*NOMARSKI INTERFERENCE CONTRAST RESOLUTION OF SUBCHROMATID STRUCTURE**

By Marjorie P. Maguire

University of Texas, Austin

Communicated by Theophilus S. Painter, April 17, 1968

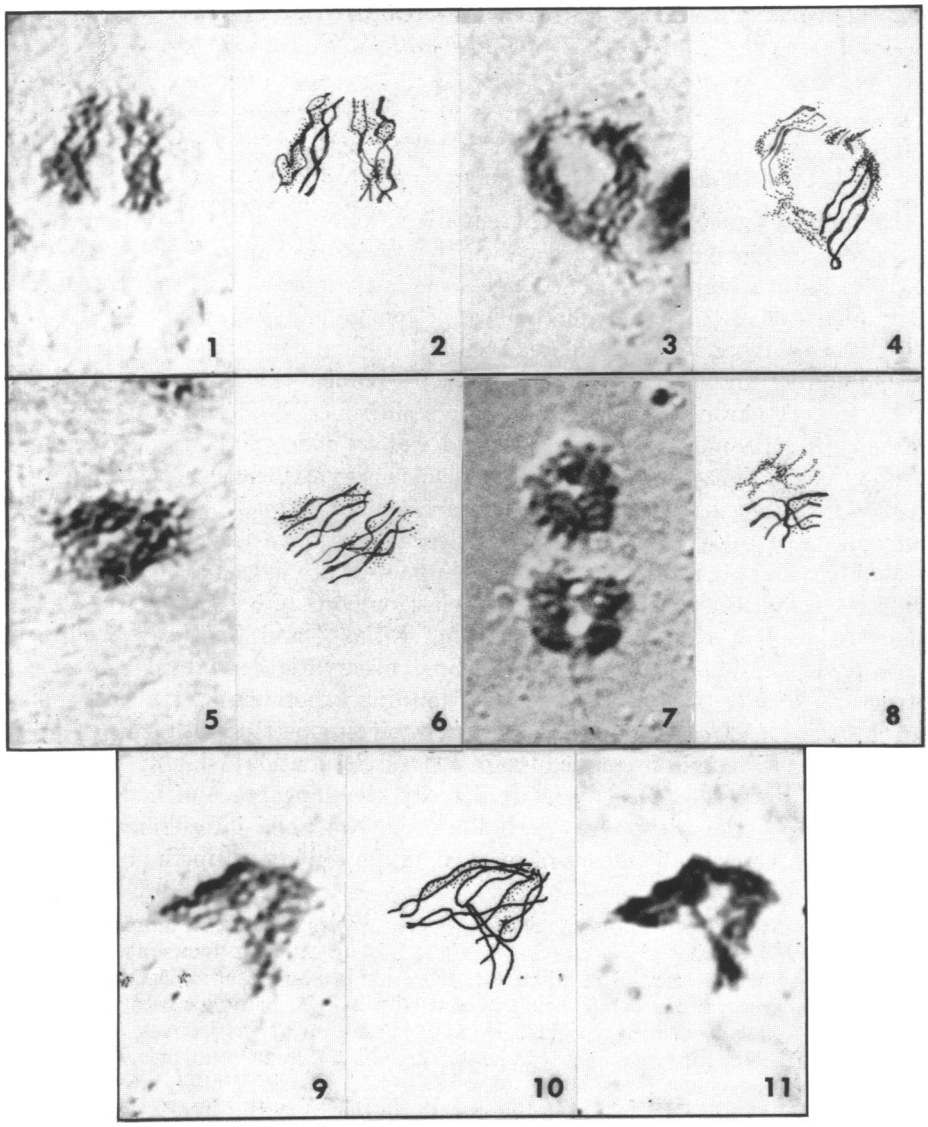
In view of abundant reports that somatic chromatids of many tissues appear to be at least bineme in structure[1–3], whether *meiotic* chromatids are structurally unineme or of a higher order of strandedness is a question of continuing concern to students of the molecular mechanism of genetic crossing-over. The hybrid-DNA-crossover models,[4] which are consistent with an impressive array of genetic recombination and conversion data, seem to require the assumption that the DNA of each chromatid (at the time of crossing-over) is organized into a single molecule or a number of these arranged end to end. Similarly, it is widely acknowledged that gene mutation and a semiconservative pattern of chromosome replications at meiosis[5] could be more simply, but not uniquely, accommodated by unineme structures. Nevertheless, evidence from a variety of observational techniques and organisms that meiotic chromatids are indeed at least bineme is difficult to dismiss. Such evidence includes: reports of half-chromatid aberrations induced at meiosis in *Tradescantia*,[6] *Lilium*,[7] and *Trillium*;[8] studies of meiotic chromatid behavior in coccids;[9] direct observations of internal chromatid structure in maize;[10] and results of N^{15}-labeling experiments at meiosis of an octet strain of *Chlamydomonas*.[11] Electron-microscope studies have so far left the question of degree of strandedness of meiotic chromatids in doubt.

It is the purpose of this report to describe the appearance of maize meiotic chromatids viewed with a new technique, the Nomarski interference contrast system.[3] A conceivable means of reconciling the conflict set forth above is also stressed.

Methods.—Normal diploid maize microsporocyte samples (from KYS inbred line) were fixed in absolute alcohol–glacial-acetic-acid (3:1) mixture at room temperature for 2 hr before storage in a freezer. The microsporocyte samples were later stained in acetocarmine squash preparations before being viewed with a Zeiss photomicroscope equipped with 100× planapochromat bright-field and phase-contrast objectives, achromatic aplanatic phase contrast and inco condenser, green-interference and polarizing filters, and interference-contrast (inco) slide. Microscope with polarizing filter, inco condenser, and inco slide with bright-field objective constitute the Nomarski interference contrast system. The Nomarski technique is suitable for optical-path differences between 0.1λ and 1λ and has the advantage that individual planes can be viewed without the disturbing effect of over- or underlapping-specimen structures.

Observations.—Photomicrographs and diagrams of bivalents at mid- to late diakinesis prepared as described above and viewed with the Zeiss Nomarski interference contrast system or phase contrast system are presented in Figures 1–11. Apparent half chromatids were almost routinely visible in this material when this technique was used. Rarely, a fraying of chromosome substance *or* elements of a higher order of strandedness were seen in short regions.

Discussion.—The following interpretations of the material illustrated in this report are at least superficially conceivable:



FIGS. 1-10.—Photomicrographs, accompanied by diagrammatic interpretations, of maize bivalents at diakinesis, viewed with Zeiss Nomarski interference contrast. Each figure represents a single bivalent, with the exception of Fig. 7, which shows two bivalents (with the lower of these in poor focus).

FIG. 11.—Photomicrograph of one of these bivalents (Fig. 9) viewed with Zeiss phase contrast.

The appearance of chromatid doubleness is most apparent in the right-hand chromatid of the left homologue of Fig. 1, in the lower half of both chromatids of the right homologue of Fig. 3, in both chromatids of the left homologue of Fig. 5, in both chromatids of the lower homologue of the upper bivalent of Fig. 7, and in the right-hand chromatid of the left homologue of Fig. 9. A chiasma is evident in the bivalent illustrated in Figs. 9, 10, and 11. Figs. 1, 3, 5, and 9 were photographed with Kodak 35-mm high-contrast copy film; Fig. 7 was photographed with Kodak 35-mm panatomic X film. Magnification is approximately 2976×.

(1) The possibility that the apparent chromatid doubleness is due to an optical illusion created by the Nomarski system should be considered. However, aside from the fact that this is optically improbable, the same appearance was visible (though less photogenically) with the standard bright-field system in each of the cases shown here and has been illustrated with bright-field photomicrography elsewhere.[10] It was also occasionally visible with phase-contrast optics.

(2) It may be suggested that the "bivalents" illustrated are in fact pairs of bivalents that happen to lie side by side. That this is not so will probably be apparent in at least some of the illustrations to those who are familiar with normal bivalent form. In any case, it should be pointed out that complete cells were examined, and the entire complement of ten bivalents was easily discernible in each. There is no possibility that an error of this type was made.

(3) If the implications are overlooked, it may seem conceivable that the chromatids seen here are folded back on themselves to give the appearance of doubleness. But if this were the case, such folding would have to be a universal or common occurrence in the bivalents of this material. Chromatid doubleness could be seen consistently in most bivalents of many cells at mid to late diakinesis. Obviously, the necessity of terminalization of chiasmata for anaphase separation renders such an explanation implausible. Furthermore, in Figure 9, there is a strong suggestion that those chromatids involved in a chiasma are each double (pairs of half chromatids can be seen to cross-over).

(4) The apparent chromatid doubleness may be due to a fixation artifact that has separated the components of a unineme structure. If this is the case, it seems improbable that chromosomal protein (or other constituent) was separated from DNA because the two strands of each chromatid appear equivalent. It has been suggested that alcohol–acetic-acid fixation may separate the two strands of the DNA helix, and it may be conceivable that each of the chromatid strands seen here represents a single polynucleotide chain (or a number of these end to end) associated with half of the chromatid protein and RNA. The finding of incorporation of tritiated cytidene into alcohol–acetic-acid-fixed chromosomes in the presence of calf-thymus DNA polymerase has been interpreted to mean that acid fixation may indeed denature chromosomal DNA.[12] A nearly complete and very wide separation of DNA double helix (as well as longitudinal splitting of whatever linkers might exist between tandem DNA molecules) from end to end of the chromatid would then be required to account for appearances illustrated in this report. Camargo and Plaut[13] found binding of tritiated actinomycin D to chromosomes following alcohol–acetic–acid fixation, and it is considered unlikely that actinomycin D binds appreciably to single-stranded DNA.[14]

(5) Maize meiotic chromatids may be longitudinally double. If this is the case, they do not become visibly so (by the technique used here) until mid to late diakinesis; at this stage, the diameter of the apparent half chromatids is only slightly greater than the theoretical limit of resolution of the light microscope. It is suggested that chromosome shortening may result in a thickening of the subunits, which may therefore become optically resolvable with light by mid diakinesis in this case.

If meiotic chromatids are indeed ordinarily bineme (but not of a higher order of

strandedness), a hybrid DNA type of crossover model may nevertheless be readily applicable, provided that crossing-over precedes the meiotic DNA replication.[10] Probably the simplest scheme incorporating these ideas and recent findings would postulate that crossover completion follows the major period of meiotic DNA replication of most of the genome as suggested by recent work of Rossen and Westergaard[15] and Henderson,[16] but precedes the DNA replication of an unknown number of short regions randomly distributed about the genetic map. These unreplicated regions in which crossovers could occur between unineme structures (by DNA hybridization) at early meiotic prophase could later become the locations of some of the meiotic prophase chromosomal DNA synthesis reported by Hotta, Ito, and Stern.[17]

* This work was supported in part by a National Institutes of Health Research Career Program Award (1-K3-GM-25,988-01) and by a Public Health Service research grant (GM 15769-01).

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