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A CYTOLOGICAL DEMONSTRATION OF THE LOCATION OF
AN INTERCHANGE BETWEEN TWO NON-HOMOLOGOUS
CHROMOSOMES OF ZEA MAYS

BY BARBARA McCLINTOCK

DEPARTMENT OF BOTANY, NEW YORK STATE COLLEGE OF AGRICULTURE

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It has been suggested (Brink,¹ Brink and Burnham²) that semisterility in maize is associated with some form of chromosomal change involving non-homologous chromosomes. Burnham³ reported the presence of a ring of four chromosomes in diakinesis in such semisterile plants which could be explained by assuming either translocation or segmental interchange. Plants showing a ring of four chromosomes in diakinesis and 50% sterility in pollen and eggs gave, when crossed with normal plants, an F_1 generation, one-half of which were normal and one-half of which were 50% sterile. A semisterile plant when selfed gave, again, one-half semisterile plants and one-half non-sterile plants, but one-half of the non-sterile plants were homozygous for the translocation or interchange. When

the latter plants were crossed to normals all the F_1 individuals were 50% sterile and showed a ring of four chromosomes at diakinesis.

The present investigation of semisterile-2 (Burnham³) was undertaken to determine which two chromosomes of the haploid set of ten were involved, and whether a simple translocation or a reciprocal one (segmental interchange) had occurred.

A comparison of the sizes of the four chromosomes constituting the ring with those of the remaining chromosomes of the complement indicated that the chromosomes involved in semisterile-2 were two of the four smallest chromosomes. To determine which two chromosomes were involved plants homozygous for the translocation were crossed with individuals

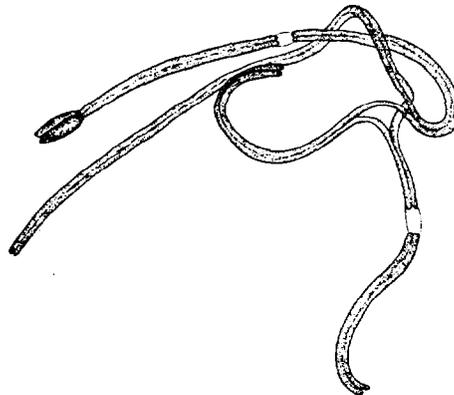


FIGURE 1

Interchange complex in mid-prophase, before opening out of four parasynapsed members to form ring; outline drawing made with the aid of a camera lucida. Magnification, 1875 \times . The clear portions represent the achromatic spindle fiber attachment regions. No attempt has been made to show the chromonemata in detail. For further explanation, see figure 2.

trisomic ($2n + 1$) for (a) the smallest and (b) the fourth smallest chromosome. Examination of meiosis in F_1 $2n + 1$ individuals showed, in both cases, a ring of four chromosomes and also a trivalent, indicating that the two chromosomes of the ring were independent of the smallest and the fourth smallest chromosomes. The chromosomes involved in what proved to be a reciprocal translocation, or segmental interchange, were, therefore, the second and third smallest chromosomes.

Open rings in late meiotic prophase do not show the nature or extent of synaptic association present in the earlier prophase

period. In consequence, early prophase were sought in which the chromosomes, as long threads, were synapsed throughout their entire length. The microsporocyte membrane in *Zea* is very delicate in the early prophase stages. Aceto-carminic smears were made, the cover glass being placed over the sporocytes after removing all excess tissue, and the slides gently heated. With this method the sporocyte flattens, the nuclear membrane disappears and the long thread-like parasynapsed chromosomes are mostly spread out in a horizontal plane. It is frequently easy, therefore, to observe the full length of a parasynapsed bivalent, or an interchange complex which in an unflattened condition would be exceedingly difficult to trace.

Fortunately, the second smallest chromosome possesses, in certain

strains of maize, a very conspicuous accumulation of stainable substance at the end of the short arm; this is more prominent in early and mid-prophases than in later stages.* It is a constant feature of the chromosome, being regularly passed on from one cell generation to another. In the material used this was the only chromosome which possessed such a terminal knob. Consequently, this chromosome, which was involved in the interchange, could be distinguished readily from all the other chromosomes

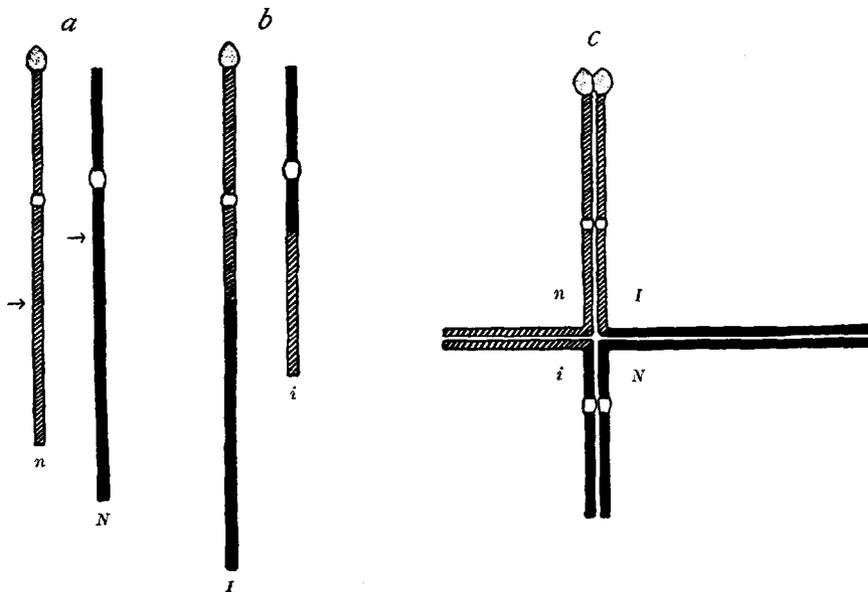


FIGURE 2

a.—Diagram of the two normal chromosomes which were involved in the segmental interchange. The clear portions in the chromosomes represent the spindle fiber attachment regions. The smaller chromosome terminated in an enlarged, deeply staining knob. The arrows indicate the places in the chromosomes at which the interchange occurred to produce the situation shown in *b*. *b*.—The two chromosomes produced as the result of the segmental interchange. *c*.—The type of synaptic complex in mid-prophase of meiosis obtained by combining a normal chromosome complement with an interchange complement through crossing. *N*, larger normal chromosome; *n*, smaller normal chromosome; *I*, larger interchange chromosome; *i*, smaller interchange chromosome.

of the prophase group. Similarly, the interchange complex in a semi-sterile plant could readily be distinguished from other chromosomes of the group.

In mid-prophase the two parental chromosomes in a normal bivalent lie side-by-side throughout their entire length. The most conspicuous structural feature of each chromosome is the spindle fiber attachment region, or so-called constriction. In general, it is a long, relatively clear region,

frequently appearing slightly swollen with the methods used. In some cases a more deeply staining spot is visible at each margin in the mid-region. This spindle fiber attachment region is achromatic; the stainable chromonemata do not pass through it. Furthermore, the relative size of this region is a constant feature of the morphology of the chromosome. In maize, the second smallest chromosome possesses a rather short spindle fiber attachment region. In the third smallest chromosome this region is nearly twice as long. When the two chromosomes are found together in an interchange complex, the contrast is evident.

If a segmental interchange had occurred one would expect, during early meiotic prophase in plants heterozygous for the interchange, a cross-shaped synaptic complex made up of two normal and two interchanged chromosomes (figure 2, *c*). The interchange point in each chromosome would be at the center of the cross. The relative length of the four arms would depend upon the location of the interchange points in the two chromosomes involved. A number of such complexes were observed (figure 1). In some of these the cross was so perfect that it could be photographed readily.

A morphological comparison of the knobbed chromosome in normal plants and in plants homozygous for the interchange showed the length of the longer arm of the knobbed chromosome to be much greater in the latter plants. This marked difference allowed the interchange chromosome (*I*, figure 2, *c*) to be distinguished from the normal chromosome (*n*) in the prophase synaptic complex in plants heterozygous for the interchange. Thus, with the aid of the knob at the end of the short arm of the second smallest chromosome (*n*) and the obvious spindle fiber attachment regions, each of the four chromosomes in the cross-shaped synaptic complex was interpretable. By means of a camera lucida, outline drawings of a number of clear figures were made and the length of each arm of each cross-shaped synaptic complex measured. A close agreement, with regard to the relative lengths of the arms, was found to exist among the figures. The diagrams in figure 2 were constructed after averaging these measurements.

It is clear that an unequal reciprocal translocation has taken place between the long arms of the two chromosomes, and that the interchanged pieces maintain the same orientation with respect to the spindle fiber attachment regions as they did in their previous, normal arrangement.

In later prophase an opening out of the members of the synaptic complex occurs, destroying the cross-like structure and forming the characteristic ring of diakinesis and metaphase *I*.

The distribution of the individual chromosomes in the ring at meiosis could not be observed directly but could be inferred from an analysis of the chromosome complements in the microspores. As a general rule, the chromosomes in the ring are distributed two-by-two in anaphase *I*. Conse-

quently, each spore contains ten chromosomes. Genetic analysis indicates that any 10-chromosome carrying spore possessing one interchange chromosome is sterile, since its nucleus lacks some part of the haploid complement.

The unequal interchange produced chromosomes of two new morphological types. The presence of the conspicuous end knob on the second smallest chromosome (n in figure 2, *a*) and the long interchange chromosome (I in figure 2, *b*) made recognition of these two chromosomes simple and sure.

If homologous spindle fiber attachment points go always to opposite poles, only four types of spores with regard to chromosome complement would be expected, two fertile and two sterile. Of the two fertile types, one would contain the normal chromosome complement (N,n) and one the interchange complement (I,i). Of the sterile complements, one would possess the long interchange chromosome with the end knob (I) plus a normal third smallest chromosome (N); the other would possess the normal chromosome with the end knob (n) and the small interchange chromosome (i). Each spore should contain, then, only one knobbed chromosome. On the contrary, many 10-chromosome-carrying spores were seen which contained the two-knobbed chromosomes (I,n) and no normal third smallest chromosome. Likewise, chromosome complements with no knobbed chromosome were observed. It is obvious, therefore, that in the distribution of the four members of the ring, chromosomes possessing homologous spindle fiber attachments can go to the same pole. Hence, there should be four types of sterile spores (I,n ; n,i ; i,N ; N,I) besides the two fertile ones (I,i ; N,n). Since the sterility is 50%, it is assumed that in half of the sporocytes any two adjacent chromosomes in the ring go to the same pole, forming sterile combinations, and in the other half of the sporocytes the adjacent members go to opposite poles, forming fertile combinations.

No numerical relationship has been established between observed and expected microspore types because of the difficulty of analyzing all types equally well. Two types of sterile combinations, those with the two knobbed chromosomes (I,n) and those without any (i,N) are easy to detect under the microscope, whereas, the other types (n,i ; N,I) are more difficult and require better figures to be properly interpreted. The two readily identifiable sterile types occur frequently enough to support the interpretation of anaphase *I* distribution given above.

Summary.—1. A case of semisterility in *Zea mays* was found to be associated with a reciprocal translocation (segmental interchange) between the second and third smallest chromosomes.

2. Through observations of chromosome synapsis in early meiotic prophase of plants heterozygous for the interchange it has been possible to locate approximately the point of interchange in both chromosomes. The interchange was found to be unequal.

3. An analysis of the chromosome complements in the microspores of plants heterozygous for the interchange indicated that of the four chromosomes constituting a ring, those with homologous spindle fiber attachment regions can pass to the same pole in anaphase *I* and do so in a considerable number of the sporocytes.

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* Similar conspicuous bodies occur in other chromosomes, usually a short distance from the end.

¹ Brink, R. A., *J. Hered.*, **18**, 266-70 (1927).

² Brink, R. A., and C. R. Burnham, *Am. Nat.*, **63**, 301-16 (1929).

³ Burnham, C. R., *Proc. Nat. Acad. Sci.*, **16**, 269-77 (1930).