# THE ASSOCIATION OF MUTANTS WITH HOMOZYGOUS DEFICIENCIES IN ZEA MAYS

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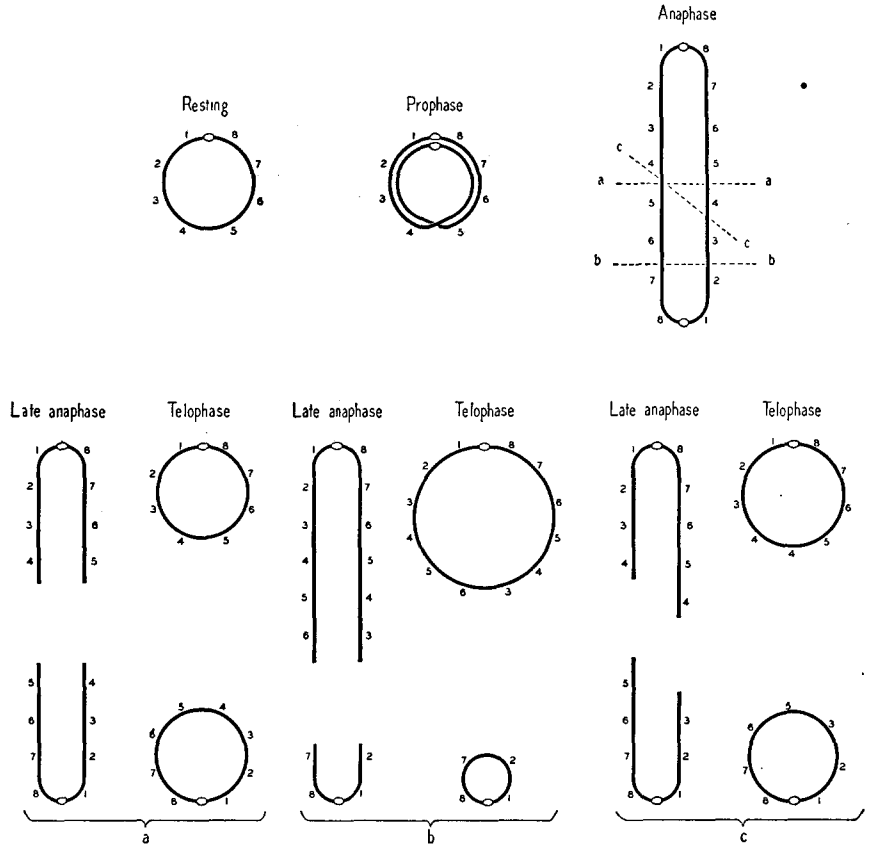
## INTRODUCTION

THE evidence to be presented in this paper supports the supposition that some recessive mutants in maize are caused by homozygous minute deficiencies. A method by which homozygous minute deficiencies may be produced in maize has been presented in a previous publication (McCLINTOCK 1938). It is related to the aberrant mitotic behavior of ring-shaped chromosomes. It has been shown both in maize and Nicotiana (STINO 1940; R. E. CLAUSEN unpublished) that a chromosome in the form of a ring does not maintain itself unaltered through successive nuclear cycles. The details of this behavior have been presented in the two mentioned papers. They may be summarized briefly as follows: (1) Ring-shaped chromosomes may decrease in size by loss of segments of chromatin from the ring. (2) Ring chromosomes may increase in size by duplications of segments of chromatin composing the ring. (3) Ring chromosomes may be completely eliminated from sister telophase nuclei during a mitotic cycle. (4) The frequency of these occurrences depends upon the length of the chromonema composing the ring—the larger the ring chromosome, the more frequent the aberrant mitotic behavior. In maize, aberrant mitotic configurations producing alterations or elimination of the ring chromosomes may occur in 17 to 20 percent of all the division figures if the chromonema composing the ring is as long as that of the longest chromosome of the complement. If the ring chromosome is small—for example, composed of only four chromomeres—the aberrant mitotic configurations are very infrequent and may occur only once in every five or six hundred divisions. In all other nuclear divisions the ring chromosome behavior is normal; the two sister halves of the ring chromosome separate freely and pass to opposite poles of the spindle figure along with the rod chromosomes of the complement.

When aberrant configurations are produced by large ring-shaped chromosomes, the chromatin content of the ring chromosome usually is altered in each resulting sister telophase nucleus. Only rarely is the ring chromosome lost to one or both sister telophase nuclei. In contrast, aberrant mitotic configurations produced by small ring-shaped chromosomes usually result in total loss of the ring-shaped chromosome from both sister telophase nuclei. Only rarely do the telophase nuclei receive altered ring-shaped chromosomes.

The method by which ring-shaped chromosomes are lost to nuclei or become altered has been described previously (McCLINTOCK 1938). It may be outlined briefly as follows. At some mitotic prophases the two sister halves of a divided ring chromosome form a continuous double-sized ring chromosome instead of two freely separating ring chromosomes (Prophase, fig. 1). It is not known whether this results from the method of reduplication of the chromonema composing the ring chromosome or from a somatic crossover between the two sister chromatids subsequent to this reduplica-




DESCRIPTIONS OF TEXT FIGURES

FIGURE 1.—Diagram illustrating a method by which a ring chromosome becomes altered in chromatin constitution. Upper left: a ring chromosome in a resting nucleus. The clear oval represents the centromere. The individual parts of the ring chromosome are designated by the numerals. Upper middle: A prophase configuration following a "crossover" between the two sister chromatids of the divided ring chromosome. A dicentric, double-sized ring chromosome is produced. Upper right: Appearance of the dicentric ring chromosome in the following anaphase. Breakage of the chromatin strands between the centromeres may occur at any position. Three possible positions, a, b, and c, respectively, are indicated by the dash lines. The resulting broken strands at late anaphase and the new ring chromosomes formed at telophase by fusions of broken ends of these strands are diagrammed below in the bracketed figures for the breaks a, b, and c, respectively.

tion. At early anaphase, the two centromeres which are present in this double-sized ring chromosome move toward opposite poles of the spindle figure (Anaphase, fig. 1). The subsequent behavior in the spindle figure depends on the size of the ring chromosome. The ring chromosomes used in the investigations to be described in this paper were small. Therefore, the following description will be confined to their behavior. With such small double-sized ring chromosomes, continued movement of the centromeres is usually suspended and the double-sized ring chromosome remains in the region of the equatorial plate during anaphase. It is consequently eliminated from both sister telophase nuclei. Very occasionally, however, the strands of chromatin between the two centromeres become broken, and a segment of the double-sized ring chromosome enters each telophase nucleus. Fusion occurs between the broken ends of the segment. Consequently, a newly organized ring-shaped chromosome is produced in each of these telophase nuclei. Illustrations of several types of ring chromosomes which may be produced following breakage of a double-sized, two-centromere ring chromosome are given in figure 1. It will be seen that ring chromosomes with duplicated segments or ring chromosomes with deficient segments may be produced by this process. In either case, the size of the segment may be minute or relatively large. Although aberrant mitotic configurations, which could result in loss or change in size of the ring-shaped chromosomes, are relatively infrequent, it has been found that the frequency of loss of the ring-shaped chromosome from both telophase nuclei is considerably greater than the frequency of breakage of the ring chromosome with inclusion of broken segments in each of the sister telophase nuclei. This relationship is of considerable importance in the study to be described.

A simple method of obtaining sectors of a plant or even whole plants which are homozygous deficient for a small segment of a chromosome may be outlined as follows. It is necessary to have a rod-shaped chromosome from which a relatively short segment has been deleted and a ring-shaped chromosome composed of the chromatin deleted from the rod-shaped chromosome. The deficient rod chromosome plus the compensating ring-shaped chromosome are thus equivalent in chromatin content to a single normal rod chromosome. (See Df-1 and R-1; Df-2 and R-2, fig. 2.) Union of two gametes, each with a complete genomic complement but possessing the deficient rod chromosome and its compensating ring chromosome (instead of the normal rod chromosome), could give rise to plants with two homologous deficient rod chromosomes and two homologous compensating ring chromosomes. However, because of their small size, the ring chromosomes would frequently be lost to telophase nuclei following the formation of aberrant mitotic configurations. Thus, in the development of such plants, some nuclei would contain only a single ring chromosome following

loss of one of the ring chromosomes. During subsequent multiplication of such cells, an aberrant configuration could eliminate the second ring chromosome. Thus, cells would be produced whose nuclei have no chromatin segment covering the deficiency in the rod chromsomes. These cells would be homozygous deficient for the full extent of the deficiency in the rod chromosomes. (For this preliminary presentation, it may be assumed that these cells are inviable or incapable of further multiplication.) Very occasionally, however, an aberrant mitotic configuration of a ring chromosome would result not in loss but in a changed composition of the ring chromosome as illustrated in figure 1. If, during development of the sporophytic tissues, one of the ring chromosomes becomes reduced in size by loss of a minute segment and subsequently, the second ring chromosome is lost to a cell at a mitotic anaphase, all the cells arising from this latter cell could be homozygous deficient for a minute segment—the segment which had previously been deleted from the remaining ring chromosome. When the loss of a minute amount of genic substance results in a change in the genotype which is neither cell lethal nor interferes greatly with the capacity of such cells to multiply, a sector of tissue could be formed with a visibly modified phenotype. Minute deletions of different segments of the ring chromosome could be produced following such aberrant mitoses. In one plant an aberrant mitosis might result in the deletion of one particular segment from the ring chromosome. In another plant, an aberrant mitosis might result in the deletion of an entirely different segment. Thus, tissues homozygous deficient for different segments within the limits of the deficiency in the rod-chromosomes could be produced. If, when homozygous, each minute deficiency results in a particular type of visible modification of the tissue, a number of different and distinguishable types of mutant sectorials should be produced in these plants, each of which should be associated with loss of a particular segment from the ring. Furthermore, sectors showing identical characters should arise independently in a number of different plants if sufficient numbers of plants are available for observation. This is because a double-sized dicentric ring chromosome (fig. 1) could be broken at the same position on a number of independent occasions resulting in the production of altered ring chromosomes from which the same segment has been deleted.

If two adjacent segments, each of which when homozygous deficient produces a mutant character, are simultaneously deleted from the ring chromosome during an aberrant mitosis, a compound mutant sectorial should be formed showing the characters caused by each deficiency—that is, comparable to a condition of homozygosity for two recessive mutants. If a mutant sector in a plant is included in the inflorescence, gametes could be formed containing the deficient rod chromosome plus the deficient ring

chromosome. Fusion of two such gametes could give rise to plants which are homozygous for the minute deficiency and thus homozygous for the phenotypic expression which it induces. In such a way, altered ring chromosomes may be isolated.

TABLE 1

*The appearance of the tissues of plants with two deficient rod chromosomes and two compensating ring chromosomes, Ring 1 and Ring 2, columns one and two, respectively. N represents a ring chromosome whose chromatin completely covers the deficiency in the rod chromosomes. a, b, and c represent ring chromosomes with minute deficiencies; each deficiency, when homozygous, gives rise to the phenotypic character a, b, and c, respectively. a b and b c represent ring chromosomes with two such minute deficiencies which, when homozygous, give rise to the compound mutant a b and b c, respectively. The appearance of the tissues when both rings are present is shown in column 3. The + indicates normal, non-mutant tissues. In columns 4 and 5 the phenotypic appearance of the tissues following somatic loss of Ring 1 and Ring 2, respectively, is indicated.*

| RING 1 | RING 2 | APPEARANCE OF TISSUES WHEN BOTH RINGS ARE PRESENT | APPEARANCE OF TISSUES FOLLOWING LOSS OF RING 1 | APPEARANCE OF TISSUES FOLLOWING LOSS OF RING 2 |
|---|---|---|---|---|
| N | N | + | + | + |
| N | a | + | a | + |
| N | b | + | b | + |
| N | c | + | c | + |
| N | a b | + | a b | + |
| N | b c | + | b c | + |
| a | b | + | b | a |
| a | c | + | c | a |
| a | a b | a | a b | a |
| a | b c | + | b c | a |
| b | c | + | c | b |
| b | a b | b | a b | b |
| b | b c | b | b c | b |
| c | a c | c | a c | c |
| c | b c | c | b c | c |
| a b | b c | b | b c | a b |

The meaning of simple and compound mutants produced by altered ring chromosomes is important for an understanding of the logic of this paper. Therefore, a table has been prepared (table 1) to illustrate this meaning. Assume three adjacent segments in the ring chromosome each of which when homozygous deficient results in the characters **a, b,** and **c,** respectively. If, in individual plants, altered ring chromosomes are isolated with the simple deficiency mutants **a, b,** and **c,** respectively, and the compound mutants **a b** and **b c**, respectively, combinations of each of these altered rings with a normal ring chromosome or with any other altered ring chromosome may be made and should give predictable results. These are shown in the table along with the characters of the tissues which would be formed following mitotic loss of one or the other of the ring chromosomes, respectively.

It is the purpose of this paper to describe the types of simple and compound mutants which arise in plants possessing two homologous deficient rod-shaped chromosomes and one or more compensating ring-shaped chromosomes and to summarize the evidence which indicates that they are caused by homozygous minute deficiencies.

## TWO CASES OF DEFICIENT ROD CHROMOSOMES PLUS COMPENSATING RING CHROMOSOMES

Two cases of deficient rod chromosomes with compensating ring chromosomes were available for this study. (For a complete description of their origin and behavior, see McCLINTOCK 1938). In both cases, segments of the short arm of chromosome 5 adjacent to the centromere were involved. The two cases are illustrated in figure 2. In the diagram, the numbers 1 to 9 in the normal chromosome 5 (first line in diagram) represent the positions of

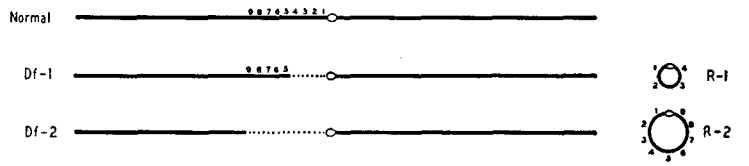



FIGURE 2.—The upper figure is a diagram of a normal chromosome 5. The clear oval represents the centromere. The region adjacent to the centromere in the short arm is designated by the numerals 1 to 9. The middle figure represents a rod-shaped chromosome 5 deficient for the segments 1 to 4 (the Df-1 rod-chromosome). The dotted line represents the extent of the deficiency. To the right of this deficient rod chromosome, a ring chromosome is diagrammed possessing the segment which has been deleted from the rod chromosome (R-1). In a similar manner, the Df-2 rod chromosome and its compensating ring chromosome have been diagrammed.

the first nine chromomeres adjacent to the centromere. The rod chromosome, Df-1, is deficient for the four chromomeres adjacent to the centromere. Its compensating ring chromosome (R-1) possesses these four chromomeres (four chromomere ring below arrow, fig. E, Plate 1). The Df-2 rod chromosome (fig. 2) is deficient for these same four chromomeres plus the next five chromomeres. The R-2 ring chromosome possesses these nine chromomeres (fig. A, Plate 1; lower arrow, fig. B and G 1, Plate 1). It should be noted that the R-1 ring chromosome does not cover the deficiency in the Df-2 chromosome but the R-2 ring chromosome does cover the deficiency in the Df-1 chromosome. The transmission through gametes of the two deficient rod chromosomes without compensating ring chromosomes or with one or more ring chromosomes is given in table 2.

The types of plants most used in this investigation and the types of tissues which result from loss of the ring chromosome during development in these plants are summarized in table 3. In all cases, the tissues are normal when a single ring chromosome is present (either R-1 or R-2) covering the deficiency in the rod chromosomes. Tissues homozygous deficient for

TABLE 2

*Transmissions through gametes of the deficient rod chromosomes without ring chromosomes or with various ring chromosomes. + represents transmission; − represents non-transmission.*

| | THROUGH ♀ | THROUGH ♂ |
|---|---|---|
| Df-1 | + | − |
| Df-1 plus R-1 | + | +* |
| Df-1 plus R-2 | + | +* |
| Df-1 plus R-1 plus R-2 | + | +* |
| Df-2 | − | − |
| Df-2 plus R-1 | − | − |
| Df-2 plus R-2 | + | + |
| Df-2 plus R-1 plus R-2 | + | + |

* In a previous publication (McCLINTOCK 1938) it was shown that Df-1 was not transmitted through the pollen even when the deficiency was covered by a ring chromosome. Since this publication, a strain was obtained which will transmit the Df-1 chromosome through the pollen when a covering ring chromosome is present.

chromomeres 1 to 9 are completely inviable; tissues homozygous deficient for chromomeres 5 to 9 are likewise inviable. In contrast, the tissues which are homozygous deficient for chromomeres 1 to 4 are viable, but the growth capacity of these cells is very poor. Only very minute sectors of such homozygous deficient tissues are found. (See McCLINTOCK 1938, text fig. 31–38 and this paper, a, fig. 3.) The cells in these sectors are small. The lignified walls of cells with a full genomic complement are white, but the walls of cells homozygous deficient for chromomeres 1 to 4 are brown in color. There are no well developed plastids in these cells; therefore, the tissues of the leaf and stalk which are homozygous deficient for chromomeres 1 to 4 are not green and contrast strikingly with non-deficient tissues which are green. On exposure to direct sunlight, these cells soon die, and the

FIGURE 3.—a. Photograph of a part of a leaf of a Df-1/Df-2 plant with ring chromosomes R-1 and R-2. A sector showing the character of the blotch mutant is evident slightly to the right of the letter a. The wide band to the left of this letter is the mid-rib. The continuous parallel fine lines are the veins of the leaf. The shorter, narrower lines between these veins are the sectors of tissue homozygous deficient for regions 1 to 4 of chromosome 5 which arise following somatic loss of both the R-1 and the R-2 ring chromosomes. b. Photograph of part of a leaf of a Df-2/Df-2 plant with a two ring chromosomes, a normal R-2 ring and the brown-blotch-dries *I* ring. Two sectors showing the blotch character are visible to the right and left of the mid-rib, respectively. The photograph was taken before the tissue had commenced to disintegrate and dry. c. Similar to b. A wide sector showing the mutant character blotch-dries is present at each edge of the leaf. The disintegration and drying process has commenced at the outer edge of each sector. d. Leaves from plants of the constitution Df-1/Df-2 plus one R-2 ring chromosome. Note the solid (non-variegated) sector of mutant tissue (pink) in each leaf.

FIGURE 4.—Appearance of a young Df-2/Df-2 plant with two R-2 ring chromosomes, a normal R-2 ring and the brown-pink *I* ring. The plant is variegated for the compound mutant character brown and pink (white sectors in the photograph). These sectors arise following somatic loss of the normal R-2 ring.

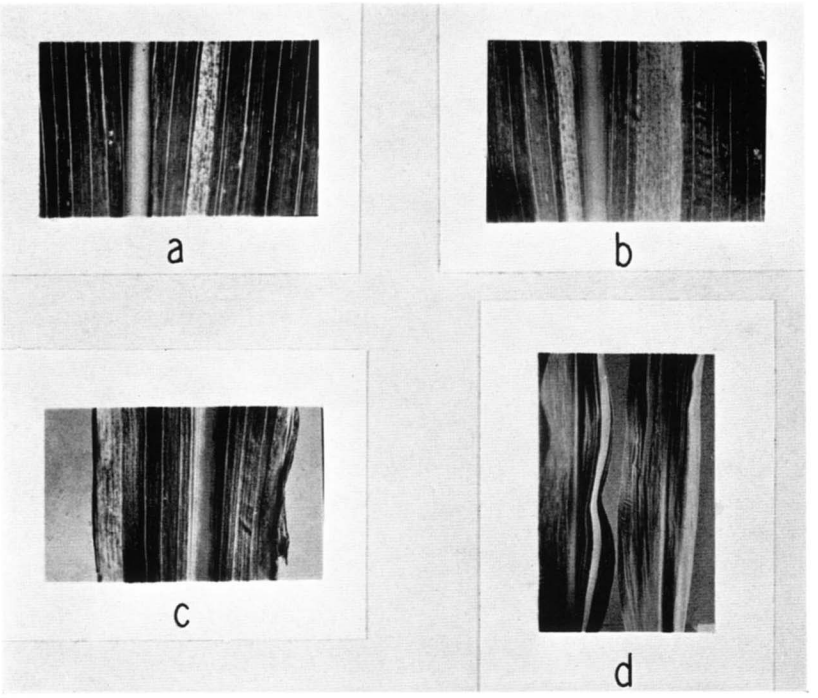



FIG. 3

FIG. 4

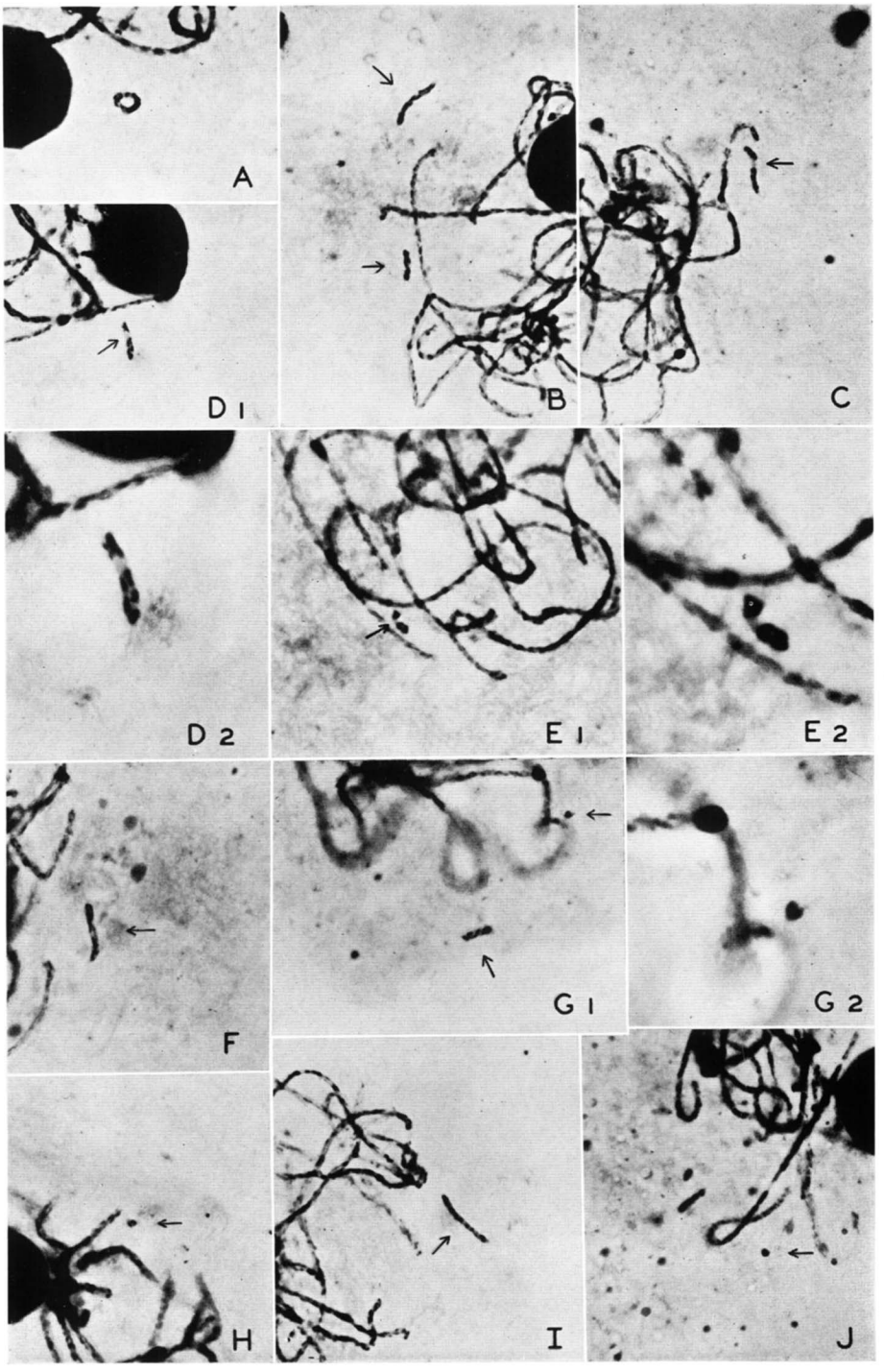


TABLE 3

*Viability of tissues following somatic loss of ring chromosomes.*
*+, normal. −, very poor growth. o, inviable.*

| CHROMOSOME CONSTITUTION OF PLANTS | | APPEARANCE OF TISSUES WITH COMPLETE COMPLEMENT | APPEARANCE OF TISSUES FOLLOWING SOMATIC LOSS OF RING CHROMOSOMES INDICATED IN THE COLUMNS | | | | | |
|---|---|---|---|---|---|---|---|---|
| DEFICIENT ROD-CHROMOSOME | RING CHROMOSOMES | | 1 R-1 | 2 R-1 | 1 R-2 | 2 R-2 | 1 R-1 plus 1 R-2 | 1 R-1 plus 2 R-2 |
| Df-1/Df-1 | 1 R-1 | + | − | | | | | |
| Df-1/Df-1 | 2 R-1 | + | + | − | | | | |
| Df-1/Df-2 | 1 R-2 | + | | | − | | | |
| Df-1/Df-2 | 1 R-1 plus 1 R-2 | + | + | | + | | − | |
| Df-1/Df-2 | 2 R-2 | + | | | + | − | | |
| Df-2/Df-2 | 2 R-2 | + | | | + | o | | |
| Df-2/Df-2 | 1 R-1 plus 2 R-2 | + | + | | + | o | + | o |

## DESCRIPTION OF FIGURES FOR THE PLATE

All photographs are of pachytene configurations in microsporocytes. All magnifications are approximately 1100× except D 2, E 2, and G 2, which are approximately 2750×.

FIGURE A.—Synaptic association of two R-2 ring chromosomes.

FIGURE B.—The lower arrow points to a collapsed normal R-2 ring. The upper arrow points to a collapsed brown-pink *II* ring chromosome which is approximately double the size of the normal R-2 ring.

FIGURE C.—Two collapsed ring chromosomes associated at their centromeres. The arrow points to the associated centromeres. The normal R-2 ring is below the arrow. Above is the reduced ring chromosome which produces the brown *I* phenotype.

FIGURE D 1.—Two collapsed ring chromosomes associated at their centromeres. The arrow points to the associated centromeres. The normal four chromomere R-1 ring (above) is slightly out of focus. The ring below the arrow is a reduced R-2 ring composed of seven chromomeres. It gives rise to the brown-pink *IV* phenotype. D 2. Enlargement of the same.

FIGURE E 1.—Two ring chromosomes associated at their centromeres. The arrow points to the associated centromeres. The normal, four chromomere R-1 ring lies below the arrow. An R-2 ring reduced to three chromomeres lies above the arrow. This latter ring gives rise to the brown-blotchdries *II* phenotype. E 2. Enlargement of the same.

FIGURES F and H.—The arrows point to the collapsed ring chromosomes in two sister cells of a Df-1/Df-2 plant with one R-2 ring chromosome. All surrounding cells possessed a normal R-2 ring. In one of these sister cells, fig. F, the R-2 ring chromosome was enlarged. In the other cell, fig. H, the R-2 ring chromosome was reduced to two chromomeres. In chromatin constitution the two rings are equivalent to two normal R-2 ring chromosomes.

FIGURE G 1.—A collapsed normal R-2 ring chromosome (lower arrow) and an R-2 ring chromosome reduced to two chromomeres (upper arrow). This latter ring chromosome gives rise to the compound mutant brown-light green-poor growth. G 2. Enlargement of the same showing this latter reduced ring chromosome.

FIGURE I.—The arrow points to the collapsed double-sized R-2 ring chromosome which gives rise to the brown-pink *II* phenotype.

FIGURE J.—Pachytene configuration in a plant with three ring chromosome. A collapsed normal R-2 ring lies to the left. A normal R-1 ring lies immediately above it. The arrow points to the reduced R-2 ring chromosome which produces the brown-light green-poor growth phenotype.

tissue disintegrates and dries. Because cells and tissues completely deficient for these four chromomeres not only are viable but also show phenotypic modifications, it is expected that tissues deficient for segments within this region would not only be viable, but could have much better growth rates and might show specific characters.

Studies of somatic mitosis have shown that the larger of the two ring chromosomes (R-2) produces a double-sized, dicentric ring chromosome (Prophase, fig. 1) approximately once in every hundred nuclear divisions. All of the other nuclear divisions are completely normal; the ring chromosome divides normally, the two free sister halves pass to opposite poles along with the rod chromosomes. In nearly all cases, when a double-sized ring chromosome is formed at prophase, it remains in the region of the equatorial plate during anaphase and telophase and is thus completely eliminated from the sister telophase nuclei. Very occasionally, however, a double-sized dicentric R-2 ring chromosome becomes broken at anaphase, and a segment of the broken double-sized ring chromosome enters each sister telophase nucleus. In both telophase nuclei fusion of broken ends occurs, reestablishing a ring-shaped chromosome, but the content of the ring-shaped chromosome is usually altered. Similar studies of the smaller ring chromosome (R-1) have shown that aberrant anaphase behavior occurs approximately once in every 600 nuclear divisions. In this case, due to its small size, the ring chromosome is nearly always lost to the sister telophase nuclei. Only rarely have altered R-1 rings been detected. The alterations in the chromatin content of the ring chromosomes to be described in this paper involve the R-2 ring.

## DETECTION OF ALTERED RING-SHAPED CHROMOSOMES BY MEANS OF MUTANT SECTORS IN THE DF-1/DF-1, DF-1/DF-2 AND DF-2/DF-2 PLANTS

As stated in the previous section, alterations of the R-2 ring are considerably more frequent than alterations of the R-1 ring. Consequently, plants with one or two R-2 rings have shown the greatest number of detectable alterations. The plants most suitable for this study have had the following constitutions: Df-1/D-2 plus one R-2 ring; Df-1/Df-2 plus two R-2 rings; Df-2/Df-2 plus two R-2 rings.

In plants of the constitution Df-1/Df-2 plus one R-2 ring, a solid sector of modified tissue should appear following an alteration in the ring chromosome which produces a visible change in the character of the tissues. The size of the sector would depend upon when the altered ring chromosome arises in the development of the plant and the subsequent rate of growth of the cells possessing this ring. If the alteration occurred in a single nuclear division early in the development of the plant, a wide mutant sector could

result. If it occurred in a nuclear division late in development, only a small mutant sector would be produced. In Df-1/Df-2 plants with two ring chromosomes (either one R-1 plus one R-2 or two R-2) or in Df-2/Df-2 plus two R-2 rings, both solid sectors and sectors which are variegated for the mutant characters should be produced. The solid sectors should arise in the tissues of the plant which have only a single ring chromosome—that is, subsequent to a mitotic loss of one of the ring chromosomes. An alteration in the remaining ring chromosome could give a solid sector with a modified appearance. Variegated mutant sectors should arise when two rings are initially present in the tissue, one of which has become altered in an earlier mitotic division. When the two rings are present, a deficiency in the altered ring chromosome would be covered by the presence of this segment in the normal ring chromosome. No character caused by the deficiency in the altered ring chromosome could appear until after the normal ring chromosome has become lost in a later nuclear division. All the cells which arise from a cell which has lost the normal ring chromosome would be able to show the mutant character associated with the deficiency in the altered ring chromosome. Since loss of the normal ring chromosome should occur independently in a number of cells, a sector which is variegated for the mutant character should be produced. The appearance of a solid sector in a plant with a single ring chromosome is illustrated in a and d of figure 3; the appearance of variegated sectors in two-ring plants is illustrated in b and c of figure 3 and in figure 4.

If a particular mutant character is associated with loss of a particular region from the ring chromosome—that is, if the character is produced by a homozygous minute deficiency—only a restricted number of mutant characters should appear in the Df-1/Df-1 and Df-1/Df-2 plants. The segment within which homozygous deficiencies may be detected is only four chromomeres long (fig. 2). If this theory is correct, the same mutant characters which appear in the Df-1/Df-1 and Df-1/Df-2 plants should likewise appear in the Df-2/Df-2 plants because homozygous deficiencies within regions 1 to 4 (chromomeres 1 to 4, fig. 2) may be produced in all three types of plants. However, other mutants which are not found in the Df-1/Df-1 and Df-1/Df-2 plants should be present in the Df-2/Df-2 plants. These characters should be related to homozygous deficiencies within the region 5 to 9 (chromomeres 5 to 9, fig. 2). Such mutant characters should not appear in the Df-1/Df-1 or Df-1/Df-2 plants because the Df-1 chromosome possesses chromomeres 5 to 9, and thus no homozygous deficiencies can be produced within this region following aberrations of the ring chromosome. These anticipations have been completely fulfilled. Since the altered ring chromosomes may be isolated following the formation of gametes with a deficient rod chromosome and an altered ring chromosome,

it has been possible to conduct tests which show that a certain group of characters is associated with region 1 to 4 and another group of characters with region 5 to 9.

The frequency of mutant sectors is greater in the Df-1/Df-2 plants with one or two R-2 rings than in the Df-2/Df-2 plus two R-2 rings. The evidence, which is conclusive but which cannot be considered here because of limitations in space, indicates that this is due to changes in the R-2 ring chromosomes which have deleted rather large segments from the ring. In a Df-2/Df-2 plant, the cells with such large homozygous deficiencies are either inviable or produce tissues with such poor growth rates that they are not suitable for precise character studies. However, removal of a large segment from the R-2 ring in a Df-1/Df-2 plant may result in quite viable tissues with readily identifiable mutant characters. As an example, removal of region 4 to 8 in the R-2 ring in a Df-2/Df-2 plant would result in cells homozygous deficient for this region. These cells are inviable. In a Df-1/Df-2 plant, only region 4 could be homozygous deficient because the segment 5 to 8 covering the deficiency in the ring chromosome is present in the Df-1 rod chromosome.

Considering the number of mitoses taking place in the development of a plant, the occurrence of an abnormal mitosis which results in an altered ring chromosome is extremely rare. Once an altered ring chromosome has been isolated, it can be maintained unchanged through as many plant generations as desired by avoiding those plants in which this ring chromosome has again become altered. There are relatively few such plants.

## MUTANT CHARACTERS ASSOCIATED WITH REGION 1 TO 4 OF CHROMOSOME 5

As stated previously, tissues which are homozygous deficient for the segments 1 to 4 of chromosome 5—the full extent of the deficiency in the Df-1 rod chromosome—are capable of multiplication but at a very slow rate. Consequently, only minute visible sectors are produced following complete loss of the ring chromosomes in Df-1/Df-1 or Df-1/Df-2 plants. The mutant characters shown by these homozygous deficient tissues are: brown cell walls, colorless plastids, very poor growth capacity, and disintegration and drying of the tissue when exposed to direct sunlight. It is to be expected that deficiencies for minute segments within this region should be viable. They may possess very much better growth capacities and could show mutant characters. Thus, relatively wide sectors showing mutant characters associated with a minute homozygous deficiency within region 1 to 4 might be produced in these plants. In the Df-1/Df-1 or Df-1/Df-2 plants, all the mutant characters observed as sectorials should be related to changes within region 1 to 4 of chromosome 5. In the Df-2/Df-2 plants, these same types of mutant characters should appear

plus additional mutant characters which are not found in the former types of plants. These latter mutants should be related to changes within region 5 to 9.

The various types of mutant characters having a clearly recognizable phenotypic expression which have appeared as sectorials in all three types of plants—that is, those mutants associated with region 1 to 4 of chromosome 5—have been divided into three groups: (1) Those which show a single recognizable character in the mutant sector (simple mutants); the tissue may have a normal growth rate. (2) Those which exhibit two or more of these characters in a single sector (compound mutants); the tissues may have a normal growth rate. (3) Sectors with distinct mutant characters. These sectors are always narrow—that is, associated with a reduced growth rate of the cells of the sector; many of these are recognizable as compound mutants.

The simple mutants to be described involve some obvious color change. Although other mutants would be expected and are probably present, their positive detection as sectorials in these plants is both difficult and uncertain. Therefore no attempt was made to isolate mutants which did not produce an unmistakable character change which could express itself as a sector in a plant. The color mutants belong to the unmistakable class.

### *Group 1. Simple mutants which may have a normal growth rate*

#### Brown cell walls

The lignified cell walls are brown in color. This character is similar in all details to the character produced by the recessive mutant brown mid-rib (symbol, *bm*) previously located within the region 1 to 4 of a normal rod chromosome 5. Henceforth the character will be designated as brown. The normal R-1 and R-2 rings are known to carry the dominant allele of *bm* (*Bm* colorless cell walls). (For a complete description of the *bm* phenotype and proof of the presence of *bm* in the normal ring chromosomes, see McCLINTOCK 1938.) Three different ring chromosomes producing the brown phenotype have been isolated. Two appear to be normal in size and could not be distinguished from normal R-2 rings. Either ring will produce the brown character in Df-1/Df-1, Df-1/Df-2 or Df-2/Df-2 plants. The third brown ring is reduced to six chromomeres (upper collapsed ring, fig. c, Plate 1). When this ring is present, brown will appear only in Df-1/Df-1 or Df-1/Df-2 plants. In Df-2/Df-2 plants the cells which have only this ring are inviable. In this case, the three chromomere deficiency in the ring chromosome probably includes a segment of region 5 to 9 which could express itself as a homozygous deficiency only in the Df-2/Df-2 plants. As explained previously, in the Df-1/Df-1 or Df-1/Df-2 plants, the cells with only this reduced ring chromosome might possess only a minute homo-

zygous deficiency and thus be quite viable. This would explain the difference in viability of the cells possessing only this ring in the three types of plants.

### Pink

Chlorophyll is absent although plastids are present. In the stalk, the number of layers of cells from the outer circle of bundles to the epidermis frequently is greater in the pink sectors than in the non-modified parts of the plant. Consequently, the pink sector produces a protuberance. On the stalk and in the upper leaves of the growing plant, the color is an intense salmon pink. As the leaves mature, the pink color gradually fades to white. Two ring chromosomes giving rise to the pink phenotype have been isolated, but the constitution of only one of these rings has been investigated cytologically. It is an R-2 ring with no clearly observable change in chromatin constitution. It produces the pink phenotype in either a Df-1/Df-1, a Df-1/Df-2 or a Df-2/Df-2 plant.

### Blotch

The chlorophyll pattern is finely speckled (a, fig. 3). No ring chromosome producing a blotch character associated with a good growth capacity has been isolated so far, although a large number of such sectorials have been observed in both Df-1/Df-2 and Df-2/Df-2 plants. Several ring chromosomes producing blotch but associated with poor growth capacities have been isolated. None of these has been investigated extensively.

### Blotch-dries

This character is similar to blotch although the chlorophyll color usually is less intense. In the young leaf, the character is first detected as a light green sector. The blotch pattern of the chlorophyll is acquired as the leaf matures. After a short time, the cells in the sector which have been exposed to direct sunlight begin to disintegrate. Finally, the whole sector so exposed becomes a mass of dead, dried tissue (see b and c, fig. 3). Two ring chromosomes producing blotch-dries have been isolated recently. Neither has been sufficiently investigated as yet.

### *Group 2. Compound mutants which may have normal growth rates*

### Brown-pink

Many sectors showing the combined characters of brown and pink have been observed in all three types of plants. Four different ring chromosomes producing this compound character have been isolated. One is an obviously reduced R-2 ring chromosome composed of seven chromomeres (lower collapsed ring, fig. D, Plate 1). Two are slightly reduced R-2 rings, and one is an enlarged R-2 ring, approximately double the size of the normal R-2 ring (collapsed ring, upper arrow, fig. B and fig I, Plate 1). The

brown-pink character produced by the seven-chromomere ring appears only in the Df-1/Df-1 or Df-1/Df-2 plants. In the Df-2/Df-2 plants, the cells with only this ring chromosome are inviable. The other three ring chromosomes will produce the brown-pink character when present in either a Df-1/Df-1, a Df-1/Df-2, or in a Df-2/Df-2 plant.

Brown-pink-dries

At the initial stage, the sectors showing this compound mutant character are strictly comparable to the compound mutant brown-pink. However, as in blotch-dries, on exposure to sunlight, the cells die and the tissue dries. These sectors appear in both Df-1/Df-2 and Df-2/Df-2 plants. Several rings causing this compound mutant character have been isolated, but none has been investigated fully.

Brown-blotch-dries

The sectors exhibiting this compound mutant are identical in appearance and behavior to the blotch-dries sectors described above. In addition, however, the lignified cell walls are brown. Two ring chromosomes producing this compound mutant have been isolated. One is a slightly enlarged R-2 ring. The other is a much reduced ring chromosome composed of only three chromomeres (ring above arrow, fig. E, Plate 1). When the former ring chromosome is present, the character will appear in either a Df-1/Df-2 or a Df-2/Df-2 plant. When the latter ring is present, the compound mutant character will appear only in a Df-1/Df-1 or a Df-1/Df-2 plant. In the Df-2/Df-2 plants, the cells which possess only this ring chromosome are inviable—probably because of the six chromomere deficiency.

*Group 3. Compound mutants whose growth rates are considerably reduced*

Brown-pink-dries-poor growth

The sectors showing this group of characters are similar in appearance to the compound mutant brown-pink-dries of group 2. However, the growth rate of the mutant tissues is always considerably reduced. Thus, only narrow sectors are formed. Three rings producing this compound mutation have been isolated, but only one has been sufficiently studied. It is a slightly reduced R-2 ring. The character which this ring produces is exactly the same in the Df-1/Df-1, Df-1/Df-2, and Df-2/Df-2 plants. Thus, the phenotypic modification which this ring chromosome induces is probably restricted to a segment within region 1 to 4 of chromosome 5.

Light green-poor growth

The chlorophyll color in these sectors is a light green, but the growth rate of the cells is so reduced that sectors are not wider than 3 or 4 mm.

Only one ring chromosome producing this character has been isolated. It possesses only two chromomeres. Thus, the character associated with this ring appears only in the Df-1/Df-1 and Df-1/Df-2 plants. It does not appear in the Df-2/Df-2 plants due to the extensive deficiency producing inviability in the cells possessing only this ring.

### Brown-light green-poor growth

Sectors showing this character are similar in appearance to those described above. In addition, the lignified cell walls are brown. Only one ring chromosome giving rise to this compound mutant has been isolated. It possesses two chromomeres (upper arrow, fig. G 1 and enlargement, fig. G 2, Plate 1). Due to the extensive deficiency in this ring chromosome, the characters associated with this ring appear only in Df-1/Df-1 or Df-1/Df-2 plants.

Many sectors exhibiting very poor growth capacities associated with a blotched chlorophyll pattern or with colorless tissues, either with or without brown, have been observed in the Df-1/Df-1, Df-1/Df-2, and Df-2/Df-2 plants. No attempt has been made to isolate the rings responsible for such characters because of the impracticability of work with characters having such poor growth rates.

## MUTANT CHARACTERS ASSOCIATED WITH REGION 5 TO 9 OF CHROMOSOME 5

The mutant characters which may be associated with alterations in the R-2 chromosome within region 5 to 9 are those which do not appear in the Df-1/Df-1 or Df-1/Df-2 plants but appear only in the Df-2/Df-2 plants. It is difficult to obtain sufficient seed for an extensive investigation of the types of mutants appearing in this latter type of plant. Only one to several kernels are likely to occur on an ear of a Df-2/Df-2 plant, since the Df-2 chromosome is not transmitted through the female gametophyte without the compensating R-2 ring chromosome. During meiosis, the R-2 rings are so frequently eliminated that few megaspores are formed possessing this ring, and thus few seed may develop. Among the several thousands of plants which have been examined, a number of mutant sectorials have been observed. Many of these are related to changes in region 1 to 4 of the R-2 ring chromosome. Only a few clear-cut mutants have been observed which may be related to changes within region 5 to 9. In these plants, many mutant sectorials are composed of tissues which grow too poorly to be useful for character studies. No attempt has been made to isolate the changed rings responsible for the mutations with very poor growth rates. When a whole plant or half of a plant was variegated for such a character, a cytological study was made to determine the types of ring chromosomes which

were present. In all examined cases, one of the ring chromosomes was decidedly reduced. Following loss of the normal ring chromosome to some cells during development, a relatively large homozygous deficiency had to be present. If these cells reproduce at a very reduced rate, only very narrow sectors of homozygous deficient tissue could be formed. The presence of a reduced ring in all examined plants showing variegated sectors with considerably reduced growth rates leads one to suspect that the cells of these sectors possess only the reduced ring chromosomes—that is, they are homozygous deficient for the segment which had been deleted from the ring chromosome.

Of the mutants which possess a good growth capacity, only three have been found which may be ascribed to region 5 to 9; and up to the present time, only two of these have been proven to be in this region. These mutants are:

### Pale green

The chlorophyll color in the leaves and the stalk is a pale green. The pale green character does not develop in the very young plants but becomes very striking in the older plants. The growth rate of this tissue is usually quite normal. Only one pale green ring has been isolated. No obvious change in the chromatin constitution of this R-2 ring was observed.

### Striate

The sectors showing this character are composed of streaks of yellow-green tissues. Only one ring chromosome has been isolated which produces this character. This R-2 ring is slightly reduced. A gamete with the Df-2 rod chromosome and the R-2 striate ring chromosome is transmitted through the female gametophyte but not through the pollen. The location of the character within region 5 to 9 has been determined by use of a third deficiency rod chromosome (Df-3) possessing a small deficiency located within the limits of region 5 to 9. Striate appears when the striate ring chromosome is present in Df-2/Df-3 plants.

### White

Sectors with a chalk white color have been observed in the Df-2/Df-2 plants but ring chromosomes producing white have not been isolated as yet. Since similar sectors have not been observed in many thousands of Df-1/Df-2 plants, it is assumed, although not proven, that this character is related to an alteration within region 5 to 9.

The pale green ring is the only one of this series which has been extensively investigated. It is not related to striate, since the pale green character does not appear in Df-2/Df-3 plants, whereas striate does appear in these plants. The following tests have shown that the pale green character

is related to region 5 to 9 and not to region 1 to 4 of chromosome 5. Following isolation of the pale green ring, plants were obtained with the constitution Df-1/Df-2 plus the pale green ring. These plants were indistinguishable from those of a similar constitution but having a normal R-2 ring. Thus, pale green does not appear in a Df-1 background. The functional male gametes produced by these plants possess the Df-2 rod chromosome plus the pale green ring chromosome. When pollen of such plants was placed on silks of plants of the constitution Df-2/Df-2 plus two normal R-2 ring chromosomes, all the resulting individuals (Df-2/Df-2 plus one normal R-2 ring plus the pale green R-2 ring) were variegated for pale green. The variegation arose as a consequence of the mitotic losses of the normal R-2 ring chromosome contributed by the female parent, thus giving rise to tissues containing only the pale green ring chromosome. It is in these tissues that the pale green character appears.

## CYTOLOGICAL OBSERVATIONS OF THE RING-SHAPED CHROMOSOMES WHICH GIVE RISE TO THE MUTANT CHARACTERS

A pachytene configuration showing the association of two normal R-2 ring chromosomes is shown in figure A, Plate 1. When a single R-2 ring chromosome is present or when two R-2 ring chromosomes which have failed to associate at the meiotic prophase are present, the ring chromosome is collapsed (lower arrow, fig. B and G, Plate 1; ring below arrow, fig. C, Plate 1). This results from non-homologous associations of the chromatin forming the ring (McCLINTOCK 1933, 1938).

Following the formation of a double-sized dicentric ring chromosome in a mitotic prophase and its breakage in the following anaphase, an enlarged ring chromosome may be formed in one telophase nucleus and a reduced ring chromosome in the sister telophase nucleus (see fig. 1). This situation is illustrated by the constitution of the ring chromosome in two sister cells (arrows fig. F and H, Plate 1). These two sister cells are in the prophase of meiosis; therefore the event which resulted in the formation of these two altered ring chromosomes occurred at the last premeiotic anaphase. In individual cases, R-2 rings have been observed with various degrees of reduction in chromatin constitution. These range from a barely observable deficiency in the ring chromosome to deficiencies so large that the ring chromosome was composed of but a single large chromomere. Likewise, in individual cases, R-2 ring chromosomes have been observed with duplicated segments varying in extent from a very small duplication to one which produced a ring chromosome eight times the size of the normal R-2 ring.

Cytological observations of the composition of the ring chromosomes which produce visible mutations are summarized in table 4. As stated pre-

viously in the description of the mutant characters, several ring chromosomes have been isolated which produce the same mutant character (simple mutant) or the same group of mutant characters (compound mutant). Although the character which each of these ring chromosomes produces appears to be the same, the event which is responsible for the character which the ring chromosome produces occurred independently in each case in a single Df-1/Df-1, Df-1/Df-2, or Df-2/Df-2 plant. The numerals *I*, *II*, and *III*, etc., have been used to distinguish ring chromosomes producing the same character or the same group of characters which have originated independently of one another. For example, brown *I* was isolated from a sector of a Df-1/Df-2 plant, brown *II* from a sector of another Df-1/Df-2 plant, and brown *III* from a Df-2/Df-2 plant. Where a photographic illustration of a particular altered ring chromosome is reproduced in this paper, the figure reference is indicated in the table. Four of the fifteen ring chromosomes showed no observable change in the constitution of the R-2 ring. All four ring chromosomes are associated with simple mutants (brown *II*, brown *III*, pink, pale green). In nine cases, the ring chromosome was reduced. In two cases, the ring chromosome was enlarged (brown-pink *II*, brown-blotch-dries *I*). The brown-pink *II* ring, when first observed, was approximately double the size of the normal R-2 ring (upper arrow fig. B and fig. I, Plate 1). The brown-blotch-dries *I* ring is only slightly enlarged. In both of these cases, the alteration in the ring chromosome, which is responsible for the character the ring chromosome produces, could have occurred subsequent to a previous alteration which had enlarged the R-2 ring chromosome. It is likewise conceivable that the enlargement of the ring chromosome could have occurred following the alteration which produces the character but before isolation of the ring. It is known that the enlargement itself is not the factor responsible for the appearance of the character which such an enlarged ring chromosome produces. This is clearly indicated by the enlarged brown-pink *II* ring. This ring, when first isolated, was approximately double the size of a normal R-2 ring chromosome. Because of the increased length of the chromonema of this ring chromosome, aberrant mitoses involving this ring chromosome occur with increased frequency. Furthermore, the added length of the chromonema of this ring chromosome brings it into the range where aberrant mitoses may frequently lead to recoverable alterations in the ring chromosome. Because of its size, it was expected that this ring chromosome would not maintain itself but would tend to become reduced to the size where aberrant mitoses would lead most frequently to elimination of the ring chromosome rather than alterations in its chromatin content. This proved to be true. Several strains have been isolated in which this ring chromosome is only slightly larger than the normal R-2 ring chromosome. At this reduced size, the

ring chromosome tends to remain stable because aberrant mitoses now tend to eliminate the ring rather than alter its chromatin composition. It should be emphasized, however, that the characters produced by the brown-pink *II* ring following its reduction in size from a double-sized ring to one only slightly larger than a normal R-2 ring have not been altered by the reduction. It is assumed that even in its enlarged state, the brown-pink *II* ring chromosome possessed a minute deficiency which was responsible for the compound mutant character it produced. No duplications of the remaining segments of the ring chromosome or subsequent deletions of these duplicated segments should alter the expression of the character, for the minute deficiency would still be present regardless of the presence or absence of these duplicated segments.

TABLE 4

*Chromatin constitution of the ring chromosomes which produce mutant characters.*

1. No observed change in constitution of the R-2 ring.
   brown *II;* brown *III;* pink; pale green.
2. Slightly reduced R-2 ring: Loss of approximately one chromomere.
   brown-pink *I;* brown-pink *III;* striate.
3. Obviously reduced R-2 ring: Loss of two or three chromomeres.
   brown-pink *IV* (fig. D, Plate 1); brown-pink-dries-poor growth; brown *I* (fig. C, Plate 1).
4. Very reduced ring: Loss of six or seven chromomeres.
   brown-blotch-dries *II* (fig. E, Plate 1); light green-poor growth; brown-light green-poor growth (fig. G, Plate 1).
5. Enlarged ring: Larger than a normal R-2 ring.
   brown-pink *II*, approximately double the size of a normal R-2 ring (fig. B and I, Plate 1); brown-blotch-dries *I*, approximately $\frac{1}{8}$ larger than the normal R-2 ring.

In 11 of the 15 cases it has been established that the ring chromosome has undergone an alteration. It would require considerable space to indicate the methods used which allow one to be certain that the ring chromosome being observed in a particular plant is the ring chromosome responsible for the character ascribed to it. The methods will be considered in a separate report.

## TRANSMISSIONS OF ALTERED RING CHROMOSOMES THROUGH THE MALE AND FEMALE GAMETES

Tests have been made of the transmissions of the various altered ring chromosomes through the female gametophyte with the Df-1 or Df-2 rod chromosomes and through the pollen with the Df-2 rod chromosome. These results are summarized in table 5. Transmissions through the pollen of the various altered ring chromosomes with the Df-1 rod chromosome have not been included in the table, since a male-transmissible strain of Df-1 has only recently been obtained, and the tests have not been completed. The cytological determination of the type of ring chromosome associated with

each particular mutant has been included in column 2 of this table, since it allows the tests to be more readily interpreted. Since many of these altered ring chromosomes are readily transmitted through the pollen, even in competition with gametes carrying normal ring chromosomes, it has been possible to obtain various combinations of the ring chromosomes in Df-1/Df-1, Df-1/Df-2, and Df-2/Df-2 plants. These will be described in the following section.

TABLE 5

*Gametic transmissions of the altered ring chromosomes with the Df-1 or Df-2 rod chromosomes. Transmissions of the altered ring chromosomes with the Df-1 rod chromosome through the pollen have not been included in the table because these tests have not been completed. + represents transmission. o represents no transmission. — indicates the test has not been completed.*

| RING CHROMOSOME | CHROMATIN CONSTITUTION OF ALTERED R-2 RING | TRANSMISSIONS THROUGH ♀ WITH: | | TRANSMISSION THROUGH ♂ WITH |
|---|---|---|---|---|
| | | Df-1 | Df-2 | Df-2 |
| brown *I* | Reduced to six chromomeres | + | o | o |
| brown *II* | No apparent reduction | + | + | + |
| brown *III* | No apparent reduction | + | + | + |
| pink | No apparent reduction | + | + | + |
| pale green | No apparent reduction | + | + | + |
| striate | Slightly reduced | — | + | o |
| brown-pink *I* | Slightly reduced | + | + | + |
| brown-pink *II* | Enlarged | + | + | + |
| brown-pink *III* | Slightly reduced | + | + | + |
| brown-pink *IV* | Reduced to seven chromomeres | + | o | o |
| brown-pink-dries-poor growth | Slightly reduced | + | + | o |
| brown-blotch-dries *I* | Slightly enlarged | + | + | + |
| brown-blotch-dries *II* | Reduced to three chromomeres | + | o | o |
| light green-poor growth | Reduced to two chromomeres | + | o | o |
| brown-light green-poor growth | Reduced to two chromomeres | + | o | o |

## THE CHARACTERS PRODUCED IN PLANTS FOLLOWING COMBINATIONS OF ALTERED RING CHROMOSOMES: PROOF THAT THE COMPOUND MUTANTS ARE COMPOSED OF TWO OR MORE OF THE SIMPLE MUTANTS

It is obvious from the description given earlier in this paper that the characters exhibited by the compound mutants bear a relation to the simple mutants. The compound mutants appear to be composed of a combination of two or more of the observed simple mutants. It was also obvious in this study that the sectorials showing compound mutant characters occurred far more frequently than those showing a simple mutant character. Sectorials showing a simple mutant character were relatively rare. The method by which ring chromosomes become reduced in size at a mitotic anaphase would suggest that adjacent blocks of chromatin should fre-

quently be deleted from the ring chromosome following an aberrant mitosis which results in an alteration in the ring chromosome. If each of the simple mutants is produced following loss of a particular minute segment from the ring chromosome, then the compound mutants should be produced following loss of two or more of these segments. It would be expected that breakage of the double-sized ring at a mitotic anaphase would delete a block of chromatin more frequently than a very minute segment. Thus, compound mutants should appear far more frequently than simple mutants. With this interpretation, one should be able to deduce the order of the various segments in the ring chromosome which produce a particular character when homozygous deficient, merely by observing the characters which are present in the compound mutants. For illustrative purposes, one may use the characters pink, brown, and blotch dries. The most frequent types of compound mutations involving these characters are: brown-pink, brown-pink-dries, and brown-blotch-dries. Since brown may be with either pink or blotch dries, the suggested order is (1) pink followed by (2) brown followed by (3) blotch dries. Loss of the first two adjacent segments would give rise to the compound mutant brown-pink; loss of the second and third adjacent segments would give rise to the compound mutant brown-blotch dries; loss of all three adjacent segments would give rise to the compound mutant brown-pink-dries.

Proof that the compound mutants are composed of two or more of the simple mutants may be obtained readily. It is known that all of the mutant types behave as recessives. It is only necessary to combine two altered rings each of which gives a character or a group of characters in a Df-1/Df-2 or a Df-2/Df-2 plant and observe the characters of the tissues which possess the two altered ring chromosomes. The method is essentially that which is diagrammed in table 1, and the results are in complete agreement with it.

Various combinations of the altered ring chromosomes have been made. Those which involve the mutants brown, pink, brown-pink, brown-pink-dries, brown-blotch-dries are given in table 6. The appearance of the cells and tissues possessing both ring chromosomes are given in column 2. In column 3, the character of the tissues which possess only one or the other of the altered ring chromosomes (following mitotic loss of one or the other ring chromosome, respectively) has been indicated. Where more than one similar combination was made, the particular altered ring chromosome (that is, *I*, *II*, or *III*) is given below the character in each specific combination. It made no difference which ring chromosome producing a given mutant type was used. In every tested case the results were the same. Combination 1 of table 6 (X plus Normal) gives the characters resulting from the combination of any one altered ring chromosome, X, with a normal R-2 ring chromosome. In all of the 15 isolated cases the normal R-2 ring chro-

mosome suppressed the character produced by the altered ring chromosome when both were present in the same cell. However, the presence of the altered chromosome in these plants was made obvious by the variegation which appeared (fig. 4). This variegation resulted from the mitotic losses of the normal R-2 ring chromosome. The character produced by the altered ring chromosome could then be expressed.

TABLE 6

*Phenotypic characters in Df-1/Df-1, Df-1/Df-2, and Df-2/Df-2 plants with various combinations of ring chromosomes together with the type of variegation which is observed in each of these plants following somatic loss of one or the other ring chromosome, respectively. + indicates normal, non-mutant tissue.*

| COMBINATIONS OF RING CHROMOSOMES | CHARACTER PRODUCED WHEN BOTH RING CHROMOSOMES ARE PRESENT | TYPES OF VARIEGATION: CHANGE IN CHARACTER OF TISSUE FOLLOWING SOMATIC LOSS OF ONE RING CHROMOSOME |
|---|---|---|
| 1. X* plus normal R-2 | + | Loss of normal R-2 ring: X tissue.<br>Loss of X ring: + tissue. |
| 2. brown *II* plus brown *III* | Plant totally brown | Loss of brown *II* ring: No change in tissue character.<br>Loss of brown *III* ring: No change in tissue character. |
| 3. brown *II* plus pink | + | Loss of brown *II* ring: pink tissue.<br>Loss of pink ring: brown tissue. |
| 4. brown plus brown-pink<br>*II* " *I*<br>*III* " *I*<br>*II* " *II*<br>*III* " *II* | Plant totally brown | Loss of brown ring: brown-pink tissue.<br>Loss of brown-pink ring: No change in tissue character. |
| 5. brown plus brown-blotch-dries<br>*II* " *I*<br>*III* " *I*<br>*III* " *II* | Plant totally brown | Loss of brown ring: brown-blotch-dries tissue.<br>Loss of brown-blotch-dries ring: No change in tissue character. |
| 6. brown *I* plus brown-pink-dries *II* | Plant totally brown | Loss of brown ring: brown-pink-dries tissue.<br>Loss of brown-pink-dries ring: No change in tissue character. |
| 7. pink plus brown-pink *I* | pink† | Loss of pink ring: brown-pink tissue.<br>Loss of brown-pink ring: No change in tissue character. |
| 8. pink plus brown-blotch-dries *I* | + | Loss of pink ring: brown-blotch-dries tissue.<br>Loss of brown-blotch-dries ring: pink tissue. |
| 9. brown-pink plus brown-blotch-dries<br>*I* " *I*<br>*I* " *II* | Plant totally brown | Loss of brown-pink ring: brown-blotch-dries tissue.<br>Loss of brown-blotch-dries ring: brown-pink tissue. |

* X represents any altered ring chromosome producing a mutant character.

† Due to lack of chlorophyll, plants that are totally pink do not survive beyond the seedling stage. The test was made by combining these two ring chromosomes with a normal ring chromosome. The character produced by the combination (column 2) and the characters indicated in column 3 were obtained from sectors of these plants which had lost the normal ring chromosome.

Combination 2 in table 6 indicates that the simple mutant brown produced by the brown *II* ring chromosome is identical with the simple mutant brown produced by the brown *III* ring chromosome. Plants with these two ring chromosomes are totally brown. No detectible alteration

occurs in the tissues following loss of either ring chromosome. Combination 3 indicates that the simple mutant brown *II* and the simple mutant pink are completely independent of one another, for the pink character is suppressed in the presence of the brown *II* ring chromosome, whereas the brown *II* character is suppressed in the presence of the pink producing ring chromosome. However, the plant is variegated for both brown and pink following mitotic losses of the pink ring or the brown ring, respectively. There were no mutant sectors with the combined character brown-pink. Combination 4 indicates that the brown in the compound mutants brown-pink *I* and brown-pink *II* are identical with the brown character produced by either the brown *II* or the brown *III* ring chromosome, for these plants are totally brown although variegated for pink, following mitotic losses of the brown *II* or the brown *III* ring chromosome, respectively. Since the character brown *II* is identical with brown *III*, the brown character in the compound mutant brown-pink *I* must be identical with the brown character in the compound mutant brown-pink *II*. Similarly, as combination 5 indicates, the brown character of the compound mutants brown-blotch-dries *I* and brown-blotch-dries *II* is identical with the simple mutant character produced by either the brown *II* or brown *III* ring chromosomes. The plants are totally brown but variegated for blotch-dries following mitotic losses of the brown *II* or brown *III* ring chromosomes, respectively. Combination 6 indicates that the simple mutant brown produced by the reduced brown *I* ring chromosome is identical with the brown produced by the ring chromosome giving the compound mutant brown-pink-dries *II*. (This latter compound mutant has not been mentioned previously in this paper.) The homology of the simple mutant pink with the pink produced by the ring chromosome giving the compound mutant brown-pink *I* is shown by combination 7. However, the pink ring covers the brown part of the compound mutant produced by the altered ring chromosome giving brown-pink *I*. Combination 8 shows that the simple mutant pink and the compound mutant brown-blotch-dries *I* have no overlapping effects. The pink ring suppresses the brown-blotch-dries *I* character. Likewise, the brown-blotch-dries *I* ring chromosome suppresses the pink character produced by the pink ring. The combination of the compound mutants brown-pink *I* with either brown-blotch-dries *I* or *II* indicates that the brown character is identical in both compound mutants, for the plants are totally brown. However, the pink character produced by the brown-pink *I* ring is suppressed by the brown-blotch-dries rings, and the blotch-dries character produced by the latter ring chromosomes is suppressed by the brown-pink *I* ring chromosome.

From the combinations given in table 6, it may be seen that the brown mutant produced by an altered ring chromosome is the same whether it

appears as a simple mutant or in combination with other mutants. A similar conclusion may be drawn regarding the pink mutant, although there is less experimental evidence for this on the basis of the combinations given in table 6. It is quite obvious from these combinations that brown, pink, and blotch-dries are independent mutants and that the compound mutants result from combinations of these simple mutants. As stated earlier one may deduce the order of the segments in the R-2 ring chromosome which are responsible for the mutant effects. This order, as stated previously, was: pink followed by brown followed by blotch-dries. The experimental evidence given in table 6 supports this interpretation.

From these tests it may be concluded that the compound mutants are produced by combinations of two (or more) of the observed simple mutants and that the mutants giving the same phenotypic appearances, although arising independently of one another, are identical.

To discuss adequately the characters which are produced in plants carrying the deficient rod chromosomes following combinations of the various altered ring chromosomes would require considerable space and cannot be undertaken within the limits of this paper. However, the results of combining an altered ring chromosome with normal, non-deficient rod chromosomes 5 should be mentioned. If a plant possesses a normal rod chromosome 5 carrying *Bm* and likewise any one of the various altered ring chromosomes, the characters which these ring chromosomes would produce if deficient chromosomes 5 were present now do not appear. The plants are normal. However, when the ring chromosome is returned to plants carrying the deficient rod chromosomes in a successive plant generation, the character produced by the ring chromosome again appears. The characters produced by the ring chromosomes are thus completely recessive to "dominant alleles" which are present in segment 1 to 9 of the normal, non-deficient rod chromosome 5. This is not true, however, if a brown producing ring is combined with normal rod chromosomes 5 carrying the recessive mutant *bm*. All the ring chromosomes which give rise to the brown character (simple or compound mutants) in plants with the deficient rod chromosomes have been placed in plants possessing normal rod chromosomes 5 carrying *bm*. In every case, the plants are totally brown. In these plants, only the brown character appears. The pink, pink-dries, blotch-dries, or light green characters are suppressed by this rod chromosome 5. From these combinations it is strikingly evident that the brown character produced by the altered ring chromosomes is homologous to the known mutant *bm* previously located within region 1 to 4 of a normal chromosome 5 (McCLINTOCK 1938). A normal chromosome 5 carrying any one of the other mutants produced by the altered ring chromosomes has not been isolated as yet.

## REVIEW OF THE EVIDENCE WHICH INDICATES THAT THE CHARACTERS PRODUCED BY THE ALTERED RING CHROMOSOMES ARE CAUSED BY HOMOZYGOUS DEFICIENCIES

Throughout this paper it has been assumed that the mutant characters are caused by homozygous minute deficiencies. It has been determined by many observations that ring chromosomes may increase in size by duplications and repeat duplications of segments composing the ring, or they may become reduced in size through loss of segments from the ring. It has also been determined that whole plants or sections of a plant which possess a ring chromosome with duplicated segments are not obviously modified in phenotypic appearance. Thus, the ring chromosomes with deficiencies are the ones which would be expected to produce an alteration in the phenotypic appearance of the tissues in the plants with the deficient rod chromosomes. Deficient ring chromosomes must be produced in some cells of some of these plants. It could be objected that such homozygous deficiencies might be expected to be cell lethal, and therefore the character produced by the altered ring chromosome could be caused by some other process than deficiency. However, this has proved not to be true. It is known that cells and tissues homozygous for the four chromomere deficiency of the Df-1 rod chromosome are viable. It is also known that these cells and tissues are decidedly modified. The lignified cell walls are brown. This brown is strictly comparable in time of development in the cell wall, in color, and in behavior to light to that produced by the known mutant *bm* which had previously been located within the limits of region 1 to 4 of a normal chromosome 5. (The character of the lignified walls produced when the altered ring chromosomes brown *I*, *II*, and *III*, brown-pink *I*, *II*, etc., are present is likewise strictly comparable to the character produced by the mutant *bm*.) No chlorophyll develops in the cells homozygous for the four chromomere deficiency. Therefore, with regard to chlorophyll, these tissues are colorless. Furthermore, on exposure to sunlight, these cells die and the tissue dries. These tissues have a very reduced growth capacity. It is known that the characters brown, pink, and blotch-dries must be related to changes within the region 1 to 4 of chromosome 5—that is, within the limits of the deficiency in the Df-1 chromosome. It has also been shown that the two very reduced rings (light green-poor growth and brown-light green-poor growth) produce tissues with a decidedly reduced growth capacity. Since the latter two ring chromosomes obviously do not have enough chromatin to cover the deficiency in the Df-1 chromosome, the reduced growth could be ascribed to the presence of the two chromomere deficiency in each case. If we add together the characters produced by the changed rings—that is, brown plus pink (no

chlorophyll) plus blotch-dries plus poor growth—we arrive at the compound character brown-colorless-dries-poor growth, which is exactly the compound character exhibited by the tissues which are homozygous deficient for the four chromomeres deleted from the Df-1 chromosome. This four chromomere deficiency expresses itself as a compound mutant just as brown-pink, brown-pink-dries, and brown-blotch-dries express themselves as compound mutants. Evidence for the compound nature of these mutants has been given in this paper (see table 6). It would be difficult to arrive at a more simple explanation of all these facts than that the characters are produced as the result of homozygous deficiencies, each individual character being produced by a relatively minute deficiency of a specific locus. This will explain both the simple and the compound mutants: the simple mutants resulting from loss of one specific locus, the compound mutants resulting from loss of two or more specific loci. It will also account for the recessive expression of these characters, for it is known that plants with one normal chromosome 5 and either the Df-1 or the Df-2 chromosome 5—that is, hemizygous for regions 1 to 4 or 1 to 9, respectively—are phenotypically normal.

Notwithstanding the cytological observations of reduction in size of many of the ring chromosomes giving mutation effects, genic mutation might be suggested as an alternative to homozygous deficiencies as the cause of the mutant characters. If so, two or more genic mutations would have to occur simultaneously in closely related loci in many instances to account for the considerably greater frequency of occurrence of the compound mutations. Furthermore, the rate of such mutations in the ring chromosome would have to be considerably greater than the rate of mutation in the same region of a normal rod-shaped chromosome 5. Our knowledge of "genic" mutations would not lead us to anticipate such behavior, for we know of no cases where supposedly genic mutations behave in exactly this manner.

Likewise, one might argue that the alterations in the ring chromosomes producing mutant effects are due to changes in the relative positions of the genes in the chromatin of the ring. One must then explain what should be expected of the reduced ring chromosomes, for they are being produced in these plants following aberrant mitoses which alter the chromatin content of the ring. Again, on this basis, one would have to explain away the cytologically obvious deficiencies in nine of the 15 isolated ring chromosomes which produce mutant effects. Since tissues homozygous deficient for chromomeres 1 to 4 of chromosome 5 are known to produce a compound mutant effect which equals the sum of the simple mutant effects located within this region, it would be hazardous to consider that smaller deficiencies within this region would produce no phenotypic effect. One might

object that not all the ring chromosomes giving mutant effects were detectibly reduced in chromatin content, for in four of them no deficiency was detected and in two cases the ring chromosome was enlarged. In all four cases where no deficiency in the ring chromosome was detected, simple mutants were produced (brown *II*, brown *III*, pink, pale green). If, in each case, the phenotypic expression is due to a minute deficiency, it is probable that detection of a single minute deficiency at the meiotic prophase in maize would be extremely difficult. In the cases of the two enlarged ring chromosomes it has been shown (table 6) that the characters produced by these two ring chromosomes, brown-pink *II* and brown-blotch-dries *I*, are identical with the characters produced by the reduced rings brown-pink *I* and brown-blotch-dries *II*, respectively. The enlargement does not mean that there is no deficiency in these ring chromosomes, for an enlargement may have occurred in an aberrant mitosis subsequent to an aberrant mitosis which deleted a segment from the ring chromosome. Following this deletion, no further duplication of the remaining chromatin could restore the original loss. Neither should subsequent deletions of duplicated segments cause any change in the expression of the character produced by such a ring chromosome. It should be emphasized that this proved to be true in the case of the enlarged brown-pink *II* ring.

The above considerations are the basis of the inferential evidence that the plant characters associated with altered ring-shaped chromosomes are produced as a consequence of homozygous deficiencies. In a previous section, the chromatin constitutions of the altered ring chromosomes have been considered briefly. It was pointed out that the brown-blotch-dries *II* ring, the light-green-poor growth ring, and the brown-light green-poor growth ring were all smaller than the normal four chromomere R-1 ring. In the Df-1/Df-1 or Df-1/Df-2 plants, the cells which contain only one such ring must be homozygous deficient for some chromatin within the limits of the deficiency in the Df-1 chromosome. All three of these reduced ring chromosomes may be distinguished from one another by differences in their chromomere constitution. Therefore, they do not have exactly the same deficiencies. Furthermore, each of these three rings produces, respectively, a particular and distinguishable type of compound mutant character. It would be difficult to escape the conclusion that the obviously different total deficiencies in these three rings are the cause of the obviously different total group of mutant characters that each of these three ring chromosomes produces. If one did attempt to account for these observations on some other bases, it would be necessary to assume that each deficiency in these three cases was without a distinct phenotypic effect—which hardly seems probable, considering the very striking compound phenotypic effect that a total deficiency of all four chromomeres produces.

On the basis of the above inferential and observational evidence, it is concluded that the characters exhibited in tissues containing an altered ring chromosome are caused by homozygous deficiencies, each character being caused by a relatively minute but specific deficiency, and that the compound mutant characters are caused by the removal of two or more such segments from the genomic complement.

## CONCLUSION

This paper presents only a summary of the evidence which has led to the conclusion that some phenotypic characters in maize may be produced by homozygous minute deficiencies. A complete account of all the evidence obtained from altered ring chromosomes—their origin, their gametic transmissions both male and female, their stability, the variation in phenotypic expression of the various mutants when combined with different genotypes, the cytological analysis of the chromatin constitutions of the various altered ring chromosomes, the appearance of plants with various combinations of three or more altered ring chromosomes, the rate of production of altered ring chromosomes, evidence showing that the mutants do not revert to normal, and other phenomena related to the behavior of these ring chromosomes—could not be included in this paper due to limitation of space. The evidence of particular interest will be reported in separate publications. It is hoped, however, that sufficient evidence has been included to indicate the nature of the method of attacking this problem and to indicate the type of evidence obtained. Again, due to limitation of space, an adequate discussion of the relation of homozygous deficiencies giving mutant effects in maize to similar phenomena in other organisms cannot be undertaken here. It should be pointed out, however, that the number of analyzed cases is very limited and is best represented by the yellow mutant in Drosophila (EPHRUSSI 1934; STERN 1935; MULLER 1935; DEMEREC 1934, 1936; DEMEREC and HOOVER 1936; KALISS 1939).

In conclusion, the author wishes to point out the possible usefulness of the evidence presented in this paper in formulating a prediction as to the nature, the location, and the crossover values to be expected of mutants not as yet obtained in the normal complement of maize. The ring mutants pink, brown, blotch-dries, and pale green are readily transmitted through the pollen, although the relative efficiency in competition with grains carrying an unmodified complement could not be determined. It is possible, however, that through natural causes or by X-ray or ultra-violet radiation, these mutants would be produced in a normal rod-chromosome 5. Pale green and brown should produce viable plants, but pink and blotch-dries would result in plants which die in the seedling stages. A mutant (brown mid-rib, *bm*, JORGENSON 1931), allelic and indistinguishable from the

brown produced by the ring chromosome mutants, has been isolated. Although it may not be concluded from observational evidence that the *bm* mutant in the normal rod-chromosome 5 is caused by a homozygous minute deficiency, it may be stated, however, that a deficiency of the locus of the dominant allele of *bm* will reproduce in all details the phenotypic expression of *bm*. Pink, blotch-dries, and pale green remain to be isolated. Following isolation, pink and blotch-dries should prove to be very closely linked to the known mutant *bm*, whereas pale green should show a small amount of crossing-over.

## SUMMARY

It is the purpose of this paper to show that viable mutants in maize may be produced by homozygous minute deficiencies. The aberrant mitotic behavior of ring-shaped chromosomes has been the method of obtaining a large number of such deficiencies. These deficiencies are located within the limits of a relatively small segment of the genomic complement composed of the proximal 9 chromomeres of the short arm of chromosome 5.

Mutants arise following changes in the chromatin constitution of the ring-shaped chromosomes. These changes are produced following aberrant behavior of the ring-shaped chromosome in some of the mitotic divisions. The types of mutants which are produced by the altered ring-shaped chromosomes are simple, composed of a single recognizable character or compound, composed of two or more of the characters recognizable as simple mutants. In a number of cases it has been possible to isolate the altered ring-shaped chromosomes which produce the simple or compound mutants.

Through appropriate tests, it has been proven that the compound mutants are the products of two or more of the simple mutants. One group of mutants has been located within the limits of the proximal 4 chromomeres of the short arm of chromosome 5. The second group of mutants has been located within the limits of the next five chromomeres.

Evidence is presented which leads to the conclusion that each mutant character, whether appearing as a simple mutant or in combination with other mutants, is produced by a homozygous minute deficiency, each mutant character being associated with loss of a particular minute segment. The simple mutants are associated with loss of one such segment; the compound mutants are associated with loss of two or more such segments.

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