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On the Karyotype of *Trichoplax* sp. (Placozoa)

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With 1 Figure

Abstract

The karyotype of *Trichoplax* sp. consists of 6 chromosome pairs, i. e. 3 pairs of bi-armed, meta- or submetacentric homologues, and 3 pairs of smaller, possibly acrocentric, chromosomes. The absolute chromosome size is not greater than 2–3 μ . The *Trichoplax* sp. karyotype is apparently similar to the karyotype of the ancestral form of Metazoa (Bilateria).

Introduction

Trichoplax adhaerens Schulze is so primitive a multicellular animal that "it may be placed only alongside the assumed ancestor of all metazoans" (IVANOV, 1973). Although *T. adhaerens* and its relative *Treptoplax reptans* Monticelli were discovered and described for the first time almost one hundred years ago (SCHULZE, 1883; 1891; MONTICELLI, 1893; 1896), up to date both of them have not yet been well studied. In 1971, K. GRELL (1971; 1972) found the sexual reproduction of *T. adhaerens* and observed the early stages of egg cleavage. This enabled him to prove that *T. adhaerens* is an independent parenchymula-like organism, and he united *T. adhaerens* and *T. reptans* in a new type which he named Placozoa. A. V. IVANOV (1973) considered these animals "living models" of the Phagocytella, METSCHNIKOFF's (METSCHNIKOFF, 1883; 1886) presumed ancestor of all metazoans. According to IVANOV, these organisms belong to the systematic group Phagocytellozoa, which is of the same range as, on the one side, the sponges, i. e. Enantiozoa (= Parazoa), and, on the other, all representatives of Enterozoa (= Eumetazoa).

Up to date, the morphology and ultrastructure of *T. adhaerens* were investigated (GRELL and BENWITZ, 1971; 1974a, b; 1981; RASSAT and RUTHMAN, 1979), and the differentiation of cells within organisms (SCHWARTZ, 1984), as well as the capability of cells to aggregate (RUTHMANN and TERWELP, 1979) were described. All these data were obtained on individuals from the culture which was kept at the Zoological Institute, University of Tübingen (FRG); the original material was brought from the Red Sea. Analogous experiments were done on the specimens of *Trichoplax* sp., which were found in Moscow marine aquaria in 1979 (IVANOV et al., 1980a; SHUBRAVYI, 1983) and then were investigated at the Chamber of Invertebrate Zoology of Moscow State University (IVANOV et al., 1980b; OKSHEIN, 1987). It was found that in the specimens of both *Trichoplax* species there are three cell layers: the ventral epithelium, consisting mainly of the flagellate and glandular cells, and the dorsal one, which includes specific "bright ball" cells, as well as the intermediate layer, in which cells having complex mitochondria are situated. In this inner layer of *T. adhaerens*, oocytes and spermatocytes form under certain conditions (RUTHMANN et al., 1981).

One of the unusual characteristics of *T. adhaerens* is the very small size of its genome, apparently the smallest in metazoans, $C \sim 0.05$ pg (RUTHMANN and WENDEROTH, 1975; RUTHMANN, 1977; RUTHMANN et al., 1981). The cells of the ventral and dorsal layers are diploid, and of the intermediate one

are possibly tetraploid, since they have an increased DNA content. The diploid number of *T. adhaerens* equals 12, but the morphology of chromosomes remained unclear because of their very small size (RUTHMANN, 1977). In this paper the karyotype of *Trichoplax* sp. is described.

Materials and methods

3-5 *Trichoplax* sp. individuals were placed in small dishes filled with artificial sea water, and cyanobacterium *Phormidium inundatum* was added as a food substratum (OKSHEIN, 1987). Colchicin, up to 0.025, 0.05, 0.1, 0.2 and 0.4% concentration, was added into the experimental dishes. Further behaviour of control and experimental animals was observed by means of a stereomicroscope. The incubation continued for two days at 16-19°C because it was shown earlier that the *Trichoplax* sp. division usually occurs in 1.5-2.0 days (OKSHEIN, 1987). Then sea water was changed for 0.9% sodium citrate solution for 1 min (if the time of the hypotonic treatment was increased, the cells of the animals became destroyed). After that, the animals were fixed with ethanol-glacial acetic acid mixture (3:1), which was changed three times. Each portion of animals was transferred into a little glass tube, where they were disaggregated in 50% aqueous acetic acid by means of a Pasteur pipette. The cell suspension was repeatedly allowed to evaporate on slides kept at 60°C on a slide warmer. The preparations obtained were stained in a 2% Giemsa solution, pH = 6.8, and after washing and air-drying they were observed in a NU-2 microscope (K. Zeiss, Jena).

Results and discussion

The results of the incubation of *Trichoplax* sp. indicates that the division of animals occurred in all cases. The presence of colchicine, even in 0.2 and 0.4% concentration, did not lead to the dissociation of the individuals to a cell mixture; this effect of 0.4% colchicin solution was seen in the case of *T. adhaerens* (RUTHMANN and TERWELP, 1979). The greatest quantity of metaphase plates was found when animals were incubated in 0.1% colchicin solution.

Fig. 1 shows photos of three metaphase plates (a-c) and a karyogram (d) of *Trichoplax* sp. In all cases one can see 12 chromosomes; on Fig. 1b, one of the chromosomes overcovers the other. Chromosomes are stained faintly, their morphology is illegible (probably because of their very small size); the best case is represented on Fig. 1c. Evidently, the *Trichoplax* sp. karyotype consists of 6 pairs of chromosomes, i. e. 3 pairs of bi-armed, meta- or submetacentric homologues of about

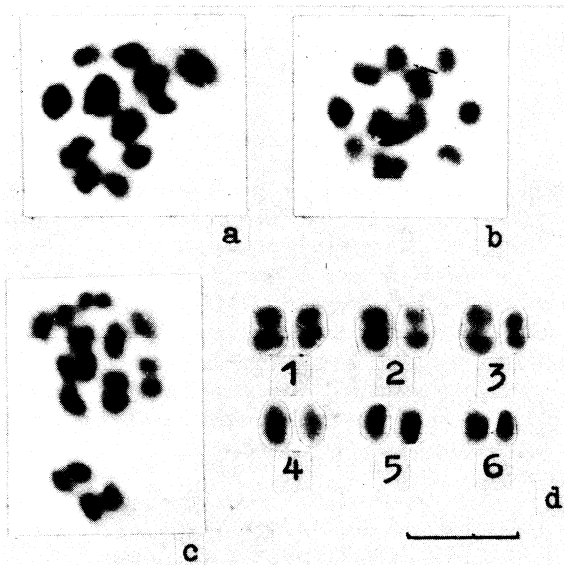


Fig. 1. Metaphase plates (a-c) and a karyogram (d) of *Trichoplax* sp. Bar represents 10 μ

same size, and 3 pairs of smaller chromosomes seem to be acrocentrics. Their absolute size depends on spiralization, since totally it is about 2–3 μ (Fig. 1d). As was already pointed out, the diploid number in *T. adhaerens* equals 12 also (RUTHMANN, 1977).

The structure of ventral epithelium of *T. adhaerens* reminds of the epidermis of Acoela representatives (Turbellaria), and the dorsal one has similar characteristics to the submerged epidermis of many turbellarians (IVANOV, 1973; IVANOV, MAMKAEV, 1973). In some representatives of Acoela investigated, the diploid numbers are similar to *Trichoplax*, being 12–20 (KIKNADZE, 1973; BENAZZI and BENAZZI-LENTATI, 1976). These data need to be examined once more, as they were obtained without use of colchicin. The chromosome size in *Convoluta convoluta*, $2n = 16$, is larger than in *Trichoplax* sp., but it depends on the stage of cell division (BIRSTEIN, in preparation). The karyotypes of other turbellarians most often consist of 10–20 relatively large, usually bi-armed (meta, submetata-, and rarely submetacentric) chromosomes (BENAZZI and BENAZZI-LENTATI, 1976; GALLENI and PUCINELLI, 1981; 1986). Thus, the chromosome number of the lower invertebrates investigated is approximately identical, $2n \sim 12-16$. The chromosome size in *Trichoplax* and most of turbellarians differs apparently because of the greater genome size in the latter; for instance, in *Dugesia lugubris* and *D. polychroa* (Tricladida), $2n = 8$, $C \sim 0.9$ and 0.4 pg, respectively, and in *D. benazzi*, $2n = 16$, $C = 1.2$ pg (BENAZZI et al., 1981; PELLICCIARI et al., 1986). The nucleus is also greater in turbellarians than in *Trichoplax* sp. (own observations). The DNA content in sponges and the polyp *Corymorpha palma* is ten-fold greater than in *T. adhaerens*, $C = 0.5-0.6$ pg (MIRSKY and RIS, 1951; CAMPBELL, 1973); in *Hydra attenuata*, it is three times greater than in these coelenterates, $C = 1.7$ pg (DAVID and CAMPBELL, 1972). The chromosome number in the few diploid sponge species investigated is two-fold that of *Trichoplax*, in *Leucosolenia complicata* (Calcarea) being 24 (ANAKINA, 1981), and in polyploid species it is greater, in *Halisarca nahantensis* $4n \sim 44$, in *H. metschnikovi* $6n \sim 66$ (CHEN, 1976).

On the other side, the genome size of *T. adhaerens* is comparable to that of protozoans. The majority of authors consider that metazoans are a monophyletic descendant group of the colonial phlagellates, most likely choanoflagellates (DOGEL, 1975; BARNES, 1985; NIELSEN and NØRREVANG, 1985). Up to date, the karyology of only parasitic flagellates, species of Protomonadida, Polymastigida, Hypermastigida, Opalinida, was studied (WALTON, 1959; RAIKOV, 1978). The chromosome number of the representatives of two first orders usually equals 6–10, and the DNA content in *Trypanosoma*, *Leishmania*, etc. is 0.1–0.2 pg per nucleus (SOPRUNOV, 1987). Therefore, in the chromosome number, the DNA content per nucleus and the DNA content per one chromosome of the *Trichoplax* species are comparable to some of the flagellates. From the comparison of the 5S RNA nucleotide sequence of different invertebrates OHAMA et al. (1984) concluded that mesozoans (*Dicyema misaiensis* was investigated) are the most ancient multicellular animals, which appeared at the same time when groups of phlagellates and ciliated infusoria formed. The lines of planarians *Dugesia* (Turbellaria) and nematodes derive from the stem of the 5S RNA phylogenetic tree somewhat later than those of the mesozoans, but earlier than the lines of other Metazoa, including sponges and coelenterates (OHAMA et al., 1984; HENDRIKS et al., 1986). Analogous future investigations of 5S RNA seem to be useful for a more exact placement of *Trichoplax* in relation to the mesozoans and lower metazoans.

From all the data discussed above it may be concluded that the karyotype of *Trichoplax* sp. is apparently similar to that of the ancestor of all metazoans. The genome increase in the phylogenetic line of Metazoa (more exactly, Bilateria) occurred evidently without any considerable increase in chromosome number. It is most likely that the DNA content increase in chromosomes during the course of evolution resulted in the increase of chromosome size.

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