

On the karyology of trematodes of the genus *Microphallus* and their intermediate gastropod host, *Littorina saxatilis*

I. Chromosome analysis of three *Microphallus* species

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Abstract

The karyotypes of three marine trematode species of the genus *Microphallus* (Microphallidae), *M. pygmaeus*, *M. piriformis* and *M. triangulatus*, the most typical parasites of *Littorina saxatilis* (Gastropoda), are similar and include 9 pairs of bi-armed chromosomes, of which two pairs are represented by large meta- and submetacentrics, and 7 pairs are smaller chromosomes. It is proposed that the ancestral trematode karyotype consisted of 20 chromosomes and, therefore, the karyotypes of the microphallids are the advanced ones.

Introduction

The diploid numbers of over 150 digenean species are known (reviews in Britt, 1947; Walton, 1959; Saksena, 1969; Benazzi & Benazzi-Lentati, 1976). But the data listed in these and earlier works are frequently incorrect because of imperfect cytological methods used (Puente & Short, 1985). At the same time the karyological information is important for clearing up the phylogenetical and taxonomical relationships between species within the Digenea (Subramanyam & Venkat Reddy, 1977). Up to date the chromosome sets of the representatives of some digenean families were studied in detail (Table 1). There were no data on the karyology of the marine family Microphallidae in the literature. Therefore in the present paper the karyotypes of three *Microphallus* species are described. These closely related digeneans, *Microphallus pygmaeus*, *M. piriformis* and *M. triangulatus*, belong to the 'pygmaeus' group of species (Galaktionov, 1983; 1984). They are very

specialized forms and only two hosts (molluscs and birds) take part in their life cycles (Belopolskaya, 1949). The karyotype of their intermediate host, the gastropod *Littorina saxatilis* from the population inhabiting the southwestern coast of the Barentz Sea, will be presented in the following paper (Birstein & Mikhailova, 1990).

Material and methods

The metacercaria of *Microphallus pygmaeus*, *M. piriformis* and *M. triangulatus* were taken from the invaded specimens of *Littorina saxatilis* (Gastropoda: Prosobranchia). The molluscs were collected on the exposed areas of the Yarnyshnaya inlet littoral near the Murmansk Institute of Marine Biology (the Kola Peninsular, USSR). Chromosome slides were prepared from the marites grown *in vitro*. For this purpose the metacercaria were taken from the sporocysts in sterile conditions and then placed into Eagle

Table 1. Chromosome numbers of the Digeneans.*

Paramphistomidae		
<i>Ceylonocotyle dicranocoelium</i>	18	Subramanyam & Venkat Reddy, 1977
<i>Cotylophoron cotylophorum</i>	16	Subramanyam & Venkat Reddy, 1977
<i>Cotylophoron</i> sp.	16	Subramanyam & Venkat Reddy, 1977
<i>Fiscoederius elongatus</i>	16	Subramanyam & Venkat Reddy, 1977
<i>Gastrothylax crumenifer</i>	18	Romanenko, 1974; Subramanyam & Venkat Reddy, 1977
<i>Gigantocotyle explanatum</i>	18	Subramanyam & Venkat Reddy, 1977
<i>Liorchis scotiae</i>	18	Romanenko, 1974
<i>Megalodiscus (Diplodiscus) temperatus</i>	20**	Grossman & Cain, 1981
<i>Paramphistomum cervi</i>	14	Venkat Reddy & Subramanyam, 1975
<i>P. epiclitum</i>	18	Subramanyam & Venkat Reddy, 1977
<i>P. ichikawai</i>	18	Romanenko, 1974
<i>P. microbothrium</i>	18	Mutafova, 1983a
<i>Stichorchis subtriquetrus</i>	18	Romanenko, 1974
Notocotylidae		
<i>Notocotylus attenuatus</i> , <i>N. imbricatus</i>	20	Petkevitiute & Baršienė, 1988
<i>N. ephemera</i>	20; 21	Petkevitiute & Baršienė, 1988
Cyclocoelidae		
<i>Cyclocoelum oculeum</i>	20	Taft & LeGrande, 1979
Fasciolidae		
<i>Fasciola gigantica</i>	20	Romanenko & Pleshanova, 1975; Subramanyam & Venkat Reddy, 1977
<i>F. hepatica</i>	20	Romanenko & Pleshanova, 1975
<i>Fasciola</i> sp.	20	Sakaguchi & Wakako, 1976; Sakaguchi, 1980
<i>Fasciola</i> sp., 3n	30	Sakaguchi & Nakayama, 1975; Sakaguchi & Wakako, 1976; Sakaguchi, 1980
Philophthalmidae		
<i>Philophthalmus gralli</i>	20	Grossman & Cain, 1981
<i>Philophthalmus</i> sp.	20	Venkat Reddy & Subramanyam, 1971
<i>-Philophthalmus</i> sp. (Georgia, USSR; Bulgaria)	20	Mutafova, 1983b
Echinostomatidae		
<i>Echinoparyphium aconiatum</i> , <i>E. recurvatum</i>	20	Mutafova & Kanev, 1984; Mutafova <i>et al.</i> , 1987
<i>Echinostoma barbosai</i> , <i>E. echinatum</i> , <i>E. revolutum</i>	22	Mutafova & Kanev, 1983; 1986
<i>Hypoderaeum conoideum</i>	20	Mutafova <i>et al.</i> , 1986
Hemiuridae		
<i>Isoparorchis euritremum</i> , <i>I. hypselobargi</i>	20(XY)	Chattopadhyay & Manna, 1987
Schistosomatidae		
<i>Austrobilharzia variglandis</i>	16	Short & Menzel, 1960
<i>Gigantobilharzia huronensis</i>	16(+ B)	Short & Menzel, 1960; LoVerde & Kuntz, 1981
<i>Heterobilharzia americana</i>		
Texas	20(ZW)	Short & Grossman, 1986
Louisiana	♂ 20(ZZ)	Short <i>et al.</i> , 1987
	♀ 19(ZWA)	Short <i>et al.</i> , 1987
<i>Ornithobilharzia huronensis</i>	16(XY)	Short & Menzel, 1960
<i>Schistosoma bovis</i> , <i>S. haematobium</i> , <i>S. intercalatum</i> , <i>S. matthei</i>	16	Short, Menzel, 1960; Grossman <i>et al.</i> , 1981a
<i>S. japonicum</i> , <i>S. mekongi</i>	16(ZW)	Grossman <i>et al.</i> , 1981b

Family, species	2n	Reference
<i>S. mansoni</i>	16(ZW, No. 1)	Short & Menzel, 1960; Short <i>et al.</i> , 1979; Grossman <i>et al.</i> , 1980
<i>S. mansoni</i>	16	Atkinson, 1980; Short & Grossman, 1981
<i>S. rodhaini</i>	16(ZW, No. 2)	Atkinson, 1980; Grossman <i>et al.</i> , 1981; Grossman, 1981
<i>Schistosomatium douthitti</i>	14(ZW, No. 1)	Short & Menzel, 1960; Short & Grossman, 1981; Puente & Short, 1985
<i>Trichobilharzia physellae</i> , <i>T. stagnicola</i>	16	Short & Menzel, 1960
Diplostomatidae		
<i>Diplostomum indisticum</i> , <i>D. mergi</i> , <i>D. pseudospathaceum</i> , <i>D. spathaceum</i>	20	Romanenko & Shigin, 1977; Mutafova & Niewiadomska, 1988
<i>Tylodelphys clavata</i>	20	Romanenko & Shigin, 1977
Strigeidae		
<i>Gogatea serpentium indica</i>	16	Subramanyam & Venkat Reddy, 1977
Opisthorchidae		
<i>Opisthorchis felineus</i>	14	Romanenko, 1973
Allocreadiidae		
<i>Allocreadium fasciatus</i> , 3n	21	Ramanjaneyulu & Madhavi, 1984
<i>A. handiai</i>	14	Ramanjaneyulu & Madhavi, 1984
Troglorematidae (Paragonimidae)		
<i>Paragonimus kellecotti</i>	22	LoVerde, 1979
<i>P. miyazakii</i>	22	Sakaguchi & Tada, 1975; 1976; Terasaki, 1977; Hirai <i>et al.</i> , 1985
<i>P. ohirai</i> , 2 geographical races (<i>P. iloktsuemensis</i> , <i>P. sadoensis</i>)	22	Sakaguchi & Tada, 1975; 1976; Terasaki, 1977; Hirai <i>et al.</i> , 1985
<i>P. westermani</i>	22	LoVerde, 1979; Hirai <i>et al.</i> , 1985; Sugiyama <i>et al.</i> , 1985
<i>P. westermani</i> , 3n (= <i>P. pulmonalis</i>)	33	Sakaguchi & Tada, 1976b; Terasaki, 1977; Agatsuma & Habe, 1985; Hirai <i>et al.</i> , 1985
Plagiorchidae		
<i>Trematorchis ranarum</i>	18	Subramanyam & Venkat Reddy, 1977
Lecithodendriidae		
<i>Ganeo tigrinum</i>	22	Subramanyam & Venkat Reddy, 1977
Microphallidae		
<i>Microphallus piriformes</i> , <i>M. pygmaeus</i> , <i>M. triangulatus</i>	18	This paper
Dicrocoeliidae		
<i>Dicrocoelium lanceolatum</i>	24	Romanenko, 1979
<i>Eurytremum pancreuticum</i>	26	Romanenko, 1979
<i>Paradistomoides orientalis</i>	28	Sharman & Nakahasi, 1974
Transversotrematidae		
<i>Transversotrema patialense</i>	20	Madhavi & Ramanjaneyulu, 1986

* Data from the works in which authors used colchicin or described chromosome morphology in detail. The systematics is given according to Brooks *et al.* (1985). B- and sex chromosomes and their numbers are shown in brackets.

** In this species the homologues of the pair No. 10 differ in size and C-heterochromatin content.

tissue culture medium, to which bovine serum (up to 10%), glutamine (3.0 mg per ml), gentamycine (0.3 mg per ml) and colchicine (0.25%) were added. The developing metacercaria were incubated *in vitro* for 8–16 hours at 37 °C. Adult worms were washed with Ringer solution, placed in distilled water for 40 min, and then fixed in ethanol-glacial acetic mixture, 3 : 1. The cell suspension was prepared in 50% acetic acid by means of a syringe. The preparations obtained were stained with 2% Giemsa solution in phosphate buffer, pH = 6.8. They were analysed with a Zeiss NU-2 microscope (K. Zeiss, Jena) with a 100× oil immersion objective.

Results

The karyotypes of the three *Microphallus* species are similar and consist of 9 pairs of chromosomes (Fig. 1). All elements of the sets are bi-armed. The absolute length of chromosomes depends in a

great degree on the metaphase substage, i.e. on chromosome spiralization (compaction). The unusual feature of these chromosomes is that in many preparations the centromeric regions were not stained and appeared as unstained gaps. One can divide the chromosomes into three size groups: two pairs of large-sized homologues, a pair of middle-sized ones, and a group of chromosomes diminishing in size. Pairs Nos. 1 and 3 are represented by metacentrics, pair No. 2 by submetacentrics. The morphology of the homologues of the other pairs is not as clear. It is evident that in *M. pygmaeus* pair No. 4 consists of submetacentrics, and in *M. triangulatus* and *M. piriformis*, of metacentrics. The homologues of pairs Nos. 7 and 8 in *M. pygmaeus* and *M. piriformis* are subtelocentrics, and in *M. triangulatus* they are most likely submetacentrics, while pair No. 9 in all three species consists of metacentrics.

Discussion

The karyotypes of three *Microphallus* species studied are very similar, including 9 pairs of bi-armed chromosomes, of which two pairs are clearly larger than the rest. The biological specifics of the trematodes investigated by us consist, firstly, in the participation of only two (not three) hosts, i.e. molluscs and birds, in the life cycle of the parasites, and, secondly, in the absence of a free-living cercaria stage (Belopolskaya, 1949); in other words, these trematodes are very specialized forms. According to the chromosome number, the microphallids examined take an intermediate place between most of the digeneans studied, $2n = 20$, and some species of a very specialized family, the Schistosomatidae, $2n = 16$ (Table 1). As the karyotypes consisting of 20 chromosomes are characteristic of species belonging (according to the phylogenetic scheme of Brooks *et al.* (1985)), to primitive families such as Echinostomatidae, as well as species of the relatively advanced family Paragonimidae and the family Transversotrematidae of unclear systematic position, one may conclude that there is an evolu-



Fig. 1. Metaphase plates and karyotypes of *Microphallus* species: *M. pygmaeus* (a), *M. triangulatus* (b) and *M. piriformis* (c). Bars represents 10 μ m.

tionary tendency in the Digenea to maintain the diploid number equal to about 20, which seems to be the ancestral one.

On the contrary, the traditional viewpoint is that the ancestral trematode karyotype included a rather low number of chromosomes. Thus, it was proposed that the ancestral karyotype of the schistosomatids consisted of 16 elements (Short & Menzel, 1960). But there are 10 pairs of homologues in the recently described karyotype of *Heterobilharzia americana* (Short & Grossman, 1986). These data seem to be in accordance with our assumption that the generalized ancestral karyotype of trematodes included 20 chromosomes.

If this is so, the karyotypes of the microphallids and schistosomatids are the advanced ones, the decrease in the diploid number in the former down to 18 and in the latter down to 16 and 14 must be connected with the extreme specialization of the life cycles of these trematodes, since in schistosomatids, like in microphallids, only two hosts take part in the cycle. The same reduction of the ancestral chromosome number in the course of the specialization of a group was found in some other invertebrates (Colombero & Lazzaretto-Colombero, 1978) and in many vertebrates (Birstein, 1987). Therefore, the Digenea is one more example of this tendency.

A comparison of the karyotypes of schistosomatids suggests that the decrease in chromosome number is due to the translocations, or fusions, of small chromosomes in the ancestral set, as species with $2n = 14$ have only one pair of small chromosomes (species with $2n = 16$ have 3 pairs), and, at the same time, the number of large and middle-sized chromosomes increases from 5 to 6 pairs (Puente & Short, 1985). Similar processes seem to occur in Paramphistomidae (Table 1). Besides, a subsequent differentiation of karyotypes has occurred in schistosomes: in some species a heteromorphic pair of sex chromosomes has formed (Table 1).

The presence of two pairs of large chromosomes must be a characteristic feature of the karyotypes of the 'pygmaeus' group of microphallids. Among other digeneans studied in detail,

two pairs of large bi-armed chromosomes were found in *Paramphistomum cervi* (Paramphistomidae), *Paragonimus kellicotti* (Paragonimidae), *Opisthorchis felinus* (Opisthorchidae), *Dicrocoelium lanceolatum* (Dicrocoeliidae) and *Transversotrema patialense* (Transversotrematidae) (Romanenko, 1973; 1979; Venkat Reddy & Subramanyam, 1975; LoVerde, 1979; Madhavi & Ramanjaneyulu, 1986). But the difference in size between the two largest chromosome pairs and the others is larger in the *pygmaeus* group than in the enumerated species. Only in *O. felinus* is this difference greater, but the karyotype of this species is unlike the karyotypes of other digeneans in general, as it consists of two pairs of very large metacentrics and 5 pairs of relatively very small chromosomes (Romanenko, 1973).

The majority of other species, mostly belonging to the families Paramphistomidae, Echinostomatidae, Schistosomatidae, Troglorematidae, have only one pair of large meta- or submetacentrics (Short & Menzel, 1960; Romanenko, 1974; Hirai *et al.*, 1985; Sugiyama *et al.*, 1985; Mutafova & Kanev, 1986; Mutafova *et al.*, 1986; Madhavi & Ramanjaneyulu, 1986). Therefore, it is possible that the reduction of the diploid number in the ancestral forms of the Microphallidae occurred as a result of the disappearance of a pair of small chromosomes. It might be translocated to another pair, resulting in the formation of a second pair of large homologues.

It is necessary to note that in some groups of the Digenea the chromosome number actually increased, possibly due to fission of some homologues of the ancestral set. Such processes seem to occur in the Dicrocoeliidae because these trematodes have karyotypes of 24–28 chromosomes (Table 1). Neither the increase, nor the formation of triploid parthenogenetic forms (which is characteristic of the Fasciolidae, Paragonimidae and Allocreadidae – Table 1) occur in so specialized digeneans as microphallids and schistosomatids.

On the basis of the data discussed one may conclude that the main trend of karyological evolution in the Digenea was the decrease in chromosome number due to fusions and translocations. These rearrangements accompanied

the process of specialization of a group of trematodes. Sometimes a contrary tendency to increase the diploid number was realized. Moreover, in three families parthenogenetic triploids were formed. But the first stages of species differentiation within the Digenea seem to be usually accompanied mostly by small inversions, duplications, deletions and minor translocations. Such rearrangements, including small changes in the C-heterochromatin content, occurred during the divergence of the *Schistosoma*, $2n = 16$ (Grossman *et al.*, 1981a, b; Short & Grossman, 1981; Puente & Short, 1985) and *Paragonimus*, $2n = 22$ (Hirai *et al.*, 1985) species. Apparently, the difference in the structure of the *Microphallus* karyotypes is a result of the same rearrangements.

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