Jap. J. Phycol. (Sôrui) 32: 1-9. March 20, 1984

Mitosis in *Spirogyra* (Chlorophyceae)

Akira HARADA* and Takaaki YAMAGISHI**

*Seiyu Senior High School, Chizuka, Yao-City, Osaka, 581 Japan.
**Biological Laboratory, College of Agriculture and Veterinary Medicine, Nihon University, Kameino, Fujisawa, Kanagawa, 252 Japan.

HARADA, A. and YAMAGISHI, T. 1984. Mitosis in *Spirogyra* (Chlorophyceae) Jap. J. Phycol. 32: 1-9.

Mitosis in three species of *Spirogyra*, *S. crassa*, *S. crassa* X and *S. hunanensis*, was observed. The chromosome numbers of *S. crassa* and *S. crassa* X were 12 and 6 respectively, and *S. hunanensis* had four chromosomes. The chromocenters and nucleoli, the origin and formation of the chromosomes, the nucleolar substance, the nucleolar organizing chromosomes, the parallel separation of chromatids, the stickiness of chromosomes and cytokinesis were observed during the mitotic cycle.

Key Index Words: *Chlorophyceae*; *chromocenter*; *chromosome*; *cytokinesis*; *mitosis*; *N.O. chromosome*; *nucleolar substance*; *parallel separation of chromosome*; *sticky chromosome*; Spirogyra.

WISSELINGH (1900) observed mitotic division in *Spirogyra* and mentioned that chromosomes are derived partly from the nucleolus and partly from the karyoplasm. Since then, special attention has been paid to the origin of the chromosomes and differing opinions regarding this point have been presented by various workers (DORAISWAMI 1946).

GEITLER (1930) and DORAISWAMI (1946) investigated the process of mitotic division and suggested that the chromosomes are derived solely from the karyoplasm and noted the existence of the nucleolar substance. WISSELINGH (1900), GEITLER (1930) and GODWARD (1950) mentioned the existence of nucleolar organizing chromosomes. GODWARD (1954) also observed bipartition of the chromosomes using the iron alum acetocarmine method (GODWARD 1948). GODWARD (1956), GODWARD and NEWNHAM (1965) published cytotaxonomical studies of *Spirogyra*. FOWKE and PICKETT-HEAPS (1969, 1969a) reported the ultrastructural observations of cell division.

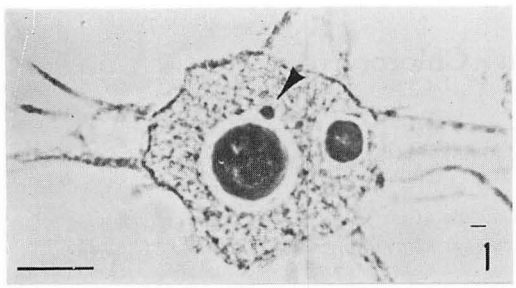
In Japan, cytological investigation of *Spirogyra* was conducted by SUEMATSU (1936), OURA (1935), UEDA (1956) and TATSUNO and IIYAMA (1971). TATSUNO and IIYAMA published a report on the chromosome numbers of some species, including three species having only two chromosomes.

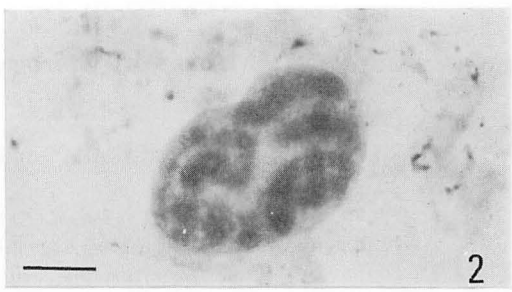
However, many questions on the mitotic process in *Spirogyra* remain, and in the present paper, mitosis in three species, *Spirogyra crassa* Kütz., *S. crassa* X and *S. hunanensis* Jao, is described.

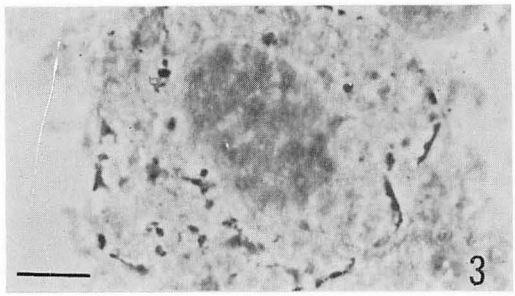
Materials and Methods

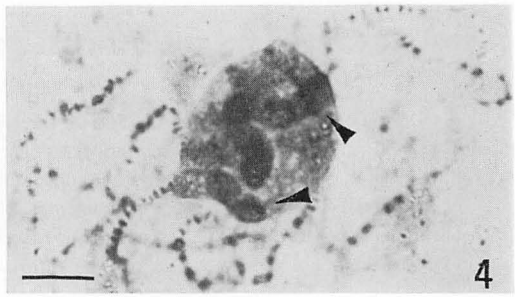
Fertile filaments of *S. crassa* were collected from rice fields of Shigisan, Nara Prefecture, in November, 1968, and from January to November, 1969. *S. crassa* X was collected from Yata, Nara Prefecture, from November 1971 till May 1972. The taxonomic characteristics of this material are similar to that of *S. crassa*, except for the smaller cell dimentions, and this material was provisionally designated as *S. crassa* X. *S. hunanensis* was collected from a pond in Fujii, Nara Prefecture, in August, 1971.

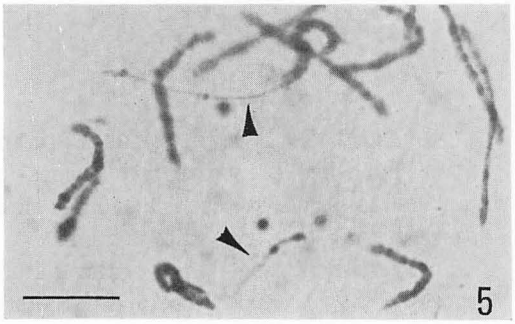
The materials were fixed with acetic-alcohol (1 : 3) mixture. By mixing the two fluids

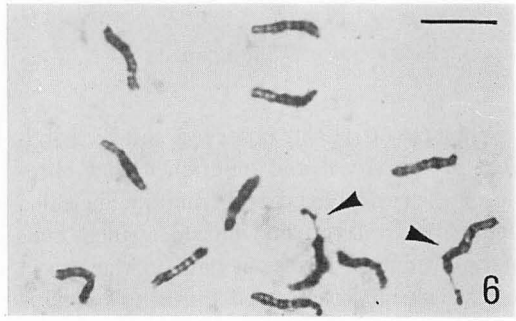

1

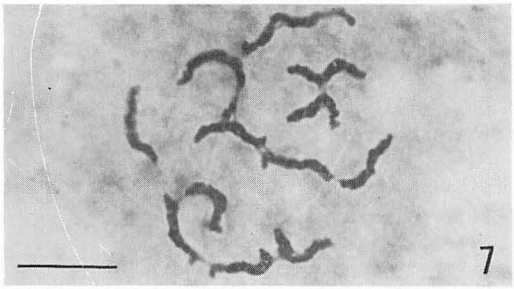

2

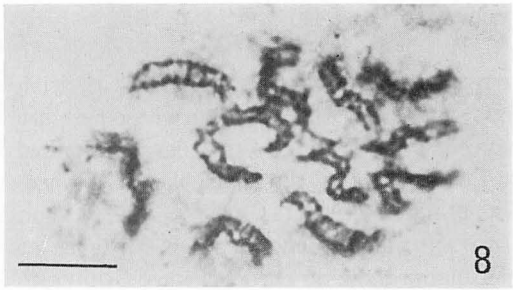

3

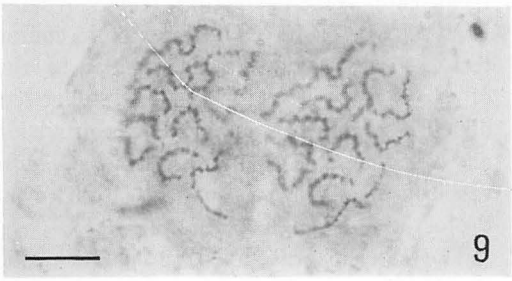

4

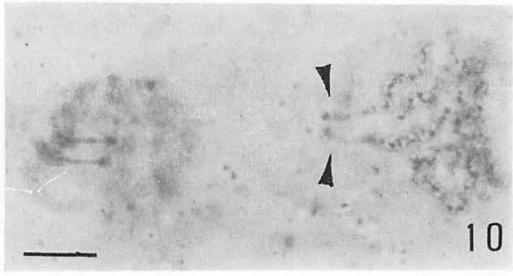

5


6


7


8


9


10

immediately before use, this fixative gave good results during staining. The fixed materials were stored in the same solution, and kept in a freezer box. Fixation was made at intervals of an hour throughout a twenty-four hour period, and the most abundant mitotic material was generally obtained from sunset to midnight.

For observation, the modified WITTMANN's (1965) method was employed, and serial treatments were carried on the slide as follows:

1) The fixative was absorbed from the material by filter paper, and the remaining alcohol was evaporated by heating.

2) The material was pretreated with 1 N HCl, and one drop of 1-4 iron alum 45% acetic acid.

3) Aceto-iron-haematoxylin-chloral hydrate was added to the material. This solution was made by throughly mixing 2.0 gm of haematoxylin, 0.5 gm of iron alum and 20 gm of chloral hydrate in 50 ml of 45% acetic acid. This mixture used immediately after melting, and gave clear chromatic results. Furthermore, the staining ability was retained for a long time.

4) Then, the material was heated, and squashed with a cover glass. By heating, the chromosomes were sharply stained and the cytoplasm became transparent.

Observations.

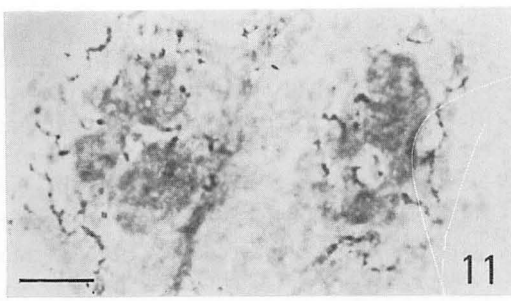
1) ***Spirogyra crassa*** **KÜTZING** (n=12)

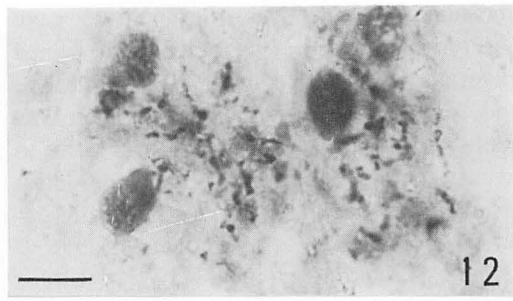
Interphase: The nucleus was lenticular, and was surrounded by cytoplasm, which spread out in all directions as cytoplasmic strands. The nucleus had one, sometimes two, large nucleoli which were seen as bright bodies in living cells (Fig. 1). In these nucleoli, two densely stained thread-like structures, called organizer tracks (GODWARD 1950) were clearly observed (Figs. 2, 3 and 4). When two nucleoli existed in a nucleus, each nuclolus had a single organizer track. Many irregular thread- or rod-shaped chromocenters were seen in the karyoplasm (Fig. 3). Near the nucleolus, there was one or two small spherical bodies which were as densely stained as the nucleolus, and had been termed Nebenkörper by GEITLER (1930) (Fig. 1). However, no activity of these bodies at any time during the division was observed.

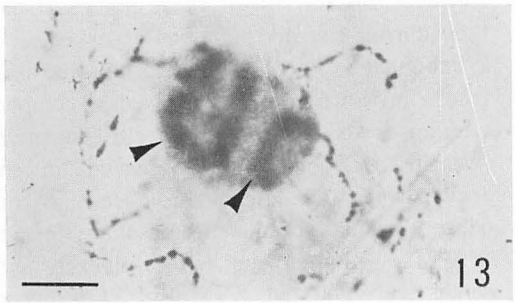
Prophase: At this stage, the organizer tracks in the nucleolus appeared as compact winding cords (Fig. 4). Many chromocenters linearly arranged were observed in the karyoplasm. Then, the chromocenters condensed gradually and chromosomes formed (Fig. 4). At mid-prophase, twelve chromosomes were counted. Two of these were nucleolar organizing chromosomes (N.O. chromosome, GODWARD 1950) that connected with each of nucleolar-organizing region (N.O. region, GODWARD 1950) in the nucleolus and a satellite ahead of the nucleolus (Fig. 5). At late prophase, the nucleolus changed into a deeply stained substance, called the nucleolar substance (GEITLER 1935), and the two N.O. chromosomes and the ten other chromosomes which originated from the chromocenters located in karyoplasm were compltely enveloped in the nucleolar substance.

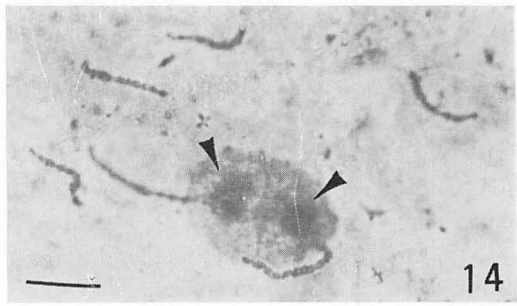
Metaphase: The nucleolar substance was lenticular in shape and located in the equatorial plate of the nucleus as in *S. crassa* X (Fig. 21). Twelve chromosomes in the nucleolar substance were clearly observed at this stage. Two of these chromosomes were the N.O. chromosomes and had a satellite at one end, about 12 μm in length (Fig. 6). The other ten chromosomes were almost the same in length, about 10 μm. By pretreatment with 1 N HCl, a banding pattern clearly

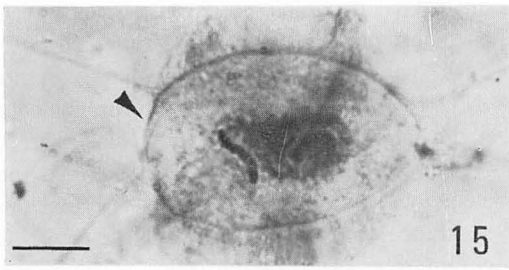
Figs. 1-10. *Spirogyra crassa* (n=12) 1-3. Interphase nucleus: 1. Two nucleoli and Nebenkörper (arrow); 2. Organizer tracks in a nucleolus; 3. Chromocenters in karyoplasm; 4-5. Prophase: 4. Two organizer tracks (arrows) in a nucleolus and bead-shaped chromosomes; 5. N.O. regions (arrows) pretreated with HCl; 6-7. Metaphase: 6. Two N.O. chromosomes with a satellite (arrow); 7. Sticky chromosomes; 8-10. Anaphase: 8. Beginning of parallel separation and ladder shaped chromosomes; 9. Symmetric arrangement of chromatids in each daughter nucleolar substance; 10. Two horn-shaped N.O. chromosomes (arrows). (Scale bars=10 μm).

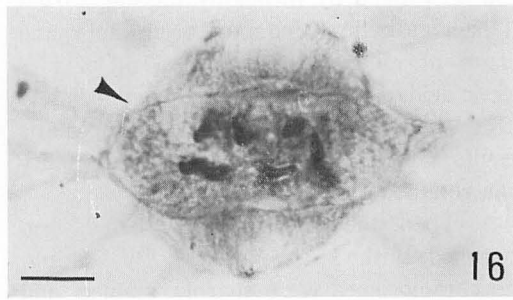

11

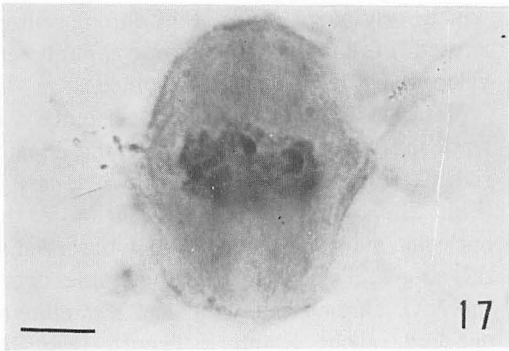

12

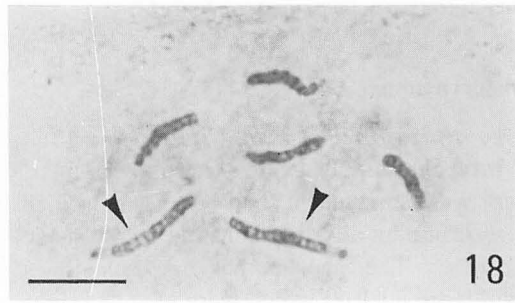

13

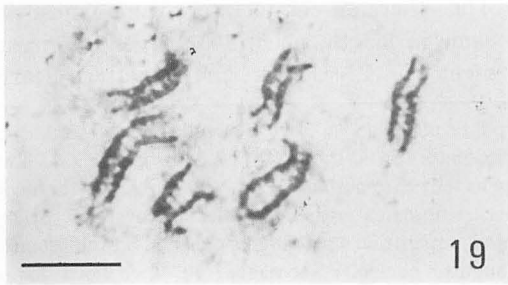

14

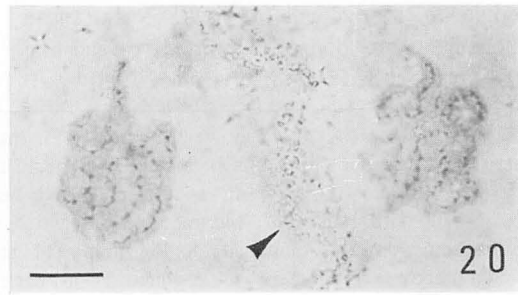

15


16


17


18


19


20

appeared on the chromosomes of this stage, and the number of bands on each chromosome was nearly constant (Fig. 6). Some of the chromosomes in metaphase showed some stickiness, and were connected with one another by a sticky substance at the end or side (Fig. 7).

Anaphase: In early anaphase, the lenticular nucleolar substance began to separate parallel to the equatorial plate of the nucleus. Twelve chromatids embedded in this substance were thus moved to the opposite poles. As a result of the separation of the nucleolar substance into two round shaped disks, all of the chromosomes began to separate in parallel. Then the densely stained portions of each chromosome began to strech and all the chromosomes assumed a ladder shape (Fig. 8). As the satellite of the N.O. chromosomes separated later than the other parts, it was often seen as two trails or horn-like processes between the two nucleolar substance (Figs. 9 and 10). The twelve chromatids in each nucleolar substance disk was assumed to have a symmetric position (Fig. 9).

Telophase: The chromatids that reached each pole became fragments or rod-shaped chromocenters. The daughter nucleolar substances changed from a round to a spongy form and then to irregular shaped masses. At the end of telophase, one or two nucleoli occured in each daughter nucleus (Figs. 11 and 12).

Cytokinesis: At late prophase, a ring consisting of minute granules appeared inside the cell wall at the middle of the cell. The ring was identified as two circles of granules which were unstainable and were not affected by heating (Figs. 20, 21 and 26). After nuclear division, the granular ring developed centripetally into a cell plate, and chloroplasts and cytoplasm were divided into two cells by the plate.

2) ***Spirogyra crassa* X** (n=6)

At the begining of prophase, the nucleus and the nucleolus began to swell up, and the organizer track in the nucleolus gradually became loose and short (Fig. 13). At midprophase, the chromocenters connected to each other and formed six bead-shaped chromosomes. Two of these chromosomes were the N.O. chromosomes (Fig. 14). The nucleolar substance was formed as in *S. crassa* (Fig. 21). The spindle was organized at four corners of the nucleus (Figs. 15 and 16). The barrel-shaped spindle developed gradually and was finally completed at late prophase (Fig. 17).

In metaphase, the two N.O. chromosomes were about 12 μm in length and the other four were the same shape and about 8 μm in length (Fig. 18). On the chromosomes, banding pattern was clearly observed.

At early anaphase, in strongly squashed preparations, by bipartition of the chromosomes, the densely stained bands stretched, and two parallel chromatids showed a ladder shape (Fig. 19). At a later stage, these chromatids separated from each other, but the satellite of the N.O. chromosomes separated later, as observed in *S. crassa* (Fig. 20). Finally, a new cross wall was formed in the same way as that in *S. crassa*.

3) ***Spirogyra hunanensis* Jao** (n=4)

At the begining of prophase, about 20 dot-shaped chromocenters were observed in the karyoplasm (Fig. 22). The chromocenters gradually joined with each other and formed four bead-like chromosomes. Two of these were connected at their terminals in the nucleoli (Fig. 23). In metaphase, four chromosomes embedded in the nucleolar substance lined up on the equatorial plate. All four chromosomes were rod-shaped and 5-6 μm in

Figs. 11-12. *Spirogyra crassa* (n=12) 11-12. Telophase: 11. Fragmented chromosomes; 12. Newly formed nucleoli.

Figs. 13-20. *Spirogyra crassa* X (n=6) 13-16. Prophase: 13-14. Two organizer tracks (arrows) and six bead-shaped chromosomes; 15-16. Spindle formation and nuclear membrane (arrow); 17-18. Metaphase: 17. Spindle and chromosomes enveloped with nucleolar substance; 18. Two N.O. chromosomes (arrows); 19-20. Anaphase: 19. Parallel separation of chromosomes showing ladder-shape; 20. Two daughter nuclei and dictyosomes (arrow). (Scale bars=10 μm).

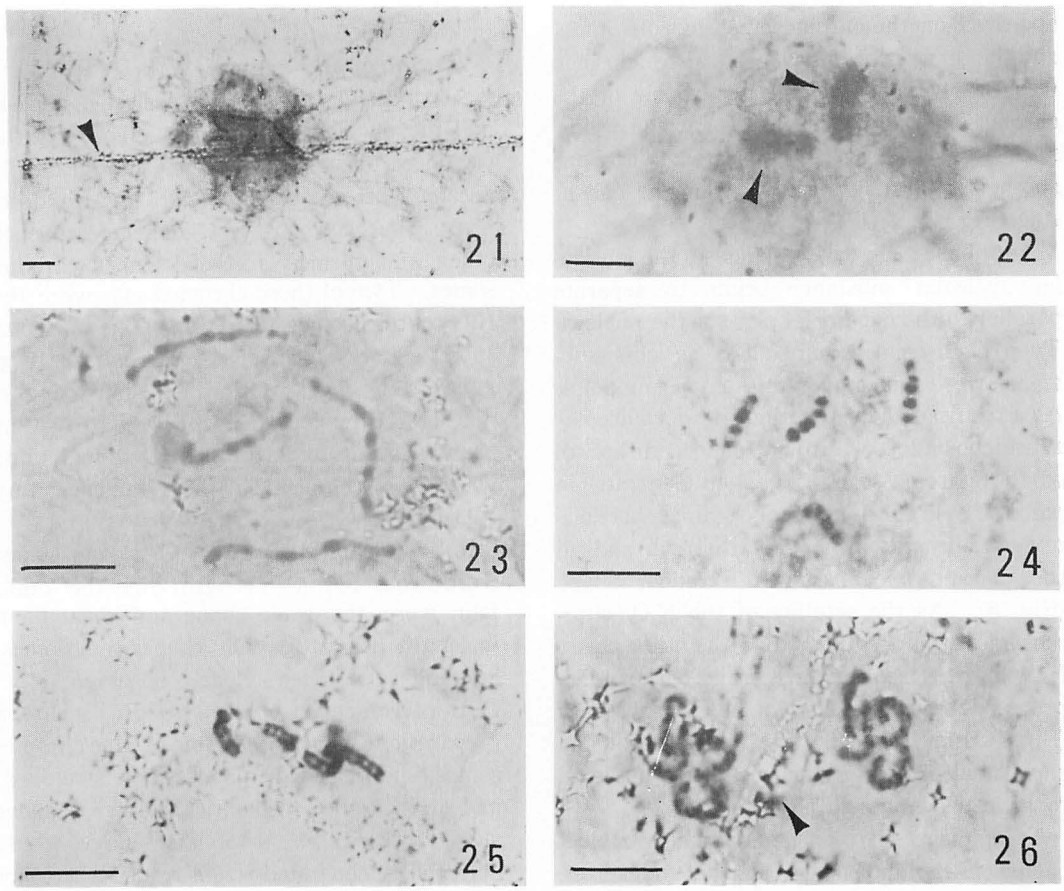


Fig. 21. *Spirogyra crassa* X. 21. Metaphase. Six chromosomes embedded in nucleolar substance and dictyosomes (arrow).

Figs. 22-26. *Spirogyra hunanensis* (n=4) 22-23. Prophase: 22. Two organizer tracks (arrow) and chromocenters; 23. Four bead-shaped chromosomes; 24-25. Metaphase: 24. Four chromosomes having bands; 25. Parallel separation of chromosomes; 26. Anaphase: 26. Symmetric arrangement of chromatids and dictyosomes (arrow). (Scale bars=10 μm).

length. The two N.O. chromosomes had a satellite at their tips. Each chromosome had clear bands (Figs. 24 and 25). At anaphase, the nucleolar substance enclosing four chromosomes split transversally into two. On the chromatids of this stage, the same banding pattern characterized metaphase chromosomes was observed (Fig. 26).

Discussion

Through the present study on three species, *S. crassa*, *S. crassa* X and *S. hunanensis*, a general mitotic cycle of *Spirogyra* can be shown in diagramatic form (Fig. 27).

Densely stained chromocenters and nucleoli in interphase nuclei are prominent features of *Spirogyra*. GODWARD (1956) mentioned that the nature of the chromocenter was regarded as a feature of cytotaxonomical significance in *Spirogyra*. However, various forms of chromocenters, thread- or dot-shaped, were observed in each of the three species investigated, and of the other species used for coromosome observation (HARADA and YAMAGISHI, in preparation). Thus, a clear specificity of the chromocenters can not be recognized. GODWARD (1950, 1956) suggested that the number of nucleoli and the organizer tracks in each nucleus was related with the

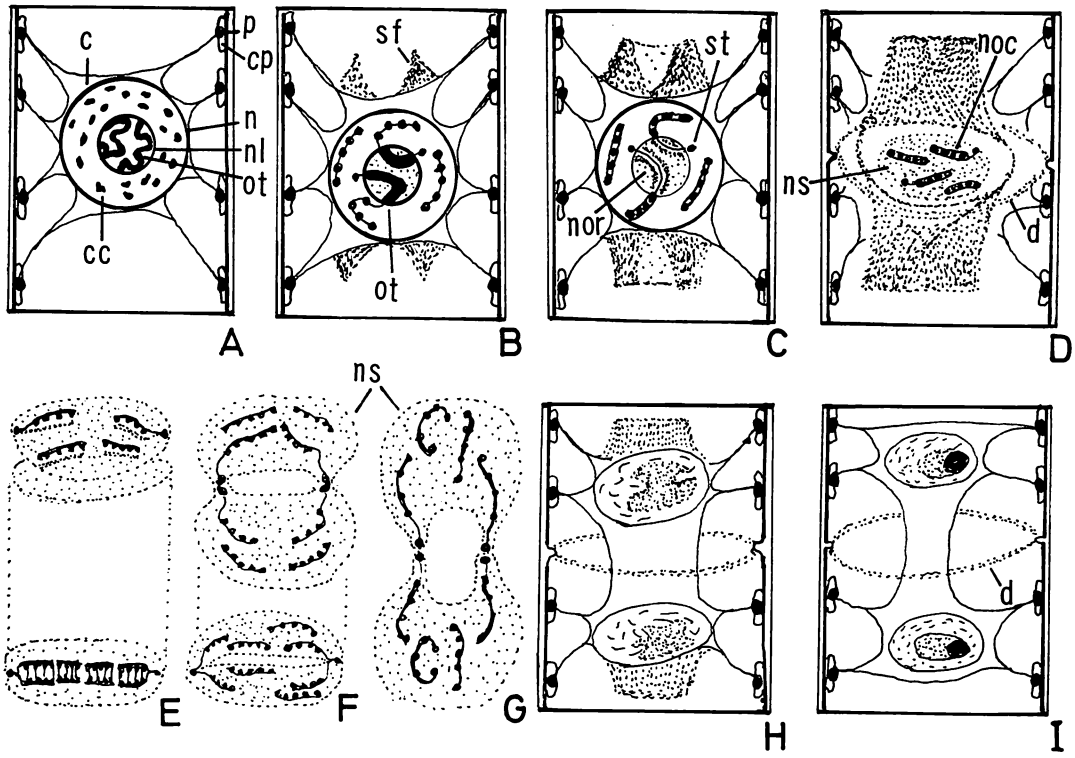


Fig. 27. Diagram showing mitosis in *Spirogyra*. A. Interphase; B-C. Prophase; D. Metaphase; E-G. Anaphase. bipartition of chromosomes enclosed with the nucleolar substance; H-I. Telophase. (c: cytoplasm, p: pyrenoid, cp: chloroplast, n: nucleus, nl: nucleolus, cc: chromocenter, ot: organizer track, sf: spindle fiber, ns: nucleolar substance, st: satellite, nor: nucleolar organizing region, noc: nucleolar organizing chromosome, d: dictyosome).

number of the N.O. chromosomes. Through the observation of the three species, it is confirmed that the number of the nucleoli coincides with the number of the organizer tracks.

GEITLER (1930, 1935) and DORAISWAMI (1946) reported that all of the chromosomes originated from the karyoplasm in *Spirogyra*. However, it was observed that all of the chromosomes except the N.O. chromosomes were formed from the iinearly arranged bead-like chromocenters in the karyoplasm, as mentioned by WISSELINGH (1900) and GODWARD (1954). The banding pattern (Figs. 6, 18 and 24) in the chromosomes at metaphase became clear in pretreated preparation by 1 N HCl. The number of the bands in each chromosome in metaphase is constant in three species investigated. Moreover, it was confirmed that the number of bands on each of the four chromosomes is equivalent to the numbers of chromocenters appeared in early prophase in *S. hunanensis* (Figs. 23 and 24).

GEITLER (1935) first described the nucleolar substance in *Spirogyra*. DORAISWAMI (1946) also suggested that the nucleolus changed into a substance, called the granullar homogenous substance, in midprophase. In *Sirogonium* (Zygnemataceae), a similar substance was also reported by WELLS (1969) and HARADA (1981). GODWARD (1953) traced the relationship of the nucleolus and the nucleolar substance in *S. crassa*. Figures 14-16 show that the nucleolus completely loses its sharp outline, then changes into the nucleolar substance. During late prophase, all the chromosomes are completely embedded inside the

nucleolar substance. This substance scatters and changes its original form in the squashed preparation of mid, and late prophase, and it remains only around the N.O. regions (Fig. 5), as GODWARD mentioned.

WISSELINGH (1900) and GEITLER (1930) observed the N.O. chromosomes in some species of *Spirogyra*. GODWARD (1950, 1953) illustrated a gradual emergence of the organizer tracks and development of the N.O. chromosomes in *S. crassa*. Throughout this investigation from early prophase to metaphase in the three species, it is demonstrated that the process of N.O. chromosome formation agrees with GODWARD's observations. Moreover, the existence of the N.O. regions in the nucleolus was ascertained by the pretreatment with 1 N HCl, which decreased the staining ability of the nucleolus (Fig. 5). GEITLER (1930) and GODWARD (1953) reported the existence of a satellite of the N.O. chromosome which projected from the nucleolar substance. In this study, the satellite at metaphase was observed as a horn-shaped projection. Moreover, it was confirmed that the satellite does not slough off and is completely enclosed in the nucleolar substance (Figs. 6 and 18).

GEITLER (1930) first mentioned the parallel separation of chromosomes in *S. crassa*, and he attributed this to the fluidity of the spindle and the rigidity of the chromosomes. GODWARD (1954) considered that the separation of the chromatids was due to the fact that the chromosomes had polycentric or diffuse centromeres instead of a localized centromere. The barrel-shaped spindle and the parallel separation of the chromosomes are observed in the three species used in this study, as has been reported by the previous workers. When the chromatids separate in parallel and move to opposite poles, the chromatids hold the same position and the same shape in each daughter nucleolar substance in polar view (Figs. 9, 20 and 26). This phenomenon may mean that the chromosome itself does not divide into two chromatids, but that the parallel separation of the chromosomes may be due to the separation of the nucleolar substance itself.

Sometimes chromosomes in metaphase are connected to one another with sticky threads at the terminals or the sides. At anaphase, each chromosome shows a ladder shaped chromatic figure at the beginning of separation (Figs. 8 and 19). GEITLER (1930) first observed the stickiness between the chromosomes in *S. crassa*. Moreover, GODWARD (1950, 1953, 1954) mentioned that the sticky matrix was the nucleolar substance itself. By the staining method used in this study, the chromosomes are distinguishable from the nucleolar substance or nucleolus by their staining ability. After pretreatment with 1 N HCl, the staining ability of the chromosomes decreases in contrast to the nucleolar substance, which is never stained. By this method, chromatic strands or sticky threads between the chromatids and the chromosomes are stained in the same degree as the chromosomes. Judging from the staining ability, it is considered that the sticky threads and sticky substance are composed the same substance as the chromosomes.

WISSELINGH (1902) observed that the spindle did not develop to extend to the karyoplasm through the nuclear membrane in *S. setiformis*. The spindle formation was also investigated in detail by GEITLER (1930, 1935) and DORAISWAMI (1946). Spindle formation was observed in *S. crassa* X (Figs. 15, 16 and 17) in material stained but not squashed, because the spindle loses its structure in squashed preparations. The process of spindle formation is similar to that described by GEITLER (1930) for *S. crassa*.

Cells which entered into division always had a circle of minute granules (Figs. 20, 21 and 26), called dictyosomes (WELLS 1969), at the area where the new wall is formed. These organelles were also observed in *Sirogonium* by HARADA (1981), and in *Zygnema* by HARADA and YAMAGISHI (1980).

References

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

FOWKE, L. C. and PICKETT-HEAPS, J. D. 1969. Cell division in *Spirogyra* I. Mitosis. J. Phycol. **5**: 240-259.

FOWKE, L. C. and PICKETT-HEAPS, J. D. 1969a. Cell division in *Spirogyra* II. Cytokinesis. J. Phycol. **5**: 273-281.

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

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

GODWARD, M. B. E. 1948. The iron alum aceto-carmin method for algae. Nature **161**: 203.

GODWARD, M. B. E. 1950. On the nucleolus and nucleolar-organizing 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. 1956. Cytotaxonomy of *Spirogyra* I. *S. submargaritata*, *S. subechinata* and *S. britannica*. J. Linn. Soc. Bot. London **55**: 532-546.

GODWARD, M. B. E. and NEWNAHAM, R. E. 1965. Cytotaxonomy of *Spirogyra* II. *S. neglecta* (Hass.) Kütz., *S. punctulata* Jao, *S. majuscula* (Kütz.) Czurda emend., *S. ellipsospora* Transeau, and *S. porticallis* (Müll.) Cleve. J. Linn. Soc. Bot. London **59**: 99-110.

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

HARADA, A. and YAMAGISHI, T. 1980. Cytological studies on *Zygnema* (Chlorophyceae) 1. Mitosis. Jap. J. Phycol. **28**: 233-240 (in Japanese).

OURA, G. 1935. On the mitosis of *Spirogyra* with special reference to the nucleolar organization and nucleolar organizing chromosome. Cytologia **18**: 297-304.

SUEMATSU, S. 1936. Karyological study of *Spirogyra* by means of nuclear-reaction. Sci. Rep. Tokyo Bunrika Daigaku. **3**- 35-40.

TATSUNO, S. and IIYAMA, I. 1971. Cytological studies on *Spirogyra*. I. Cytologia **36**: 86-92.

UEDA, K. 1956. Structure of plant cells with special reference to lower plants. I. Mitosis in *Spirogyra setiformis*. Cytologia **21**: 384-390.

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

WISSELINGH, C. 1900. Über Kernteilung bei *Spirogyra*. Flora **87**: 355-377.

WISSELINGH, C. 1902. Untersuchung über *Spirogyra*. Bot. Zeitg. **60**: 115-138.

WITTMANN, W. 1965. Aceto-iron-haematoxylin-chloral hydrate for chromosome staining. Stain Technol. **40**: 161-163.

原田　彰*・山岸高旺: アオミドロ属（緑藻類）の体細胞分裂**

奈良県下の水田，池から採集したアオミドロ属の3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，静止核の仁内構造，前期での仁内構造の変化に伴う仁形成染色体と仁物質の形成，核質内での染色体の形成過程が観察された。また前期の仁形成染色体が塩酸処理で観察された。中期の染色体が平行に分離し，染色体相互の形態を維持したまま後期の染色分体を生じ，遅れて分離する付随体の部分が突出した形を示すが，これらは仁物質に包まれたままであることが観察された。*S. crassa* は12，*S. crassa* X は6，*S. hunanensis* は4個の染色体数をもち，3種ともそのうち2個は仁形成染色体である。(*581 八尾市千塚 102大阪府立清友高校，**252 藤沢市亀井野 日本大学農獣医学部)