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THE CHROMOSOMES OF SPIRORCHIS MAGNITESTIS BYRD (TREMATODA, DIGENEA)[1]

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INTRODUCTION

Chromosomes have been described in about fifty species of trematodes. Britt (1947) established certain correlations between cytological and taxonomic details, thereby strengthening some existing systems of classification and weakening others in the groups studied. Spermatogenesis has been described for many species (see Willey and Godman, 1951), and oogenesis for a few (Jones, Mounts and Wolcott, 1945). Details of chromosome structure and behavior, however, have not been revealed by many of the studies since the chromosomes are relatively minute (as in most of Britt's, 1947, species) or the cytological methods used were not reliable.

Spirorchis magnitestis, the trematode studied by the present authors, has large chromosomes. The material was fixed in cytologically useful reagents (Carnoy's, Nawaschin's), cut at 12 microns, and stained with either iron haematoxylin or crystal violet according to the methods recommended by Darlington and LaCour (1947). The following is a report of chromosome morphology and number, behavior in spermatogenesis, and the peculiar pachytene chromosomes which exhibit chromomeric patterns.

DESCRIPTION

Spirorchis magnitestis has 18 chromosomes, diploid. These include one pair of metacentrics, three pairs of long acrocentrics, one pair of intermediate acrocentrics (the nucleolar chromosomes), and four pairs of short acrocentrics. At mitotic metaphase in the spermatogonia the longest chromosomes are about 5-6 microns, the shortest about one micron, in length. At prometaphase the chromosomes are somewhat longer, with distinct centromeres (Figs. 1-7).

Spermatogenesis proceeds in the usual manner. A primary spermatogonium divides, retaining cytoplasmic connection between the daughter cells, and the latter divide, forming a group of four tertiary spermatogonia. These divide, forming eight primary spermatocytes, which undergo meiosis. Two exceptional groups of 16 primary spermatocytes were observed; possibly the number of spermatogonial divisions is not completely determined in this organism. Meiosis is not unusual. Chiasmata are clearly visible from diplotene until anaphase I, and show no evidence of shift (terminalization). After anaphase II, spermatids form, becoming spermatozoa with the loss of considerable cytoplasm.

A study of chiasmata in over 100 cells at diplotene or diakinesis showed (1) that in Spirorchis magnitestis at least one chiasma is formed by each pair of homologous chromosomes, (2) that in the shorter chromosomes there is never more than one chiasma per bivalent, (3) that in the longer chromosomes up to four chiasmata may form, two being of common occurrence, and (4) that in the metacentric chromosome two chiasmata invariably form. Figure 8 illustrates these points. Their possible significance will be discussed below.

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Oogenesis could not be studied in this material because usually few eggs (for trematodes) mature at one time, and there were not enough specimens. Meiosis begins in the ovary, however, as in other trematodes, proceeding through pachytene to a "diffuse" diplotene. In pachytene the bivalents are extremely clear, revealing details not before reported from trematode material (Fig. 5). The nucleolus organizing chromosomes have an unstained (heteropycnotic) region adjacent to the nucleolus. This region may correspond to the secondary constriction seen in many other organisms. Perhaps further study will disclose characteristic patterns of heteropycnotic regions in each pachytene bivalent.

Discussion

The cytology of *Spirorchis magnitestis* presents several points of interest. First, the chromosomes are large enough to provide information (such as centromere location, chiasma frequency and localization,

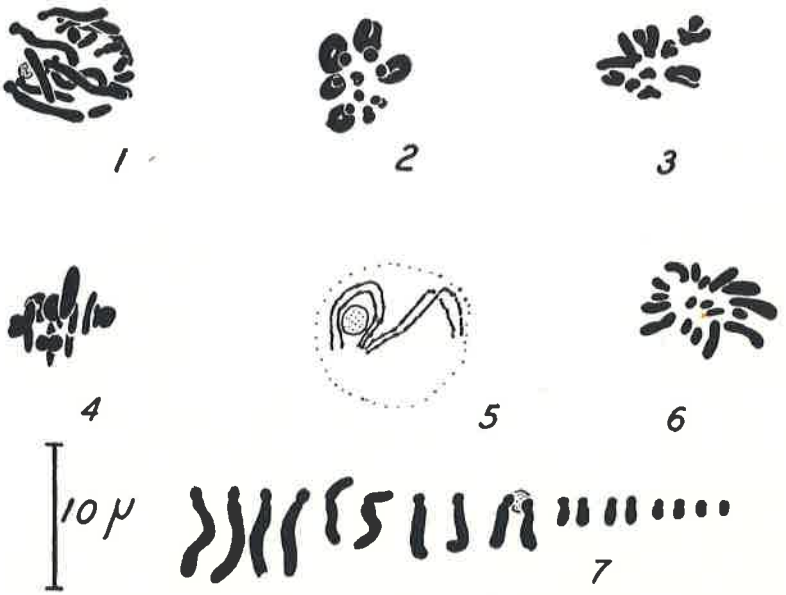

Plate I. Fig. 1. Prometaphase, spermatogonium. Fig. 2, spermatocyte metaphase I, polar view. Fig. 3, metaphase II, polar view, showing metacentric chromosome at upper right. Fig. 4, metaphase I, side view, with metacentric (ring) bivalent left of center, partly obscured. Fig. 5, oocyte nucleus, showing nucleolar chromosomes and one other pair at pachytene. Fig. 6, spermatogonial metaphase. Fig. 7, chromosomes of Fig. 1 arranged according to size and form, in pairs. Figs. 1-7 drawn with the aid of a camera lucida, and reproduced at the magnification indicated.

relatively precise determinations of chromosomal changes during mitosis and meiosis, for instance) that is lacking for chromosomes of trematodes in general. Thus much may be learned from this species, presumably, about trematode cytology, assuming that the chromosomes of other species in the group resemble those of *S. magnitestis* except in size. Second, the analysis of chiasmata in spermatogenesis upholds White's (1948) suggestion that chiasmata

mechanically insure regular disjunction. Thus at least one chiasma occurs in the shortest bivalent, although the long bivalents, which may be four to six times the length of the short ones, seldom contain as many as three chiasmata, and rarely show four. While there is, then, correlation between chromosome arm-length and number of chiasmata, there must be an added factor which insures at least one chiasma per bivalent. This factor may be, as White (1948) has suggested, the presence of heterochromatin "blocks," near which crossing over is likely to occur.

The condition of the metacentric chromosomes is suggestive. These (Fig. 8) always form a bivalent with two—neither more nor fewer—chiasmata. This fact might be explained by supposing that the metacentric had been formed from two rather small acrocentrics by translocation and centromere loss, and that of its two arms therefore each

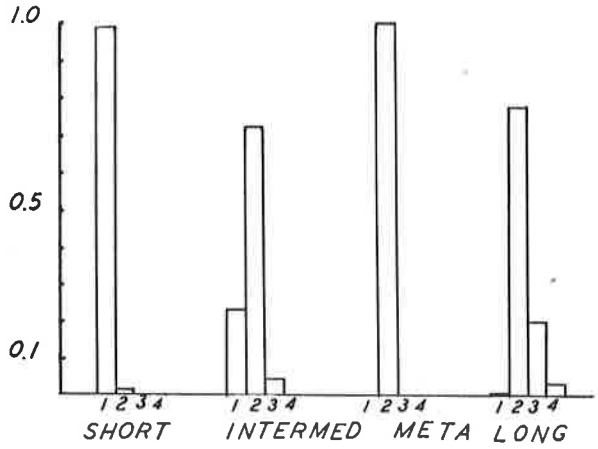

Fig. 8. Chiasma frequencies in the four classes of bivalents, based on totals of 426 short, 90 intermediate, 89 metacentric, and 345 long bivalents.

possesses a heterochromatin block near which a chiasma always occurs. In opposition to this hypothesis—which might find support in studies of the chromosomes of other species of the genus—is the practical difficulty of recognizing any bivalent as having been formed by metacentrics unless it exhibit the classical ring, or two-chiasma, form; the present authors may have excluded unintentionally all non-ring bivalents from their data on the metacentric chromosome.

The presence of heterochromatin can be detected in the chromosomes of *Spirorchis magnitestis*. At pachytene, especially in the oocytes, the beaded or banded appearance (Fig. 5) is probably due to alternating regions of heavily staining euchromatin and lightly staining heterochromatin. It should be possible to show whether or not special regions of these chromosomes are associated with chiasma formation. Further study of the pachytene chromosomes is being planned, with a view to reexamining the taxonomy of the genus. Evolution of its species may be traceable in cytological terms, using

detectable arrangements and rearrangements of the bands of the pachytene chromosomes.

Summary

The chromosomes of *Spirorchis magnitestis* Byrd consist of one pair of metacentrics, three pairs of long acrocentrics, one pair (the nucleolar chromosomes) of medium acrocentrics, and four pairs of small acrocentrics.

Spermatogenesis involves three, rarely four, spermatogonial divisions, followed by normal meiosis.

Chiasma frequencies, studied in meiosis in the testis, are related to length of chromosome arms, the relatively short-armed metacentric having always two chiasmata only. The small bivalents always form chiasmata.

Pachytene chromosomes of the oocyte show chromomeric banding, which may serve to identify specific chromosomes or regions.

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