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*A CORRELATION OF RING-SHAPED CHROMOSOMES WITH  
VARIATION IN ZEA MAYS*

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It has been concluded from cytological and genetical investigations that ordinary rod-shaped chromosomes perpetuate themselves through nuclear cycles by splitting into two similar halves, which pass freely to opposite poles of a mitotic spindle figure. Through such behavior the same genetic constitution in every nucleus of the plant is maintained. The evidence presented here indicates that the size and genetic constitution of ring-shaped chromosomes are not the same in all the nuclei of the plant. In one cell or a group of cells it may be larger or smaller than the original ring chromosome contributed by one of the gametes. The method by which a ring-shaped chromosome becomes reduced in size by loss of a section, or even totally eliminated from the cell, has not been determined. Nevertheless, somatic elimination of a section of a ring-shaped chromosome or a loss of the ring altogether should result in variegation if a genetic marker is present in the ring. If a normal chromosome carrying a recessive gene has as its homologue a ring-shaped chromosome carrying the dominant allelomorph, sporadic loss during development of the ring chromosome or a section of it which carries the dominant will result in groups of cells possessing only the recessive gene.

Eight cases of ring-shaped chromosomes have been studied. In all of these a diminution in size or loss of the ring chromosome was observed. One case was found in untreated material. The other cases arose in the progeny of x-rayed pollen. In five of these instances genetic markers were present in the chromosomes concerned.

All the variegated plants described in this paper appeared in the cultures of Dr. L. J. Stadler. The author is indebted to Dr. Stadler for the privilege of examining these plants for the presence of ring-shaped chromosomes.

The first case to be described involved the gene *B*, which is associated with sun-red plant color. The normal *B-lg* chromosome is easily dis-

tinguishable from the others of the complement by its relative size, the position of the insertion region and the position and size of its knob. The regional location of the gene *liguleless* (*lg*) and the probable locations of the genes *B* and virescent seedling (*v<sub>4</sub>*) on this chromosome are given in figure 1.\*\*\*

Pollen containing the genes *Lg*, *B* and *V<sub>4</sub>* was x-rayed and then placed upon silks of untreated plants possessing the recessive allelomorphs, *lg*, *b* and *v<sub>4</sub>*. The progeny included a seedling which was classified as *liguleless* (*lg*) and probably virescent (*v<sub>4</sub>*). As the plant developed, stripes of red and green tissue were noted on the stalks and leaf sheaths. The red tissue indicated the presence of the dominant gene *B*; the green tissue indicated the loss of this gene during development. Examination of the mid-prophase of meiosis showed, in many cells from different anthers, a large ring-shaped chromosome synapsed with the normal *b-lg* chromosome. The ring was deficient to the extent indicated by the regions beyond the arrows in figure 1. The breaks in the normal chromosome to produce this ring probably occurred a short distance from the end of the

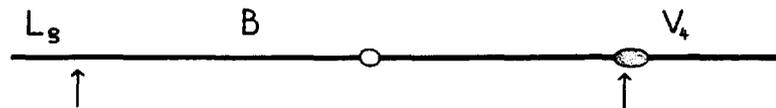


FIGURE 1

short arm, thus removing the *Lg* gene, and through the knob on the long arm possibly removing the *V<sub>4</sub>* gene. In many sporocytes, however, the size of the ring was obviously diminished, this frequently involving a loss of the remaining portion of the knob. Very few sporocytes showed a total loss of the ring chromosome.

This evidence strongly suggests that the loss of the *B* gene, indicated by the green regions in the stalk and leaf sheaths, is due to the absence of that section of the ring chromosome which carries the *B* gene.

A second case of variegation involving the gene *B* was found to be associated with a ring-shaped *B-lg* chromosome. In this case, *lg* and *v<sub>4</sub>* were not marked. Here, also, diminished rings were observed.

A third case of variegation involving the *Pl* gene associated with purple plant color. X-rayed pollen from a plant homozygous for the dominant gene *Pl* was placed upon silks of a plant carrying only the recessive allelomorph, *pl*. The variegation appeared as stripes of purple and sun-red plant color. The locus of the *Pl* gene is toward the middle of the long arm of the satellited chromosome. In the sporocytes of this plant there were 9 normal appearing bivalents, a single satellited chromosome and a small ring-shaped chromosome. Anthers from different regions of the tassel differed in the size of the rings predominating in their

sporocytes. In each anther there were patches of related cells with rings much smaller than the predominating type for the anther. Moreover, the chromosome affected is the one known to carry the gene which is involved in the variegation.

The other two cases examined involved the brown mid-rib gene ( $bm_1$ ) which is associated with brown color in the veins of the leaves and leaf sheaths. This effect is due to a brown coloration in the walls of the cells. Pollen from  $Bm_1$  plants was x-rayed and placed upon silks of  $bm_1$  plants. Variegation expressed itself as patches of brown and of colorless cell walls visible in cross-sections of the stem, or as regions with brown veins on the stalk and leaf.

In the first of these two cases metaphase and anaphase *I* figures showed 10 bivalents plus a fragment chromosome, a total of 21 chromosomes. Examination of mid-prophase of meiosis revealed the fragment as a small ring-shaped chromosome. Furthermore, a deletion in the short arm of the  $bm_1$  chromosome comparable in extent to the most frequently observed size of the ring chromosome was also present. There was one extra insertion region to be accounted for. It is difficult to avoid the conclusions that the insertion region was divided in the formation of the ring and the deleted rod and that both sections of the insertion region were capable of functioning. The ring chromosome did not synapse with the homologous region in the normal chromosome (see McClintock, 1932).<sup>1</sup> The position of the buckling in the normal rod chromosome which compensates for the deficiency in the deleted rod chromosome usually appeared at a little distance from the insertion region in the short arm of the  $bm_1$  chromosome. Evidence recently obtained makes it difficult to interpret these figures with regard to homologous association and thus to be sure of the location of the deficiency. This being so, no decisive statement can be made at this time regarding the origin of the ring and the division of the insertion region.

Diminution in size as well as loss of the ring-shaped chromosome occurred in this plant. Therefore, it is likely that the  $Bm_1$  gene was included in the ring chromosome at the time of its formation from the normal rod chromosome and that the diminution in size or loss of the ring chromosome was responsible for the variegation.

The second case of variegation involving the brown mid-rib gene was similar in some aspects to the one described above. At metaphase and anaphase *I* there were 10 bivalents plus a tiny fragment chromosome, or 21 chromosomes in all. In the mid-prophase of meiosis the fragment chromosome was seen to be a very small ring composed of not more than several chromomeres. In synapsis of the normal  $bm_1$  chromosome derived from the female with the  $Bm_1$  chromosome derived from the male there was no indication of buckling in the normal chromosome to compensate

for a deletion. Since the ring representing the deleted portion was so small, it is not improbable that the deficient chromosome was slightly stretched to compensate for the small loss. Pollen examination indicated that the plant was approximately 50 per cent sterile. It could be concluded, therefore, that a deficiency was present in one of the rod chromosomes. A deficiency in the  $Bm_1$  chromosome corresponding to the ring fragment would account for this sterility in view of the fact that complete absence of the ring fragment was noted in groups of sporocytes or even large sections of the tassel. Furthermore, anaphase *I* was characterized by the frequent loss of the ring fragment in those sporocytes possessing it. The evidence, taken as a whole, suggests that in this, as in the other  $Bm_1$  case, a deletion process involving the  $Bm_1$  region of the chromosome bisected the insertion region producing a ring fragment and a deficient rod chromosome each with a functional insertion region.

Large sections of an anther or even whole branches of the tassel were found to lack the ring-shaped chromosome. It is probable, therefore, that variegation in this case was more commonly associated with complete elimination of the ring chromosome than with diminution in size.

Of a total of 9 variegated plants which were obtained from x-ray treatment, 6 were examined cytologically. One of these gave such poor cytological figures that no definite conclusions could be drawn; in the small amount of material available, no evidence of a ring chromosome was visible. It is possible that the material examined represented a region in which the ring had been lost. It is also possible that some other chromosomal condition was responsible for the variegation, but the material was too poor to yield suggestive evidence on this point. Four of the other five plants showed a ring-shaped chromosome which was obviously derived from the chromosome known to be associated with the gene involved in the variegation. The fifth case, the second  $Bm_1 - bm_1$  variegation, possessed a tiny ring-shaped chromosome but cytological evidence of its origin from the  $Bm_1$  chromosome was not available. The presence of the ring chromosome in some cells and its absence in others, coupled with the appearance of variegation, indicated its origin as being similar to that in the other four cases.

Decrease in size of the ring-shaped chromosome through loss of constituent sections was strikingly manifest in all of the 8 cases of such chromosomes examined. In the ring with a knob marker, the decrease was frequently associated with a loss of the knob. The extent of loss ranged from a relatively small to a very large section of the chromosome. Complete elimination of the ring was observed also, this being especially frequent in those cases having the smaller rings. The evidence from the cases with genetic markers in the affected chromosomes suggests that these losses are responsible for the variegation observed in the plants.

Two other points not directly connected with variegation are of interest with regard to the behavior of ring-shaped chromosomes. These rings may increase in size and in number during the course of somatic divisions. Evidence for increase in the size of the ring was obtained not only in measurements showing such increases in cases where a more or less accurate estimate of the size of the original ring could be made, but also in the duplication of a visible marker within the ring. The ring chromosome in the first case described possessed a fragment of a knob. If the ring maintained itself unchanged throughout successive nuclear divisions, only one knob should be present regularly. In very rare cases 2 or even 4 knobs have been observed in the same ring chromosome. Here, again, where such duplication is evident in the rings, related cells in a group show similar conditions. Occasional accumulation of two or more rings in the same cell was observed in isolated cells or in groups of related cells. The several rings may or may not be of like size.

Investigations which should lead to an understanding of the mechanism responsible for the frequent decrease and occasional increase in size of the rings or for their loss have not as yet been conducted. Lack of uniformity in the splitting plane could give rise to a double sized ring with two insertion regions or cause split halves of the ring to become interlocked. Subsequent movement of the two insertion regions toward opposite poles at anaphase would cause breaks in the ring chromosomes and thus produce changes in their size and constitution. The figures of two interlocking rings in a somatic metaphase in *Crepis* given by Nawashin<sup>2</sup> are suggestive. It is noteworthy that in the decrease or increase in size of the rings in *Zea* nothing but rings have been observed to come from rings.

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\*\*\* A deficiency involving  $V_4$  showed this gene to be in the long arm of the *B-1g* chromosome. Its assignment to a locus between the knob and the end of the chromosome is based only on the case described here. This plant was classified as a probable  $v_4$ . More evidence is necessary before a definite locus within the long arm can be given.

<sup>1</sup> McClintock, B., *Proc. Sixth Internat. Cong. Genet.*, 2, 126-128 (1932).

<sup>2</sup> Nawashin, M., *Univ. Calif. Pub. Agr. Sci.*, 6, 95-106 (1930).