosomes

The mitotic behavior of small ring-shaped chromosomes differs from that of large ring-shaped chromosomes in (1) the reduced frequency with

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EXPLANATION OF PLATE II

PLATE II.—All photographs are of pachytene configurations in microsporocytes. ×1100.

Photograph 17. Synaptic association of a normal chromosome V, a Def2 chromosome V and an R2 chromosome. See text figure 25.

Photograph 18. Similar to photograph 17. See text figure 26.

Photograph 19. For description, see text figure 27.

Photograph 20. Synaptic association of a normal chromosome V and a Def2 chromosome V. See text figure 28.

Photograph 21. From a sporocyte of a plant with a normal chromosome V, a Def2 chromosome V and two R2 chromosomes. The two rod-chromosomes have associated with one another (note buckle at spindle fiber attachment region). The two ring chromosomes have associated with one another.

Photograph 22. Synaptic association of two R2 chromosomes.

Photograph 23. Late pachytene. The arrow points to the tiny R1 chromosome.

Photograph 24. Collapsed R1 (upper) and R2 (lower) chromosomes associated at their spindle fiber attachment regions.

Photograph 25. Collapsed R1 (left) and R2 (right) chromosomes associated at their spindle fiber attachment regions.

Photograph 26. R1 chromosome (arrow). Its spindle fiber attachment region is stuck to that of bivalent chromosome VIII.

Photograph 27. Collapsed R1 chromosome (arrow) whose spindle fiber attachment region is associated with that of a normal chromosome V bivalent.

Photograph 28. Synaptic association of a normal chromosome V and a Def1 chromosome V. See text figure 30.

which double-sized or interlocked rings arise; (2) the more frequent loss of the ring chromosomes from the nuclei; (3) the considerably less frequent occurrence of changes in size of the ring chromosomes and (4) the occasional increase in the number of rings in a nucleus.

The two small rings in cases I and II, figure 1, have been used to study the behavior of small ring-shaped chromosomes in mitosis. In the subsequent discussions these two ring chromosomes will be referred to as R 1 and R 2 respectively. Cytological examination of the sporocytes in different branches of the tassel in plants with either of these rings had indicated that loss of the ring chromosome was occurring far more frequently than changes in size of the ring. This is in direct contrast to the behavior of large ring chromosomes, where changes in size are more frequent than loss. To obtain evidence on the method of loss, examinations of the meristematic regions of the roots of such plants were made. The tiny R 1 ring chromosome is clearly visible in the prophase nuclei of these cells. However, the description will confine itself to the behavior of the larger of these two rings, R 2, and one of its enlarged derivatives, since anaphases showing aberrant configurations of the R 1 chromosome are found only very rarely.

The aberrant anaphase and telophase configurations are characterized by the median or nearly median position of the double-sized (or interlocked) ring chromosome in the spindle figure (photograph 15 for the normal R 2 and photograph 11 for the enlarged R 2, Plate I). However, they occasionally lie some distance from this position (figure 22 and photograph 12, Plate I). The ring chromosome in these configurations, as with the large ring chromosomes, frequently appears to be double-sized. In roots in which most of the nuclei contained the normal R 2 chromosome, 14 of the 1209 anaphase and telophase figures counted, or 1.1 percent showed these aberrant configurations (B, table 1). They were observed many times in roots where counts were not made.

The fate of the delayed double-sized ring depends upon its position in the spindle figure as the cell-plate appears. If it is in the middle, the cell plate passes through it, dividing it into relatively equal or decidedly unequal segments (figures 19 and 20). If it is not medially placed, the cell-plate passes to one side and the double-sized ring remains in the cytoplasm of one of the daughter cells (figures 21 and 22, and photographs of same, 12 and 16, Plate I). If it lies rather far away from the mid-region, it may be included in one of the nuclei. If this occurs and if normal splitting of this double-sized ring with two spindle fiber attachment regions follows in the next division, two double-sized rings, each with two spindle fiber regions should then be found lying close together in the spindle figure when the two spindle fiber regions on the same chromatid pass to opposite

poles. Several configurations have been observed in which two rings were lying very close together (figures 23 and 24, and photographs of the same, 13 and 14, Plate I). The exact contours of the individual rings could not be accurately followed and therefore have not been shown in the drawings. Since the contours of the two ring chromosomes could not be accurately

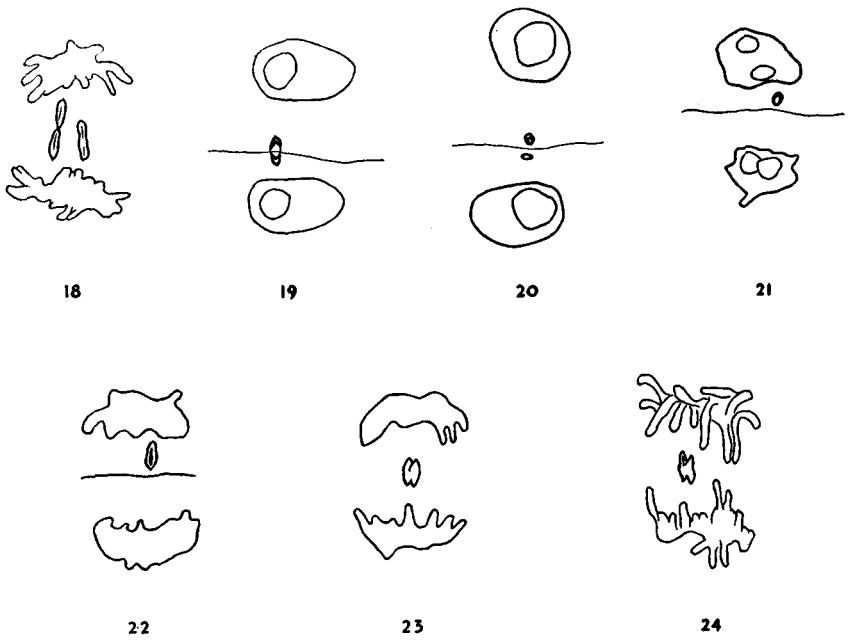


FIGURE 18.—Simultaneous loss at late anaphase of two enlarged R2 chromosomes.
FIGURE 19.—Late telophase appearance after loss of an R2 chromosome. The double-sized ring chromosome has been intercepted by the cell-plate.
FIGURE 20.—Similar to figure 19 but a later stage.
FIGURE 21.—Telophase. The excluded R2 chromosome has not been intercepted by the cell-plate. See photograph 16, Plate I.
FIGURE 22.—Telophase. The excluded enlarged R2 chromosome was not medianly placed in the spindle figure. See photograph of the same, 12, Plate I.
FIGURE 23.—Late anaphase. Two enlarged R2 chromosomes lying close together at the cell-plate region. For description, see text. See photograph of the same, 13, Plate I.
FIGURE 24.—Similar to figure 23. See photograph of the same, 14, Plate I.

followed, such figures could represent two interlocked ring chromosomes from a similar condition to that described above, if instead of a double-sized ring, two interlocked sister ring chromosomes had been included in the preceding telophase nucleus.

Since anaphases and telophases with the ring chromosome lying in the middle of the spindle figure are the most frequent types of aberrant configurations, and since these rings are subsequently excluded from the

telophase nuclei, the frequently observed absence of the ring chromosome from branches of the tassel or from groups of cells in an anther can be explained.

In connection with the problem of the mechanism of movement of chromosomes in the spindle it would be of interest to explain why these small double-sized rings do not appear to be under greater tension as their spindle fiber regions pass toward opposite poles. It is possible that movement toward opposite poles is initiated at the spindle fiber region of the chromosome at early anaphase but that continued movement is made possible by other forces exerted on the chromosomes when they have reached a region in the spindle which is some distance away from the equatorial plate. These double-sized ring chromosomes may be too small to reach this region by the pull exerted at the spindle fiber region. The behavior of intermediate sized rings lends support to this assumption since these sometimes remain in the equatorial plate either with or without evidence of tension.

From cytological examination of sporocytes it was known that changes in size of the small ring chromosomes do occur though with relatively low frequency. The R 2 chromosome has been observed to decrease to several chromomeres and also to increase to seven or eight times its original size. Photograph 15, Plate I, represents a normal R 2 chromosome, photographs 11 and 12, an enlarged R 2 chromosome. It has also been seen that an increase in the number of these rings in a nucleus, usually with alterations in size, sometimes occurs. As many as six ring chromosomes in the nuclei of a sector of a plant which very probably possessed but one ring in the zygote have been observed in a single instance.

If a double-sized ring is included in one of the daughter nuclei, as described above, a start in the direction of increase in number of rings has been made. The chance of loss of this ring chromosome in subsequent divisions is great. However, as already shown, the ring chromosome may be directly broken in two by opposed poleward forces at anaphase, or the poles of the spindle may lie so close together that the spindle fiber regions of the ring are readily included in the two nuclei, after which the resulting chromatin bridges are cut through by the cell-plate and drawn into the nuclei. When two double-sized sister ring chromosomes each with two spindle fiber regions are present in an anaphase figure, and when each of these is subsequently broken and the broken ends drawn into the telophase nuclei, the initial event in the production of a sector of tissue with two altered ring chromosomes has occurred. It is apparent, on this basis, why increase in number of these small ring chromosomes is relatively rare: one infrequent event must be followed by another.

Conclusions regarding ring chromosome behavior

The foregoing account has indicated the probable method by which rings are altered in size and genic constitution or are lost from the nuclei altogether. From both cytological and genetical observations it has been concluded that the rate at which this occurs is dependent upon the length of the chromonema composing the ring: the longer the chromonema, the more frequent the occurrence of aberrant mitoses involving the ring. Counts of the aberrant ring chromosome configurations in late anaphase and very early telophase are given in table 1. The counts are from roots in which the ring chromosome was present in most of the cells. In A, the R 1 chromosome of case I, figure 1, is represented. One aberrant configuration of the ring chromosome was observed in these roots which were counted. Judging from the relatively small amount of *bm 1* tissue shown by plants with this R 1 chromosome, loss of the ring chromosome must be relatively infrequent. In B, the small R 2 chromosome of case II is represented. The observed aberrant configurations of the ring chromosome in these roots amounted to 1.1 percent of all the figures recorded. As stated above, these aberrant configurations result mainly in loss of the ring chromosome from both nuclei. Variegation, expressed by the *bm 1* tissue in these plants, is considerably greater than in the plants from which the counts of table 1, A, were obtained. Table 1, C, represents the counts from a plant which possessed two ring chromosomes, a normal R 2 and an R 2 enlarged approximately three times. In the roots from which the counts were made, both rings were present in many of the nuclei. Aberrant configurations involving the enlarged R 2 chromosome were more frequent than those involving the normal R 2 chromosome. The size of the enlarged R 2 chromosome lies at the border line between those rings whose aberrant configurations lead mainly to exclusion from the telophase nuclei (small rings) and those whose aberrant configurations lead mainly to changes in size of the ring chromosomes (large ring chromosomes). For the enlarged R 2, both types of configurations were frequently encountered. In table 1, D, from a plant with a ring chromosome approximately twice the size of the enlarged R 2 chromosome, the aberrant configurations amounted to 8 percent of the total number recorded. In this case, as described above, the figure is possibly too low. The telophase figures here were characterized mainly by changes in size of the ring chromosome rather than loss from the nuclei.

If a ring chromosome in a given individual carried a dominant gene and the two normal rod chromosomes carried a recessive gene, the expression of variegation produced by losses or changes in constitution of the ring chromosome would depend upon (1) the size of the ring chromosome and

(2) the position of the gene with respect to the spindle fiber attachment region. In relatively small ring chromosomes, which are mainly lost from the nuclei, the expression of variegation is directly dependent upon the actual size of the ring chromosome: the larger the ring chromosome the greater the amount of variegation exhibited. This is strikingly illustrated by the two ring chromosomes, R 1 and R 2. The variegation produced by R 1 is very much less than that produced by R 2. To check this conclusion without prejudice, cultures were obtained in which either R 1 or R 2 or both R 1 and R 2 chromosomes were expected to be present in individual plants

TABLE 2

Comparisons of the predicted and observed ring chromosome constitutions in cultures segregating plants with one, two and three ring chromosomes.

PREDICTION: 1R1		PREDICTION: 1R2		PREDICTION: 2 rings		PREDICTION: 3 rings	
Correct	Deviation	Correct	Deviation	Correct	Deviation	Correct	Deviation
14	1(R1+R2)	15	3(R1)	24	5 (1 ring)* 1(3 rings)†	3	0

* Three showed one R1; one showed one R2 in an estimated two R2 plant; one showed one R2 in an estimated R1 plus R2 plant. Complete agreement in all cases could not be expected in two- and three-ring plants from sporocyte examinations since loss of one of the ring chromosomes in the developmental stages of the tassel is expected in some cases. This particularly applies to the R2 chromosome.

† The estimate for this plant was two R1. Some of the two R1 plants have practically no *bm1* tissue. A two R1 plant could be difficult to distinguish from a three R2 plant.

of the culture. In some of these cultures plants with three ring chromosomes were expected. From the expression of the variegation exhibited by each plant a prediction was made as to the ring chromosome constitution of the plant. Cytological observations were subsequently made to determine the correctness of these predictions. Table 2 shows the correlation of these observations with the predictions. Cultures of plants with the R 1 chromosome can readily be separated from cultures whose individuals possess the R 2 chromosome through observations of the variegation alone (see following section for more complete discussion).

With relatively large ring chromosomes, which are characterized mainly by changes in size of the ring chromosomes, the expression of variegation would depend upon the nearness of the locus of the gene to the spindle fiber region. The farther away the locus, the greater is the amount of variegation that should be expressed.

The method of alteration of the ring chromosomes as suggested by the somatic anaphase and telophase configurations should produce rod-shaped chromosomes. Although thousands of microsporocytes have been examined in many of which an alteration of the ring chromosome has been ob-

served, no rod-fragments have been recognized. It can not be stated that they do not occasionally occur, but certainly their frequency must be exceedingly low. If the method by which ring chromosomes change in size has been correctly interpreted from the study of somatic anaphase and telophase figures, one is forced to conclude that the broken ends of the chromosomes unite, thus reëstablishing a ring.

It might be stated here that when two ring chromosomes are present in the nuclei of a plant, it is rare that both rings show aberrant configurations in the same cell (figure 18). Each ring chromosome apparently acts independently with regard to the formation of double-sized or interlocked rings.

That the behavior of ring chromosomes in maize is a consequence of their form and not of their genic constitution can be definitely stated, since a number of different ring chromosomes, each involving segments of chromosomes not strictly comparable, have been found so far. These include segments from chromosomes II, V, VI, VIII and IX. Most of them were detected by the variegation which they produced but three were isolated independently of any visible effect.

III. THE NATURE OF THE *Bm 1–bm 1* VARIEGATION

The gene *bm 1* when homo- or hemizygous produces a brown color of the cell walls. The color appears in the walls as soon as lignification sets in. It is not present before this period. The depth of color, on external examination of *bm 1* plants, is greatest in those tissues which are composed largely of thickened cell walls, such as the midrib of the leaf, the veins in the leaf sheath and the stalk tissue. The brown color is not easily detected in the leaf tissues other than the midrib since the cell walls are thin and the color is masked by the chlorophyll.

As the plant matures in the field, the brown color has been noted to fade considerably in exposed regions of the plant but remains deep in those regions which are well protected from light. It was suspected that direct sunlight was causing a change in the structure of the brown pigment which resulted in loss of color. To determine if this was correct, black paper was placed about exposed parts of several *bm 1* plants when the brown color was intense. The bands of black paper remained about these parts for a period of three weeks. When the paper was removed, the tissues protected from light had retained their original deep brown; the brown color in the tissues above and below the protected region had faded considerably.

In plants possessing two normal chromosomes V with *bm 1* (or one normal chromosome V with *bm 1* and one of the deficient chromosomes V) and a ring chromosome with *Bm 1*, streaks of *bm 1* tissues are pro-

duced and can be seen by external examination of the plants (figure 1). Over 7000 variegated plants have been examined in the progeny of the two original variegated plants. Cytological observations have indicated that loss of the ring chromosome carrying *Bm 1* is the primary cause for the appearance of the *bm 1* tissues. Losses can occur anywhere in the ontogeny of the plant. The patterns of the *bm 1* tissues should give some indication of where and when these losses occurred. Although wide or narrow bands on the stalk (figure 1) indicate an early or late loss of the ring chromosome, respectively, cross sections of the stem, where most of the cell walls are heavily lignified, give even a better indication of the time of loss. If loss occurred early in ontogeny, the whole plant would be *bm 1*. If the first loss occurred in one of the cells which is to give rise to the part of the plant above the ground, a wide sector of *bm 1* would be produced. Still later losses would produce sectors of various widths in the stem. Very late losses would produce streaks or patches composed of a few cells only. All of these types of variegation patterns have been observed.

When a stalk with a relatively wide external band of *bm 1* tissue is cross-sectioned and examined with low magnification, the brown-walled tissue is seen to be composed of a V-shaped sector with the tip of the V pointing toward the center of the stalk. Many narrow surface streaks are produced by similar sectors but the V is smaller and the tip considerably removed from the center of the stalk. Very narrow streaks may be composed of only a few cells. Such streaks are visible on external examination of the stalk only if they lie at or close to the surface. Patches of *bm 1* cells not close to the surface cannot be seen by external examination.

Dilution of color in the brown (*bm 1*) cell walls on the side of the wall adjacent to the white (*Bm 1*) cell walls was a striking feature of the variegation in all plants. That it is a dilution produced by the adjacent *Bm 1* cells and not a spreading of the brown color from the *bm 1* cell walls is suggested by the considerable reduction in intensity of color in the brown walls of the very small patches composed of only a few cells, and by the dilution of color of a row of *bm 1* epidermal cells on the side adjacent to inner *Bm 1* cells.

The variegation in plants possessing an R 2 chromosome is expressed by a few totally *bm 1* plants where the ring chromosome has been lost before the cells which are to produce the stem meristem have been differentiated, to plants which are composed of many *bm 1* streaks of different widths. Cross sections of the stems of the average variegated plant show wide V-shaped sectors, smaller V-shaped sectors and many irregular patches of *bm 1* composed of few to many cells.

The variegation patterns in plants with the R 1 chromosome were similar

to those produced by plants with the R2 chromosome but the total amount of *bm 1* tissue was very much less. There were fewer sectors of all types in these plants, making cultures of the two types of variegated plants readily distinguishable. This is expected from the cytological examinations since the smaller ring chromosome is lost less frequently in somatic divisions than the larger ring chromosome. The extent of variegation is a direct expression of the rate of loss of the ring chromosome.

Plants with two ring chromosomes show considerably less variegation than plants with one ring chromosome. Loss of one ring chromosome followed later by loss of the second ring chromosome or simultaneous losses of both ring chromosomes must occur before the *bm 1* tissue could be produced. The patterns of the *bm 1* tissues in cross-sections of the stem clearly show this relationship. These fall into three main types of sectors: (1) solid V-shaped sectors, (2) spotted V-shaped sectors, and (3) small patches of *bm 1* tissue.

The solid V-shaped sectors are interpreted as relatively early losses of one ring followed slightly later by loss of the second ring chromosome or by occasional simultaneous losses of both rings. The spotted V-shaped sectors reveal more closely the relationship between loss of one ring followed considerably later by losses of the second ring. They are detected as a cluster of *bm 1* patches in an isolated region of a stem which otherwise shows very few *bm 1* patches. When each of the brown patches in such a cluster is traced with a camera lucida and lines drawn joining the outer boundaries of the outermost patches, the lines converge in the direction of the center of the stem. They clearly define a V-shaped sector. Such spotted V-shaped sectors would be expected if loss of one ring carrying *Bm 1* is followed later in development by losses in different cells of the the second ring chromosome carrying *Bm 1*.

The small patches of brown walled tissue, usually composed of only a few cells, can be interpreted as relatively late, successive, or occasionally simultaneous, losses of the two rings.

There are three types of plants with two ring chromosomes: (1) those with two R1, (2) those with one R1 and one R2 chromosome, and (3) those with two R2 chromosomes. Since somatic loss of the R2 chromosome is considerably more frequent than the R1 chromosome, the amount of *bm 1* tissue produced in each of these plants is progressively greater. Plants with two R2 chromosomes have considerable amounts of *bm 1* tissue; those with one R1 plus one R2, very much less, and those with two R1 chromosomes exceedingly little *bm 1* tissue. In this latter type of plant it is often necessary to examine cross-sections of the stem to determine if any *bm 1* tissue is present. Such tissue, when not close to the surface, cannot be detected from field examinations of the plants.

Plants with three ring chromosomes of the constitution two R 2 plus one R 1 chromosome or one R 2 plus two R 1 chromosomes, have been obtained. These plants frequently show no external evidence of *bm 1* tissues. In all cases, however, careful examinations of the stalks have revealed small patches of *bm 1* cells. The *bm 1* cells could arise only after loss (mainly successive) of all three ring chromosomes from the nuclei.

For the sake of comparison, the stalks of a number of plants with a normal chromosome constitution carrying *Bm 1* in one chromosome V and *bm 1* in its homologue were examined. In no case was there any evidence of *bm 1* tissue.

In conclusion it can be emphasized that the genetic expression of variegation is in full agreement with expectation on the basis of the cytological observations given in the previous section. In these plants with small ring chromosomes whose aberrant mitotic configurations are followed mainly by loss of the ring chromosome from the nucleus, the extent of variegation is a direct indication of the length of the chromonema composing the ring chromosome, the larger the ring chromosome the higher the rate of loss and thus, the greater the amount of exhibited variegation. Loss of the ring chromosome can occur at any stage in the development of the plant, early loss giving rise to a totally *bm 1* plant, later loss to wide sectors of *bm 1* and very late losses to small patches of *bm 1* cells. The patterns exhibited by two and three ring chromosome plants are those expected from the cytological observations where it has been shown that simultaneous loss of the several ring chromosomes from a nucleus is rare. The cause of the aberrant mitotic configuration arises independently in each ring chromosome.

Knowledge gained from a study of variegation in these plants has been utilized in the analysis of tissues of plants mosaic for homozygous deficiencies (section V).

IV. TYPES OF FUNCTIONAL GAMETES PRODUCED BY THE TWO ORIGINAL VARIEGATED PLANTS

Each of the two original variegated plants possessed one normal chromosome V with *bm 1*, one deficient chromosome V and a ring-shaped fragment chromosome corresponding in size to the deficiency in the rod chromosome (figure 2). In case II (Def 2, R 2) prophase meiotic associations had indicated the homology of the ring chromosome with the region in the rod chromosome which had been deleted (figures 25 and 26; photographs of the same, 17 and 18, Plate II). Most frequently, the ring chromosome did not associate with its homologous section in the normal rod

chromosome but remained separate and collapsed (for meiotic prophase behavior of ring-shaped chromosomes, see McCLINTOCK 1933). In all cells the deficient rod chromosome V and the normal chromosome V were associated. The normal V had to buckle to compensate for the deletion in the deficient V. Figures 27 and 28, and photographs of the same, 19 and 20, Plate II, illustrate this association. In the plant from which figure 27

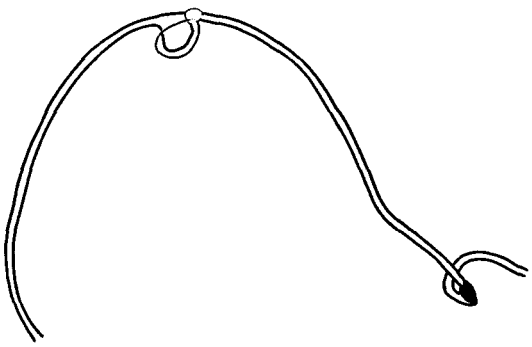


FIGURE 25.—Pachytene association of a normal chromosome V, a Def2 chromosome V and its compensating R2 chromosome. The ring chromosome has been drawn with a finer line. The spindle fiber attachment region has been drawn as a slight bulge. The dark bodies toward the end to the right are the knobs. See photograph of the same, 17, Plate II.

was drawn, two ring chromosomes were present. They are separate and collapsed. Another figure from the same plant, photograph 21, Plate II, shows the not infrequent association of the two R2 chromosomes to form a true ring-shaped configuration and also the association of the normal and Def 2 chromosomes.

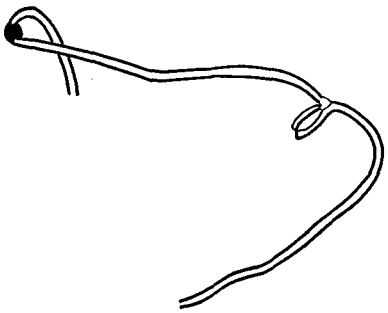


FIGURE 26.—Similar situation to that shown in figure 25. See photograph of the same, 18, Plate II.

In case I (Def1, R1), no figures were observed showing the association of the ring chromosome with its homologous section in the normal chromosome. It is probable that it occurred in a small percentage of the cases but

would be difficult to detect except in the most favorable figures because of the smallness of the deficiency and the ring chromosome. Figures 29 and 30 illustrate the pachytene association of the Def1 chromosome with

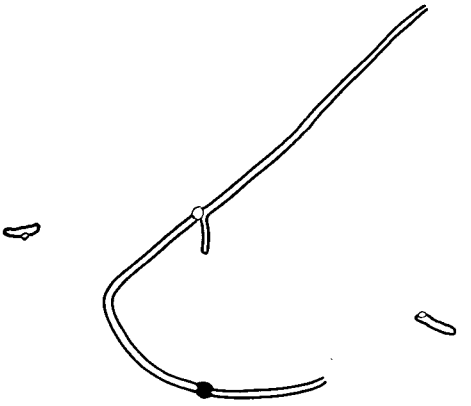


FIGURE 27.—Pachytene association in a microsporocyte of a plant with one normal chromosome V, one Def2 chromosome V and two R2 chromosomes. Note the buckle in the normal chromosome V at the spindle fiber attachment region and the two unassociated, collapsed ring chromosomes. The ring chromosomes have a similar chromatin constitution to that of the buckle. See photograph of the same, 19, Plate II.

a normal chromosome V. The small ring chromosome (R1) lies free and is collapsed. In figure 30 and photograph of the same, 28, Plate II, non-homologous associations about the deficient region have resulted in a

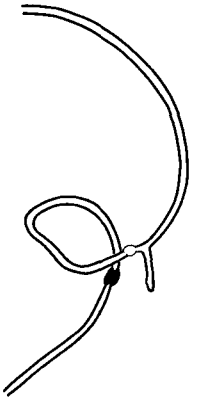


FIGURE 28.—Pachytene association of a normal chromsome V and a Def2 chromosome V. See photograph of the same, 20, Plate II.

separation of the spindle fiber attachment regions of the two chromosomes (for expected non-homologous associations, see McCLINTOCK 1933). The small buckle below the lower spindle fiber region or the distance between

the two spindle fiber regions represents the extent of the deficiency. Photographs 23, 26 and 27, Plate II, illustrate the appearance of the R1 ring at meiotic prophase. In photograph 23, very early diplotene, the

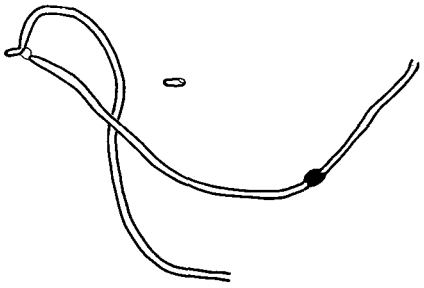


FIGURE 29.—Pachytene association in a microsporocyte of a plant with one normal chromosome V, one Def1 chromosome V and an R1 chromosome. The ring chromosome is collapsed and is not associated with its homologous region (buckle) in the normal chromosome V.

ring shape of the chromosome is clear. In photograph 26 the spindle fiber region of the R1 chromosome is stuck to that of chromosome VIII. In photograph 27, the spindle fiber region of the collapsed R1 chromosome is adjacent to that of a normal chromosome V bivalent. Photographs 24

FIGURE 30.—Pachytene association of a normal chromosome V and a Def1 chromosome V. Through non-homologous associations, the buckle which compensates for the deficiency, has shifted into the long arm of the normal chromosome V. Note the displacement of the spindle fiber attachment regions. See photograph of same, 28, Plate II.

and 25, Plate II, illustrate the relative sizes of R1 and R2 when both are present in the same sporocyte. In both photographs the collapsed ring chromosomes are associated by their spindle fiber attachment regions. In photograph 24 the R1 chromosome is above, the R2, below. In photograph 25 the R1 chromosome is to the left, the R2 to the right.

In plants heterozygous for either deficiency and its compensating ring

chromosome, the deficient rod chromosome and its normal homologue proceed quite normally during the meiotic mitoses, two spores of a quartet receiving the deficient chromosome, two the normal chromosome. The behavior of the ring chromosome, on the other hand is irregular. In the case of R1 chromosome the split halves separate and pass to opposite poles at anaphase I along with the disjoining bivalents (except where double-sized or interlocked ring chromosomes are formed). The split halves of the R2 chromosome likewise separate at I, either at the same time that the bivalents disjoin or slightly later. They are nearly always included in the first division telophase nuclei.

In the second meiotic mitosis, the behavior of the ring chromosome is variable. They do not divide again but either pass to one of the poles along with the other chromosomes of the complement or remain in the spindle figure and are excluded from the telophase nuclei. The behavior of the rings in the two sister cells is not always the same.

As a result of meiosis, four types of spores are to be expected. They carry the following chromosomes:

1. Normal chromosome V.
2. Deficient chromosome V.
3. Normal chromosome V plus the ring fragment.
4. Deficient chromosome V plus the ring fragment.

The percentage of each type in an anther would depend upon (1) the proportion of the sporocytes which lacked a ring chromosome, giving only types 1 and 2 above, and (2) the percentage of cases in which the ring chromosome, when present, was included in the second meiotic telophase nucleus.

Examination of the pollen has given some indication of the percentage of each of these four types which are present in an anther. Pollen from a *bm1* sector of a plant known to have a normal chromosome V (carrying *bm1*), a deficient chromosome V (Def1), and an R1 chromosome, showed three types of grains: (1) large well filled grains, (2) small partially filled grains and (3) small totally empty grains. The proportions of each type are shown in table 3, A. In these *bm1* sectors it is assumed on good evidence (see sections I and II) that the ring chromosome has been lost. Equal proportions of type 1 and type 2 grains should be present. If the small partially filled grains represent those with the Def 1 chromosome, the large filled grains, those with the normal chromosome V, they should be present in equal proportions. A total of 5535 normal pollen grains to 5547 small partially filled grains clearly indicates this association. The 347 small empty grains represent 3.3 percent of the total. In all samples of

pollen from normal maize plants there is a small percentage of these empty grains. They probably represent the products of abnormalities in meiosis which are not infrequently observed in normal plants.

Anthers from *Bm 1* regions of the plant, in which the ring chromosome is present, give a higher proportion of normal well-filled grains (table 3, B). The difference is interpreted as due to the presence of the ring chromosome in some of the grains which have a deficient rod chromosome (type 4, above). Since the ring chromosome, if unaltered, covers the deficiency, a normal appearing pollen grain is expected. On this interpretation, the number of each type in a particular anther can be estimated. Pollen types 1 and 2 should be present in equal numbers. Likewise, types 3 and 4 should be present in equal numbers. Type 2 grains can be directly recorded. An equal number of the normal appearing grains should belong to type 1. When this number is subtracted from the total number of normal appearing grains, the remainder can be equally distributed to types 3 and 4. The estimates of each type of grain from the *Bm 1* anthers in table 3 B, are: type 1, 1994; type 2, 1994; type 3, 743; type 4, 743, or 36.5 percent each of types 1 and 2 and 13.5 percent each of types 3 and 4.

In one plant, heterozygous for Def 1 R 1, four types of pollen grains were present, 619 large well-filled grains, 170 small but well-filled grains, 426 small partially-filled grains (type 2) and 49 small empty grains. If it is assumed that the small well-filled grains represent type 4 with an altered ring chromosome which does not completely cover the deficiency, pollen types 2 and 4 can be directly recorded. If, on the other hand, these grains are included in the normal appearing class, and calculations made as above, the proportion of types are: type 1, 426; type 2, 426; type 3, 184; type 4, 184. It is obvious that there is a close agreement in the calculated number of 184 for type 4 grains and the 170 grains which have been assumed to represent this type.

In plants heterozygous for Def 2 and R 2, the type 2 pollen grains are large but almost completely empty. These grains cannot be distinguished from the few empty grains produced by other causes than the presence of the deficiency in chromosome V. However, if these latter grains are assumed to represent two percent of all the grains, an approximate estimate can be made of the number of grains with each of the four chromosomal constitutions. The counts from the *bm 1* anthers are given in table 3 C, and similar counts from the *Bm 1* anthers, with estimates of proportions of types, in table 3 D.

The functional capacity of each of the four types of gametes can best be illustrated by reference to the types and numbers of individuals resulting from the crosses given in table 4. Section A in the table represents the

TABLE 3

A. Pollen counts from bm1 anthers of plants with the constitution Def 1/*bm1*/R 1.

PLANT	LARGE FILLED GRAINS	SMALL PARTIALLY FILLED GRAINS	EMPTY GRAINS	% EMPTY GRAINS
598A-2	1181	1148	49	2.0
598A-2	832	917	38	2.1
597A-2	775	751	66	4.1
597A-2	610	611	21	1.6
598A-16	692	678	42	2.9
598A-3	523	526	70	6.2
597B-6	922	916	61	3.2
Totals	**5535**	**5547**	**347**	**3.3**

B. Pollen counts from Bm1 anthers of a plant with the constitution Def 1/*bm1*/R 1.

PLANT	LARGE FILLED GRAINS	SMALL PARTIALLY FILLED GRAINS	EMPTY GRAINS	% EMPTY GRAINS	ESTIMATES OF FOUR TYPES OF GRAINS IN PERCENT			
					1	2	3	4
598A-2	1431	802	47	2.0	36	36	14	14
598A-2	1094	561	26	1.5	34	34	16	16
598A-2	956	631	28	1.7	40	40	10	10
Totals	**3481**	**1994**	**101**	**1.8**				

C. Pollen counts from bm1 anthers of a plant with the constitution Def 2/*bm1*/R 2.

PLANT	NORMAL GRAINS	EMPTY GRAINS
953B-6	681	733
953B-6	943	918
953B-6	864	962
953B-6	761	779
953B-6	1036	1079
Totals	**4285**	**4471**

D. Pollen counts from Bm1 anthers of plants with the constitution Def 2/*bm1*/R 2.

PLANT	NORMAL GRAINS	EMPTY GRAINS	ESTIMATE OF FOUR TYPES OF GRAINS IN PERCENT			
			1	2	3	4
1009-10	906	864	48	48	2	2
1009-8	955	623	38	38	12	12
1009-8	1281	847	38	38	12	12
1009-8	1109	822	41	41	9	9
Totals	**4251**	**3156**				

progeny from crosses of the two original variegated plants. Section B represents crosses of plants from A of this table, which were heterozygous for the deficiency and compensating ring, with normal *bm 1* plants. It can be seen that all four gametes produced by plants heterozygous for Def1 R1 can be transmitted through the eggs. Through the pollen, gametes of type 1 and 3 are readily transmitted but gamete type 2 is not transmitted and gamete type 4 only rarely in competition with gametes 1 and 3.

TABLE 4

	TYPE OF CROSS (Female parent to left)	CONSTITUTION OF PLANTS RESULTING FROM CROSSES *bm1* PLANTS		VARIEGATED PLANTS	
		bm1/bm1	Def/*bm1*	*bm1/bm1*/R	Def/*bm1*/R
A.	Def1/*bm1*/R1×*bm1*	218	9	16	12
	bm1×Def2/*bm1*/R2	38	0	0	8
B.	Def2/*bm1*/R2×*bm1*	352	1*	13	11
	reciprocal	696	4*	38	154
	Def1/*bm1*/R1×*bm1*	300	13	29	17
	reciprocal	74	0	17	1
C.	Def1/*bm1*×*bm1*	155	6	0	0

* These *bm1* plants probably arose through very early loss of the ring chromosome. The Def2 chromosome is not transmitted without the R2 chromosome.

In plants heterozygous for Def2 R2, gametes 1, 3 and 4 are transmitted through the eggs but gamete 2 is not transmitted. Through the pollen, gametes 1 and 4 are readily transmitted. The frequent transmission of the Def2 R2 combination through the pollen contrast with the very infrequent transmission of the Def1 R1 combination through the pollen. Both cases are in agreement in the lack of transmission of the deficient chromosome minus its compensating ring chromosome through the pollen.

To obtain high counts on transmission of the deficiency carrying gametes, the gene *bt* (brittle endosperm) was introduced into the normal chromosome. The *bt* gene is located in the long arm of chromosome V (RHOADES 1936) and gives very little crossing over with *bm1*. No positive case of crossing over between *bt* and the two deficiencies of chromosome V has been found. Thus, plants with a deficient chromosome carrying *Bt* and a normal chromosome with *bt*, when crossed to normal *bt* should give the percentage of deficiency-carrying gametes (*Bt* kernels) directly, without the necessity of growing large progenies and testing each indi-

vidual plant for the presence of the deficient chromosome. The results of such crosses are given in table 5. In many of the crosses *bm1* was likewise involved. The deficiency carrying plants were Def *Bt/Bm1 bt* with or without a ring chromosome carrying *Bm1*. These were crossed with normal *bm1 bt* individuals. The *Bt* kernels should give rise to variegated (*Bm1–bm1*) plants when the ring chromosome is present or totally *bm1* plants when the ring chromosome is absent; the *bt* kernels should give rise to totally *Bm1* plants. The results of a test of the *Bt* and *bt* kernels are summarized in table 6. If no crossing over occurred between the deficiency and *Bt*, *bm1* could appear in these crosses only when a deficient chromosome was present. 177 of the 358 individuals showing *bm1* were tested for the presence of the deficient chromosome. It was present in every case. Thus, the data in table 5 can be used as a direct means of determining the functioning of the gametes which carry a deficient chromosome.

TABLE 5

Bt kernel test of transmissions of Def 1 *and* Def 2 *chromosomes.*

CROSS*	*Bt*	*bt*
1. +*Bt*/+*bt*×*bt*	2893	2746
2. reciprocal	797	889
3. Def 1 *Bt*/+*bt*/R 1×*bt*	1217	6055
4. reciprocal	4	5544
5. Def 1 *Bt*/+*bt* (no ring)×*bt*	927	12682
6. reciprocal	0	323
7. Def 2 *Bt*/+*bt*/R 2×*bt*	187	5735
8. reciprocal	363	1951
9. Def 2 *Bt*/+*bt* (no ring)×*bt*	0	1319
10. reciprocal	0	4065

* A non-deficient chromosome V is represented by +. The pollen parent is placed at the right in each cross.

The results given in lines 3 and 5 of table 5 are particularly interesting with respect to the functioning of the Def 1 chromosome through the eggs. If there had been no megaspore selection in favor of the spore carrying the normal chromosome and if all the eggs (or zygotes) which carried the Def 1 chromosome functioned, the ratio of *Bt* to *bt* should be equal. In all cases the number of *bt* kernels was greater than *Bt* and in all cases the ears were incompletely filled. The presence of the abortive grains on the ear indicate that there has been little if any selection of normal chromosome carrying megaspores. Therefore, the percentage of *Bt* kernels,

when the *bt* kernels are taken as the standard of expectancy, indicates the extent of functioning of the deficiency carrying eggs (or zygotes). The 927 *Bt* kernels in line 5, table 5, represent 7.2 percent of the deficiency carrying eggs (or zygotes) which functioned. In the 74 ears which contributed to this count, the percentages ranged from 0 to 23.2 with half of the ears falling within the range of 2 to 8 percent. When the ring chromosome (R 1) was present (line 3, table 5) the *Bt* kernels on the 30 ears contributing to this count ranged from 1 to 40 percent of expectancy on the individual ears and averaged 20.1 percent. That this increase can be attributed to the presence of the ring chromosome covering the deficiency in many of the eggs can be seen from line 1, table 6.

TABLE 6

CROSS	PLANTS FROM *Bt* KERNELS		PLANTS FROM *bt* KERNELS	
	Bm1–bm1 variegated	*bm1*	*Bm1*	*bm1*
Def 1 *Bt/Bm1 bt*/R1×*bm1 bt*	40	23	not grown	
Def 1 *Bt/Bm1 bt*×*bm1 bt*	0	85	328	0
Def 2 *Bt/Bm1 bt*/R2×*bm1 bt*	106	7	342	0
bm1 bt×Def 2 *Bt/Bm1 bt*/R2	95	2	24	0

That pollen containing the Def 1 chromosome without its compensating ring chromosome does not function in competition with normal pollen can be concluded from line 6, table 5. By use of a 170 wire mesh screen these grains, which are small and partially filled with starch, can be segregated from the normal grains and the possible factor of competition with the grains carrying the normal chromosome eliminated. Some of the ears pollinated with sifted pollen gave no kernels at all, others a few *bt* kernels through passage of a few small but normal chromosome pollen grains through the wire mesh. It can be definitely stated, therefore, that these grains are incapable of producing an effective pollination when placed upon normal silks.

Line 4, table 5, suggests that the grains with a Def 1 and an R 1 chromosome normally do not effect a pollination in competition with normal chromosome carrying grains. The four *Bt* kernels were grown to determine the chromosome constitution of the resulting individuals. Two of these *Bt* kernels were definitely produced through contamination, one through functioning of a Def 1 R 1 grain and one could have been a crossover between *Bt* and the deficiency although contamination could not be excluded definitely. If this kernel represents a crossover, it is the only evidence so far obtained of crossing over between the deficiency and *bt*.

From this evidence it could be concluded that (1) the ring-shaped chromosome, R1, does not completely cover the deficiency due to a loss of a small section either at the time of irradiation or during the development of the original plant or that (2) a mutation affecting pollen tube growth appeared at the time of irradiation or (3) the particular chromosomal modification (position effect) is responsible for the reduced pollen tube activity. If (1) above is correct, the deficiency, when homozygous, does not produce a visible effect in the tissues of the mature plant (see section V).

That the gametes with Def2 function only when the ring chromosome, R2, is present is evident from tables 5 and 6. The Def2 gametes with a complete R2 chromosome have an equal chance in competition with normal chromosome carrying gametes. The discrepancy in the percentages of *Bt* kernels in the reciprocal crosses, lines 7 and 8, table 5, can be understood when it is realized that the ear arises from a definite sector of tissue which originally may or may not have had the ring chromosome in its nuclei (6 of the 36 ears had no *Bt* kernels) whereas the pollen is shed from all parts of the tassel which is usually a mosaic of sectors with and without the ring chromosome. The percentage "expected" *Bt* kernels on the 36 individual ears in the cross summarized in line 7, table 5, ranged from 0 to 8.9 percent, those on the six ears summarized in line 8, from 4.5 to 21.6 percent.

As stated in section II, changes in size and genic constitution of the ring chromosomes occasionally occur during ontogenesis of a plant. This being so, it could be objected that the two original ring chromosomes could not be kept constant through successive generations. Small duplications within a ring chromosome are not phenotypically detectable. They must be determined through cytological examination. In contrast, the deficiencies within the ring chromosome can be detected through phenotypic appearances of certain plants (see section V) and through pollen transmissions which tend to eliminate gametes with the deficient ring chromosome. However, change in size of the ring chromosome is not frequent and with proper care, it is not difficult to maintain stocks with unaltered ring chromosomes.

V. PRODUCTION OF PLANTS MOSAIC FOR HOMOZYGOUS DEFICIENCIES

As shown in the previous section, the progeny of the crosses of the two original variegated plants by normal *bm1* included a number of individuals with chromosomal constitutions similar to the two original plans. Plants heterozygous for Def1 and R1 when crossed by plants heterozygous for Def2 and R2 should produce twelve types of plants, each with a different chromosomal constitution:

1. *bm1/bm1*
2. *bm1/bm1*/R2
3. Def2/*bm1*/R2
4. Def1/*bm1*
5. Def1/*bm1*/R2
6. Def1/Def2/R2
7. *bm1/bm1*/R1
8. *bm1/bm1*/R1/R2
9. Def2/*bm1*/R1/R2
10. Def1/*bm1*/R1
11. Def1/*bm1*/R1/R2
12. Def1/Def2/R1/R2

In the cross of heterozygous Def1 R1 by normal *bm1* plants, a number of individuals with the constitution of plant type 4, above, were obtained. When these, in turn, are crossed by plants heterozygous for Def 2 R2, the first six types of plants listed above should be produced.

All of the plants except 6 and 12 from the first cross, and all the plants except 6 from the second cross can be distinguished by the presence or absence of variegation, the type of variegation exhibited and the type of pollen shown by each plant. Cytological examinations of a number of these plants were in agreement with the field determinations.

The appearance of plants of type 6 and 12 could not be predicted. In actual experience it proved very simple to identify them. Both types of plants possess two deficient chromosomes, Def1 and Def2. Plant 6 has one ring chromosome, R2, plant 12, two ring chromosomes, R1 and R2. These two types of plants will be designated R2 double-deficient and R1 R2 double-deficient.

In the R2 double-deficient plants, the ring chromosome covers both deficiencies. Its loss in somatic nuclear divisions should result in cells homozygous deficient for the extent of the deficiency in the Def1 chromosome. If these cells were viable and continued to multiply at the same rate as the surrounding heterozygous deficient cells, which are close to normal in growth rate, both wide and narrow sectors of homozygous deficient tissues should be produced through early and late losses, respectively, of the ring chromosome during ontogeny. In the R1 R2 double-deficient plants, both ring chromosomes cover the homozygous deficient segment in the rod chromosomes. Simultaneous loss of both ring chromosomes or loss of one ring chromosome followed later by loss of the second ring chromosome must occur in order that homozygous deficient tissue can be produced. The total amount of homozygous deficient tissues produced in the R2 double-deficient plants should be considerably greater than that produced by the R1 R2 double-deficient plants. If tissues homozygous deficient for the extent of the deficiency in Def1 were visibly modified, the two types of plants should be readily distinguishable. However, no prediction as to the nature of the homozygous deficient tissue was possible before the appearance of these plants.

In addition to the *bm1* and variegated plants resulting from the cross of Def1/*bm1*×Def2/*bm1* R2, one plant appeared (table 7) which was not *bm1* and did not show the ordinary *Bm1–bm1* variation. This plant was stunted in growth habit, the leaves and leaf sheaths were uniformly

TABLE 7

Def 1/*bm 1* (*no ring*)×Def 2/*bm 1*/R 2.

CULTURE	*bm1* AND VARIEGATED	Def1/Def2/R2
692	220	1
693	23	0
36–30	97	0
Totals	340	1

streaked with fine bands of colorless tissue, as shown in figure 32 (compare with figure 31, a normal leaf). In the cross of Def1/*bm1*/R1×Def2/*bm1*/R2, besides the *bm1* and variegated plants, two new types of plants appeared (table 8). One type was similar to the plant just described. The other type was considerably larger, approaching normal in growth habit, but was not a typical *bm1* or variegated plant, and presented the same fine streaks of colorless tissues in the leaves and leaf sheaths as the first new type. However, the total amount of such tissue was markedly less and the pattern of this tissue was not uniform, figures 35 and 36.

TABLE 8

Def 1/*bm1*/*R1*×Def 2/*bm1*/*R2*.

CULTURE	*bm1* AND VARIEGATED	Def1/Def2/R2	Def1/Def2/R1/R2
694	40	1	1
695	41	1	2
35-9	126	0	0
35-10	243	1	0
35-11	134	5	1
36-24	172	2	2
Totals	856	10	6

It was suspected that the two new types of plants represented the two expected types of double deficients, and that the colorless streaks represented the homozygous deficient tissues. Since these streaks of colorless tissue were not wide, as some of them theoretically should be from homologies with the *bm1* streaks in normal variegated plants, it was suspected that the cells of the homozygous deficient tissue were unable to multiply at the same rate as the surrounding heterozygous deficient cells. As stated in section II, loss of the R2 chromosome occurs much more frequently than loss of the R1 chromosome. If the rate of loss for each of these two chromosomes is uniform throughout development, a uniform distribution of colorless streaks should be present in the R2 double-deficient plants.

In the case of the R1 R2 double-deficient plants, early loss of the R1 chromosome should give a sector of tissue with the pattern of colorless streaks characteristic of the R2 double-deficient plants, since the chromosome constitution within the sector is the same. If the R2 chromosome were lost early in ontogeny, a sector of tissue with a different pattern of colorless streaks should result. These colorless streaks should be considerably less frequent since the R1 chromosome is lost from the nuclei less frequently. Nevertheless, the distribution of such streaks should be uniform. If this hypothesis were correct, the small, uniformly but heavily streaked plants should be the R2 double-deficient plants, the larger, nonuniformly streaked plants, R1 R2 double-deficient plants. That these two types represented the expected R2 and R1 R2 double-deficient plants was established through cytological observations and confirmed by pollen examinations and appropriate crosses.

In the intercrosses of plants heterozygous for Def2 and R2, the union of a Def2 R2 gamete with a similar gamete results in a plant with two Def2 chromosomes plus two R2 chromosomes. (It is of theoretical interest to point out that the chromosome number has been increased in these plants without changing the genome complement, that is, these 22-chromosome plants are genomically equivalent to normal 20-chromosome plants.) Since these plants are markedly different from the double-deficient plants, a description will be postponed until section VII. The functional gametes produced by these plants contain the Def2 chromosome plus one or two R2 chromosomes. The gamete most frequently transmitted through the pollen contains but one R2 chromosome. In the crosses of Def1/*bm1* and Def1/*bm1*/R1 by plants homozygous for Def2 and R2, all the eggs which carry the normal chromosome with *bm1* should give rise to normal variegated plants, all those that carry a deficient chromosome to double-deficient plants. The results of these two types of crosses are given in tables 9 and 10. The test for the functioning of deficiency-carrying eggs is similar to the *Bt* and *bt* tests described in the previous section. The correlation between the proportions of functional

TABLE 9

Def1/*bm1* (*no ring*)×*Homozygous* Def2, R2.

CULTURE	NORMAL *Bm1*—*bm1* VARIEGATED*	Def1/Def2/R2
814	76	0
36-28	61	8
36-29	135	20
37-56	13	0
37-57	64	0
Totals	349	28

* A few plants were *bm1*, see page 364.

TABLE 10

Def 1/*bm 1*/*R 1*×*Homozygous* Def 2, R 2.

CULTURE	NORMAL *Bm 1*—*bm 1* VARIEGATED*	Def 1/Def 2/R 2	Def 1/Def 2/R 1/R 2
36-25	106	18	15
36-26	56	9	4
37-52	154	17	12
37-53	175	22	31
37-54	107	2	5
37-55	162	5	18
Totals	**760**	**63**	**85**

* A few plants were *bm 1*. See page 364.

eggs with a normal chromosome V, a deficient chromosome V, and a deficient chromosome V plus its ring chromosome, respectively, is similar in the two tests.

It remains to be shown that the colorless streaks in the double-deficient plants represent the homozygous deficient tissues produced after loss of the ring chromosomes during somatic mitosis. Adequate confirmation of this relationship is obtained from the patterns of such tissues in double-deficient plants with the following ring chromosomes: one R 1, one R 2, two R 2, one R 1 plus one R 2, two R 1 plus one R 2, one R 1 plus two R 2. Double-deficient plants with different combinations of ring chromosomes can be obtained from crosses of R 2 and R 1 R 2 double-deficient plants by plants homozygous for Def 2 R 2 and from intercrosses of the double-deficient plants. The results of the respective crosses are given in tables 11, 12, and 13. It should be noted that only plants homozygous for Def 2 R 2 and double-deficient plants result from these crosses.

Classification of these plants into the two categories of homozygous

TABLE 11

Def 1/Def 2/R 2×*Homozygous* Def 2, R 2.

CULTURE	HOMOZYGOUS Def 2, R 2	Def 1/Def 2 one R 2	Def 1/Def 2 two R 2
823	0	1	0
987	0	2	5
988	2	3	1
989	9	11	13
990	4	18	2
991	7	4	4
992	3	20	6
37-85	1	7	1
37-86	3	17	5
37-87	0	15	1
37 88	1	9	1
37-89	4	20	7
37-90	4	8	3
37-91	4	18	2
Totals	**42**	**153**	**51**

TABLE 12

Def 1/Def 2/R 1/R 2 × *Homozygous* Def 2, R2.

CULTURE	HOMOZYGOUS Def 2, R 2*	Def 1/Def 2 ONE R 2	Def 1/Def 2 TWO R 2	Def 1/Def 2 ONE R 1, ONE R 2	Def 1/Def 2 THREE RINGS
37-58	11	30	9	14	3
37-59	6	10	11	16	2
37-60	5	8	2	4	0
37-61	1	0	0	8	0
37-62	1	11	11	0	0
37-63	3	14	3	30	2
37-64	3	14	0	33	4
37-65	5	5	0	4	3
37-66	0	7	0	8	2
37-67	4	11	2	13	8
37-68	0	2	1	6	2
37-69	1	6	1	12	5
37-70	3	1	1	6	5
37-71	2	8	6	14	2
37-72	14	16	3	20	6
37-73	12	16	5	27	17
37-74	5	8	5	19	3
37-75	6	15	1	20	3
Totals	**82**	**152**	**61**	**254**	**67**

* Some of these plants had, in addition, an R 1 chromosome.

Def 2 R 2 and double-deficient was simple since the former type of plant has a peculiar growth habit (see section VII) and does not show the particular streaks which are always present in the double-deficient plants. The double-deficient plants, in turn, were classified as to their ring chromosome constitution on the basis of the patterns of the colorless streaks. If the colorless streaks represent the homozygous deficient tissue, then the pattern exhibited by the double-deficient plants with the R 1 or R 2 chromosome or various combinations of two or three of these rings should

TABLE 13

Plants obtained from sib crosses of Def 1/Def 2/R 1/R 2.

CULTURE	HOMOZYGOUS Def 2, R 2*	Def 1/Def 2 1 RING	Def 1/Def 2 2 RINGS	Def 1/Def 2 3 RINGS†
37-76	4	15	15	9
37-77	1	14	27	11
37-78	3	0	19	5
37-79	5	6	20	8
37-80	0	3	4	6
37-81	2	0	9	1
37-82	6	17	21	17
1000	0	8	27	11
1001	9	1	14	9
1003	2	7	22	10
Totals	**32**	**71**	**178**	**87**

* Several of these plants had, in addition, an R 1 chromosome.

† Several of these plants were suspected to have four rings.

be predictable from the knowledge of their behavior in somatic mitosis (section II) and from the knowledge gained from a study of the patterns of *bm1* tissues in normal variegated plants with these same combinations of ring chromosomes (section III). A number of these plants were examined cytologically to establish the value of the prediction. The results are summarized in table 14. The agreement between predicted and observed is obvious from the table.

TABLE 14

Comparisons of predicted and observed chromosomal constitutions.

PREDICTED CONSTITUTION FROM APPEARANCE OF PLANT	NO. PLANTS EXAMINED	DEVIATION FROM EXPECTATION
Homozygous Def2, R2	17	0
Def1/Def2+one R2	18	0
Def1/Def2+two R2	10	1 (1 R1)
Def1/Def2+one R1 and one R2	24	1 (1 R1)
Def1/Def2+two R1 and one R2	5	2 (2 R2; 1 R1+2 R2)
Def1/Def2+one R1 and two R2	6	1 (R1+R2)
Totals	80	5*

* See footnote *, table 2.

The patterns of colorless tissue exhibited by the various double-deficient plants will be briefly described. Photographs of parts of leaves of double-deficient plants with various ring chromosome combinations are given in figures 31 to 38. To conserve space only a small part of a leaf is shown, which considerably limits the effectiveness of the demonstration.

The colorless streaks in the one R2 double-deficient plants are uniformly distributed throughout the leaf area and are relatively closely spaced (figure 32). Double-deficient plants with the R1 chromosome have been produced only by very early loss of the R2 chromosome in plants originally possessing both the R1 and R2 ring chromosomes. There is considerably less streaking but the distribution of these streaks is uniform (figure 34). Plants with two R2 chromosomes are clearly distinguishable from those with an R1 and R2 chromosome. In both types of plants the streaking is not uniformly distributed. The two R2 chromosome plants have a considerably greater total amount of colorless tissue. There are numerous sectors of various widths with a pattern similar to that shown by the one R2 plants (figure 33, sector to right). This is to be expected since loss of either ring chromosome would give rise to cells with the same chromosome constitution as the one R2 plants. Sectors with the R1 pattern, figure 34, are not found. The R1 R2 double-deficient plants have fewer streaks than the two R2 plants. The sectors in these plants are either of the one R2 type (figure 36, sector to left), or of the R1 type (figure 35, sector to right of mid-rib), through early loss of the R1 or R2 chromosome, respec-

tively. In the three ring double-deficient plants, very few colorless streaks were observed. This is particularly evident in the two R1 plus one R2 plants. Many of the leaves in these plants have no well defined sectors but only scattered streaks here and there. Sectors, when present, are

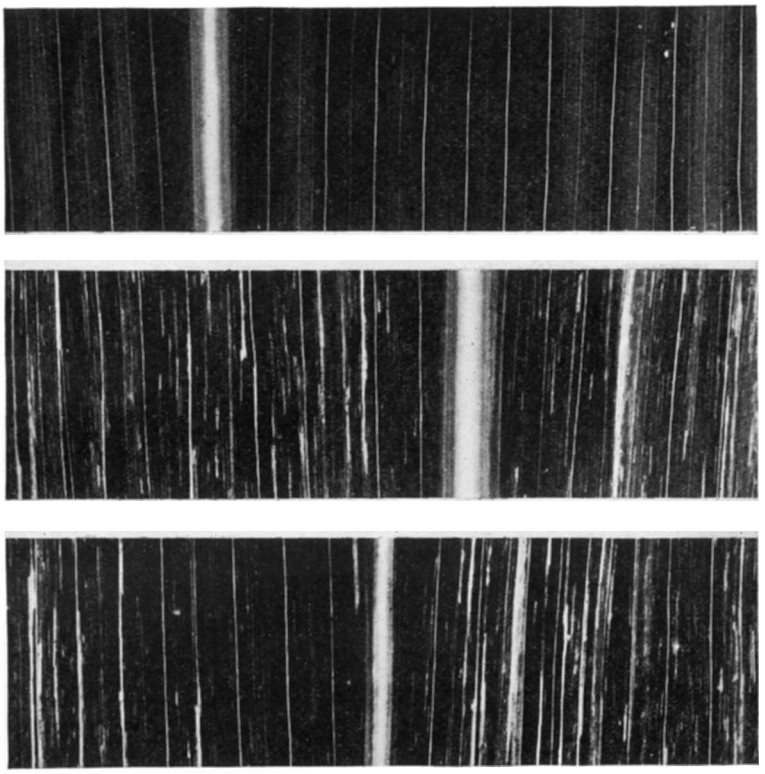


FIGURE 31.—(upper) Surface view of a small region of a leaf of a plant with a normal chromosome constitution. The wide clear band is the midrib. The finer parallel bands are the veins.

FIGURE 32.—(middle) Surface view of a small region of a leaf of a plant with Def1, Def2 and an R2 chromosome. The wide clear band is the midrib. Note the many fine streaks of colorless homozygous deficient tissue.

FIGURE 33.—(lower) Surface view of a small region of a leaf of a plant with Def1, Def2 and two R2 chromosomes. Note the sector to the right of the midrib composed of many fine streaks (one ring sector) and the sector immediately to the left of the midrib with comparatively few streaks (two-ring sector).

usually narrow (figures 37, 38). The two R2 plus one R1 plants have more streaking and more well defined sectors.

That the colorless streaks represent the homozygous deficient tissues resulting from loss of the ring chromosome from the nuclei seems certain from the correlations of the patterns of this tissue in the six types of plants.

The amount of homozygous tissue in a double-deficient plant bears an

inverse relation to the size of the plant, the greater the total amount of homozygous deficient tissue present, the smaller the plant. The one R2 double-deficient plants are smaller than the two R2 plants, which in turn, are smaller than the R1 R2 plants. The three ring plants are practically equal in size and vigor to plants with a normal chromosome constitution.

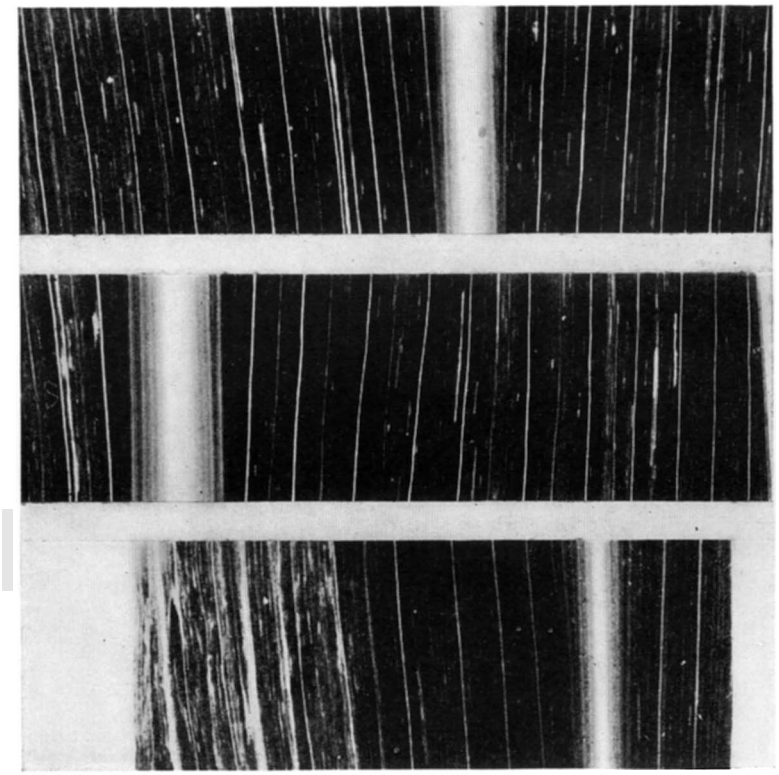


FIGURE 34.—(upper) Surface view of a small region of a leaf of a plant with Def1, Def2 and one R1 chromosome. Note the distribution of streak of homozygous deficient tissue. Compare with figures 32 and 33.

FIGURE 35.—(middle) Surface view of a small region of a leaf of a plant with Def1, Def2 an R1 and an R2 chromosome. Note the R1 sector in the middle of the region to the right of the midrib and the sectors to either side of it which are comparatively free of streaks of homozygous deficient tissue.

FIGURE 36.—(lower) Surface view of a leaf of a plant with the same constitution as that in figure 35. Note the R2 sector (left) and the comparatively small number of homozygous deficient tissue streaks in the tissue to the right.

From homologies of the *bm1* sectors in normal variegated plants (section III), wide sectors of homozygous deficient tissues in the double-deficient plants would be expected to be found if the cells of such tissues could grow and multiply at the same rate as the surrounding cells. It is

reasonable to assume that these cells, with a deficient chromosome complement, would be incapable of an equal growth rate. The juxtaposition of two tissues with unequal growth rates should cause considerable distortion of the cells about the boundaries of the two tissues. This is obvious from the microscopic observations of the two types of tissues in the double-deficient plants. The normal tissues appear to be pulling in the direction of the homozygous deficient tissues. The more rapid growth of the normal

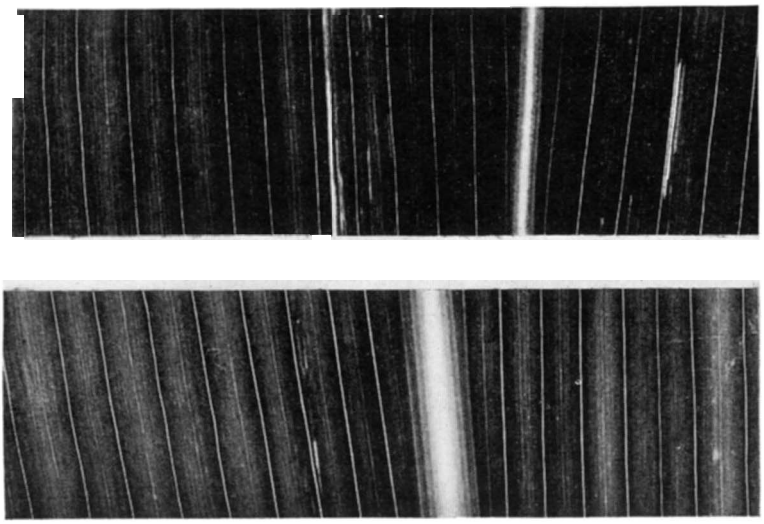


FIGURE 37.—(upper) Surface view of a small region of a leaf of a plant with Def1, Def2, two R2 and one R1 chromosomes. Note the two narrow sectors with streaks of homozygous deficient tissue.

FIGURE 38.—(lower) Surface view of a small region of a leaf of a plant with Def1, Def2, two R1 and one R2 chromosomes. Note the narrow sector to the left of the midrib with a few streaks of homozygous deficient tissue.

tissues may exert enough pull upon the homozygous deficient cells to cause separation at cell boundaries and the production of a hole in the midst of a patch of homozygous deficient tissue. In the one R2 double-deficient plants, which have the most homozygous deficient tissue, the unequal growth rates of the two types of tissue is reflected in a roughened, finely corrugated surface of the considerably reduced and narrowed leaf.

The evidence for considering the colorless streaks as tissues homozygous deficient for the extent of the deficiency in the Def1 chromosome can be briefly summarized:

1. Plants which show these colorless streaks must contain two deficient rod chromosomes, one of which must be the Def1 chromosome. This has been proven by cytological examination, pollen examination and appropriate crosses. Plants homozygous for Def2 R2 do not show the colorless streaks characteristic of the double-deficient plants. It will be shown in

section VII that cells homozygous for the full deficiency in the Def2 chromosome (which is more than twice as long as that in the Def1 chromosome) are incapable of surviving.

2. Plants with these colorless streaks can arise only in crosses where double-deficient plants are expected. For confirmation, see tables 7 to 13.

3. When streaked plants are crossed to normal plants, no streaked plants should appear in the progeny. This has been fully confirmed.

4. The amount of streaked tissue present in a plant and the pattern exhibited should be correlated with the number and kinds of ring chromosomes present. (See table 14 and previous discussion.)

5. A reduced growth rate in the cells homozygous deficient for such a *relatively* large section of the chromosome is to be expected. This is reflected in the small size of the colorless streaks and the distortion of tissues produced by the juxtaposition of tissues with unequal growth rates.

In mentioning the crosses given in tables 11, 12 and 13, little was stated concerning the functional gametes produced by the double-deficient plants. The one R2 double-deficient plants produce four types of gametes (1) Def1, (2) Def2, (3) Def1 R2, (4) Def2 R2. As shown previously, the gamete with Def2 (2 above) will not function in pollen or ovule; (1) above will function in some but not all of the eggs in which it is present but will not function through the pollen. Gamete (4) is equally viable through the eggs and pollen (tables 11 to 13). Gamete (3) is a new type the functioning of which had to be tested. It was found to function readily through the eggs. That it does not function through the pollen in an appreciable amount in competition with (4) is shown by the following test. Pollen of R2 double-deficient plants was placed upon silks of normal *bm1* plants. The resulting plants should be variegated (*Bm1* and *bm1*) and heterozygous for either Def1 or Def2. Since both types of plants can be distinguished through pollen examinations (see section IV), the pollen of 171 plants resulting from this cross was examined. All showed the presence of the Def2 chromosome. Cytological verification was obtained from 28 of these plants. From this evidence, it can be concluded that type (4) pollen grain is the only normally functioning grain produced by these plants.

The functional gametes of the R1 R2 double-deficient plants have been determined. Of the eight possible gametes: (1) Def1, (2) Def1 R1, (3) Def1 R2, (4) Def1 R1 R2, (5) Def2, (6) Def2 R1, (7) Def2 R2, (8) Def2 R1 R2, the egg can transmit all except (5) and possibly (6). The pollen transmits only (7) and (8).

The dissimilarity in functional capacity of the different types of gametes in pollen and ovules is reflected in the *Pr* and *pr* ratios (*Pr*, purple aleurone; *pr*, red aleurone) in reciprocal crosses (table 15). *Pr* is located in the long arm of chromosome V, 18–24 units from *Bm1* (EMERSON, BEADLE and

FRASER, 1935; the percentage of crossing over varies within this range in different strains). As is obvious from the discussion in this paper, *Bm1* is very close to the spindle fiber region in the short arm of chromosome V. A measure of the crossing over between the deficiency (or spindle fiber region) and *Pr* can be obtained directly from the crosses shown in table

TABLE 15

Dissimilarities in reciprocal crosses.

CROSS	*Pr*	%	*pr*	%
Def1 *pr*/Def2 *Pr*/R2×*pr*	60	31.5	130	68.5
reciprocal	5192	81.3	1198	18.7
Def1 *Pr*/Def2 *pr*/R2×*pr*	85	61.2	54	38.8
reciprocal	495	19.0	2106	81.0
Def1 *pr*/Def2 *Pr*/R1/R2×*pr*	177	27.8	458	72.2
reciprocal	4964	83.7	973	16.3
Def1 *Pr*/Def2 *pr*/R1/R2×*pr*	165	66.6	83	33.4
reciprocal	313	18.0	1422	82.0

16. Since no ring chromosome is present, only the pollen grains carrying the normal chromosome function. Crossing over is not altered in plants heterozygous for Def1 and very little in plants heterozygous for Def2.

TABLE 16

CROSS	*Pr*	*pr*	% CROSSING OVER
pr×Def1 *Pr*/+*pr*	2789	9175	23.3
pr×Def1 *pr*/+*Pr*	1286	425	24.8
pr×Def2 *Pr*/+*pr*	562	2201	20.3
pr×Def2 *pr*/+*Pr*	823	184	18.2

In double-deficient plants, with one Def1 and one Def2 chromosome, crossing over is similar to that in plants heterozygous for the Def2 chromosome, "reciprocal" crosses (table 15).

VI. SIMULATION OF THE *bm1* PHENOTYPE THROUGH LOSS OF THE *Bm1* LOCUS

When one-ring and two-ring double-deficient plants were closely examined, fine streaks of brown tissue were seen in the leaf sheath, the midrib and the veins of the leaf. These fine streaks were many times more frequent in the one-ring plants than in the two-ring plants. The shade of color and its association with cells having thickened walls were strikingly similar to the effect produced by brown midrib (*bm1*). In some of the streaks it was obvious that the brown color of the vein was associated

with adjacent parenchyma cells lacking chlorophyll. Since it was known that normal *bm 1* produces a brown color in the lignified cell walls, which can be seen in sections of any lignified tissue, the leaf sheaths with brown streaks were removed, sectioned fresh and examined microscopically. It was immediately observed that the brown color was in the cell walls. It was similar in its range of color and in its deposition in the cell wall to that of ordinary *bm 1*. When brown patches appeared in regions where plastids are normally lacking, that is, heavy-walled schlerenchyma cells, the adjacent parenchyma cells, when too thin-walled to show the brown clearly, frequently lacked plastids. When the adjacent plastid deficient parenchyma cells possessed thickened walls, a brown color could be seen in these walls. The brown-walled and plastid deficient cells formed a definite unit of tissue. It was suspected that these small sectors represented homozygous deficient tissues and that a brown pigmented cell wall would accompany all such tissues. However, the walls of the cells in the colorless streaks in the leaf, except about the veins, are too thin, and the concentration of brown pigment insufficient for a visible effect. If the homozygous deficient cells, produced by loss of the ring chromosome from the nuclei, have brown cell walls, cross-sections of the stem of the single-ring double-deficient plants should show many small, uniformly distributed patches of cells with brown cell walls, just as the leaf is uniformly streaked with fine stripes of colorless tissue. This proved to be true for each of the many R 2 double-deficient plants examined. The sections of the stem were advantageous in relating the brown-walled cells to those which lacked plastids since the normally plastid-carrying parenchyma in the outer region of the stem is thick walled and has a sufficient concentration of brown to be readily seen. Considerable distortion of the bundles and cells was present, particularly in the regions about relatively large patches of brown-walled cells. The types of distortion suggested that the brown-walled cells had grown at a slower rate than the surrounding white-walled cells.

In contrast to the single-ring plants, cross-sections of stems of the two-ring plants showed fewer brown patches. Such would be expected if the brown color were limited to cells which were homozygous deficient; loss of one ring followed by loss of the second ring must occur before the homozygous deficient cells could be produced. Frequently a cluster of brown patches was present in a restricted region of the stem. When the extent and position of these brown patches were traced with a camera lucida and a boundary drawn about the cluster, it was clear that they formed one continuous sector. In the two R 2 double-deficient plants, the number and distribution of the brown patches in such a sector were similar to those of the one R 2 plants. In the R 1 R 2 plants, some of such sectors were similar to the above and some had considerably fewer patches in a cluster.

In the former plant, the sectorial clusters of brown patches correspond to the one-ring sectors seen in the leaf through early loss of one of the R 2 chromosomes. In the latter plant, the two types of sectorials correspond to early losses of the R 1 or R 2 chromosome, respectively.

If the brown-walled, plastid-deficient patches of cells represent the homozygous deficient tissue, the three-ring double-deficient plants should show very few such patches. When present, their distribution should correspond to that observed in the leaves of these plants. The results obtained were in complete agreement. There were very few such patches in these three-ring plants. This is especially true of the two R 1 plus one R 2 plants.

It might be stated here that plants homozygous for Def 2 R 2 do not show these patches of brown-walled, plastid-deficient cells. The homozygous deficient cells in these plants, as will be shown in the next section, are inviable.

(1) The correlation of the brown cell walls with the cells lacking plastids, (2) their presence in narrow streaks, (3) the restricted growth capacity of these cells, (4) their uniform distribution in one-ring double-deficient plants, (5) their reduced frequency in two R 2, R 1 R 2, and three-ring plants, respectively, and (6) the distribution patterns in sectorials in these two- and three-ring plants summarize the homologies of the brown-walled cells with the streaks of colorless cells in the leaf which were shown to be homozygous deficient in the previous section.

The brown-walled cells are homozygous deficient for the full extent of the deficiency in Def 1. At this point it should be emphasized that the locus of *Bm 1* is carried by the ring chromosome and that there is no locus for *Bm 1* or *bm 1* in the deficient rod chromosomes. If the brown color in the walls of these homozygous deficient cells is similar to *bm 1*, it should appear in the development of the wall at the time lignification sets in, as has been shown for normal *bm 1* (section III). Furthermore, the brown color of the walls in such cells, when adjacent to white-walled cells, should be diluted on the side adjacent to the white-walled cells, as observed in normal variegated plants. Thirdly, when exposed to intense light, the brown color should fade just as normal *bm 1* fades on exposure to light.

Immature cells are present at the base of each leaf sheath. These cells rapidly merge into fully mature cells just above this region. If a leaf showing a prominent brown streak is removed and serial sections made to trace the brown streak as it emerges from the immature cells, it becomes obvious that the brown color appears as the cell walls become lignified in a manner fully comparable to normal *bm 1*. Thus, the time of appearance of the color in the development of the wall is similar to that in normal *bm 1* plants.

When examining the brown patches in sections of the stem it was ob-

served that the color of the brown in the walls of the plastid deficient cells was considerably diluted on the side adjacent to the white-walled, plastid containing cells. This, in turn, is comparable to the observations in normal variegated plants.

To test the third correlation, fading of the color when exposed to light, black paper was placed over part of a conspicuous brown streak in a leaf sheath or midrib of a leaf. Upon removal, several weeks later, the brown color under the paper had retained its intensity, that above and below had lost much of its intensity. In this respect, the brown of the homozygous deficient tissues is similar to normal *bm1*.

The range in color of normal *bm1* varies in some plants from a deep wine red to a light orange, the deep red color being present in the stem toward the basal nodes, the light orange in the regions toward the top of the plant. This same gradation of color in comparable regions was found in the brown patches of the double-deficient plants.

To summarize: The two browns, the normal *bm1* and the brown produced in cells possessing no locus for this gene, are comparable in (1) time of appearance of the color in the development of the cell walls, (2) in dilution of the color in regions adjacent to white-walled (*Bm1*) cells, (3) in loss of intensity of color when exposed to light, and (4) in range of color variations in specific regions of the plant. No differences could be detected in the expression and behavior of the brown color in the two cases. Although it has not been proven that *bm1* is due to a deficiency in chromosome V, it can be stated that absence of the locus of *Bm1* will duplicate the phenotypic expression of *bm1*.

VII. THE PRODUCTION AND APPEARANCE OF PLANTS HOMOZYGOUS FOR Def2 R2

Mention of plants homozygous for Def2 R2 has been made in the previous section. The first of these plants appeared in the progeny of sib crosses of Def2/*bm1*/R2 through the union of two gametes each containing Def2 and R2. Such plants were to be expected. However, as in the case of the double-deficient plants, no prediction could be made as to appearance other than that they should not be typical *bm1* or variegated plants. Nevertheless, they are readily recognizable. They are short, usually deep green in color, with thickened, erect leaves and do not show normal *Bm1*–*bm1* variegation. The streaks of colorless tissue, so characteristic of double-deficient plants are absent from the leaves.

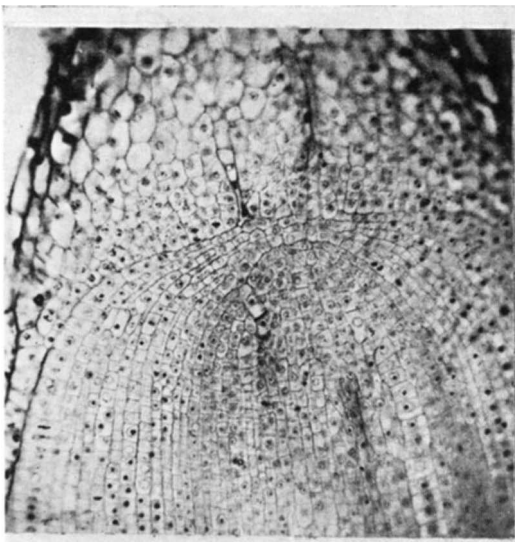
In later generations many plants homozygous for Def2 R2 were obtained. Cytological examination of microsporocytes at pachytene has confirmed the accuracy of the phenotypic classification (table 14). The two deficient chromosomes V synapse homologously throughout their length.

The changed arm ratio produced by the deficiency is clearly evident. In the normal chromosome V, the chromomeres adjacent to the spindle fiber region on the short arm are relatively large and deep-staining. With the removal of this section in the production of the ring chromosome, small, light-staining chromomeres are brought adjacent to the spindle fiber region, making this deficient chromosome V readily recognizable at pachytene. The two ring chromosomes either synapse to form a ring-shaped bivalent, similar to the rings in photographs 21, 22, Plate II, or remain unsynapsed and appear as two collapsed rings, similar to the rings in photograph, 19, Plate II. There is no tendency for the ring chromosomes to synapse with any part of the deficient rod chromosomes V.

The pollen of these plants is always highly abortive. Only those grains possessing a ring chromosome are normal in appearance and capable of functioning.

The loss of one ring chromosome followed by loss of the second ring chromosome should give rise to cells homozygous deficient for the full extent of the deficiency in the rod chromosomes. If these cells were viable and capable of multiplying, evidence of such tissue would be expected in the leaves of these plants from homologies with the double-deficient plants described in the previous two sections. Since evidence of such tissue did not appear in the leaf, it was suspected that the cells whose nuclei were homozygous deficient for this relatively long section were inviable or incapable of further multiplication. Longitudinal sections of growing points of roots of these plants were made with the view of finding evidence of the fate of these cells since such cells necessarily are formed.

In all root meristems of plants homozygous for Def 2 R 2, the following peculiar cell type was found. It was confined to roots of these plants, not being present in normal or double-deficient plants. Very much enlarged, heavily vacuolate groups of two or more cells in positions indicating relation in descent, were observed in regions of the root where enlarged cells are not normally encountered (figures 39, 40, 41). When a mitotic figure was observed in one of these cells, the chromosomes were short, thickened and sometimes irregularly placed in the spindle. Daughter telophase nuclei were sometimes joined by a connecting nuclear bridge. In older regions of the root, degeneration processes in these cells, depicted first by an aberrant staining reaction of the nucleolus followed by a pycnotic condition of the cytoplasm (figure 42), and finally by a collapse of these cells due to the pressure of the normal surrounding cells (figure 43). Should the space formerly occupied by these cells be extensive, the surrounding cells divide in planes other than normal, filling in this space which might otherwise have remained as a hole in the tissue (figure 43). The numbers of such patches of enlarged cells varied considerably in different roots. Some had many, others relatively few.



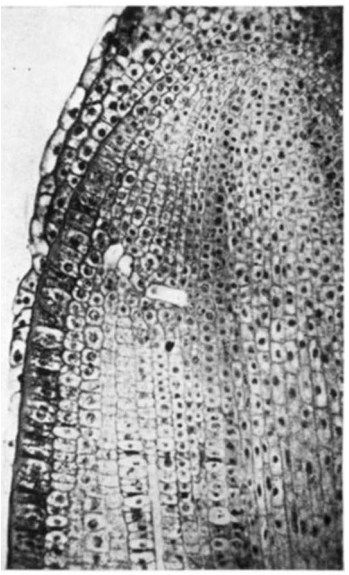


FIGURE 39.—(left) Longitudinal section of a root tip of a plant homozygous for Def2 and R2. Note the two adjacent, enlarged (homozygous deficient) cells near the tip of the growing point and the row of enlarged, degenerating (homozygous deficient) cells in the root cap directly above. Mag. ×160.

FIGURE 40.—(right) Similar to figure 39. Note the transverse row of four enlarged (homozygous deficient) cells. Mag. ×160.

The conditions depicted conform strictly to expectancy if these cells represent the homozygous deficient cells resulting from loss of the ring chromosomes from their nuclei. Loss of one ring chromosome would cause no obvious tissue alteration since tissues heterozgyous for Def2 are normal in appearance. Loss of the second ring chromosome, such loss taking place at anaphase by being left at the equatorial plate of a mitotic figure, would result in two adjacent cells whose nuclei would be homozygous deficient for the extent of the deficiency in the Def2 rod chromosomes. The occurrence of pairs of enlarged cells has been mentioned. In many cases, it was possible to see the cast-out ring chromosome in the cytoplasm of one of these cells but also in many cases, degeneration processes in the cytoplasm had advanced too far for such a determination. No ring chromosome was observed in the few cells which had mitotic figures but the small ring chromosome could have been obscured by one of the rod chromosomes so that evidence of the homozygous deficient conditions of these cells from direct observations of the chromosome constitution was not considered satisfactory.

The numbers of such patches of cells and their distribution in the different roots lend strong supporting evidence for the homozygous deficient

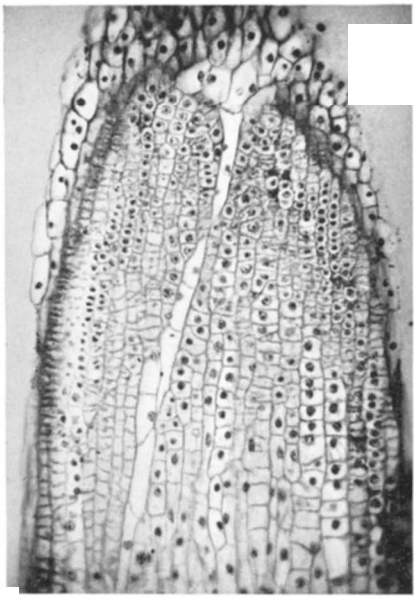


FIGURE 41.—Longitudinal section of a root tip of a plant homozygous for Def2 and R2. Note the row of very much enlarged (homozygous deficient) cells. Mag. ×160.

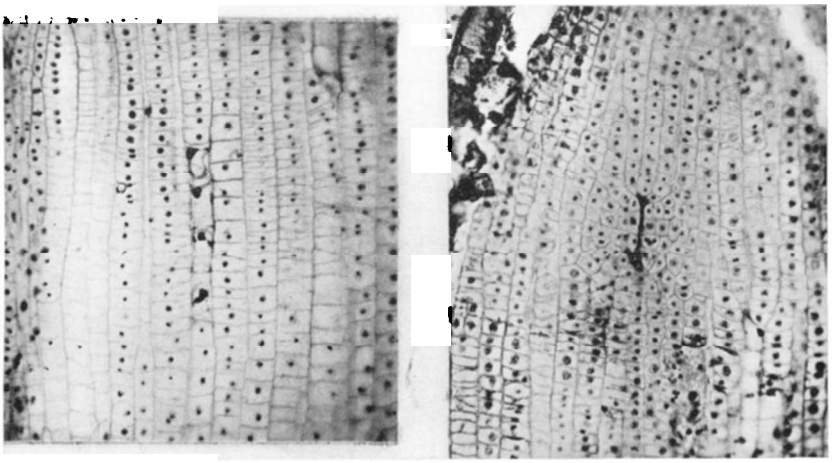


FIGURE 42.—(left) Longitudinal section immediately below the actively meristematic region of a root of a plant homozygous for Def2 and R2. Note the row of four enlarged, degenerating (homozygous deficient) cells. Mag. ×160.

FIGURE 43.—(right) Longitudinal section of a root tip of a plant homozygous for Def2 and R2. Note that there has been a proliferation of cells about the degenerated streak. Mag. ×160.

nature of these cells. They were present in all roots but the frequency was variable. In a root in which only one ring chromosome is present in the nuclei of the normal cells, a high frequency of such patches should be

observed. In this latter case, every loss of the ring chromosome would be reflected in a patch composed of two or more such cells. In roots where two ring chromosomes are present in many of the nuclei, loss of one ring chromosome followed by loss of the second ring chromosome or the very occasional simultaneous loss of both ring chromosomes would have to occur to produce such a patch. Thus, the frequency of such patches in these roots would be considerably less than in the former type of root. Since a root could contain but one ring chromosome in its normal cells or be a mosaic of one- and two-ring sectors of various sizes, variations in the numbers of patches of abnormal, enlarged (homozygous deficient) cells in the different roots of the homozygous Def 2 R 2 plants is to be expected.

(1) The presence of these patches of abnormal cells only in plants homozygous for Def 2 R 2 and their absence in double-deficient and normal plants, (2) the observed presence of the R 2 ring in the cytoplasm of one of these cells in many cases, (3) the frequency and distribution of these patches in different roots, (4) their arrangement in descent, that is, rows of two or more, (5) the rapid distintegration of these cells, and finally (6) the absence of evidence of homozygous deficient tissues in the mature cells of the stalk and leaves of these plants as contrasted with double-deficient plants, strongly support the view that they represent the homozygous deficient cells since such cells *must* be produced in these plants. Since early death is the fate of these cells, it is clear why the leaves of these plants are not streaked with modified tissues which could be interpreted as representing the homozygous deficient tissues.

At this point it might be mentioned that a third deficiency (Def3), not previously considered in this paper, which is outside the limits of Def1 but within the limits of Def2 and therefore covered by the R2 chromosome, produces the same pattern of homozygous deficient tissues as that exhibited by the R2 double-deficient plants when the constitution of the plant is Def2/Def3/R2. However, in this case, the homozygous deficient tissue has an even poorer growth capacity than tissues homozygous for the deficiency in the Def1 chromosome. To return to the Def2 chromosome, the deficiency is apparently too long, the loci deleted too important in cell physiology for survival of cells which are homozygous deficient for this region.

When pollen of plants homozygous for Def 2 R 2 is placed upon silks of normal *bm 1* plants, the progeny should all be variegated for *Bm 1* and *bm 1* except in those cases where the ring chromosome has been lost sufficiently early in development to be absent from that part of the embryonic tissue which produces the visible part of the plant.[2] In this latter case,

[2] Functional male gametes without a ring chromosome, which would give rise to *bm 1* plants, might be produced as the result of a loss of the ring chromosome in the second microspore mitosis.

the plant would be *bm1*. The progeny of 13 such crosses totalled 1,829 variegated to 68 *bm1* plants. To exclude the possibility that this 3.5 percent of *bm1* plants represented contaminations, 42 of them were examined for the presence of the deficient chromosome. In 41 of these plants the deficient chromosome was present; one represented a contamination.

The number of kernels which develop on the ear of a plant homozygous for Def2 R2 is always very small, ranging from 0 to 30 kernels with the average about 10. This would be expected since only those gametes which possess a ring chromosome are functional. Many of the ears should arise from one-ring sectors and others should be composed of both one- and two-ring sectors. Since, in the two-ring sectors, the two small ring chromosomes frequently do not synapse, of if so, do not remain together at the first meiotic mitosis, their elimination in the two meiotic mitoses is frequent. Relatively few ovules with eggs containing a ring chromosome would be expected and thus only a few kernels should be expected on an ear. Since the exsertion of anthers and shedding of pollen is dependent upon the presence of a number of well-formed grains in the anther sac, pollen collected from such plants usually contains enough functional grains (those with a ring chromosome) to produce a complete set of seed when placed on silks of normal plants. Since both deficient chromosomes are similar, there is no selection in favor of one or the other chromosome either through the pollen or eggs. Reciprocal backcrosses of *Pr/pr* plants give 1:1 ratios.

VIII. PHENOTYPIC EFFECTS OF ALTERED RING CHROMOSOMES

As stated in section II, ring-shaped chromosomes not only are lost from nuclei during somatic mitoses but also change in size. They may increase in size through duplications of segments composing the ring or decrease in size through losses of segments. The frequency of such changes depends upon the size of the ring chromosome. In the case of the small R2 chromosome, such changes are relatively infrequent. Cytological examinations of large numbers of plants and many nuclei within each plant have given abundant evidence, however, of such changes. The R2 chromosome has been seen to increase to approximately seven times its original size and to have decreased to several chromomeres. The duplicated segments do not result in tissues showing extensive modification. Slight modifications are visible with higher multiples resulting in smaller plants with thickened, erect leaves.

In the case of the double-deficient plants, loss of the ring chromosome

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In this case, the tube nucleus would contain the ring chromosome and could be expected to function normally in pollen germination. Thus, sperm nuclei lacking an R2 chromosome, could be introduced into the embryo sac.

results in viable tissues which are homozygous deficient for the extent of Def 1, as shown in the previous sections. This tissue is poor in growth capacity, has brown cell walls similar to normal *bm 1*, possesses no plastids and, on continuous exposure to sunlight this tissue in the leaves dries and shrivels. In Def 1 the four chromomeres adjacent to the spindle fiber attachment region on the short arm of chromosome V have been removed. The R 2 chromosome includes these four chromomeres plus the next five chromomeres.[3] Should changes in size of the ring chromosome delete one or more of the four chromomeres covering the deficiency in Def 1 or fractions of them, tissues homozygous deficient for sections within the limits of the deficiency in Def 1 should result. Since tissues homozygous for the full extent of the deficiency in Def 1 are viable, fractional deficiencies within this region might be expected to have even better viabilities and should reveal themselves by wider sectors of homozygous deficient tissue having a specific modification, this modification should be repeatedly encountered as a sectorial in large populations of such plants. In other words, the nature of these sectorials should vary depending upon the fraction of the four chromomeres which has been deleted from the ring chromosome. The same region should be independently deleted in a number of instances and therefore the same type of sectorial should be produced in a number of different double-deficient plants.

A large number of sectorials have been found. These are classified as simple mutant sectorials, involving a single recognizable change, and compound mutant sectorials, which are combinations of the simple mutant types. Only those sectorials which are readily recognizable because of their good growth capacity are included in this classification. The simple mutant sectorials are as follows: (1) *translucent white* ("onion skin") with colorless cell walls, no plastids; (2) *opaque white*, with colorless cell walls, colorless plastids; (3) *deficiency-brown-midrib*, similar in detail to tissues homozygous for the *bm 1* gene; (4) *pink* colored tissues with colorless cell walls, colorless plastids; (5) *blotched* chlorophyll pattern. The following types of compound mutant sectorials have been found: (1) pink, deficiency-*bm*, viable in sunlight; (2) pink, deficiency-*bm*, dries in sunlight; (3) opaque white, deficiency-*bm*; (4) blotch, dries in sunlight; (5) blotch, deficiency-*bm*, dries in sunlight. The compound mutant sectorials are far more frequent than the simple mutant sectorials. The summation of the characters exhibited by these sectorials is equal to the characters present in tissues homozygous for the full extent of the deficiency in Def 1, with the exception that the tissues with the total deficiency are incapable of growing at

[3] This is an estimate of the number of chromomeres. The chromomeres in maize appear to be compound and may show more or less in a particular region depending upon the state of contraction or the degree of stretching in a particular preparation.

a rate which will result in a large sector, whereas the simple mutant sectorials outlined above more nearly approximate the normal growth rate.

The theory is proposed that the simple mutant effects are due to losses of one particular region from the ring chromosome, each mutant effect being related to one particular region. The compound mutant effects are then produced by losses of several adjacent regions within the ring chromosome. On this theory, a linear arrangement of the mutant effects can be referred to the chromosome in the following order: pink, deficiency-*bm*, dries in sunlight and blotch. Translucent white must be removed from deficiency-*bm* and opaque white close to it. The presence of a ring chromosome in the cells of a sectorial has been determined cytologically when the sectorial included a part of the tassel. If an altered ring chromosome was present in the mutant sectorials, streaks of tissue homozygous deficient for the full extent of the deficiency in Def1 should appear within these sectors through loss of this altered ring chromosome. This is especially easy to confirm in sectorials of deficiency-*bm* and blotch.

In double-deficient plants with two ring chromosomes, the same types of sectorials are encountered. Here, however, all of the sectors are not solid; many of them are variegated. The explanation is similar to variegated patterns of *Bm1* and *bm1* in plants which have two normal chromosomes V carrying *bm1* and two ring chromosomes carrying *Bm1* and to the patterns of homozygous deficient tissues in double-deficient plants with two ring chromosomes. One ring chromosome has suffered a deletion. Production of homozygous deficient tissues showing the character of the deletion can occur only after loss of the second normal ring chromosome. Thus the sectorial is a variegate of normal and mutant tissues. Should the altered ring chromosome be lost in a somatic mitosis many generations later than that which produced the alteration, the tissues resulting after this loss will appear as normal one-ring double-deficient tissue inserted into variegated tissue since only the one normal ring chromosome is left in this tissue. These variegated sectors, mosaics of two types of patterns, are frequently encountered in the two-ring double-deficient plants.

If the theory that the mutant sectorials are due to losses of specific regions within the ring chromosome is correct, plants homozygous for Def2 R2 should show mutant sectorials similar to those exhibited by the double-deficient plants plus a number of types not exhibited by these latter plants since the extent of the deficiency in Def2 includes the same four chromomeres plus five more. This has been fully realized. Simple and compound mutants of pink, deficiency-*bm*, blotch, and opaque white plus an additional number of chlorophyll and growth types have been found.

Should a change occur in the ring chromosome, it should remain un-

altered for most of the cell generations and thus should be capable of being passed from one generation to the next, provided the original alteration occurred in tissues which give rise to the gametes. A female gamete containing a deficient rod chromosome plus an altered ring chromosome united with a male gamete with the deficient rod chromosome and a normal ring chromosome would give rise to plants which are complete mosaics of normal and changed tissues. The changed tissues should be of the same type in an individual plant. In some cases there should be many female gametes in the original plant with this same altered ring chromosome if the alteration occurred early in the ontogeny of the ear. Thus there should be many individuals in a culture resulting from the outlined cross each exhibiting the same type of variegated pattern involving the identical mutant characters. This has been realized in one culture. These plants are useful for cytological determinations of the nature of the modification in the ring chromosome but a detailed account of this will appear in a later paper. It is not the purpose of this paper to consider all the evidence concerning changed rings and their mutant effects. It is too extensive. It is necessary to mention it, however, since a comprehension of the mitotic behavior of ring chromosomes would lead one to anticipate the presence of such changed rings with phenotypic effects.

DISCUSSION

The presence of ring-shaped chromosomes and a suggestion as to their inconstant behavior in somatic tissues was first published by NAWASHIN (1930; see also, 1936) for a single plant of *Crepis tectorum*. Since this time, ring-shaped chromosomes have been found or produced in Drosophila (L. V. MORGAN 1933; STURTEVANT and BEADLE 1936; SIDOROV, SOKOLOV and TROFIMOV 1936; SCHULTZ and CATCHESIDE 1937), Trillium (HUSKINS and HUNTER 1935), Locusta (WHITE 1935), Pisum (ATABEKOWA 1936), Tradescantia (HUSTED 1936), Tulipa (UPCOTT 1937) and Nicotiana (CLAUSEN, unpublished). In none of these cases has an intensive cytological study been made to determine the mitotic behavior of the ring chromosomes. In Zea a number of ring-shaped chromosomes have been found (MCCLINTOCK 1932, 1933, and unpublished; RHOADES and MCCLINTOCK 1935; CREIGHTON, unpublished; CAMERON, unpublished). In all cases studied, the mitotic behavior of these ring chromosomes has been similar. Deletions of sectors from the ring, duplication of sectors, additions in numbers of ring chromosomes of varying constitutions and total loss of the ring chromosome from the nuclei have been observed. In Drosophila, phenotypic effects which could be definitely ascribed to alterations in constitution of the ring-shaped X chromosome have not been described. The chances of detecting such an altered ring chromosome would depend

on the rate at which aberrant anaphase figures would be formed. As pointed out in section II, the rate at which alterations occur in ring chromosomes in maize depends upon the length of the chromonema composing the ring. Since the primary cause of double-sized and interlocking rings, the first step in the production of altered ring chromosomes, has not been determined, it is difficult to argue that the behavior found in Crepis, Nicotiana (R. E. CLAUSEN, unpublished) and Zea would likewise be found in Drosophila.

HUSTED (1936) reported double-sized, continuous ring-shaped chromosomes, in some of which the two chromonemata made a half turn around each other, and interlocked rings in the microspores of Tradescantia following irradiation. Such configurations should be expected if, before irradiation, the chromosomes were split and the two chromatids were relationally coiled about one another. Although the Tradescantia cases were not followed beyond the microspore stage, HUSTED attempted to explain the method by which such figures could be produced throughout the life of a plant. The presence of a continuous ring with two spindle fiber attachment regions or two interlocked sister ring chromatids at somatic anaphase, whatever the method by which it originally arose, would result in a chromatin bridge the strands of which would eventually break. HUSTED assumes that each chromosome is split at somatic anaphase. "A broken end of an anaphase chromatid (caused by breakage of continuous or interlocking rings) may unite as often with the broken end of its sister as with its other broken end. Ring chromosomes which are continually breaking might persist in this way. Whenever the two broken ends of one chromatid unite, however, and the two sister strands are *not twisted*, a 'disjunctional' [two sister halves free to disjoin] ring-shaped chromosome would result. There would be a trend toward displacement of the 'continuous' and 'interlocked' types by 'disjunctional' rings which separate without breaking unless relational coiling of chromatids is increased before each union of broken ends" (page 551). The disjunctional rings, once established, should remain free from further complications. Such a theory of the continuous appearance of double-sized and interlocked ring chromosomes throughout the life of a plant cannot account for the origin of such configurations in maize, although it may contribute to some of the cases. This arises from the following consideration. In plants homozygous for Def 2 and R 2, the two split halves of the ring chromosomes separate freely from one another at anaphase I in most of the sporocytes, that is, are "disjunctional," not continuous or interlocked. Thus, the ring chromosome in the majority of the spores has been derived from a ring chromosome whose two split halves have separated freely in the previous division. As seen in section VII, only those gametes which possess a ring chromosome

are functional. If HUSTED'S theory of the continuous appearance of double-sized and interlocked rings were correct for maize, most of the individuals resulting from the cross of normal *bm1* by homozygous Def2 R2 should be totally *Bm1* through elimination of the cause of loss of the ring chromosomes in somatic mitoses. As shown on page 364, all plants resulting from the cross are variegated for *Bm1* and *bm1*. The cause of the interlocked or double-sized rings arises anew and is dependent upon the length of the chromonema in the ring for its frequency. The primary cause of these configurations may be related to the occurrence of a crossover between the two split halves of a ring chromosome. The high frequency of normally disjoining ring chromosomes, even when the chromonema of the ring is long, leads one to conclude that the plane of splitting or method of reproduction of a new chromonema from an old, is definitely predetermined along a given plane and that trouble might arise only during or after the split had occurred at some point of tension or torsion in the chromosome, that is, a tension relieved by an interchange of segments at this point, resulting in a somatic crossing over between the two chromatids. Unless two crossovers occurred in a chromosome, only continuous, double-sized rings would result. As noted in section II, there was a high frequency of such continuous, double-sized rings as compared to other possible complicated configurations. The maize chromosomes are too small in somatic cells to give a clear picture of the configurations in each cell other than in those with simple continuous rings. On this theory, interlocked rings could arise (1) after two somatic crossovers in which the second crossover did not counteract the first, or (2) following a previous anaphase break and reunion of broken ends in which a twist in the chromonema was present before the union occurred. In this latter case, it is not necessary to assume that the chromosome is split before union of broken ends occurs as interlocked or continuous rings could arise depending upon the plane of splitting or reproduction of the chromonema assumed. On this hypothesis, the proportion of interlocked rings to continuous rings would be expected to be greater with large ring chromosomes than with small ring chromosomes. Likewise, sister nuclei in these plants should show aberrant ring configurations more frequently than sister nuclei in plants with small ring chromosomes.

Since no rod-shaped fragment chromosomes have been observed to arise from ring-shaped chromosomes through breakages in somatic anaphase and telophases, it has been assumed that union of broken ends must occur. In an effort to determine if two broken ends which enter a nucleus would unite, the following experiment was outlined. A plant was made heterozygous for two inversions on two different chromosomes. The inversions did not include the spindle fiber attachment regions and therefore, should give

bridges at anaphase I (or II) and free fragments after a crossover within the inverted segment (McCLINTOCK 1933; MÜNTZING 1934; SMITH 1935; RICHARDSON 1936; DARLINGTON 1936; UPCOTT 1937; SAX 1937 and others). With normal crossing over, the size of the fragment is constant for any particular inversion. In the two inversions chosen, the size of the fragment produced by each was readily distinguishable. In many sporocytes of the plants with the inversions, two chromatin bridges with their respective fragments produced by a crossover in each of the two inversions, were present at anaphase I. In most cases, the bridges of chromatin had broken by late telophase and the broken ends had been drawn into the telophase nuclei. Thus broken ends from two chromosomes entered the same nucleus. If fusion of these broken ends occurred, the product of this fusion should be obvious in some of the second division figures in the cells of which two recognizable fragments were present. In maize, the two second meiotic anaphase figures are oriented in one plane and thus can be observed together. However, the evidence for fusions was negative. It was then considered that the broken ends might be too far apart from one another, in many cases, to join together before the second meiotic mitosis. Therefore, cases of double-crossing over in plants heterozygous for a single inversion were investigated. When a four-strand double crossover occurs within the inverted region, a double bridge involving all four chromatids, and two free fragments of similar size are found in anaphase I. The strands composing these bridges break and the two broken ends, lying side by side, enter the same nucleus. If fusions of these broken ends occurs in each telophase I nucleus, each sister cell in anaphase II should show a chromosome involved in an anaphase bridge. The sister cells to be examined can be distinguished by the two fragments of recognizable dimensions. The evidence for fusions of broken ends entering the same nucleus was likewise mainly negative in this case. On the supposition that each chromatid might already be split in anaphase I and that fusions occurred between broken ends of the two split halves of a chromatid rather than between the broken ends of each chromatid, anaphase configurations in the microspores of these plants were examined. Such fusions should give rise to a chromatin bridge at anaphase of the first mitosis in the spore (see Sax 1937). Upon examination, a chromosome showing a bridge configuration was found in a number of spores. By a method which will be described in a separate publication, it was possible to show that the spores which have a bridge configuration likewise possess a chromosome which was broken during the meiotic mitoses. The examinations indicated, also, that such fusions probably always occur. Such evidence illustrates, directly, the tendency of broken ends to fuse. One would be tempted to use this information and transfer the process to the somatic chromosomes. How-

ever, the evidence at present indicates that one is not justified in doing so. Until the contradictory features of this evidence are completely analysed, the author is unwilling to interpret the ring chromosome behavior on this basis.

Viable homozygous deficiencies in Drosophila giving effects similar to "genes" known to be located in the region which has been made deficient, have been described by MULLER (1935) for yellow and achaete, EPHRUSSI (1934), STERN (1935) and DEMEREC and HOOVER (1936) for yellow, STURTEVANT and BEADLE (1936) for scute, EMMENS (1937) for roughest–2, and OLIVER (1937) for facet. Viable individuals homozygous deficient for a region possessing no known genes have been described by DEMEREC and HOOVER. Homozygous deficiencies producing immediate or delayed effects in zygotes and embryos, which eventually result in death to the individual, have been described by POULSON (1937). As far as the author is aware, homozygous deficiencies in plants which simulate a gene known to be located in the deleted segment, have been found only in the *bm1* case described in this paper. Evidence that the known genes in the Drosophila cases might be due to deficiencies in the regions involved, has been given only for the gene roughest–2. Simulation of the known gene by a region deficient for its locus has been claimed for the other cases and applies likewise to the *bm1* case in Zea. It is unprofitable at present to estimate to what extent homozygous deficiencies are responsible for known genic effects. That some of these may be related to position effects seems possible from the accumulating evidence in Drosophila. In how many of these cases the factor of a minute deficiency can be eliminated, remains to be decided, granting that the presence of a deficiency introduces the possibility of a position effect. In the case described in this paper, the two independent segments of chromosome V, the deficient rod and the unaltered ring fragment, produce effects, with regard to the *Bm1* character, which are indistinguishable from that produced by a normal chromosome V carrying *Bm1*. The only evidence so far obtained of a "position effect" is derived from the appearance of plants homozygous for Def2 and R2 and from the lack of expected transmission through the pollen of gametes with Def1 R1 or Def1 R2. In neither case can the factor of a minute deficiency be eliminated, the deficiency affecting plant growth in the former case and pollen transmission in the latter case.

The method of producing phenotypic effects by alterations in ring chromosomes in plants with two deficient chromosomes plus a covering ring fragment, briefly described in section VIII, should prove useful in analysing in considerable detail the genetic composition of small sections of chromosomes. The effects produced have been ascribed to minute homozygous deficiencies rather than to position effects since homozygous

deficiencies must be produced by alterations in the ring chromosomes. If individual regions within the deficient segment produce particular effects independent of their neighbors, combinations of these effects should be produced by loss from the ring chromosome of two or more of these regions. Since the method by which the ring chromosomes become altered should delete adjacent segments from the ring chromosome, an orderly arrangement of compound effects should result. The order of the particular regions within the ring chromosome which have specific effects could be developed from analyses of the individual effects contributing to the compound effects. Since the results obtained so far substantially correspond to the requirements of this theory, the notion of a particulate nature of the composition of this region of the chromosome has been retained. The development of this method of analysing the composition of sections of chromosomes has just been started. It would be premature to draw rigid conclusions from the results so far obtained. Three deficiencies of chromosome V, each of which can be covered by a ring fragment, are available for this study. Two of these fall within the range of the third. Correlations of results from all three deficiencies should conform to a predicted pattern if the above theory is correct. Until the evidence from these studies has accumulated, no attempt will be made to force a particulate theory of the organization of the chromosome in contradistinction to a continuum theory. The former will be retained as a working hypothesis until the evidence definitely requires a modified view.

SUMMARY

1. Two cases of a deficiency adjacent to the spindle fiber attachment region in the short arm of chromosome V involving approximately 1/20 (Def 1) and 1/7 (Def 2) the length of the chromosome, respectively, were produced by X-ray treatment. The piece deleted in each case formed a small ring-shaped chromosome, R 1 and R 2, respectively. In each case a section of the spindle fiber attachment region was removed to the ring chromosome and a section was retained by the deficient rod chromosome. Since the deficient rods and compensating ring chromosomes possessed a functional section of the spindle fiber attachment region, each was capable of participating successfully in the mitotic process. The ring chromosome in each case carried the locus of *Bm 1* (allele of *bm 1*, brown mid-rib, producing a brown color in the lignified cell walls). The rod chromosomes lacked the locus for *Bm 1*.

2. Plants with two normal chromosomes V carrying *bm 1* (or one normal chromosome V carrying *bm 1* and a deficient chromosome V) plus either ring chromosome are variegated for *Bm 1* and *bm 1* through frequent losses of the ring chromosome from the somatic nuclei.

3. Somatic loss of ring-shaped chromosomes is described.

4. The abnormal mitotic behavior of large and small ring-shaped chromosomes is contrasted. Large ring-shaped chromosomes frequently change in size and chromatin constitution during somatic mitotic cycles. Small ring-shaped chromosomes are more frequently lost from nuclei during mitotic cycles although changes in size and chromatin content sometimes occur.

5. The frequency of aberrant mitotic configurations of the ring chromosomes, leading to loss or change in size, is related to the length of the chromonema composing the ring. The longer the chromonema the more frequent the aberrant configurations. With small ring-shaped chromosomes, whose aberrant mitotic configurations usually lead to loss of the ring chromosome from the nuclei, the extent of variegation (2 above) is proportional to the size of the ring chromosome.

6. Functional gametes with Def1, Def1 plus R1, and Def2 plus R2 were obtained. The functional capacities of these two deficiencies with various combinations of the ring chromosomes were tested. Some of these were functional, others were not.

7. Plants with Def1/Def2/R2 (R2 covers the deficiencies in the rod chromosomes) were a uniform mosaic of tissues heterozygous and homozygous for the full extent of the deficiency in Def1 (the smaller deficiency) through losses of the ring chromosome during somatic mitosis. The patterns of homozygous deficient tissues in plants with these two deficient rod chromosomes plus various combinations of ring chromosomes (R1, R2, two R2, R1 plus R2, two R1 plus one R2, two R2 plus one R1) have been compared and agree with expectancy on the basis of the cytological analysis of ring chromosome behavior in mitosis and the analysis of variegation (2 above) produced in plants with these same combinations of ring chromosomes.

8. The homozygous deficient tissues, lacking a locus for *Bm1*, have the phenotypic expression of *bm1* in their cell walls.

9. Plants homozygous for Def2 R2 have 22 chromosomes in their zygotes. Although the chromosome number has been increased, there has been no increase in the genome. Losses of the R2 chromosomes during development produce cells homozygous deficient for the extent of the deficiency in Def2. These cells are abnormal in appearance and are short lived, degenerating before maturity of the surrounding cells.

10. In plants with two deficient chromosomes and one or more compensating ring chromosomes, somatic alteration in constitution of a ring chromosome is reflected in modified tissues having mutational characteristics. A number of repeatedly encountered, distinct types are briefly described. One type is indistinguishable from normal *bm1*.

ACKNOWLEDGMENTS

Much of the investigation described in this paper was undertaken at CORNELL UNIVERSITY with the aid of a grant from the ROCKEFELLER FOUNDATION. The original material for this investigation was supplied by Dr. L. J. STADLER to whom the author is very grateful. To Dr. R. A. EMERSON of CORNELL UNIVERSITY the author is particularly indebted for his generosity in placing the facilities of the Department of Plant Breeding at her disposal and for his continued encouragement.

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