GENOME SIZE AND CHROMOSOMES IN MARINE SPONGES *[SUBERITES DOMUNCULA, GEODIA CYDONIUM]*

Georg Imsiecke[1], Marcio Custodio, Radovan Borojevic[2], Renate Steffen[1], Mohamed A. Moustafa[3] and Werner E.G. Muller[1]*

1 Institut für Physiologische Chemie, Universität, Duesbergweg 6, D-55099 MAINZ, GERMANY; 2 Departamento de Bioquimica, Instituto de Quimica, Universidade Federal do Rio de Janeiro, Caixa Postal 68021,21944-970 RIO DE JANEIRO, BRAZIL; 3 Institüt für Molekulargenetik, University, Becherweg 32, D-55099 MAINZ, GERMANY

Key words: Sponges; *Suberites domuncula;* karyotype; genome size.

ABSTRACT

The genome size of the marine sponges *Suberites domuncula* and *Geodia cydonium* has been determined by flow cytofluorometric analysis using diamidino-phenylindole [DAPI]. Using human lymphocytes as reference the amount of DNA in cells from *S. domuncula* has been determined to be 3.7 pg and that of G. *cydonium* 3,3 pg. While no chromosomes could be identified in G. *cydonium,* the karyotype of the *Suberites domuncula* is 32 chromosomes in the diploid state, The size of the chromosomes was between 0.25 and 1.0 μm. No pronounced banding pattern was visible.

INTRODUCTION

Porifera (Sponges) are the oldest metazoan phylum and known since the early Cambrian (600 million years) (Finks, 1970; Knoll, 1994)]. In one view the Porifera are considered simply as colonies of choanoflagellata which have derived from a separate protist lineage and evolved independently from the deuterostomes, protostomes and cnidarians [Barnes, 19SO]. In contrast, according to Weissenfels [cited in: Mehlhorn, 1989], some sponge cells are already organized into epithelia-like tissues which form simple organs or organ-like assemblies.

Recent sequencing data from 28S rRNAs of members of Porifera revealed unexpected relationships [Lafay *et al.,* 1992]. Based on these data the phylogenetic relationships showed a separation of the sponges into three groups with different correspondence to the phyla Cnidaria and Ctenophora hence, allowing no conclusive branching order. Moreover, 18S rRNA sequence analyses also proved not to be suitable to resolve deep branching in the phylogenetic tree including Porifera [Rodrigo *et al.,* 1994]. Our DNA sequencing data analyzing cell adhesion/receptor (lectins [Pfeifer *et al.,* 1993] and tyrosine kinase receptor [Schacke *et al.,* 1994a and 1994b]) and a nuclear receptor [Kruse *et al.,* 1994] from the marine sponge *Geodia cydonium* revealed that sponge genes have - in spite of a calculated age of 800 million years basing on amino acid exchanges [Muller *et al.,* 1994] - close homology with corresponding genes from other metazoan organisms. These findings strongly suggest a monophyletic origin of all metazoan [Muller *et al.,* 1995].

Despite the fact that we are beginning to learn basic facts about gene organization in marine sponges, no conclusive data are known about the DNA content and the chromosomes in marine sponges. Recently we reported for the first time the karyotype in a freshwater sponge, *Spongilla lacustris* [Imsiecke *et al.,* 1993]. In the present study we report on the genome size of the two marine sponges *Suberites domuncula* and *Geodia cydonium.* In a further effort to visualize chromosomes from marine sponges we failed up to now with one exception which is described here, *Suberites domuncula.*

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Materials and Methods

Animals

The marine sponges *Suberites domuncula* Olivi (Porifera, Demospongiae, Hadromerida) and *Geodia cydonium* (Porifera, Demospongiae, Homosclerophorida) were collected in the Northern Adriatic near Rovinj (Croatia) and then kept in seawater basins in Mainz for 2 weeks at a temperature of 16ºC. The sponge cells were mechanically dissociated and subsequently used for chromosome preparation and determination of DNA content.

Flow cytofluorometric analysis of DNA

Sponge cells were suspended in natural seawater [Sigma, St. Lois, MO (S 9148)) supplemented with 0.5% (w/v) of Triton X-100 and 0.2% (w/v) of Nonidet NP-40 for 15 min at 20ºC. After centrifugation (300 x g; 10 min) the pellet was suspended in 1 M NaC1 solution and incubated (10 min, 20ºC). After a further centrifugation step (350 x g; 15 min) the cells were treated with a solution of 0.3% (w/v) of 4',6-diamidino-2-phenylindole [DAPI; Sigma (D-9542)] and subsequently analyzed by the cytofluorometer ICP-22 [Ortho, Neckargemund; Germany]. Ten independent samples have been evaluated.

Chromosome preparation

The cells were collected by centrifugation (1,000 x g; 10 min) and treated in a hypotonic solution composed of natural sea water (Sigma, St. Louis, Mi; S 9148) diluted with the same volume of 75 mM KCl. Then the cells were Axed with methanol: glacial acetic acid (1: 1) for two cycles of 10 and 20 min, respectively. The chromosomes were spread onto an ice-cold and wet microscopic slide. Staining of chromosomes was performed with 7 ml of Giemsa solution (E. Merck, Darmstadt) in 100 ml of Sorensen-phosphate buffer (pH 6.8). After air drying the specimens were examined microscopically (Olympus AH-3 microscope).

RESULTS AND DISCUSSION

Dissociated cells

Single cells were obtained by mechanical dissociation and consisted to more than 50% of choanocytes and to approximately 20% of spherulous cells; here the cells from *S. domuncula* are shown (Fig. 1).

DNA content

The DNA content of cells from the two marine sponges, *S. domuncula* and *G. cydonium* has been determined cytoflurometrically after staining them with DAPI. Using human lymphocytes as reference [DNA content: 7.2 pg/cell], the amount of DNA in *S. domuncula* was 3.3+0.1 pg/cell and the one in G. *cydonium* 3.7+0.1 pg/cell (Fig. 2).

Chromosomes during mitosis

In the prophase (Fig. 3a and b) the chromosomes are very thin (0.25 μm in maximum) and condense with time (0.5 μm; Fig. 3b). With transition to metaphase (Fig. 3c and d) the chromosomes reach their maximum density and thickness; they showed a spheric to rod-like shape (0.75 to 1.0 μm). In the early anaphase the chromosomes are obviously arranged into two groups of chromatides suggesting a spindle apparatus (Fig. 3e). In the late anaphase the chromosomes are separated into two different nuclei (Fig. 3f).

In comparison with chromosomes of the freshwater sponge *Spongilla lacustris* which have sizes between 0.7 and 2.1 μm (Imsiecke et al. 1993) the dimensions of the chromosomes from *S. domuncula* are smaller. We could not identify unequivocally centromers in the chromosome preparations from *S. domuncula;* the same difficulty was noticed already with the description of the chromosomes from *S. lacustris.* A distinct banding pattern of the sponge chromosomes is not visible.

Karyotype

The diploid number of chromosomes of *S. domuncula* is 32, Hence the karyotype is larger compared to that of *S. lacustris* (18 chromosomes; Imsiecke et al. 1993).

Mitotic index

The mitotic index determined in the dissociated single cell suspension was found to be 3.5%. 500 cells have been inspected for this analysis.

Figure 1. Dissociated cells from the marine sponge *S. domuncula.* Arrow head: choanocytes and arrow; spherulous cells. Magnification: x 400.

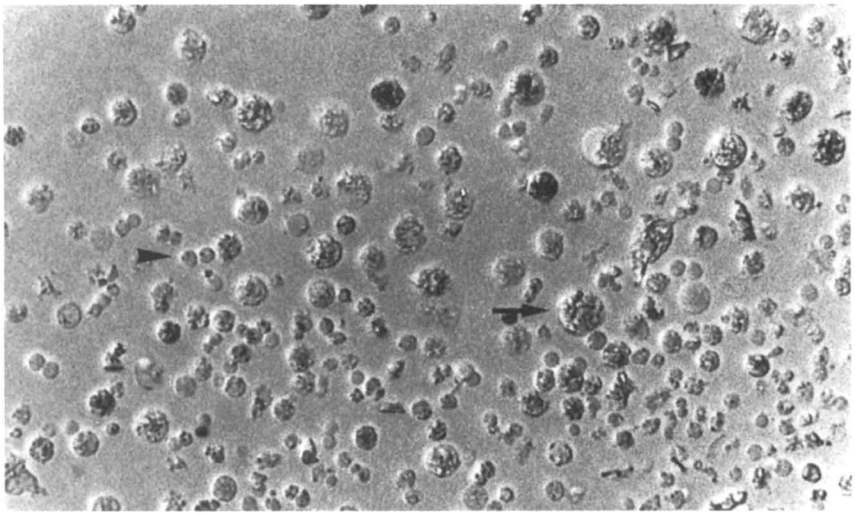


Figure 2. DNA content of cells &am the sponges G. *cydonium* and *S. domuncula* as determined by flow cytometry after staining the DNA with DAPI. Human lymphocytes have been used as reference.

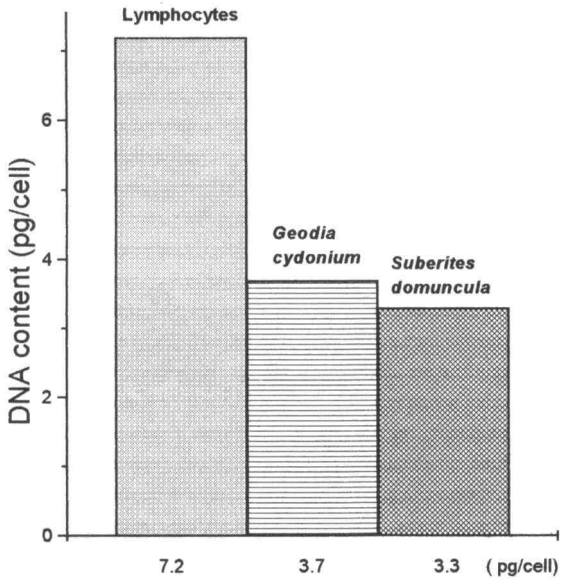
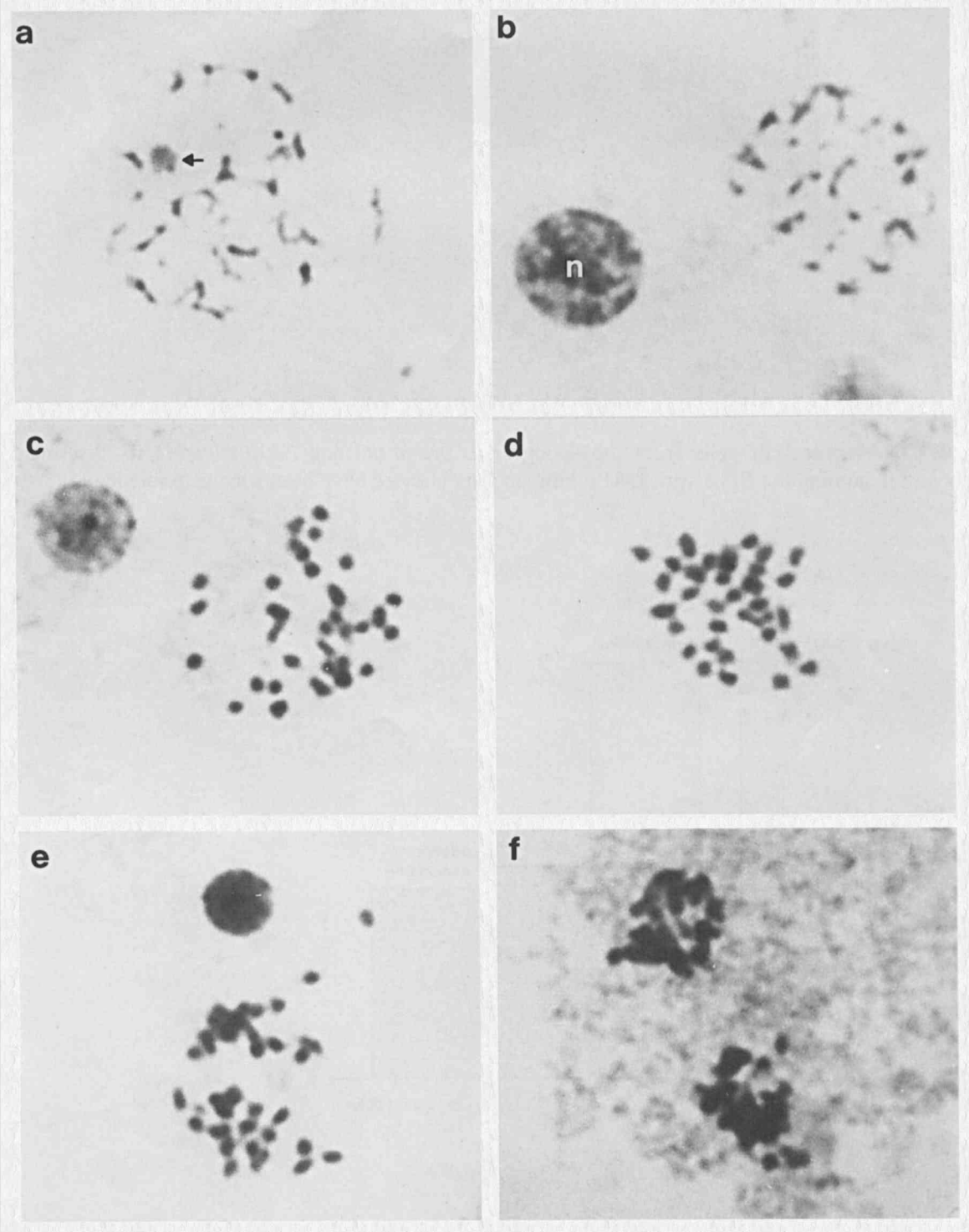


Figure 3. Chromosomes of *S. domuncula*. The specimens have been spread after hypotonic treatment. **a:** prophase (the arrow points to the nucleolus), **b:** interphase nucleus (n) [left] and a prophase [right], **c:** and **d:** condensed metaphases, **e:** early anaphase and **f:** late anaphase/early telophase, The structures are visualized by bright field microscopy. Magnification: x 4,000.



CONCLUSION

Until now only preliminary data about the DNA content per sponge cell are available. Using Feulgen staining the amount of DNA per cells has been estimated with 0.11 pg DNA in one sponge species, *Dysidea crawshagi* [Fasman, 1976]. Applying the technique of flow cytometry and using DAPI as dye to stain the DNA quantitatively the DNA content in two species of marine sponges was determined to be 3.3+ 0.1 pg/cell in *S. domuncula* and 3.7+0.1 pg/cell in G *cydonium.* Until now the DNA content during the cell cycle could not been determined. Considering the finding that only a low percentage of cells of *S. domuncula* are in mitosis and none of the cells G. *cydonium* are in this cell cycle phase we assume that these values represent the DNA content per diploid genomic set. Consequently the genomic size of the haploid genome [C value] of both sponges is approximately 1.7 pg, corresponding to 1,670,000 kb. This value is in the range of those found in some vertebrates, e.g. *Gallus domesticus* (chicken) [1,200,000 kb] or *Cyprinus carpio* (carp) [1,700,000 kb]. In comparison, the size of the human haploid genome is 3,400,000 kb (reviewed in Li and Graur, 1991).

Chromosomes could only be visualized in the sponge *S. domuncula.* Their number is in the range of that found previously in the freshwater sponge *Spongilla lacustris* (Imsiecke *et al.*, 1993), No chromosomes could be detected in cells from G. *cydonium.* This ending confirms our observation that dissociated cells from G. *cydonium* do not proliferate *in vitro,* under the conditions used at present (to be published).

ACKNOWLEDGEMENTS: This work was supported by grants from the Deutsche Forschungsgemeinschaft and the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie (under the coordination of KFA Jülich - Internationales Büro).

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Paper received 04.08.95. Revised paper accepted 25.11.95.