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CHROMOSOMES OF *FASCIOLOPSIS BUSKI* TREMATODA: FASCIOLIDAE

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ABSTRACT

Mitotic chromosomes of *Fasciolopsis buski* were studied using aceto-orcein squash preparations made from cercarial embryos which were obtained from experimentally infected snails, *Segmentina hemisphaerula*. There were 14 (=2n) chromosomes consisting of 7 homologous pairs. Chromosomes fixed in Carnoy's solution were shorter than those fixed in Newcomer's fluid; the anaphase chromosomes observed were more condensed than the metaphase chromosomes. At metaphase, the average total lengths of 14 chromosomes were 65.9 (Carnoy's) and 86.0 (Newcomer's) microns. The average lengths of each homologue were 7.1, 6.3, 6.1, 5.4, 4.7, 4.4 and 3.7 microns. The arm-length ratios in order of diminishing length of 7 homologues were 1 : 1.3, 1 : 1.6, 1 : 1.3, 1 : 1.8, 1 : 1.3, 1 : 1.1 and 1 : 3.2.

Among the trematodes in the family Fasciolidae, only the chromosome numbers of *Fasciola hepatica* have been studied (Schubmann, 1905; Schellenberg, 1911; Dehorne, 1911; John, 1953; Sanderson 1953). Since the establishment of the life cycle of *Fasciolopsis buski* in the laboratory (Kuntz and Lo, 1967), various stages of this fluke have become available. This report contains observations on the number, size and morphology of the chromosomes of *F. buski*,

MATERIALS AND METHODS

F. buski was maintained in the laboratory at the University of Michigan using *Segmentina hemisphaerula* as the intermediate host. The original stocks of both the snails and the parasites were introduced from Taiwan. The second generation rediae were obtained from the snails eight weeks after exposure to miracidia, and fixed in Carnoy's solution (3 parts 95 percent ethanol: 1 part glacial acetic acid), and in Newcomer's (1953) fluid. Temporary aceto-orcein squash preparations (La Cour, 1941) were made within a week after fixation, and mitotic cells in the cercarial embryos were studied. Chromosomes at various stages of mitosis were recorded by photographs taken at 1500 X and by camera lucida drawings (2600 X). Lengths of chromosome arms were then measured on the drawings to the nearest millimeter, and arm-length ratios calculated. The widths of chromosomes were not considered.

OBSERVATIONS

Various stages of mitosis are shown in Figures 1 to 4. The diploid chromosome number of *F. buski* was found to be 14, and the number could be determined in cells ranging from late prophase to anaphase. In many preparations it was possible to construct 7 matching pairs according to the chromosome length and the position of centromere. In Fig. 5,

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these chromosomes are assigned numbers from 1 to 7 in order of diminishing length. The average length of each chromosome, and the position of the centromere, expressed as arm-length ratio, are shown in Table 1. An idiogram (Fig. 6) was then constructed from this table.

TABLE 1

Chromosome lengths and arm-length ratios of *F. buski*[1]

Chromosome No.	1	2	3	4	5	6	7
Average length (microns)							
Metaphase (6 plates)	7.1	6.3	6.1	5.4	4.7	4.4	3.7
Anaphase (3 plates)	5.6	5.0	4.5	3.9	3.6	3.0	2.8
Arm-length ratio (1 :)[2]	1.3	1.6	1.3	1.8	1.3	1.1	3.2

[1] Fixed in Carnoy's solution.

[2] Length of shorter arm is considered as 1. Both metaphase and anaphase cells are used for calculation.

The condensation of chromosomes was greatest at the end of metaphase when the chromatids began to separate to opposite poles (Fig. 3). This stage apparently was very short, since only 3 cells in this stage were encountered while 42 favorable cells at metaphase and anaphase were available for analysis.

Table 1 shows that the longest chromosome is about twice as long as the shortest chromosome in both metaphase (7.1 microns vs. 3.7 microns) and anaphase (5.6 microns vs. 2.8 microns). The total lengths of 14 chromosomes are shown in Table 2, which includes the data contained in Table 1. These data indicate that the anaphase chromosomes were about 10 microns shorter than the metaphase chromosomes. Furthermore, the chromososomes fixed in Carnoy's solution were about 20 microns shorter than those of comparable stages fixed in Newcomer's fluid. Although all of the squash preparations were made within a week after fixation, the rediae in Newcomer's fluid were processed first. Therefore, it remains to be determined whether the difference in chromosome length was due to the kind of fixative, which appears likely, or due to the different length of fixation time.

TABLE 2

Total lengths of 14 chromosomes of *F. buski* in different fixatives

Fixative	Carnoy's		Newcomer's	
	Metaphase	Anaphase	Metaphase	Anaphase
Length in microns	65.9	54.8	86.0	77.6
No. of plates studied	18	9	10	5

Chromosomes 1 to 5 have submedian centromeres. They always appeared as V-shaped in both metaphase and anaphase, thus the centromeres could be located easily. Chromosome 6 has a nearly median centromere. At metaphase it sometimes resembled a slightly bent rod. During anaphase, however, it was always the smallest V-shaped chromosome with 2 arms of nearly equal length. The determination of the centromere position of chromosome 7, the smallest, was most difficult. It was often rod-shaped or only slightly bent near one end.

DISCUSSION

Chromosome numbers of *Fasciola hepatica*, another member of the family Fasciolidae, have been studied by various workers. The diploid numbers reported were 8 (Schubmann, 1905), 12 (Schellenberg, 1911; John, 1953) and 20 (Dehorne, 1911; Sanderson, 1953). Sanderson pointed out that 2n=12 was erroneous, and showed photographs in which individual chromosomes were clearly identifiable. Thus in the members of the Fasciolidae studied to date the diploid number of chromosomes seems to range from 14 (*F. buski*) to 20 (*F. hepatica*). Such variation in chromosome number within a family has also been reported for several other groups of trematodes (Britt, 1947; Short and Menzel, 1960). According to Walton's list (1959), which includes 24 families and 69 species of digenetic trematodes, the chromosome numbers of these trematodes range from n=6 to 14, lacking n=12 and 13. The haploid number of 7 is seen in *Zygocotyle lunata* and *Gastrothylax crumenifer*, both of Paramphistomidae (Willey and Godman, 1951; Dhingra, 1955), *Notocotylus filamentis* of Notocotylidae (Ciordia, 1950), *Gorgoderina attenuata* of Gorgoderidae, *Bunodera luciopercae* of Allocreadiidae (Britt, 1947) and *Schistosomatium douthitti* of Schistosomatidae (Nez, 1954; Nez and Short, 1957).

The usefulness of chromosomal data in solving taxonomic problems varies from group to group. Because of overlapping in chromosome numbers on diverse groups of trematodes, Walton (1959) pointed out that the chromosome morphology and behavior must be given emphasis in elucidating the phylogenetic relations among these animals. Britt (1947) studied 35 species in 8 families of Digenea and found that in some cases genera could be distinguished by karyotypes. In Schistosomatidae, Short and Menzel (1960) showed that karyotype of each genus (6 genera studied) was distinct, while it was difficult or impossible to separate species within a genus (2 genera studied). Cytological data available to date indicate that aneuploidy and other minor chromosomal rearrangements rather than polyploidy seem to play a major role in the chromosomal evolution of digenetic trematodes. Therefore, it was not unexpected that the karyotypes of *F. buski* did not show resemblance to any one of the species having the same chromosome number. It would be of interest to examine the phylogenetic relations between *F. hepatica* and *F. buski* with the techniques of karyotype analysis.

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PLATE I. CHROMOSOMES OF *FASCIOLOPSIS BUSKI*

Fig. 1. Late prophase
Fig. 2. Metaphase
Fig. 3. Early anaphase
Fig. 4. Anaphase
Fig. 5. Camera lucida drawings of the chromosomes shown in Fig. 2.
Fig. 6. Idiogram. The lengths are based on metaphase cells shown in Table I.

Figs. 1-5 have the same magnification.

PLATE I