The Chromosomes of *Cotylogaster occidentalis* and *Cotylaspis insignis* (Trematoda: Aspidogastrea) with Evolutionary Considerations

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ABSTRACT: The chromosome number and morphology for two species of Aspidogastrea were studied. The chromosome number of *Cotylogaster occidentalis* is $2n = 12$ and for *Cotylaspis insignis* is $2n = 22$. *Cotylogaster occidentalis* has two pairs of metacentric chromosomes, two pairs that are subcentric and two pairs that are acrocentric. The largest pair of chromosomes are 6 $\mu$m long, while the shortest are 2 $\mu$m. Only meiotic chromosomes were observed for *Cotylaspis insignis*. Phylogenetic implications of chromosome numbers in the Aspidogastrea are discussed.

Among the Trematoda, the Monogenea and Digenea have been subjected to cytological analysis, while in the Aspidogastrea incidental observations have been made in two cases and a definite chromosome number report in one instance (Table 1).

Osborn (1905) depicted an anaphase cell and several cells at the pachytene stage of meiosis (pl. 14, figs. 42-45, p. 241; pl. 15, fig. 52, p. 252, respectively) of *Cotylaspis insignis*, but referred to them as “chromatine” masses in the cytoplasm. Brinkman (1957) described “with safety 6 pair of daughter chromosomes” from *Macraspis elegans* but concluded the chromosome number to be at least “6 probably 8 diploid.” Rohde (1976), the first to report an actual chromosome number, observed $2n = 14$ for *Lobatostoma manteri*.

This report is a study on the chromosome numbers and morphology of two aspidogastrid species.

Materials and Methods

*Cotylogaster occidentalis* was obtained from the pericardial cavity of *Legumia recta* collected near Douglas Lake, Michigan; *Cotylaspis insignis* from the gills of *Anodonta corporulentum* from Lake Pepin, Minnesota.

The parasites were fixed in a modified Carnoy's fluid (3 parts glacial acetic acid; 1 part 95% ethanol) for approximately 4 hr, after which they were stained with either acetic-orcein (LaCour, 1941) or Gomori's chrom alum hematoxylin (Short and Menzel, 1960). The ovaries and testes usually with surrounding tissue were removed by dissection and handled separately from the remaining tissue which was cut into small pieces. Pieces of tissue (ovaries, testes, etc.) were placed on a clean slide, rinsed with 45% acetic acid to remove excess stain, and squashed under a coverslip. Pressure from the thumb was sufficient to adequately spread the chromosomes. The slides were sealed temporarily with paraffin and later were made permanent by the method of Conger and Fairchild (1953).

Observations

In *Cotylogaster occidentalis* eight cells in mitosis showed metaphase chromosomes suitable for detailed observations. Each cell had 12 chromosomes ($2n = 12$) (Figs. 1–3). There were two pairs of acrocentrics, two pairs of submetacentrics, and two pairs of metacentrics. The longest chromosome measured ca. 6 $\mu$m in length while the shortest was ca. 2 $\mu$m long. No meiotic chromosomes were observed for *Cotylogaster occidentalis*. However, in *Cotylaspis insignis* 20 different meiotic metaphase cells obtained from testes had the haploid number 11 ($2n = 22$) (Fig. 4). Mitotic metaphase chromosomes observed in *Cotylaspis insignis* were judged as not reliable for chromosome number determination or characterization.

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Table 1. Chromosome number determinations for the Trematoda.*

<table>
<thead>
<tr>
<th>Mongenea</th>
<th>Digenea</th>
<th>Aspidogastrea†</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 families</td>
<td>26 families</td>
<td>1 family</td>
</tr>
<tr>
<td>3 genera</td>
<td>60 genera</td>
<td>3 genera</td>
</tr>
<tr>
<td>3 species</td>
<td>85 species</td>
<td>3 species</td>
</tr>
</tbody>
</table>

* See Walton, 1959; Shott and Menzel, 1960; Pickel and Jones, 1967; Rohde, 1973; Filippo and Fried, 1974; LoVerde, 1974; Fried, 1975.
† Includes this report.

Discussion

The generally accepted phylogenetic hypothesis is that a rhabdocoele stock of turbellarians (the Dalyellioidea) gave rise to the Trematoda (see Walton, 1959; Llewellyn, 1965; Stunkard, 1967; Rohde, 1972, 1973). It is also generally assumed that the Monogenea and Digenea were derived independently most likely from different rhabdocoele stocks.


Figure 4. Meiotic metaphase of Cotylaspis insignis, n = 11.
Table 2. Chromosome numbers for three families of trematodes.

<table>
<thead>
<tr>
<th>Family</th>
<th>Species</th>
<th>Haploid number*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heronimidae</td>
<td><em>Hermonius chelydra</em></td>
<td>10</td>
</tr>
<tr>
<td>Microscaphiidae</td>
<td><em>Gigantocotyle bathocotyle</em></td>
<td>8, 9</td>
</tr>
<tr>
<td>Paramphistomidae</td>
<td><em>Gastrothylax crumenifer</em></td>
<td>6</td>
</tr>
<tr>
<td></td>
<td><em>Zygocotyle lunata</em></td>
<td>7</td>
</tr>
<tr>
<td></td>
<td><em>Cotylpheron elongatum</em></td>
<td>8</td>
</tr>
</tbody>
</table>

* See Walton, 1959 for exact references.

Evidence presented by Stunkard (1963) and more recently by Rohde (1971, 1972, 1973) indicates that the Aspidogastrea are more closely allied to the Digenea than to the Monogenea, and that the Aspidogastrea exhibit archaic features; Rohde (1972, p. 143) suggests that they "stand close to the root of the Digenea, i.e., to the hypothetical Prodigenea." Both authors agree that the Aspidogastrea and Digenea descended from a common ancestor. Cable (1974), on the other hand, has suggested that the Aspidogastrea could as well have been derived from "heteroxenous ancestors." All agree that it is unlikely the Aspidogastrea and Digenea evolved independently from a common turbellarian stock.

The chromosomal data presented here have a bearing on the phylogeny of these groups. In the dalyellioi d rhabdocoel es the haploid chromosome number ranges from 2–4 (Ruebush, 1937, 1938). For the three species of Monogenea whose chromosome numbers are known, haploid numbers of 4 and 6 have been reported (Pickel and Jones, 1967). In the Digenea, the haploid chromosome number ranges from 6 to 14, with 11 being the most common (Britt, 1947; Walton, 1959). The three species of Aspidogastrea exhibit 6, 7, and 11 as haploid numbers. In a comparative study of the Turbellaria, Ruebush (1938) found that the acocelids and allocoelids, considered to be the most primitive families on noncytological grounds, consistently possessed the highest number of chromosomes. Conversely, in the Trematoda, where chromosomal evolution seems to be proceeding by aneuploidy also, it appears to be correlated with phylogenetic advancement. The aspidogastrids studied so far exhibit the haploid chromosome numbers 6, 7, and 11 which are within the upper range of the Dalyellioidea and the lower range of the Digenea. According to the scheme of Cable (1974), the Aspidogastrea would be derived from an evolutionary line leading to the Heronimidae, Microscaphiidae, and Paramphistomidae. The chromosome numbers that have been reported for these families are in Table 2. Cable argued (p. 188) that "whether the heronimid cycle is primitive or not, it is just one "step" from the cycle of the aspidobothrians as far as generations are concerned and equally close in its host–parasite relationships." The chromosome data indicate that it is plausible that the Aspidogastrea were derived from paramphistome stock as suggested by Wootton (1966).

Nevertheless, the chromosome data do support the notion that members of the Aspidogastrea stand close to the root of the Digenea. It is hoped that further studies of chromosome number and morphology in the Aspidogastrea will aid in establishing the relationships between them and the dalyellioi d rhabdocoele group of turbellarians and digenea on one hand, and their interspecific relationships on the other.

Acknowledgments

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Literature Cited


### ANNOUNCEMENT

The Division of Systematic Zoology of the American Society of Zoologists will sponsor a symposium entitled, “Contemporary Methods in Systematic Parasitology” which will be presented during the joint meetings of the American Society of Zoologists and American Microscopical Society December 27–30, 1978 in Richmond, Virginia. Numerical and non-numerical approaches will be presented and their applications discussed. For further information contact either Daniel R. Brooks, Department of Biology, University of Mississippi, University, Mississippi 38677 or W. Wayne Moss, The Academy of Natural Sciences of Philadelphia, Department of Entomology, Nineteenth and the Parkway, Philadelphia, Pennsylvania 19103.