

On the karyotypes of the Neorhabdocoela species and karyological evolution of Turbellaria

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Abstract

The karyotypes of 10 species belonging to the Neorhabdocoela order (Turbellaria) are described: *Proxenetes flabelliger*, $2n = 6$ (Trigonostomidae), *Promesostoma rostratum*, $2n = 12$ (Promesostomatidae), *Castrada* sp., $2n = 6$, *Rhynchomesostoma rostratum*, $2n = 6$, *Bothromesostoma essenii*, $2n = 10$, *Mesostoma lingua*, $2n = 8$ (Typhloplanidae), *Provortex karlingi*, $2n = 8$ (Provorticidae), *Halamovortex macropharynx*, $2n = 16$ (Dalyellidae), *Macrorhynchus crocea*, $2n = 16$, and *Gyratrix hermaphroditus*, $2n = 4$ (Polycystidae). It is proposed that the karyotypes of the ancestral marine neorhabdocoel forms, as well as those of the other groups of turbellarians, must have consisted of 16–20 biarmed chromosomes. The processes of adaptation and speciation in each group seem to be accompanied by a gradual decrease in chromosome number to $2n = 8–4$, this being due to Robertsonian fusions and translocations. In some freshwater turbellarians the diploid number increased by polyploidisation. The same processes evidently took place in different groups of parasitic worms.

Introduction

The various turbellarian groups have been studied karyologically to a different extent. The Paludicola (Tricladida) were investigated best of all (reviews in Benazzi and Benazzi-Lentati, 1976; Benazzi, 1982; De Vries, 1984; 1986; De Vries *et al.*, 1984; Ribas *et al.*, 1988). However, in the past few years papers on the karyology of the Terricola (Minelly, 1977; Ball & De Vries, 1983; Oki *et al.*, 1988), the Maricola (Sluys, 1989) and the Polycladida (reviews of Galleni & Puccinelli, 1981; 1986), as well as of the Proseriata (Curini-Galletti *et al.*, 1984a, b; 1985; 1989; Martens & Curini-Galletti, 1987; Martens *et al.*, 1989 a, b), the Acoela (Birstein, 1990) and the Lecithoepitheliata (Galleni & Canovai, 1988) appeared. Karyologically the order Neorhabdocoela is known poorly in comparison to the others, as the data on the diploid numbers of these animals were obtained mainly in the 1930s (Table 1). The only species of this order that has been investigated relatively recently and in detail, is *Gyratrix hermaphroditus* (Reuter, 1961; Heitkamp,

1978; L'Hardy, 1986; Puccinelli & Curini-Galletti, 1987; Curini-Galetti & Puccinelli, 1989; Puccinelli *et al.*, 1989).

The order Neorhabdocoela is very interesting. First, it is the most phylogenetically advanced order within the class Turbellaria (Bresslau & Reisinger, 1928–1933; Ax, 1963). Second, a few classes and groups of parasitic flatworms have originated from this order (Bresslau & Reisinger, 1928–1933; Ehlers, 1985; Ioffe, 1987). And last, taking into account the central position of the turbellarians in the process of the emergence of the Metazoa (Ivanov & Mamkaev, 1973), the investigation of the karyological trends within the Turbellaria, especially within its most advanced group, the Neorhabdocoela, may be useful for understanding possible ways of karyological evolution in the early metazoans.

In this work the karyotypes of 10 species belonging to all three suborders of the Neorhabdocoela are described. The results obtained lead to conclusions about the tendencies and mechanisms of the karyological evolution within the class Turbellaria.

Table 1. Chromosome numbers in the Neorhabdocoela.

Species	2n	Reference*
Typhloplanoida		
Trigonostomidae		
Proxenetinae		
<i>Proxenetes flabelliger</i>	8	This paper
Trigonostominae		
<i>Trigonostomum lillei</i>	6	Ruebush, 1938
Promesostomatidae		
<i>Promesostoma marmoratum</i>	12	Ruebush, 1938
<i>P. rostratum</i>	12	This paper
Typhloplanidae		
Protoplanellinae		
<i>Amphibolella virginata</i>	4	Kepner & Ruebush, 1937
<i>Protoplanella minuta</i> (?)		
New Haven	6	Ruebush, 1938; 1938
Pine Orchard	4	Ruebush, 1938; 1939
Typhloplaninae		
<i>Castrada brevispina</i>	6	Papi, 1959
<i>C. cristatispina</i>	6	Papi, 1951
<i>C. infernalis</i>	6	Papi, 1951
<i>C. i. breviorispina</i>	6	Papi, 1954
<i>C. montana</i>	6	Papi, 1959
<i>C. subsala</i>	6	Papi, 1950
<i>C. sp. I, II</i>	6	Valkanov, 1939
<i>C. sp.</i>	6	Ruebush, 1938
<i>C. sp. 6</i>	This paper	
<i>Mesocastrada fuhrmanni</i> (?), 6n	18	Papi, 1959
<i>Tetracelis marmorosa</i>		
Italy (Pisa)	4	Papi, 1954
Finland (4n?)	8	Luther, 1950
<i>Typhloplana viridata</i>	6	Ruebush, 1938.
Rhynchomesostominae		
<i>Rhynchomesostoma rostratum</i>		
European form		
Italy, Finland	6	Valkanov, 1938; Papi, 1950
USSR	6	This paper
American form	4	Ruebush, 1938
Olistanellinae		
<i>Krumbachia minuta</i>	4	Ruebush, 1938
<i>Olisthanella sp.</i>	4	Valkanov, 1938
<i>O. virginiana</i>	4	Senn, 1935
Mesostominae		
<i>Bothromesostoma essenii</i>		
Finland	10	Papi, 1950
USSR	10	This paper
<i>B. evelinae</i>	4	Marcus, 1946
<i>B. personatum</i>	8	Valkanov, 1938
<i>Mesostoma benazzi</i>	8	Papi, 1950
<i>M. chuskolensis</i>	8	Ruebush, 1939
<i>M. ehrenbergii ehrenbergii</i>		
Europe, different populations	10	Bresslau, 1904; Luther, 1904; Voss, 1914; Valkanov, 1938; Husted & Ruebush, 1940; Papi, 1950; Keyl & Göltenboth, 1972
South America	10	Marcus, 1946
<i>M. e. wardii</i> (North America)	8	Husted <i>et al.</i> , 1939
<i>M. georgianum</i>	4	Darlington, 1959

Species	2n	Reference*
<i>M. lingua</i>		
Western Europe, different populations	8	Valkanov, 1939; Holmquist, 1967; Heitkamp, 1972; Heitkamp, Schrade-Mock, 1977
USSR	8	This paper
<i>M.l.bronensis</i> (India)	6	Ruebush, 1938
<i>M.rhynchotum</i> (?)	16	Valkanov, 1938
<i>M.sp.</i>	6	Ruebush, 1938
<i>M.sp.</i> I, III	8	Valkanov, 1938
<i>M.sp.</i> II	4	Valkanov, 1938
<i>M.togarmensis</i>	8	Ruebush, 1939
<i>M.virginianum</i>	8	Kepner <i>et al.</i> , 1938
Phaenocorinae		
<i>Phaenocora kepneri</i>	4	Ruebush, 1938
<i>Ph.lutheri</i>	4	Ruebush, 1938
<i>Ph.unipunctata</i> (2n)	6	Valkanov, 1938
(<i>Ph.jucunda</i>) (4n)	12	Cognetti de Martiis, 1922
<i>Ph.virginiana</i>	4	Ruebush, 1938
Opistominae		
<i>Opistomum pallidum</i>	4	Papi, 1952
<i>O.sp.</i> I, II	8	Ruebush, 1938
Dalyellioida		
Provorticidae		
<i>Provortex affinis</i>	6	Ruebush, 1935
<i>P.karlingi</i>	8	This paper
Dalyelliidae		
<i>Gastrella truncata</i>	4	Ruebush, 1938
<i>Dalyellia abursalis</i>	4	Ruebush, 1938
<i>D.amiger</i>	4	Ruebush, 1938
<i>D.rossi</i> var. <i>maxima</i>	4	Ruebush, 1938
<i>D.rossi</i> var. <i>virginata</i>	4	Ruebush, 1938
<i>D.sp.</i> I-IV	4	Ruebush, 1938
<i>D.virginata</i>	4	Ruebush, 1938
<i>D.viridis</i>	4	Ruebush, 1938
<i>Halamovortex macropharynx</i>	16	This paper
<i>Microdalyellia gilesi</i>	4	Jones & Hayes, 1941
Graffillidae		
<i>Paravortex gemellipara</i>	8	Patterson, 1912; Ball, 1916
<i>P.cardii</i>	4	Hallez, 1908
Kalyptorhynchia		
Polycystidae		
Acrorhynchinae		
<i>Acrorhynchus reprobatus</i>	4	Ruebush, 1938
Polycystidinae		
<i>Polycystis goettei</i>	16	Ruebush, 1938
Macrorhynchinae		
<i>Macrorhynchus crocea</i>	16	This paper
Gyratricinae		
<i>Gyratricella attemisi</i> (marine)	6	Puccinelli, Curini-Galletti, 1987
<i>Gyratrix hermaphroditus</i>		
Marine populations:		
Roscoff (France)	6	L'Hardy, 1986; Curini-Galletti, Puccinelli, 1989
List (Germany)	6	Puccinelli, Curini-Galletti, 1987
Livorno (Italy)	6	Puccinelli, Curini-Galletti, 1987
Corsica (France)	6	Puccinelli, Curini-Galletti, 1987
Freshwater:		
Different populations of Western Europe, 2n	4	Ruebush, 1938; Reuter, 1961; Heitkamp, 1978; Puccinelli, Curini-Galletti, 1989

Species	2n	Reference*
USSR	4	This paper
Population L, Finland (3n)	6	Reuter, 1961
Different populations of Western Europe, 4n	8	Reuter, 1961; Heitkamp, 1978
<i>G. proavus</i> (marine)	6	Puccinelli, Curini-Galletti, 1987

* Papers dated 1904-1922 are cited according to Benazzi, Benazzi-Lentati (1976). The systematic position of species described by Ruebush as *Brysophlebs* sp. (2n = 8), *Trigonostomum lillei* (2n = 6), *Solenopharynx* sp. (2n = 4) and *Microrhynchus virginatus* (2n = 6) should be reexamined (Dr. B. I. Ioffe, pers. commun.).

Material and methods

The Barentz Sea species were collected in the Dalnezelenetskaya Inlet near the Murmansk Institute of Marine Biology (the Kola Peninsula) in autumn 1987 and spring-summer 1988. The animals were small, about 1.5-2.0 mm. The freshwater turbellarians were caught in lakes and other reservoirs near the Institute of Inland Water Biology (Borok, Yaroslavl District) in the summer of 1988. These animals were bigger than the marine ones, *Bothrosomostoma essenii* and *Mesostoma lingua* specimens being about 5-6 mm long.

In all, the following species were investigated (the systematics is given according to Luther (1962; 1963):

(1) Typhloplanoida: suborder

Trigonostomidae

Proxenetes flabelliger (marine species)

Promesostomatidae

Promesostoma rostratum (marine species)

Typhloplanidae

Typhloplaninae

Castrada sp. (freshwater species)

Rhynchomesostomatinae

Rhynchomesostoma rostratum (freshwater species)

Mesostominae

Bothrosomostoma essenii (freshwater species)

Mesostoma lingua (freshwater species)

(2) Dalyellioida: suborder

Provorticidae

Provortex karlingi (marine species)

Dalyelliidae

Halamovortex macropharynx (marine species)

(3) Kalyptorhynchia: suborder

Polycystidae

Macrorhynchinae

Macrorhynchus crocea (marine species)

Gyratricinae

Gyatrix hermaphroditus (freshwater species)

The chromosome preparations were obtained in the following manner. 5-10 turbellarians were incubated in a 0.3% colchicine solution in marine or fresh water for 8-10 hours. In the cases of *G. hermaphroditus* and *Castrada* sp. only two and three individuals respectively were investigated. After incubation marine turbellarians were placed in a 0.56% KCl solution for 20 min and in a 1% sodium citrate solution for the same time. For the hypotonic treatment of freshwater turbellarians distilled water was used for 40 min. Animals were fixed by three changes of a cold ethanol-glacial acetic acid mixture, 3:1. They were homogenized by means of a Pasteur pipette in 60% acetic acid. The suspension obtained was dropped on glass slides and air-dried at 60 °C. The preparations were stained with a 2% Giemsa solution in phosphate buffer, pH = 6.8. A Zeiss NU-2 microscope (K. Zeiss, Jena) with a 100 × oil immersion objective was used for the analysis of chromosome preparations.

Results

Judging from the appearance of the metaphase plates described below it is evident that all of them were obtained from dividing spermatogonia (2n) or spermatocytes (n). Regrettably, the chromosome morphology was not always seen clearly, therefore in such cases it is possible to discuss the diploid number only.

(1) Typhloplanoida

Proxenetes flabelliger, 2n = 8 (Trigonostomidae)

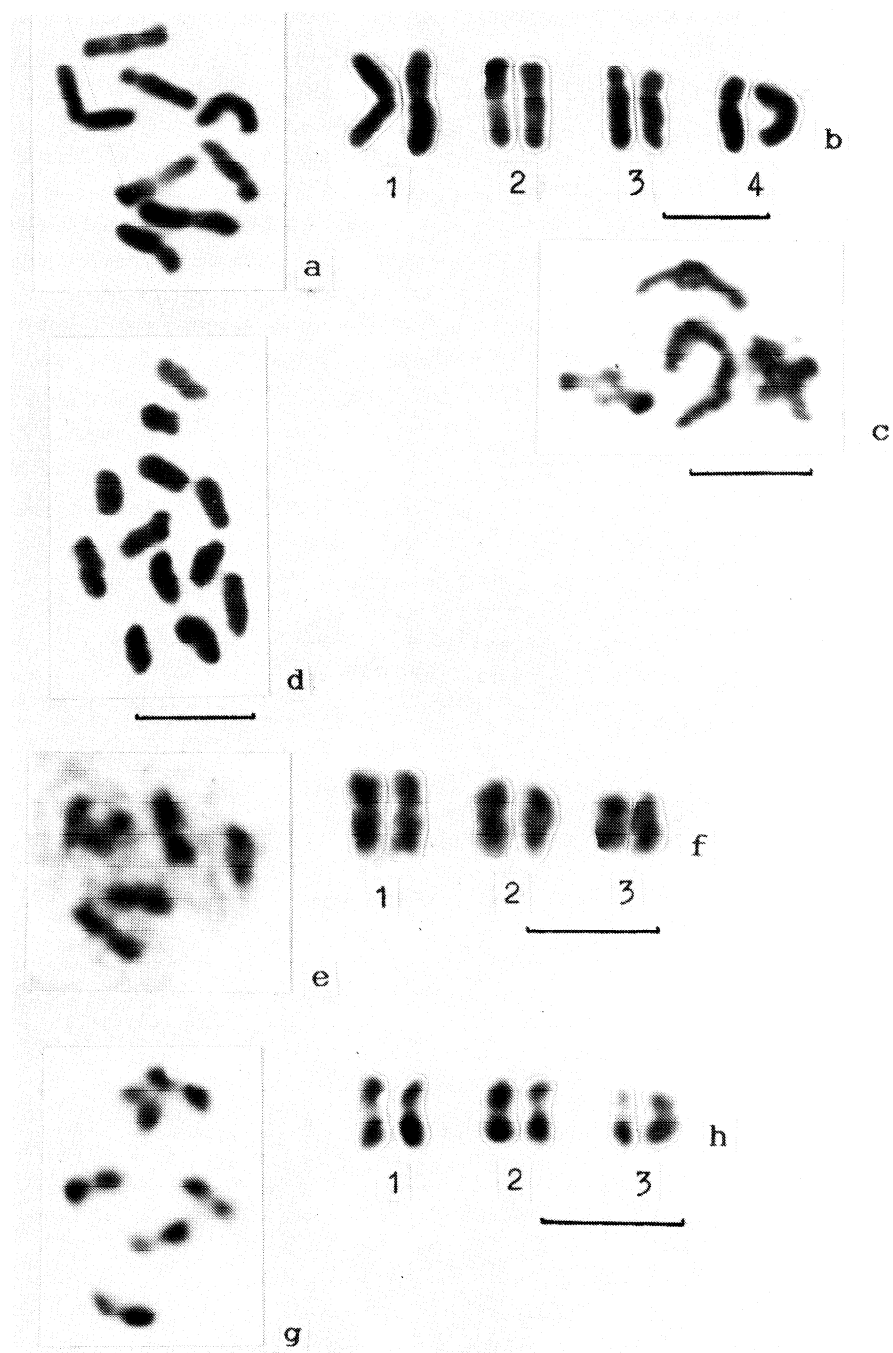


Fig. 1. Metaphase plate (a), karyotype (b) and diakinetic chromosomes (c) of *Proxenetes flabelliger*; metaphase plate of *Promesostoma rostratum* (d); metaphase plates and karyotypes of *Castrada* sp. (e, f) and *Rhynchomesostoma rostratum* (g, h). Bars: 10 μ m.

The karyotype of this species includes 4 pairs of biarmed chromosomes (Fig. 1a-c): the largest pair consists of metacentrics, the 2nd and 4th, of submetacentrics, and the 3rd, of subtelocentrics.

Promesostoma rostratum, $2n = 12$ (Promesostomatidae)

The karyotype consists of 12 evidently biarmed chromosomes. (Fig. 1d). Plates with a good chromosome morphology were not obtained in this case. It seems possible that a pair of large chromosomes (the 1st or 2nd) is represented by subtelocentrics. The diploid number in *P. marmoratum*, which was investigated earlier, is also 12 (Ruebush, 1938; Table 1).

Castrada sp., $2n = 6$ (Typhloplanidae, Typhloplaninae)

Rhynchomesostoma rostratum, $2n = 6$ (Typhloplanidae, Rhynchomesostominae)

The karyotypes of these two species consist of three

pairs of meta- or submetacentrics (Fig. 1e-f). The other *Castrada* species are characterized by the same diploid number, 6 (Table 1). $2n = 4$ was found in the American form of *R. rostratum* (Ruebush, 1938).

Bothromesostoma essenii, $2n = 10$ (Typhloplanidae, Mesostominae)

Mesostoma lingua, $2n = 8$ (Typhloplanidae, Mesostominae)

The diploid numbers in these two species belonging to the same subfamily Mesostominae differ, as the karyotype of *B. essenii* consists of 3 pairs of large biarmed chromosomes (Nos. 1 and 2 are meta- and submetacentrics, No. 3, subtelocentrics) and two pairs of relatively small subtelocentrics (Fig. 2c-d), although the karyotype of *M. lingua* includes only 4 meta- or submetacentrics (Fig. 2a-b). These numbers are in accordance with the data of the other authors (Table 1).

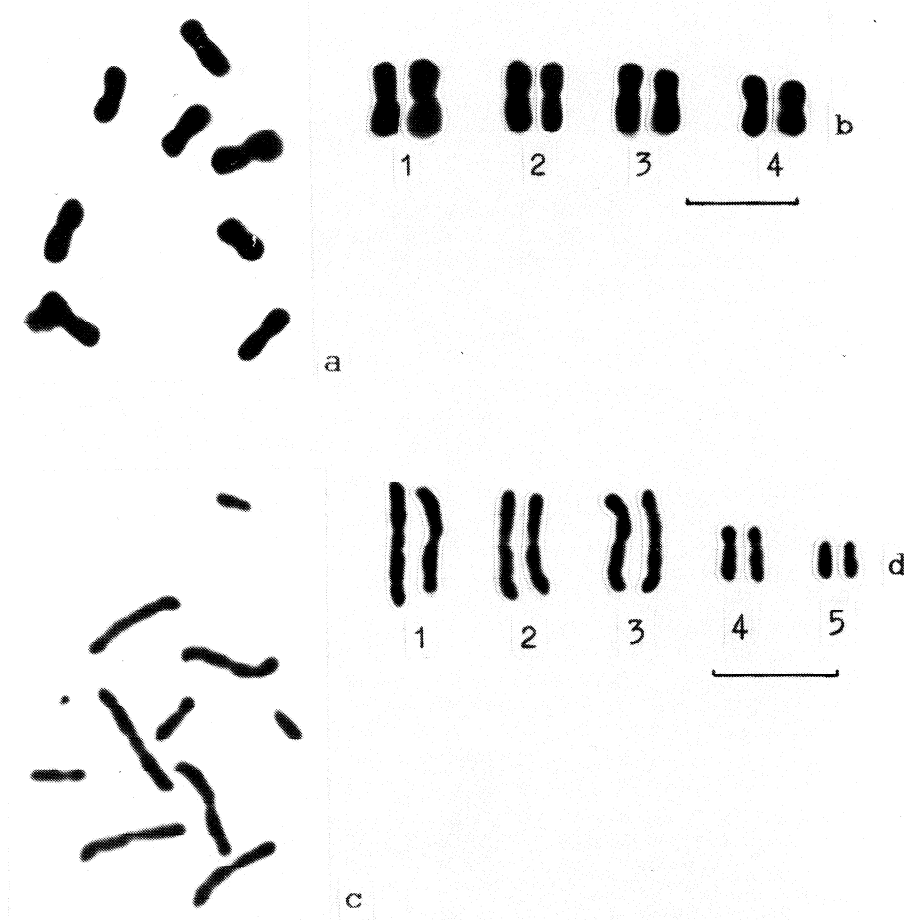


Fig. 2. Metaphase plates and karyotypes of *Mesostoma lingua* (a, b) and *Bothromesostoma essenii* (c, d). Bars: 10 μ m.

(2) *Dalyellioida*

Provortex karlingi, $2n = 8$ (Provorticidae)

The karyotype consists of four pairs of biarmed chromosomes, two of them being meta/submetacentrics (Nos. 1, 2) and two, subtelocentrics (Nos. 3, 4).

Halamovortex macropharynx, $2n = 16$ (Dalyelliidae)

The karyotype consists of 8 chromosome pairs (Fig. 3b). All elements of the set seem to be biarmed, although plates with a good chromosomal morphology were absent in the preparations. The species investigated of the other Dalyelliidae genera have karyotypes of 4 chromosomes (Table 1).

(3) *Kalyptorhynchia*

Macrorhynchus crocea, $2n = 16$ (Polycystidae)

There are 8 pairs of chromosomes in the karyotype

of the marine *M. crocea* (Fig. 4a-b). Nos. 1, 6, 7 consist of submetacentrics, Nos. 2 & 3, of subtelocentrics, and Nos. 4, 5 & 8, of acrocentrics with a very small short arm. Within the Kalyptorhynchia species studied the same diploid number is known in *Polycystis goettei* (Table 1).

Gyratrix hermaphroditus, $2n = 4$ (Polycystidae)

The karyotype of this species consists of only two pairs of meta- or submetacentrics differing considerably in size: the first chromosomes are almost twice as long as the second ones (Fig. 4c-d). Earlier, the analysis of freshwater populations of Western Europe showed that there are three chromosomal types within this species: the diploid, $2n = 4$, the tetraploid, $4n = 8$, and the triploid, $3n = 6$ (Rueter, 1961; Heitkamp, 1978; Puccinelli *et al.*, 1989). Besides, marine populations of individuals having $2n = 6$ were found in the Channel (Brittany, France), the North Sea (Germany) and the Mediterranean (Corsica, France; Leghorn,

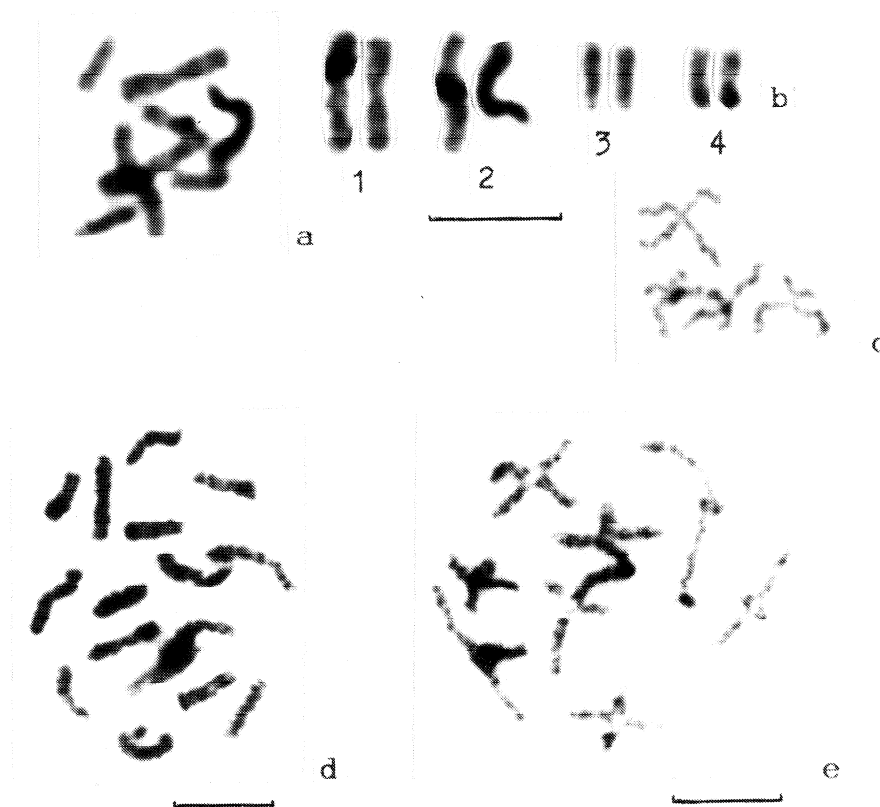


Fig. 3. Metaphase plate (a), karyotype (b) and haploid set of chromosomes (c) of *Provortex karlingi*; metaphase plate (d) and diakinetid chromosomes (e) of *Halamovortex macropharynx*. Bars: 10 μ m.

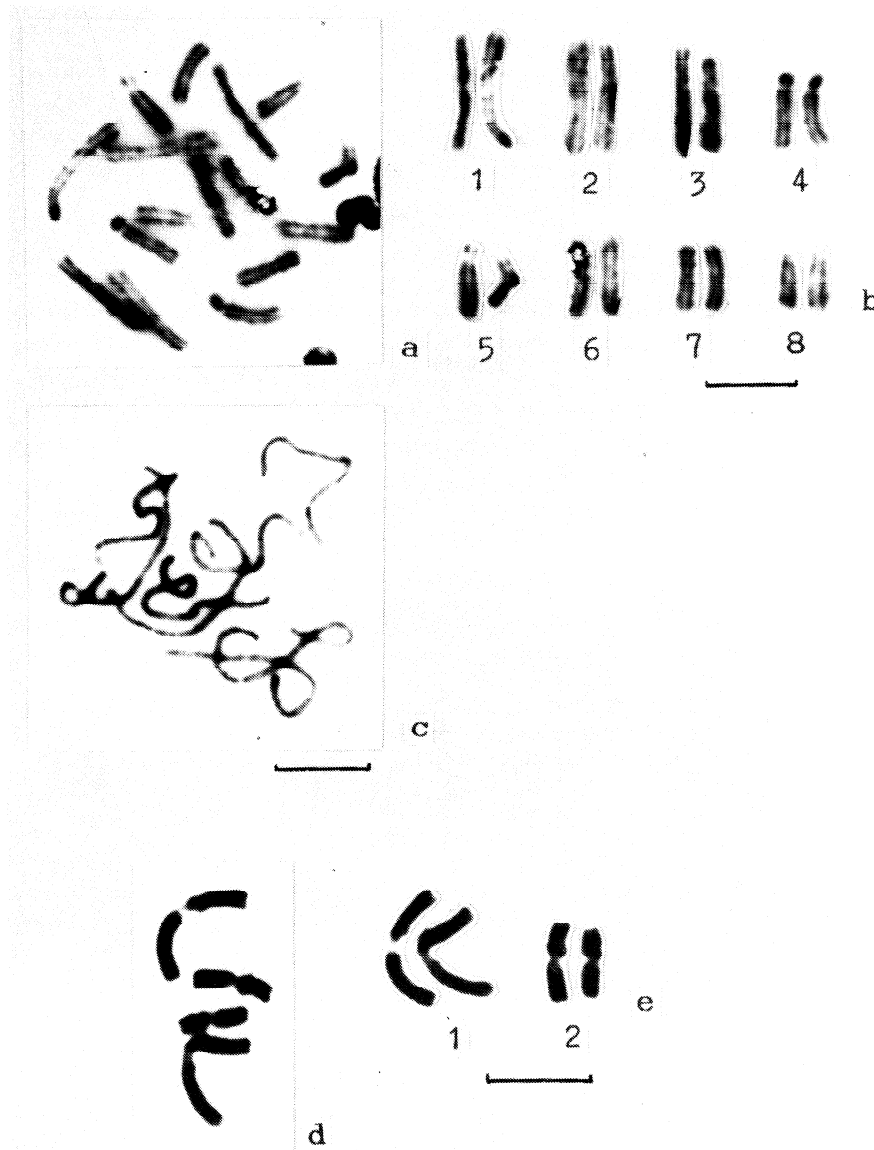


Fig. 4. Metaphase plate (a), karyotype (b) and zygotenic chromosomes (c) of *Macrorhynchus crocea*; metaphase plate (d) and karyotype (e) of *Gyratrix hermaphroditus*. Bars: 10 μ m.

Italy) (L'Hardy, 1986; Puccinelli & Curini-Galletti, 1987; Curini-Galletti & Puccinelli, 1989).

Discussion

Our results and those in the literature point to the high variability of the diploid number within the Neorhabdocoela, $2n = 4$ to 16. It is necessary to say that in all groups investigated the karyotypes of marine species

frequently consist of a greater number of chromosomes than those of freshwater species. Thus, in *Macrorhynchus crocea* and *Halamovortex macropharynx* the diploid number equals 16, and in the *Promesostoma* species, 12 (but in *Provortex karlingi* and *Proxenetes flabelliger* $2n = 8$). As freshwater neorhabdocoels have originated from marine forms (Bresslau & Reisinger, 1928-1933), and there are species with $2n = 16$ in all three Neorhabdocoela suborders, it seems probable that the karyotypes of the

ancestral forms must be of about 16 chromosomes. It is necessary to note that the ancestral forms of worms which possibly have diverged from the Neorhabdocoela seem to have also sets of 16-20 chromosomes (see below).

In the course of evolution of different phyletic Neorhabdocoela lines the decrease in diploid number evidently occurred mostly due to Robertsonian fusions, in which small elements of the sets took part, although some other types of translocations and rearrangements may have happened as well. For the same decrease in chromosome number due to fusions in the vertebrates the term 'karyotypic symmetrization' is used (review in Birstein, 1987). Probably, as a result of such a symmetrization the karyotypes of *Proxenetes flabelliger* and *Provortex karlingi*, $2n = 8$, have been formed.

The process of the decrease in diploid number due to fusion of small chromosomes is clearly seen in freshwater species of the suborder Typhloplanoida. In particular, in the subfamily Mesostominae all chromosome numbers from 10 to 4 are present, but a tetraploid subspecies (?) *Mesostoma lingua bronensis* with 16 chromosomes was formed (Table 1). From the comparison of the karyotypes of two species of this subfamily, *Bothromesostoma essenii*, $2n = 10$, and *Mesostoma lingua*, $2n = 8$, described above it may be inferred that the karyotype of the latter species was formed from karyotypes resembling that of *B. essenii* by a fusion of the two smallest chromosome pairs.

The same changes took place in the Kalyptorhynchia suborder. Although the karyotype of *Macrorhynchus crocea* consists of 16 chromosomes, one may consider that it is an 'advanced' one, as many rearrangements seem to have occurred and been fixed in it. Thus, three pairs of acrocentrics must be a result of preceding inversions. Because of these peculiarities the *M. crocea* karyotype appears to be in the process of 'preparation' for the next evolutionary stage of changes, especially to fusion of chromosome pairs.

The *Gyratrix hermaphroditus* karyotype, $2n = 4$, seems to be a result of a series of such fusions. Evidently, the rate of karyotypic symmetrization in freshwater neorhabdocoels is higher than that in marine species, as the latter seem to be characterized by a tendency to preserve a high diploid number (Table 1). The karyotypes of the Mediterranean and

North Sea marine forms of *G. hermaphroditus* consists of six instead of four chromosomes: a pair of large meta-, a pair of middle-sized submeta- and a pair of slightly smaller subtelocentrics, or three pairs of meta-centrics (L'Hardy, 1986; Puccinelli & Curini-Galletti, 1987; Curini-Galletti & Puccinelli, 1989). L'Hardy proposed that such karyotypes must have given rise to that of the freshwater forms by a loss of the smallest pair, this, therefore, being an example of aneuploidy. But it seems more probable that the $2n = 4$ karyotype originated from the $2n = 6$ one by a fusion of homologues of two pairs (the 2nd and 3rd) in the latter, as the size difference between the 1st and 2nd chromosomes in the $2n = 4$ karyotype is great (Fig. 4e). The same conclusion was drawn by Puccinelli and Curini-Galletti (1987). Moreover, they showed that the diploid number $2n = 6$ could be considered basic for the entire subfamily Gyratricinae (Table 1).

At the next stage of the karyological evolution, tetraploidization seems to occur in *G. hermaphroditus* after reaching $2n = 4$. Thus, tetraploid forms, $4n = 8$, were found in different European populations of this species (Reuter, 1961; Heitkamp, 1978). Besides that, triploid parthenogenetic forms may appear: a Finnish population L, consisting of individuals having three pairs of short submetacentrics, was described by Reuter (1961).

Relatively high diploid numbers are characteristic not only of the marine neorhabdocoels, but also of marine species belonging to the other turbellarian orders. Such is the case in the majority of Polycladida species ($2n = 16-20$) (Galleni and Puccinelli, 1979; 1981; 1984; 1986). The polyclads are of a great interest because from the embryological viewpoint they seem to be the most conservative group within the Turbellaria (Galleni & Gremigni, 1988). A karyometric analysis showed that three main types of karyological changes occurred in these turbellarians: a change in centromere position which came about as a result of a pericentric inversion, a decrease in arm size, and a decrease in the diploid number by chromosome fusions.

Acrocentric homologues seem to be rather rare in the karyotypes of most marine turbellarians. Besides *M. crocea*, these chromosomes were found in *Thysanozoon brocchi* (No. 8) and *Yunga aurantica* (Nos. 8, 9) (Polycladida), both having $2n = 18$ (Galleni &

Puccinelli, 1985), as well as in *Cercyra hastata*, $2n = 14$ (No. 2; besides that, in this species the short arms in the homologues 6 and 7 are practically absent) (Galleni & Puccinelli, 1982).

But acro- or subtelocentrics are usually present in many Monocelididae species, $2n = 4-10$ (Proseriata), the majority of them having $2n = 6$ (Curini-Galletti *et al.*, 1989; Martens *et al.*, 1989a). The basic monocelid karyotype is considered to consist of one large and one medium-sized metacentric, and one small acro- to subtelocentric chromosome. All other monocelid karyotypes ($2n = 4, 8, 10$) were explained as being derived from the basic one by pericentric inversions, translocations, fissions, and fusions (Curini-Galletti *et al.*, 1985, 1989; Martens & Curini-Galletti, 1987; Martens *et al.*, 1989a, b). The diploid number of the nonmonocelidid proseriates, the Archinocelididae, Coelognoporidae, Otoplanidae, Ungiophora representatives equals 8-24 (Martens *et al.*, 1989b). Martens and his co-workers hypothesized that doubling of the $n = 3$ genome has taken place and a karyotype with $n = 6$ must be the ancestral one for the nonmonocelidid taxa. According to our assumption, the monocelidid karyotype $n = 3$ must be considered as evolutionarily advanced and reduced and all processes of karyological changes seem to be the same in the Proseriata and Neorhabdocoela. To our opinion, neither the data on haploid length, nor on DNA content may be an absolute proof of the former hypothesis. Particularly, there is no evidence that chromosome packing is the same in different species investigated.

As for the freshwater triclads, the whole picture is complicated because of many cases of polyploidy, although the principal trends here are similar to those described above. Thus, the Terricola diploid species have $2n = 6-16$ (Minelli, 1977; Winsor, 1981; Ball & De Vries, 1983; Oki *et al.*, 1988), and within the *Bipalium* genus there are two 10-chromosome species, while *B. kewense* has 16 chromosomes (Oki *et al.*, 1988). This situation seems to be an example of a chromosome number decrease.

It is more difficult to interpret data on the diploid numbers of the dendrocoelids. The karyotypes of the majority of the representatives of this family consist of 28-32 and even 44 banded chromosomes (Benazzi & Benazzi-Lentati, 1976; Umylina, 1976; Benazzi, 1982). But there are also species having $2n = 10, 14, 16$ and 20

within this family. In other words, most of the dendrocoelids with high diploid numbers must be of tetraploid origin (Dahm, 1961). It is probable that the diploid ancestral forms already had reduced karyotypes of 14-16 chromosomes and in some cases a decrease in a doubled chromosome number took place. Tetraploidization and subsequent symmetrization were postulated for the explanation of the origin and evolution of the *Neodendrocoelum* subgenus species (genus *Dendrocoelum*) inhabiting the lake Ochrid (Paunovic, 1977).

The *Dugesia* karyotypes may be considered as a good illustration of the karyological processes occurring in the planarians (reviews in Benazzi & Benazzi-Lentati, 1976; Benazzi, 1982). This complex genus consists of three subgenera, *Dugesia*, *Girardia*, and *Schmidtea*. Although the diploid number in the majority of the *Dugesia* and *Girardia* species equals 16, there are a few peculiar situations. Thus, the *D. (D.) gonocephala* group of the European, Asian and African species includes also forms with $2n = 18$, *D. sicula* (Gourbault, 1981; Ribas *et al.*, 1988) and *D. biblica* (Bromley, 1977), as well as with 14 chromosomes, *D. hepta* (Pellicciari *et al.*, 1986) and *D. japonica ryukyensis* (Kawakatsu *et al.*, 1976; Oki *et al.*, 1981). Moreover, numerous Mediterranean populations, which morphologically belong to the *gonocephala* group are fissiparous; usually these planarians are triploids having 24 or 27 chromosomes (Gourbault *et al.*, 1981; De Vries, 1986a, b; Pala *et al.*, 1985; Ribas *et al.*, 1988). In individuals of many populations B-chromosomes are present. It was concluded that the fissiparous populations of 24-chromosome animals belong to *Dugesia (D.) iberica*, while the populations of 27-chromosome planarians, to *D. (D.) sicula* (Ribas *et al.*, 1988). Many types of unusual karyotypes, for instance, mixoaneuploid triploid and orthoploid diploid ones, were found in the Far Eastern *D. japonica japonica* and *D. j. ryukyensis* (Kawakatsu *et al.*, 1976; Oki *et al.*, 1981; Tamura, 1986; Tamura *et al.*, 1988, etc.). The karyotypes of the *Girardia* species (the *Dugesia* of the New World) are very similar to those of the *D. gonocephala* group (Benazzi & Benazzi-Lentati, 1976; Benazzi, 1975; 1982). The diploid number in almost all of them equals 16, although in two species it is 8. There are triploid populations within *D. (G.) dorotocephala* and *D. (G.) tigrina*.

The karyotypes of the *Schmidtea* species (or the '*Dugesia polychroa*' group) differ from the described ones since they consist of 4 chromosome pairs, while *D. nova* has $2n = 6$ (Benazzi & Benazzi-Lentati, 1976; Benazzi, 1982; De Vries *et al.*, 1984). Within *D. (S.) polychroa* polyploid forms are known: the A-D biotypes form an autopolyploid series, $2n$, $3n$, $4n$ and $3n-6n$. The *D. (S.) nova* karyotype, $2n = 6$, seems to have been formed by a centromeric fusion of the 1st and 3rd acrocentrics of *D. lugubris* (biotype E) (Benazzi & Puccinelli, 1973). Although *D. (S.) polychroa* and *D. (S.) lugubris* have the same diploid number, $2n = 8$, the DNA content differs considerably in these species, being 0.70 and 1.75 pg respectively (Benazzi *et al.*, 1981; Pellicciari *et al.*, 1986). Moreover, this difference is not correlated with C-heterochromatin content or distribution, since in *D. polychroa* C-bands are present in the pericentromeric regions of all chromosomes, and in the telomeric and paracentromeric regions of some of them, while in *D. lugubris* there are centromeric C-bands only (Sluys & De Jong, 1984; Galleni *et al.*, 1986; 1989). In the former species these bands are considerably larger than in the latter one.

Therefore, there are two main trends in the *Dugesia* karyological evolution: the decrease in the chromosome number due to translocations, and the polyploidization. Polyploid forms are characteristic of many planariids (Benazzi, 1982). It seems evident that the fissiparous triploid forms appear frequently only in freshwater turbellarians. The highest degree of ploidy was found in *Phagocata velata*, $8n = 96$ (Ball *et al.*, 1981). Besides chromosome changes, there are considerable changes in the DNA and/or heterochromatin content in related *Dugesia* species. On the whole the data on the triclads, especially on the *Maricola* (Ball, 1979a; Galleni & Puccinelli, 1979; 1984; Galleni *et al.*, 1984; Sluys, 1989) and parasitic ones (Kawakatsu *et al.*, 1988) do not contradict our assumption that the ancestral turbellarian karyotypes must have consisted of approximately 16-20 chromosomes, since the diploid number in the majority of these species equals 14-16.

One more proof of our assumption is the data on the karyology of parasitic flat worms which diverged from the turbellarians. All these groups (the classes Digenea, Monogenea, Aspidogastrea, Cestoda, the

order Temnocephala) originated from ancient neorhabdocoels belonging to the Dalyellioida suborder (Ehlers, 1985) or having the typhloplanoid organization (Ioffe, 1987). Thus, the majority of living digenean species have karyotypes of 20 chromosomes, and it is possible that the ancestral digeneans must have had karyotypes of 20 biarmed chromosomes (Birstein & Mikhailova, 1990). In the process of specialization of different phyletic lines of this class the chromosome number decreased to 18-14 mainly due to Robertsonian fusions. In a few groups centric fissions seem to have occurred, since these animals have $2n = 22-28$. As in the planarians, triploid forms also appeared in three digenean families.

From the data discussed above the following course of turbellarian karyological evolution can be inferred. Originally the hypothetical ancestral marine forms had karyotypes consisting of 16-20 biarmed chromosomes. This diploid number was evidently an optimal one and in many cases it has been conserved unchanged (or almost unchanged) during the course of further evolution (this situation may be considered as an example of a stabilizing selection process). During the adaptation and specialization a gradual decrease in chromosome number to 8-4 due to Robertsonian and other types of chromosomal fusions occurred. This stage was preceded by that of formation, fixation and accumulation of subtelo- and telocentrics in the karyotypes. Many freshwater forms acquired a capability of increasing the chromosome number by polyploidization. In some cases a reduplication of a rather great ancestral number of chromosomes took place; however, the reduplication of a karyotype reduced to 4-6 chromosomes was a more frequent event. Sometimes a row of polyploid forms emerged. If the diploid number grew over 30 due to polyploidization, a subsequent symmetrization by chromosome fusion possibly occurred in some groups. Evidently, the same processes took place in the descendant parasitic flat worms.

The question of the mechanisms of the karyological evolution of the Turbellaria was discussed in the literature since Ruebush's work (Ruebush, 1938). This author considered the ancestral turbellarian karyotypes as consisting of a low number of large homologues which were fragmented or fissioned during the course of evolution. The karyological data on the

Proseriata are treated in accordance with this hypothesis (Curini-Galletti *et al.*, 1985; 1989; Martens & Curini-Galletti, 1987; Martens *et al.*, 1989a, b). Our opposite opinion, based on the analysis of more numerous data seems to be better grounded. Earlier Benazzi and Benazzi-Lentati (1976) hypothesized that no general conclusions about 'the evolution of the karyotypes in the major taxa' of the turbellarians should be drawn. However, our findings, as the data in this paper described, point to some trends of karyological evolution in the Neorhabdocoela and the Turbellaria on the whole.

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