Jap. J. Phycol. (Sôrui) 29: 23-29. March 10, 1981

Cytological studies on *Sirogonium* (Chlorophyceae) 1. Mitosis in *S. sticticum* and *S. melanosporum*.

Akira HARADA

Seiyu Senior High School, Chizuka, Yao-City Osaka, 581 Japan

HARADA, A. 1981. Cytological studies on *Sirogonuim* (Chlorophyceae) 1. Mitosis in *S. sticticum* and *S. melanosporum*. Jap. J. Phycol. 29: 23-29.

Cell division and chromosomes behavior in two species of *Sirogonium*, *S. sticticum* and *S. melanosporum*, were studied, with special emphasis on, the behaviour of the nucleolar organizing chromosomes, the composition of the nucleolus, and the parallel separation of chromosomes embedded in the nucleolar substance at anaphase, and during cytokinesis. The chromosome numbers of *S. sticticum* and *S. melanosporum* were 52 and 8 respectively.

Key Index Words: *Chlorophyceae*; *cytology*; *mitosis*; *N.O. chromosome*; Sirogonium.

The mitosis and chromosomes in some species of *Sirogonium* were observed by WAER (1966), HOSHOW and WAER (1967), and WELLS (1969). However, there are as yet no reports on meiosis. In a series of papers, cytological studies on *Sirogonium* will be presented with special attention paid to the chromosomal behaviour in both mitosis and meiosis. In this paper, mitosis in two spicies of *Sirogonium*, *S. sticticum* (J. E. SMITH) KÜTZING and *S. melanosporum* (RANDHAWA) TRANSEAU, is discribed.

Materials and methods

The vegetative filaments of *S. sticticum* used in this investigation were first collected from a rice field in Ikaruga, Nara Prefecture, in December 1970. In April of the next year, fertile filaments were obtained from the same rice field. Both vegetative and fertile filaments of *S. melanosporum* were collected from a rice field in Ikebe, Nara Prefecture, in August 1975.

Collection of specimens of the two species was continued at frequent intervals. Most of each collection was fixed for karyological investigation, and the rest were kept in culture in the laboratory.

The materials were fixed in 1 : 3 acetic and ethyl alcohol and were preserved in the same solution. Many dividing cells were obtained in materials fixed at sunset. For staining, the aceto-iron-haematoxyrin-chloral method (Wittmann, 1965) was employed. The materials were pretreated with 1 N HCl and 4% iron-alum-45% acetic acid for a clear distinction in staining between the chromosomes and the nucleolar substance.

Both pre- and post-treatment as well as staining procedure, were carried out on glass slide. After staining cells were heated, if necessary, to facilitate clear observation of the nuclei. Cells were treated with 45% acetic acid to reduce overstaining of the nucleolar substance, and squashed on the glass slide.

Observations

Sirogonium sticticum

Interphase: The interphase nucleus was elliptic to spherical in shape and usually had one, or rarely two, nucleoli which appeared as bright spheres in living cells

(Fig. 1). Two deeply stained, curved thread-like structures were observed in the nucleolus (Fig. 5). Approaching prophase, many chromatins were seen scattered in the karyoplasm.

Prophase: At the beginning of prophase, the nucleus was bounded with a well-defined membrane. It was slightly enlarged, and many short chromatic threads (chromonemata) with bead-like chromatins were seen in the karyoplasm around the nucleolus (Fig. 6).

In the nucleolus, the compact thread-like structures, which were called the nucleolar organizer tracks by Godward (1950), were visible as two densely stained and curved strings connecting with a chromatic thread in the karyoplasm (Figs. 7, 8).

As prophase proceeded, chromatins condensed to become short rod-like chromosomes with lightly stained portions in the contral area (Fig. 9), and a spindle was formed (Fig. 10).

Fifty-two chromosomes were counted at this stage (Figs. 13, 14). Two of these were nucleolar organizing chromosomes, (N-O-chromosomes: Godward, 1950) connecting with each organizer track embedded in the nucleolus.

At the end of prophase, the nucleolus lost its sharp outline was transformed into a deeply stained mass, called as nucleolar substance by Geitler (1935). All the chromosomes were completely embedded inside the nucleolar substance. The nuclear membrane disappeared during later prophase.

Metaphase: At this stage, 52 chromosomes were recognized. The smallest chromosomes measured 0.5 μm in length, while the largest was 1.0 μm in length. They were arranged regularly and embedded inside the nucleolar substance. When the spindle developed fully, the nucleolar substance became a deeply stained, disk-shaped mass at the equatorial plate of the nucleus (Fig. 10).

Anaphase: The nucleolar substance divided into two parallel disk-shaped daughter plates, and all the chromosomes also separated transversely at early anaphase (Fig. 11).

Each disk-shaped plate (daughter nucleolar substance) included a set of chromatids. The arrangement of the chromatids in each plate was symmetric with in the other in polar view in strongly squashed preparation (Fig. 12). Fifty-two chromo somes were also confirmed at this stage. The two disk-shaped daughter plates containing the chromatids began to separate and move toward the opposite poles.

Telophase: At the beginning of telophase, the chromatids fragmented into small pieces, and their shapes were gradually obscured. The nucleolar substance was reformed in the nucleus as a darkly stained amorphous mass.

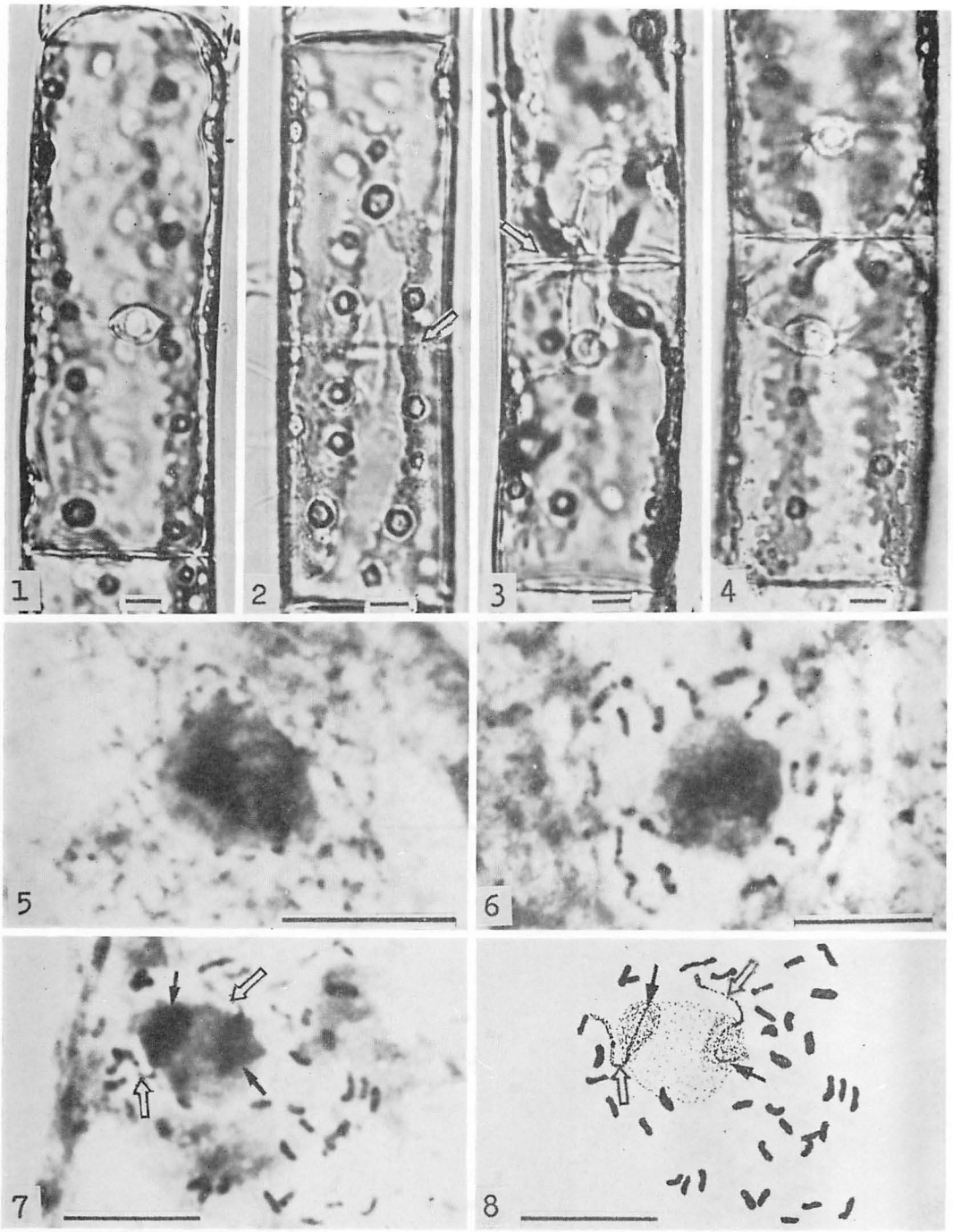
Cytokinesis: At the beginning of prophase, a remarkable granular particles (Fig. 2, arrow) could be seen along the cell wall at the middle portion of a cell. These particles arranged themselves into a circle and developed gradually into a cell plate (Fig. 3). After telophase, when the new nuclei and nucleoli were formed, the chloroplasts and cytoplasm were divided by the cell plate which had developed centripetally (Fig. 4).

Sirogonium melanosporum

Interphase: The interphase nuclei usually had one nucleolus, or rarely two. In the nucleolus, organizer tracks were clearly observed, and small deeply stained globules were seen at the end of these tracks (Fig. 15). When two nucleoli existed in a nucleus, each of the two had one such globule (Fig. 16). Many chromatins appeared in the karyoplasm as observed in S. *sticticum*.

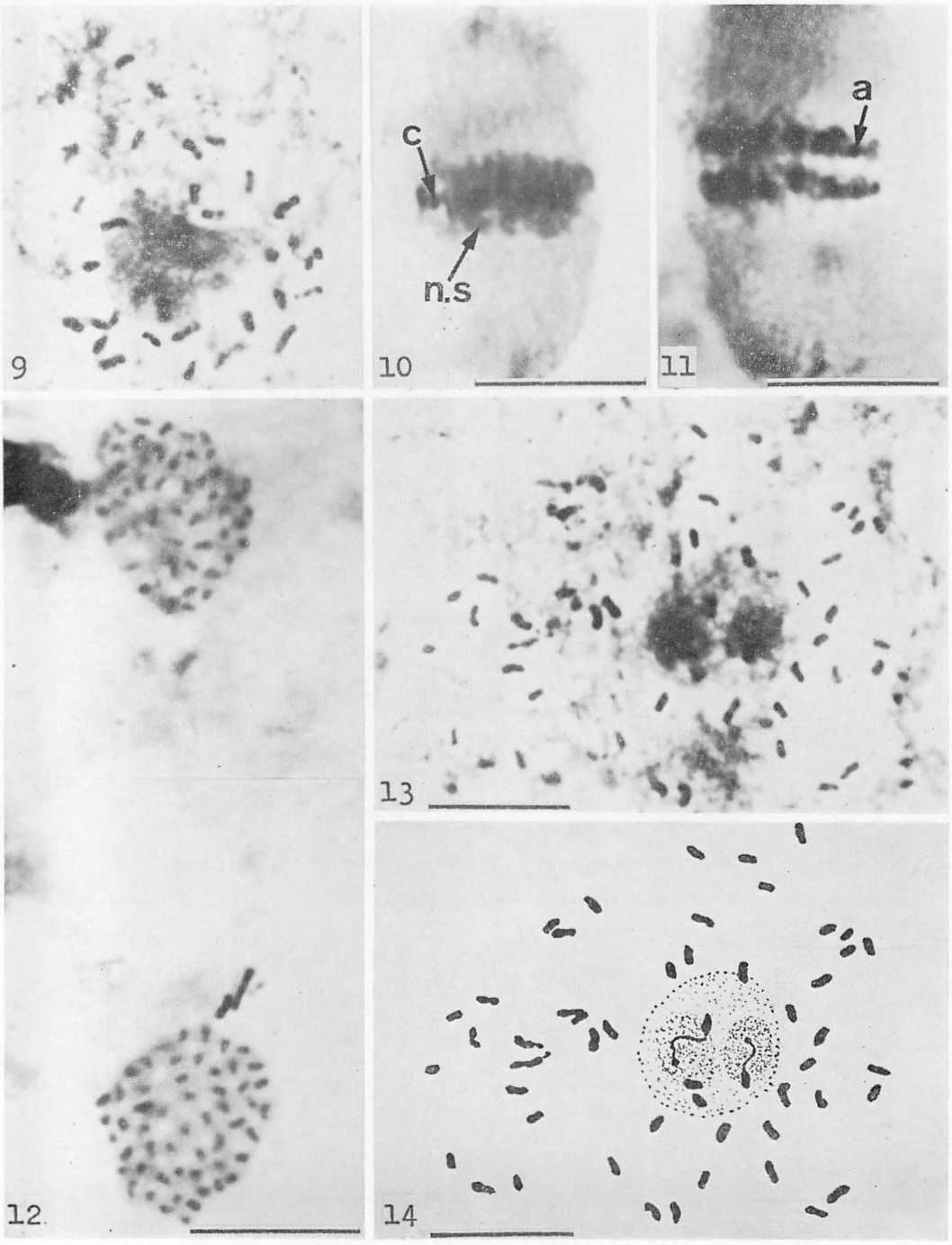
Prophase: In early prophase, bead-like chromonemata were seen in the karyoplasm. These chromonemata began to condense into chromosomes. In the nucleolus, one or two organizer tracks connecting to the chromonemata were observed. At later prophase, the nucleolus was transformed into nucleolar substance (Fig. 17), and all the chromosomes moved into the substance as occurred in *S. sticticum*.

Metapnase: Eight chromosomes were

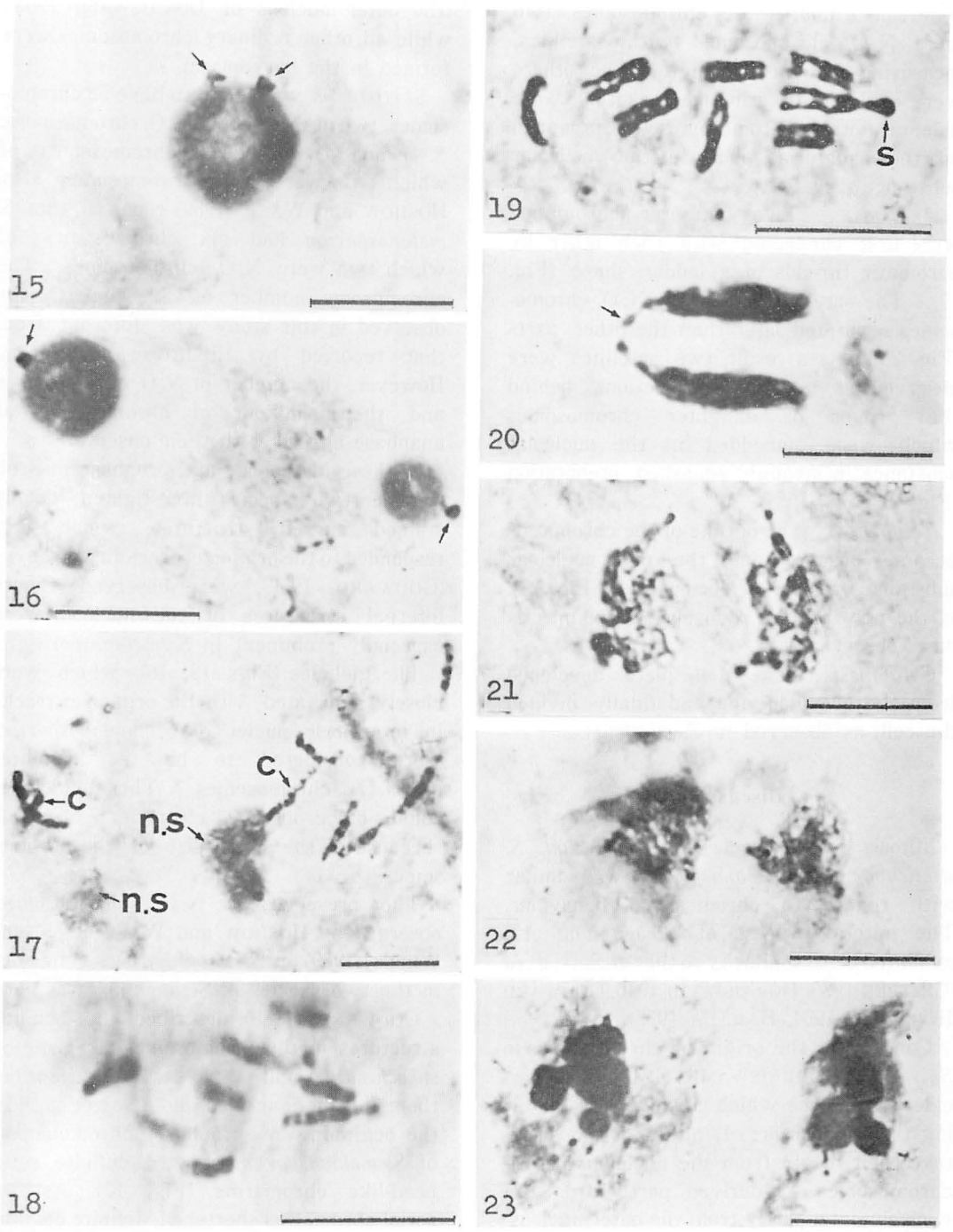


Figs. 1-4. Cell division in living material of *S. sticticum*. (scale 10 μm) 1. Interphase: a nucleus with a prominent nucleolus. 2. Metaphase: a granular ring (which arrow). 3. Late telophase: division of chloroplasts by cell plate (white arrow). 4. Two daughter cells.

Figs. 5-8. Nuclear division of *S. sticticum*. (Scale 10 μm) 5. Early prophase: two organizer tracks in the nucleolus and thread-like chromonemata in karyoplasm. 6. Prophase: chromonemata with bead-like chromatins. 7. Prophase: two organizer tracks (arrows) and chromatic threads connected with the tracks (white arrows). 8. Diagram of Fig. 7.



Figs. 9-14. Nuclear division of *S. sticticum*. (Scale 10 μm) 9. Late prophase: chromosomes inside the nucleolar substance. 10 Metaphase: chromosomes (c) embedded inside the nucleolar substance (n.s.) at equatorial plate. 11. Early anaphase: parallel separation of chromatids (a). 12. Anaphase; chromatids embedded inside the nucleolar substance in polar view, at the same stage as Fig. 11 in strongly squashed preparation. 13. Late prophase. 14. Diagram of Fig. 13 showing 52 chromosomes.



Figs. 15-23. Nuclear division of *S. melanosporum*. (Scale 10 μm) 15. Interphase: two organizer tracks in the nucleolus and two globules (arrow). 16. Interphase: nucleus with two nucleoli and globules (arrow). 17. Mid prophase: two N.O. chromosomes (c) attached to the nucleolar substance (n.s.). 18. Later prophase: eight chromosomes. 19. Early anaphase: ladder-shape chromosomes and satellite (s). 20. Anaphase: a satellite of N.O. chromosome divided into two. 21. Telophase: chromatins in each chromatid. 22. Telophase. 23. Later telophase: two daughter nuclei.

counted. Among these chromosomes, two were N. O. chromosomes with satellites, measuring 6.0 μm in length. The others were of the same form and measured 3.0 μm in length. Eight chromosomes were arranged on the equatorial plate in the nucleolar substance (Fig. 18).

Anaphase: Two daughter chromatids were first connected with each other by chromatic threads in a ladder shape (Fig. 19). The satellite part of the N. O. chromosomes separated later than the other parts (Fig. 20). As a result, two satellites were observed as horn-like projections behind the groups of daughter chromosomes which were embedded in the nucleolar substance in strongly squashed preparation (Fig. 21).

Telophase: The outline of the chromatids became obscure and the old nucleolar substance gradually disappeared (Fig. 22). In the new nucleoli, densely stained masses were observed (Fig. 23).

Cytokinesis: The cell plate developed from the granular ring and finally divided the cell, as observed in *S. sticticum.*

Discussion

Mitosis in two species of *Sirogonium, S. sticticum* and *S. melanosporum,* was similar with regard to chromosomal behaviour. The mitotic process of *Sirogonium* also exhibited close similarity to that of *Spirogyra* (GEITLER 1930, DORAISWAMI 1946, GODWARD 1950, 1953, 1954, HARADA 1972).

Concerning the origin of chromosomes in *Spirogyra,* DORAISWAMI (1946) reviewed older literatures which advanced the following three patterns: 1) all the chromosomes take their origin from the nucleolus; 2) the chromosomes are derived partly from the nucleolus and partly from the outer nucleus; and 3) all the chromosomes are derived solely from the outer nucleus. The present studies on mitosis in *Sirogonium* revealed that N. O. chromosomes were derived partly from the organizer tracks in the nucleolus and partly from the karyoplasm (the outer nucleus of DORAISWAMI 1964), while all other ordinary chromosomes were formed in the karyoplasm.

S. sticticum was found to have 52 chromosomes, two of these being N. O. chromosomes. *S. melanosporum* had 8 chromosomes, of which two were N. O. chromosomes also. HOSHOW and WAER (1969) reported that *S. melanosporum* had six chromosomes, of which two were N. O. chromosomes. The chromosome number of *S. melanosporum* observed in this study was different from that reported by HOSHOW and WAER. However, the number of N. O. chromosomes and the behaviour of chromosomes at anaphase agreed with their observations.

In these interphase and prophase nucleoli of the two species investigated, deeply stained curved structures, which corresponded to the organizer track in *Spirogyra* (GODWARD, 1950), were observed. These internal structures of the nucleoli were especially prominent in *S. melanosporum*

The globules (Figs. 15, 16), which were closely associated with the organizer tracks in interphase nuclei of *S. melanosporum,* were considered to be the satellites of N. O. chromosomes. These globules could not be observed clearly in *S. sticticum* because of the small size of the chromosomes.

The presence of N. O. chromosomes, observed by HOSHOW and WAER (1969) and WELLS (1969) in *Sirogonium,* was confirmed in the two species of *Sirogonium* used here.

GODWARD (1966) described the banding structures in the chromosomes of *Spirogyra subechinata,* and WELLS (1969) described them in some species of *Sirogonium.* At the beginning of prophase, chromonemata of *S. melanosporum* showed definite serial bead-like chromatins (Fig. 17). As the serial chromatins shortened, definite banding patterns were formed in the chromosomes. In *S. sticticum,* however, such banding patterns could not be clearly observed, and small rod-shaped chromosomes had only one discontinuous portion in the middle (Fig. 6). These chromatic patterns of chromosomes

disappeared in metaphase and in chromosomes homogeneously stained.

The nucleolar substance of *Spirogyra* was mentioned by GEITLER (1935), DORAISWAMI (1964), and GODWARD (1950). A similar material was also observed by WAER (1966) in *S. melanosporum* and by WELL (1969) in ten strains of *Sirogonium*. The special features of the nucleolar substance, enclosing all the chromosomes during mitosis, were also observed clearly in the two species.

At anaphase, the beginning of separation into sister chromatids occurred, each chromosome showing a ladder-shape which was composed of daughter chromatids connected by many chromatic threads. The ladder-shape chromosomes were observed in the mitosis of *Spirogyra crassa* by GEITLER (1930) and GODWARD (1954, 1966).

GODWARD (1954, 1966) pointed out the presence of the polycentric chromosomes in some species of *Spirogyra*. WAER (1966) and WELLS (1969) also found that the chromosomes of *Sirogonium* lacked of localized centromeres. This chromosomal feature was shown by the characteristic parallel separation of chromatids, which might suggest the presence of polycentric-chromosomes during metaphase to anaphase in the two species investigated in the present study.

Acknowledgements

The author wishes to express his sincere thanks to Prof. Dr. T. YAMAGISHI, Nihon University, and Prof. Dr. K. UEDA, Nara Women's University, for their valuable suggestions and kind encouragement.

References

DORAISWAMI, S. 1946. Nuclear division in *Spirogyra*. J. Indian Bot. Soc. **25**: 19-36.

GEITLER, L. 1930. Über die Kernteilung von *Spirogyra*. Arch. Protistent. **71**: 79-100.

GEITLER, L. 1935. Neue Untersuchungen über die Mitose von *Spirogyra*. Arch. Protistenk. **85**: 10-19.

GODWARD, M.B.E. 1950. On the nucleolus and nucleolar organising chromosomes in *Spirogyra*. Ann. Bot. **14**: 39-53.

GODWARD, M.B.E. 1953. Geitler's nucleolar substance in *Spirogyra*. Ann. Bot. **17**: 403-415.

GODWARD, M.B.E. 1954. The diffuse centromere or polycentric chromosomes in *Spirogyra*. Ann. Bot. **18**: 143-156.

GODWARD, M.B.E. (ed.) 1966. The Chromosomes of the Algae. Edward Arnold Publ. Ltd., London.

HARADA, A. 1972. Mitosis and meiosis in *Spirogyra crassa* No. 91 (n=6). Seiyu High School Bull. **3**: 2-30 (in Japanese).

HOSHOW, R.W. and WAER, R.D. 1976. Polycentric chromosomes in *Sirogonium melanosporum*. Can. J. Bot. **45**: 1167-1171.

WEAR, R.D. 1966. A cytological investigation of cell division in the filamentous green alga, *Sirogonium melanosporum* (RANDHAWA) TRANSEAU. (M.S. Thesis, Univ. Arizona) (*fide* WELLS 1969)

WELLS, C. 1969. Cytology of the green algae *Sirogonium*. (Ph. D. Thesis, Univ. Arizona)

WITTMANN, W. 1965. Aceto-Iron-Haematoxyline-Chloral Hydrate for chromosome staining. Stain Technol. **40**: 161-163.

原田　彰：シロゴニウム属（緑藻類）の細胞学的研究　1. *S. sticticum* と *S. melanosporum* の体細胞分裂

奈良県下の水田から採集したシロゴニウム属の2種を材料として，糸状体細胞にみられる体細胞分裂の経過を調べた。その中で，静止核にある仁の内部構造，仁形成染色体と仁物質の形成などが観察された。また，中期の染色体は濃染する仁物質塊に包まれて赤道面に配列し，後期に入ると染色体を包んでいる仁物質塊が2個の円板状塊に分離するのに伴って平行に分離して染色分体になること，前期に細胞壁の内側に沿って環状に配列した顆粒体が出現し，それが後期の終り頃までに次第に求心的に成長して細胞板に発達する。この間に細胞質や葉緑体もこの細胞板によって分けられることなどが観察された。前期末や中期の分裂像から，*S. sticticum* は52個 *S. melanosporum* は8個の染色体をもち，両種とも，それらの中の2個は仁形成染色体であることがわかった。（581 大阪府八尾市千塚 102 大阪府立清友高等学校）