Murmansk Institute of Marine Biology, USSR Academy of Sciences

First Contribution to Karyology of Two Acoels (Turbellaria) and a Dinophilid (Annelida)

By V. J. Birstein

With 2 Figures

Abstract

The karyotypes of two acoels, *Convoluta convoluta* and *Baltalimania agile* (Convolutidae, Acoela, Turbellaria), consist of 16 and 14 chromosomes respectively. All chromosomes of *C. convoluta* are biarmed. A low diploid number in the Acoela contradicts the earlier data. The karyotype of *Dinophilus taeniatus* (Dinophilidae, Polychaeta, Annelida) also includes 16 biarmed chromosomes. The karyological data confirm the view of the ancient origin of the Acoela and Dinophilidae.

Introduction

The origin and systematic position of such groups of primitive invertebrates as the Acoela and Dinophilidae is not clear (for instance, Ax, 1963; Karling, 1974; Ehlers, 1985; 1986; Mamkaev, 1985; Smith and Tyler, 1985). According to the traditional view, the Acoela is the most ancient order within the Turbellaria, which must be considered as being closely related to the ancestral forms of the Bilateria (Hyman, 1951; Ivanov and Mamkaev, 1973; Ivanov, 1976). Other authors suppose the Turbellaria to be of polyphyletic origin, and consist of three groups, the Acoela (more exactly, the "Acoelomorpha") being one of them and representing an independent specialized offshoot (Rieger, 1985; Smith and Tyler, 1985; Smith et al., 1986). Data on egg cleavage also contradict the opinion that the Acoela is the most primitive order of the Turbellaria (Galleni and Gremigni, 1988). There are also different viewpoints in the literature on the Dinophilidae's origin: some investigators consider this family to be a primitive group closely related to the ancestral annelids, and include it in the order Archiannelida, while the others beleave it to be a family of neotenic, secondary il simplified forms (Mamkaev, 1985).
Karyologically these groups were investigated poorly; separate available data on the diploid numbers are mostly rather old and incorrect (the reviews in Benazzi and Benazzi-Lentati, 1976; Christensen, 1980). But the karyological analysis may be useful for solving the problems of the systematic position of these invertebrates. Therefore the aim of this paper is to investigate the karyotypes of two acoels, *Convoluta convoluta* and *Baltalimania agile* (Convolutidae), and of a dinophilid, *Dinophilus taeniatus*. That the diploid number of *C. convoluta* equals 16 was determined earlier (Drobysheva and Mamkaev, 1971; Kiknadze, 1963), but no attempts to obtain and describe karyotypes of the acoels were made, and no data on the karyology of *D. taeniatus* are known. An approximate chromosome number of 16–20 was published relatively recently for *D. gyrociliatus* (Traut, 1970), which belongs to a distant species group of the same family Dinophilidae (Donworth, 1986).

Materials and methods

The individuals of *Convoluta convoluta* and *Baltalimania agile* (Convolutidae, Acoela, Turbellaria), as well as of *Dinophilus taeniatus* (Dinophilidae, Polychaeta, Annelida) were collected in the Dalnezelenetskaya Inlet near the

Murmansk Institute of Marine Biology (Kola Peninsular, the Barentz Sea) in spring-summer 1988. Usually the animals were incubated in a 0.3 % colchicin solution in marine water for 12–24 hours at 4–6 °C. In further experiments the *C. convoluta* individuals were cut by a razor blade and kept in marine water for a few days for the regeneration of the lost fragments (ISAEVA, 1972) before being incubated in the colchicin solution. After that they were fixed immediately in a cold ethanol-glacial acetic acid mixture (3:1), while the other animals investi-

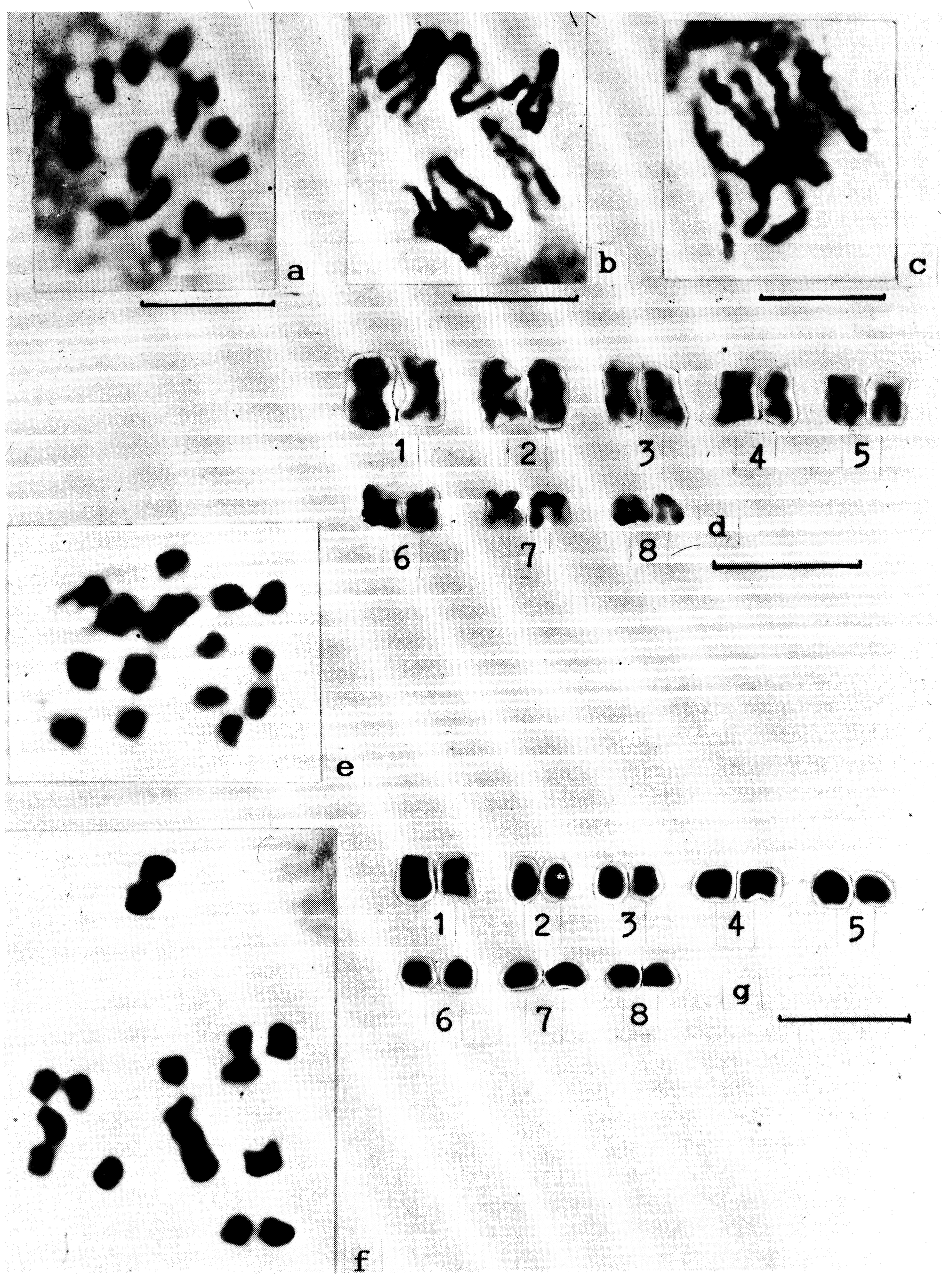

Fig. I. Metaphase plate (a), zygo- (b), and pachytenic (c) chromosomes, and karyotype (d) of *Convoluta convoluta* (parenchymal cells); metaphase plates (e, f) and karyotype (g) of the same species (regenerating tissue cells). Bar: 10 μm gated were preliminarily hypothenized in 0.56% KCl and 1% natrium citrate solutions for 20 min each. After fixation the animals were homogenized by means of a Pasteur pipette in 60% acetic acid. The suspension was dropped on glass slides and air-dried at 60 °C. The preparations were stained with a 2% Giemsa solution, *p*H-6.8, and observed in a NU-2 microscope (K. Zeiss, Jena).

Results and Discussion

In the course of the preparation of metaphase plates of *C. convoluta* we had to ommit the usual hypotonic treatment, as these animals, if placed in the hypotonic solution, at first began to secrete mucilage very fast and intensively, and then completely disaggregated. In the slides prepared without this procedure the metaphase plates were seen in rare dividing nuclei of parenchymal cells (Fig. 1a). Part of the plates seems to consist of meiotic bivalents (Fig. 1b, c), as the sexual products are formed in the parenchyma in this species (Drobysheva and Mamkaev, 1971).

A much greater quantity of mitoses was found in the preparations obtained from regenerating individuals (Fig. 1e, f). In most cases the diploid number equalled 16, but there were also plates of 14 and even 12 chromosomes, especially in preparations from the regenerating parts of animals. It is difficult to conclude if the latter data are artifacts obtained during the procedure of slide preparation or a result of aneuploidy in fast dividing cells.

The karyotype of *C. convoluta* consists of 8 pairs of biarmed, mostly meta- and submetacentric chromosomes (only the pair No. 5 is of subtelocentrics) (Fig. 1d). Their size decreases gradually, but the 8th is much smaller. Chromosomes in the plates from the regenerating tissue seem to be more condensed than those from parenchymal cells and therefore have unclear morphology (Fig. 1g)

In the case of *Baltalimania agile* metaphase plates with a good chromosome morphology were not obtained (for instance, Fig. 2a). But from the analysis of these plates and the plates of meiotic divisions (Fig. 2b, c) it can be inferred that the diploid number in this species is 14.

The karyotype of *Dinophilus taeniatus* consists of 16 small chromosomes (Fig. 2d, e). All elements seem to be biarmed, although the homologues of the 6th and 7th pairs are possibly subtelocentrics (or even acrocentrics).

The results described above are the first contemporary data on the karyology of two ancient invertebrate groups, the Acoela and Dinophilidae. In the earlier papers the diploid number in the Convolutidae species seems to be usually overestimated, equalling 20–34 (Gardiner, 1898; Ruebush, 1938; Costello, 1970), and only in *Convoluta thauma* a very low number, 2n = 6, was determined (Marcus, 1952, cit. in Benazzi and Benazzi-Lentati, 1976). Besides the convolutids, the only other Acoela representative investigated, *Otocelis dichona* (Otocelidae), has 2n = 12 (Marcus, 1954, cit. in Benazzi and Benazzi-Lentati, 1976). Therefore, the living acoels studied can be characterized by 2n = 12–16, and the case of *C. thauma* should be reinvestigated. As for *Dinophilus taeniatus*, similar chromosome numbers, 2n = 16–20, were found earlier in *D. gyrociliatus* (Shearer, 1911; 1912; Traut, 1970) and in *D. apatris* (Nachtsheim, 1920), distant species of the same genus.

Judging from the chromosome number, the species studied by us are similar to the most primitive and generalized forms of invertebrates. Thus, in *Trichoplax adhaerens* and *Trichoplax* sp. 2n = 12 (Ruthmann, 1977; Birstein, 1989), in the sponge *Leucosolenia complicata* (Calcarea) 2n = 24 (Anakina, 1981), and in most of marine turbellarian species 2n = 10–18 (Benazzi and Benazzi-Lentati, 1976; Galleni and Puccinelli, 1986; Birstein, in press) were observed.

In the advanced turbellarian groups the specialization is usually accompanied by a chromosome number decrease and, after achievement of 2n = 4–6, tetraploidization events frequently take place in freswhater species; in some orders of freshwater turbellarians the reduplication of a high chromosome number set may occur (Benazzi and Benazzi-Lentati, 1976; Benazzi, 1982).

As for the annelids, the karyotypes of the most ancient Polychaeta representatives studied (species of the Archiannelida families, the Protodrillidae, Saccorridae, Nerilidae) are rather small, of 8–16 chromosomes (review in Christensen, 1980). The representatives of relatively advanced and evolutionary more young orders, *Errantia* and *Sedentaria*, usually have sets of a higher number of elements, 2n = 18–38 and even more, although in a few species belonging to the Syllidae, Tomopteridae and Dorvilleidae families, 2n is 6–14 (Christensen, 1980; Vitturi et al., 1984;

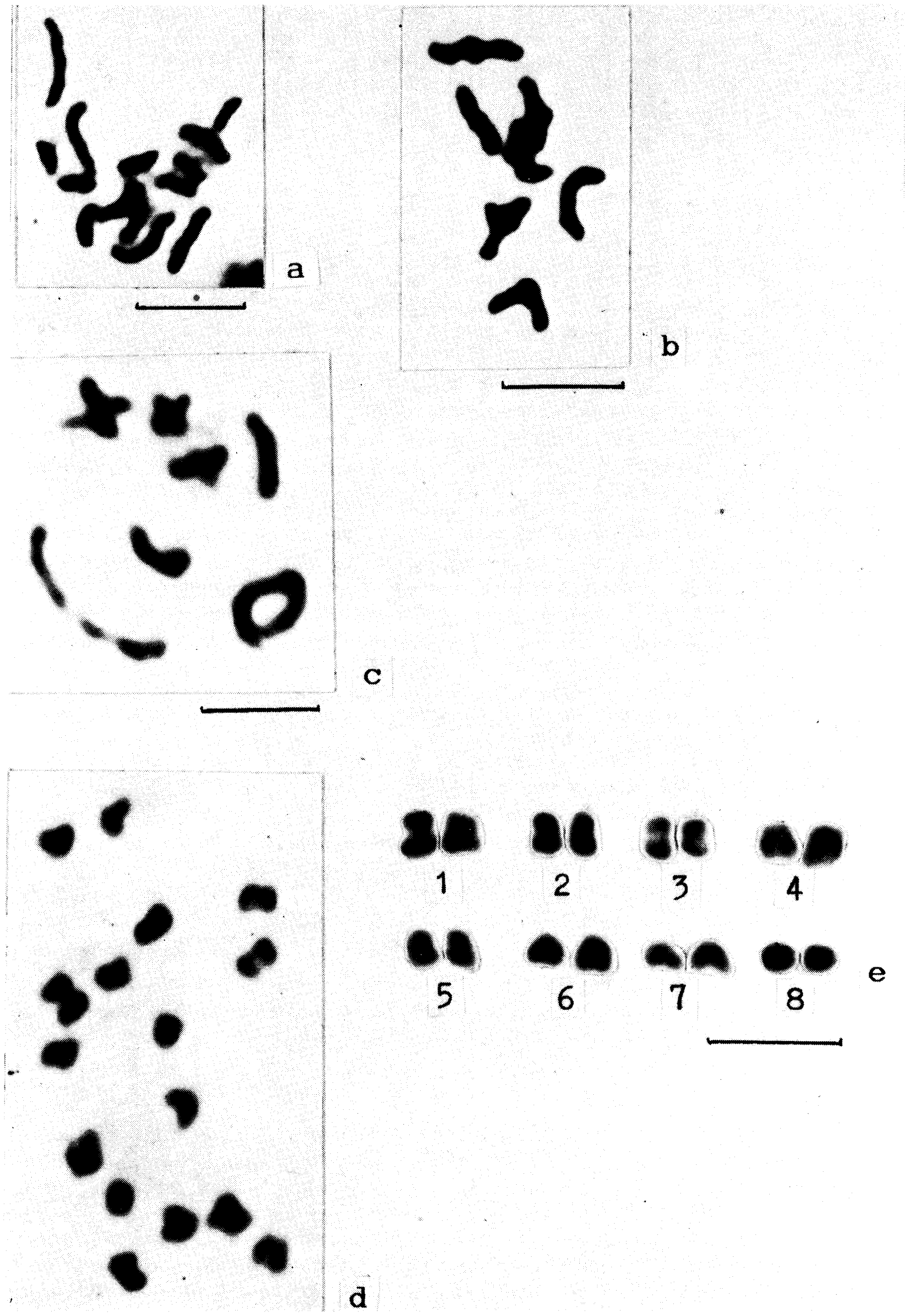

Fig. 2. Metaphase plate (a) and diakinetic chromosomes (b, c) of *Baltalimania agile*; metaphase plate (d) and karyotype (e) of *Dinophilus taeniatus*. Bars: 10 µm

Curini-Galletti et al., in press). The karyotypes of the latter species seem to be evolutionary advanced. The ancestral diploid number in the Oligochaeta is considered to have been 32–36 (Christensen, 1980), and polyploidy is characteristic of many families of this class (Christensen, 1980;

Bulatova et al., 1987). Thus, according to the diploid number the dinophilids are closer to the archiannelids and can be placed within this class.
A comparison of chromosome numbers in the species studied by us with the data on related invertebrates suggests that the acoels and dinophilids, as well as the archiannelids must be on the whole ancient forms, which confirms the view on the ancient origin of these groups. It is necessary to emphasize that the karyotypes of such primitive living animals as *Trichoplax*, acoels and other archoophors, as well as dinophilids consist mostly of biarmed chromosomes. Therefore the data described strengthen the assumption (Birstein, 1989) that the ancestral metazoan karyotypes must have been composed of about 20 (12–20) biarmed chromosomes.

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Buchbesprechung

Autorenkollektiv (PETER HANKE, Leiter), *Biotechnologie.* Herausforderung und Entwicklungschancen. 241 S., 38 Übersichten. Akademie-Verlag, Berlin 1988. Preis: brosch. M 18,–.

Der Biotechnologie wird heute große Bedeutung für die Entwicklung der Produktivkräfte beigemessen. Den Autoren des vorliegenden Buches geht es darum, die Etablierung dieser zukunftsweisenden Technologie und ihre Möglichkeiten darzustellen.

Nach einem kurzen historischen Abriß werden in geraffter Form und populärwissenschaftlicher Darstellungsweise die vielfältigen Techniken der Biotechnologie beschrieben. Leider wird dabei die entscheidende Rolle der Gentechnik in der modernen Biotechnologie nicht herausgearbeitet. Im zusammenfassenden Abschnitt 2.3. fehlen Erläuterungen zu den dargestellten Übersichten.

Im zweiten Teil des Buches werden Möglichkeiten der Anwendung biotechnologischer Verfahren, die von der Lebensmittelherstellung und Pharmazeutik über die chemische Industrie und Landwirtschaft bis zum Bergbau und der Umweltgestaltung reichen, besprochen. Vollständigkeit kann auf Grund des begrenzten Umfanges des Buches nicht erwartet werden. Trotzdem demonstrieren die ausgewählten Beispiele deutlich die Potenzen der neuen Technologie.

Im dritten Teil des Buches diskutieren die Autoren Probleme bei der Gestaltung biotechnologischer Innovationsprozesse. Die hier dargestellten Probleme und Einschätzungen dürften besonders für Leiter in Forschung und Industrie von Interesse sein.

Das Buch wird bei einem breiten Leserkreis zum Verständnis der strategischen Bedeutung der Biotechnologie beitragen. Gleichzeitig regt es zu Diskussionen über die Realisierungsmöglichkeiten biotechnologischer Verfahren an.

J. ENGEL (Gatersleben)