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Meiosis in *Spirogyra* (Chlorophyceae)

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Meiosis in four species of *Spirogyra*, *S. crassa*, *S. crassa* X, *S. hunanensis* and *S. lacustris*, was investigated. The time of occurrence of meiosis, the pairing of homologous chromosomes in young zygospores, the bivalents which formed in each conjugated nucleus, nucleolar organizing chromosomes, sticky chromosomes, parallel separation of chromosomes, and abortion of three nuclei were observed.

Key Index Words: *meiosis*; *pairing of homologous chromosomes*; *bivalent formation*; *chromosome*; *N.O. chromosome*; *sticky chromosome*; Spirogyra; *Chlorophyceae*.

Meiosis in *Spirogyra* has been reported previously by only a few workers, KARSTEN (1908), TRÖNDLE (1911) and GODWARD (1961), but these reports have dealt with only part of meiotic division. In the present study, the entire meiotic cycle in four species, *Spirogyra crassa* Kützing, *S. crassa* X, *S. hunanensis* Jao and *S. lacustris* Czurda, is presented.

Materials and Methods

S. crassa, *S. crassa* X and *S. hunanensis* were collected from various rice fields or ponds in Nara Prefecture. The places and dates of collection were the same to the materials used for our previous observation on mitosis (HARADA and YAMAGISHI 1984). Fertile filaments of *S. lacustris* were collected from a rice field in Ikebe, Nara Prefecture, in August, 1975.

Materials were fixed with acetic-alcohol (1 : 3) mixture. For observation of the meiotic cycle, the zygospores were isolated from fixed filaments. Then, the contents of the zygospore were squashed on slides. Then, the zygospores were pretreated and stained using the same methods as reported for observations of the mitotic process (HARADA and YAMAGISHI 1984).

Observations

1) *Spirogyra crassa* Kützing (n=12)

Zygospore formation and meiosis: A zygospore was formed in a female cell. The zygospore wall immediately after conjugation was thin and soft. Inside the young zygospore, the remainders of the choroplast were seen as light green coloured contents. Shortly after formation of zygospore, the green colour of the zygospore faded away, and oil drops accumulated. The zygospore wall became gradually hard and thick, and finally developed characteristic ornamentation on the surface. Meiosis began early in the green zygospore and was already finished when the zygospore wall fully ripened.

The fusion of two gametangial nuclei: In the young zygospore, the two nuclei which originated from the gametes stayed in contact with each other without fusion, and the

nuclear membrane remained intact. It was very difficult to stain the nucleus at this stage, and moreover, the nucleolus was not as distinct as the interphase nucleolus in the mitotic cycle (Fig. 1). In the karyoplasm, a densely stained amorphous substance and short thread-like bodies were observed (Fig. 1).

The first division. Prophase: In early prophase, the nucleoli in each of the contacting two nuclei enlarged gradually and stained homogenously. Many stained thread-like bodies were observed in the karyoplasm. This stage seemed to be leptotene. Meanwhile, the many thread-like bodies which consist of granular chromatins began to form a line and constructed chromonemata. This stage was considered to be pachytene (Figs. 2 and 3). These granular chromatins on the thread-like body condensed gradually and formed twelve chromosomes in which two were the nucleolar organizing chromosomes (N.O. chromosome, GODWARD 1950) (Fig. 4). In each nucleus, homologous chromosomes, including two N.O. chromosomes paired in parallel and formed six bivalents (Fig. 5). In this stage, which seemed to be diplotene, densely stained chromatined arranging on each chromosome were cleary observed (Figs. 4 and 5). The bivalents at this stage were seen as two groups and gathered around each nucleolus of the two contacted nuclei (Fig. 6). The N.O. chromosomes attached to densely stained regions in the nucleolus at one end (Fig. 5).

The thread-like structure in the nucleolus during mitotic division, illustrated by GODWARD (1950), was not observed, and only a stained portion was recognized in the nucleous.

At late diplotene, each chromosome contracted. The terminal of the bivalents appeared to separate into four chromatids, and almost all of the bivalents seemed to consist of two parallel homologous chromosomes (Fig. 7). Chiasma was not observed. At this stage, the membrane of the two conjugated nuclei, which were still in contact with each other, disappeared and the two nuclei fused into one. At diakinesis the bivalents began to move to the equatorial plate of the cell, and the nucleoli attached to the N.O. chromosomes became invisible (Fig. 8).

Metaphase: Each of the twelve bivalents on the equatorial plate consisted of four chromatids, was either 0 shaped or quadrangular which were connected at both ends and separated in the middle (Figs. 8 and 9). The nucleolar substance that enveloped all the chromosomes at metaphase in mitosis could not be recognized.

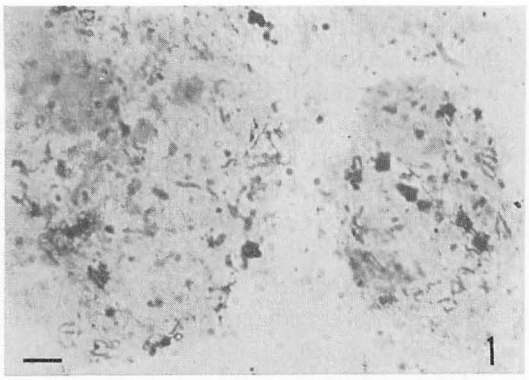
After a short time, the spindle was formed from both poles and the whole nucleus was enveloped as observed in *S. hunanensis* (Fig. 23). Spindle fibers that were observed at prophase in mitosis could not be observed in meiosis. When all the bivalents were arranged at the equatorial plate, the completed spindle had a barrel shape (Fig. 11).

The sticky thread-like stainable substance between the chromosomes, seen in mitotic metaphase, was also observed on the bivalents (Fig. 10).

Anaphase: The bivalents divided into two, and these half-bivalents moved in parallel towards the poles, being pulled by the spindle fibers. The half-bivalents did not change their form or position at metaphase (Fig. 13), as in mitotic anaphase. The tooth or woolly shaped sticky substance appeared between the half-bivalents that began to divide (Fig. 12).

The second division. Interphase, prophase and metaphase: The half-bivalents that reached to each pole immediately began the second division. Therefore, the interphase between the first and second division which occurs in higher plants was not observed. When the first division was complete, two nuclei were distinguishable, but the membrane around them could not be observed. In these two nuclei, the second division occured at the same time. The half-bivalents were formed with two parallel or 0 shaped chormatids which were connected at the ends. In some cases, a nucleolus-like body at the end of one, or two half-bivalents was observed (Fig. 13).

Anaphase: At second anaphase, these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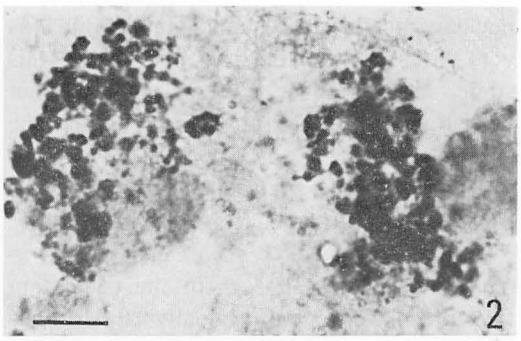
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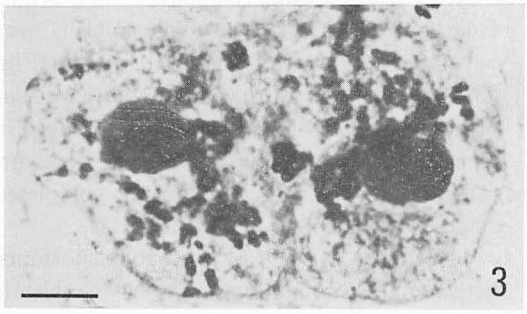
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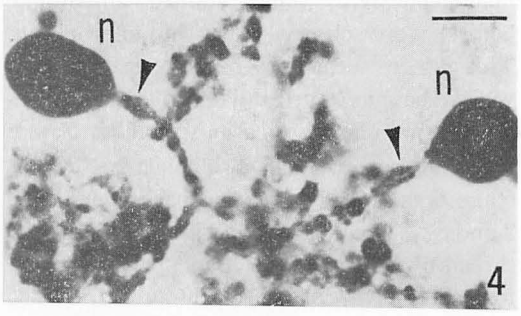
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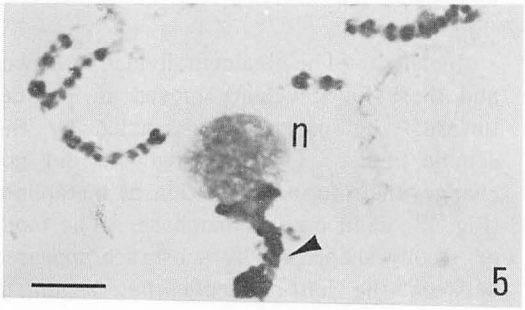
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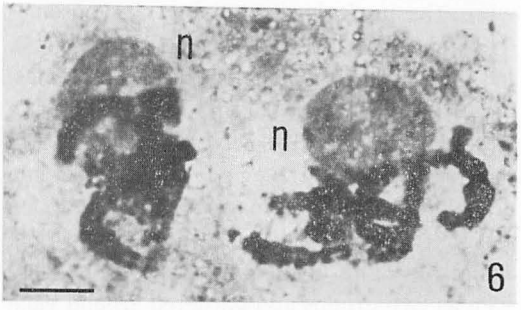
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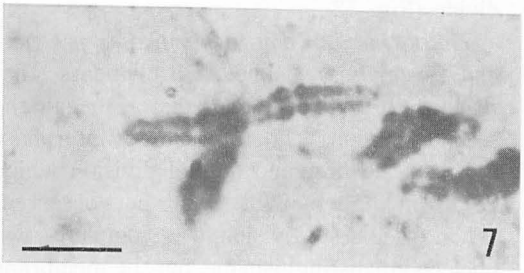
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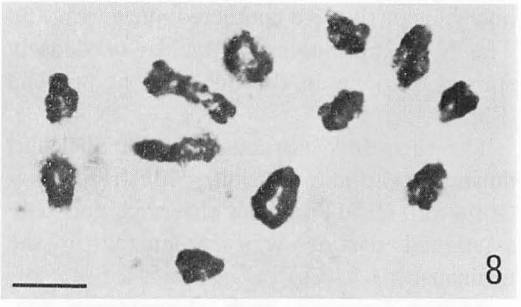
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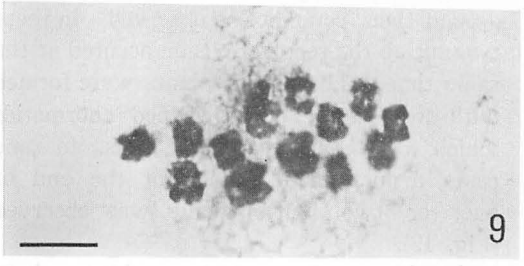
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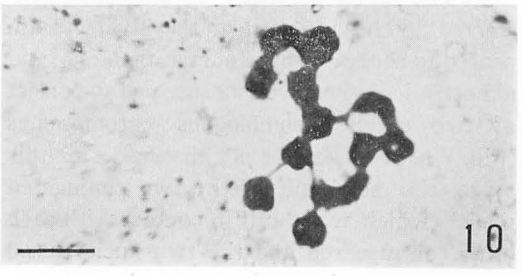
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chromatids divided in parallel in the same way as at first metaphase. As a result of the second division, four nuclei were formed. The twelve chromatids in each of four nuclei remained in the same shape and arrangement in polar view (Fig. 14).

Telophase: The chromatids gradually became fuzzy and broke down, and then changed into many short irregular fragments. At the same time, the nuclear membrane appeared. Two or more globular or irregular shaped nucleoli were reformed in each nucleus, but finally they became one or two in number. On the other hand, individual chromatids changed to rod-shaped or thread-like chromatins scattered in the karyoplasm (Fig. 15).

Degeneration of three nuclei: Only one nucleus of the four nuclei which formed in telophase had one or sometimes two large nucleoli and many thread-like chromatins. The other three nuclei changed to densely stained irregular bodies and finally disappeared (Fig. 16).

The fully matured zygospores had a hard and coloured wall, and only one large nucleus with many short zigzag thread-like chromatins, and one or two large nucleoli. Moreover, one or two small globular bodies, called Nebenkörper (GEITLER 1930), were observed together with the nucleolus (Fig. 17).

In the dormant zygospore, the nucleus had no thread-like chromatins, and showed a rather homogenous condition. The nucleoli also stained homogenously and contained no structure, as had appeared in mitotic interphase.

Germination of the zygospore: At germination, the zygospore wall split and only one new cell appeared. In it one nucleus and one clearly stained nucleolus were found. Then the cell began to divide. The basal cell elongated, but did not divide further. Another top cell divided repeatedly and the filaments elongated, but there was no synchronous division.

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

This species showed a process similar to that of *S. crassa* mentioned above, except having smaller and fewer chromosomes.

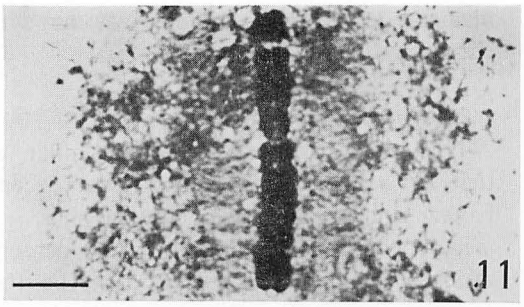
In prophase of the first division, a densely stained region that was assumed to be the organizer track (Fig. 18), as in mitosis, appeared in the nucleolus, but the relationship of this region to the N.O. chromosome was not observed clearly. At diakinesis, each bivalent was contracted and three bivalents appeared in each nucleus. Six bivalents arranged at the equatorial plate were observed in metaphase (Fig. 19). At anaphase, the six half-bivalents separated towards the both poles. In some cases, one of the half-bivalents had a nucleolus-like body at its end. The process of the abortion of three nuclei was similar to that in *S. crassa* (Fig. 20).

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

This species had four chromosomes and size of each was smaller than that of the two other species observed. Therefore, it was diffcult to clearly observe the relationship of the N.O. chromosomes to the nucleolus and the process of bivalent formation. The outline of meiotic division of this species was similar to that of the two other species studied.

At prophase of the first division, each of the two contacted nuclei in the young zygospore had a prominent nucleolus. In the nucleolus, there was a large globule having an inner reticular structure (Fig. 21). At diplotene, the nuclei became indistinct and four bivalents having a banded pattern were observed (Fig. 22). At metaphase and anaphase, the four bivalents showed similar behaviour to the two former species, except for their smaller size (Fig. 23). At anaphase,

Figs. 1-10. *Spirogyra crassa* (n=12) 1-10: First division. 1. Two contacted nuclei in a young zygospore; 2-3. Pachytene: Granular chromatins and nucleoli in each of contacted nuclei; 4-7. Diplotene: 4. bivalents, N.O. chromosomes (arrows) and nucleoli (n) in two nuclei; 5. Five bivelents and a N.O. chromosome (arrow) in one nucleus; 6. Two groups of bivalents around each nucleolus (n); 7. Parallel structure of bivalents; 8. Diakinesis; 9-10. Metaphase: 10. Sticky chromosomes. (Scale bars=10 μm).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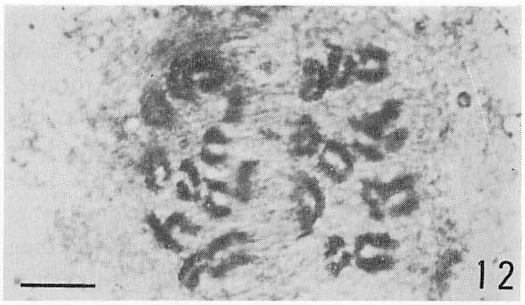
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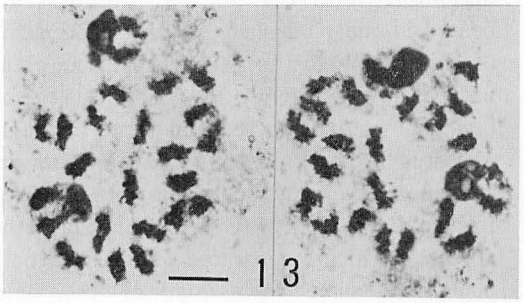
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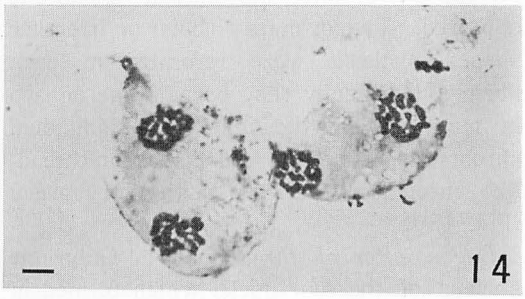
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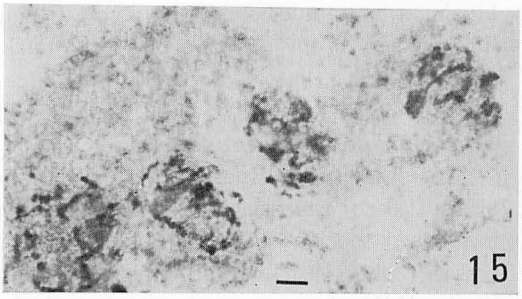
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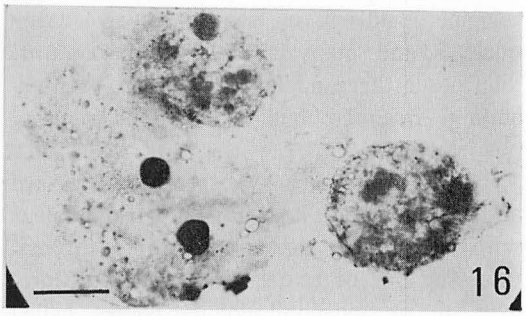
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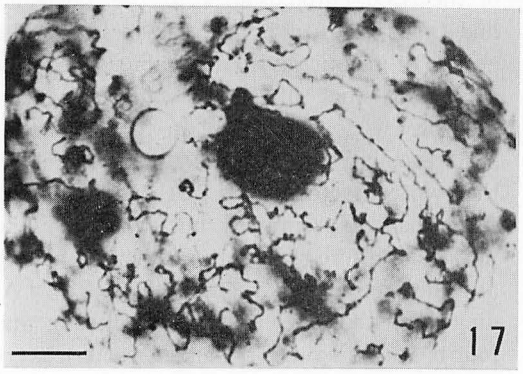
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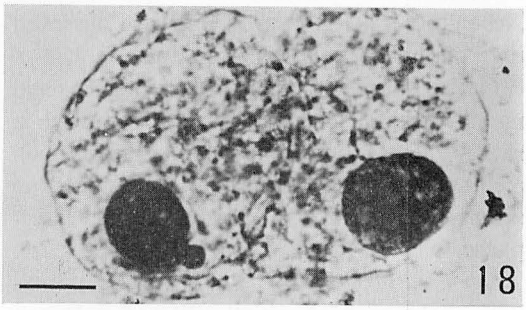
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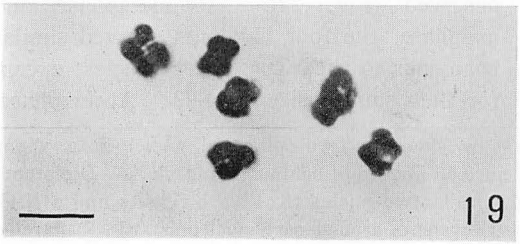
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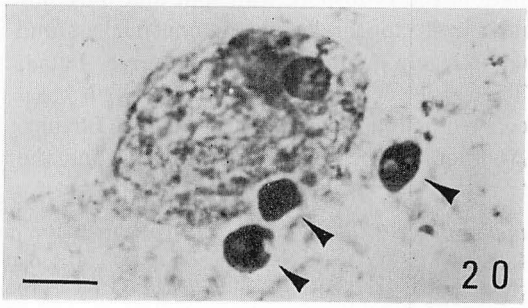
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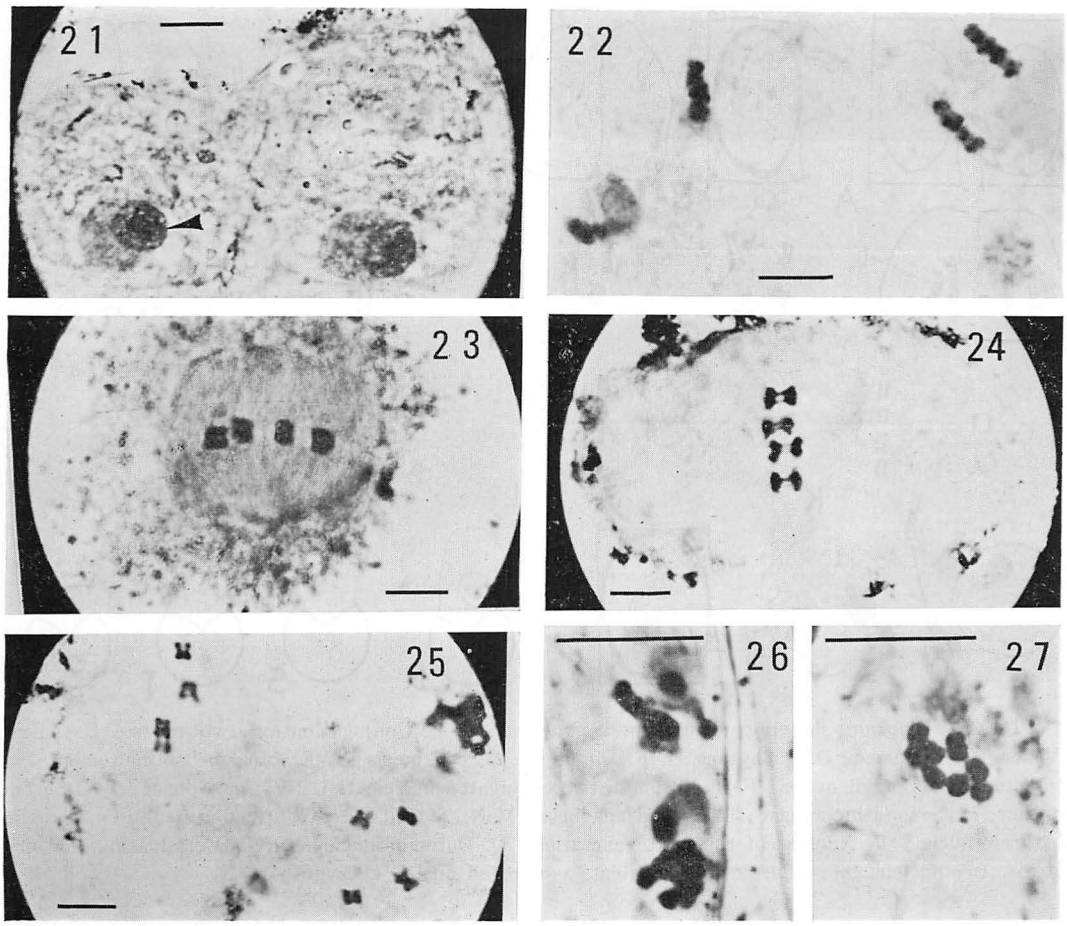

Figs. 21-25. *Spirogyra hunanensis* (n=4) 21. Leptotene. Two contacted nuclei and reticular structure in nucleoli (arrow); 22. Diplotene. Four bivalents; 23. First metaphase. Spindle and chromosomes; 24. First anaphase; 25. Second metaphase.

Figs. 26-27. *Spirogyra lacustris* (n=8) 26. Diakinesis; 27. First metaphase. (Scale bars=10 μm).

sticky chromosomal fibers connecting the separated half-bivalents were observed (Fig. 24). The fibers were also observed at anaphase of the second division (Fig. 25). After the second division, in the daughter nuclei, two, or rarely four, nucleoli were observed.

4) ***Spirogyra lacustris* Czurda** (n=8)

The behaviour of the nucleolus and chromosomes in the first division in this species was similar to that of the former three species. At diplotene, in each of the two connected nuclei, one N.O. chromosome and three other chromosomes gathered around the nucleolus (Fig. 26). At first metaphase, the eight bivalents arranged on the equatorial plate consisted of two short rod-shaped chromosomes (Fig. 27).

Figs. 11-16. *Spirogyra crassa*. 11-12: First division; 11. Metaphase: A barrel-shaped spindle; 12. Anaphase; 13-16. Second division: 13. Metaphase; 14. Anaphase; 15. Four nuclei after meiosis; 16. Two aborted nuclei.

Figs. 17-20. *Spirogyra crassa* X (n=6) 17. Leptotene; 18. Chromatids arranged in a line and nucleoli in two contacted nuclei; 19. First metaphase; 20. Three aborted nuclei (arrows). (Scale bars =10 μm).

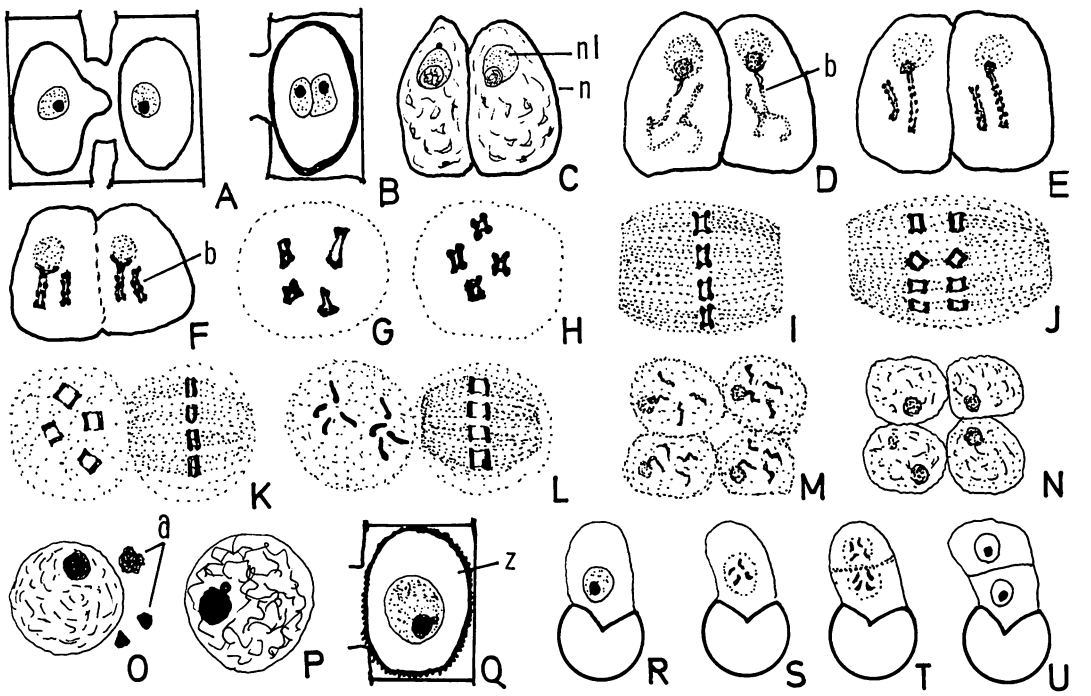

Fig. 28. Diagram showing meiosis in *Spirogyra*. A-B. Conjugation of two nuclei; C-J. First division; C. Leptoene; D. Pachytene; E-F. Diplotene; G. Diakinesis; C-E. Bivalent formation in two contacted gametangial nuclei; F-H. Fusion of two conjugated nuclei; H-I. First metaphase; J-N. Second division; K. Second metaphase; L. Second anaphase; M-N. Tetrad, newly formed nucleoli; O. Abortion of three nuclei; P. Nucleus of the next generation; Q. Dormant nucleus; R-U. Germination and first mitosis. (n: nucleus, nl: nucleolus, b: bivalent, a: aborted nuclei, z: zygospore).

Discussion

The meiotic cycle of *Spirogyra* observed during this study is diagrammatically shown in Figure 28.

From observation of meiosis in four species of *Spirogyra*, the most prominent feature is the pairing of homologous chromosomes. After the conjugation of two gametangial cells, the two nuclei still remain in contact with each other without fusion in the young zygospore (Figs. 3, 18 and 21). During first prophase, the pairing of chromosomes occures in each nucleus (Figs. 4 and 5). The first observation of meiosis in *S. jugaris* was by KARSTEN (1908). He reported that the chromosomes paired at first metaphase, but he did not mention the continued contact of two nuclei nor bivalent formation in each nucleus. TRÖNDLE (1911) reported the process of meiosis in three species of *Spirogyra*. He observed, in *S. neglecta*, that the pairing of chromosome occured in ech of the two contacted nuclei. However, he stated that such a pairing might occur probably for some artificial effect.

The pairing of chromosomes before fusion of two nuclei has been confirmed in four species studied. In *S. crassa* X and *S. hunanensis*, which have smaller number of chromosomes, the process of bivalent formation was clearly observed in each nucleus. This pairing of the homologous chromosomes in each nucleus originated from two gametangial cells has not been reported for any haplont plants. However it seems to be common in *Spirogyra*. Moreover, a similar feature has been previously observed in two species of *Sirogonium* at first prophase in meiosis (HARADA and YAMAGISHI 1981).

Bivalent formation in *Spirogyra* can explained as the following: considering the

example of *S. crassa* X (n=6), the two N.O. chromosomes and four ordinary chromosomes (autosomes) appear in pachtene, then the three bivalents occur in each nucleus in the diplotene stage, It is thougt that the six chromosomes are composed of three homologous pairs, The authors conclude that the chromosome composition in the nucleus of *Spirogyra* is acutually diploid, not haploid. Therefore, the bivalents that are arranged on the equatorial plate in first metaphase have a different origin from those of higher plants.

GODWARD (1961) mentioned chiasmata at the formation of bivalents, but in diplotene and diakinesis, chiasmata could not be observed. The paired chromosomes of each bivalent are parallel and they reach maximum contraction at first metaphase.

The existence of N.O. chromosomes in the mitotic cycle were reported by WISSELINGH (1900), GEITLER (1930) and GODWARD (1950). However, there is no report of the N.O. chromosomes in meiosis. Regarding the chromosome formation in the meiotic cycle, KARSTEN (1908) mentioned that the chromosomes originated from the nucleolus in the zygospore, and TRÖNDLE (1911) observed the existence of the nucleous, but did not mention the relationship between the nucleolus and the chromosomes. GODWARD (1961) paid special attention to the behaviour of the nucleolus in both mitosis and meiosis. However, she could not clearly trace it in the meiotic cycle.

In this study, the relationship between the N.O. chromosomes and the nucleolus is confirmed for the first time, through observation of first prophase and second metaphase in meiosis. In *S. crassa* (n=12), there are two N.O. chromosomes in each conjugated nucleus. At diplotene, these two chromosomes, forming a bivalent,are attached terminally to the nucleolus (Figs. 4 and 5). It is difficult to distinguish the N.O. chromosomes from the other chromosomes at first metaphase. In second metaphase, nucleolus-like bodies were observed at the terminal of two half-bivalents. It is suggested that the nucleous is reformed at the terminal of the N.O. chromosome.

GODWARD (1961) observed the stainable woolly-shaped or fiber-like threads between, or around the separating half-bivalents at late diakinesis and first anaphase, and she mentioned that these stainable structure were derived from the stickiness of the nucleolar substance enclosing the chromosomes. KUSANAGI (1962) also observed similar stainable fibers in *Luzula* (Juncaceae) which appeared on the chromosomes at anaphase of the first division.

During prophase of the first division, each chromosome was observed as a clear outline, somewhat rigid, and such a woolly structure was not observed. At first metaphase and anaphase, the fiber-shaped threads between the chromosomes and dividing chromatids were observed (Figs. 8, 9, 12 and 13). As mentioned in the previous paper (HARADA and YAMAGISHI 1984), the sticky substance is considered to be homogeneous with the chromosomes from the view point of staining ability.

TRÖNDLE (1911) first reported that *S. calospora* and *S. longata* had diploid chromosomes at first metaphase, and four nuclei having haploid chromosomes were formed in the second division, and the three of them aborted and only one developed. From observation of four species, the process of the abortion of three nuclei agrees well with that noted by TRÖNDLE.

KARSTEN (1908) mentioned that the meiosis occured at the time of the germination of zygospores. TRÖNDLE stated that the meiosis completed at about the same time as the maturation of zygospores. Moreover, GODWARD (1961) observed that the meiosis finished a week after conjugation. In all four species investigated in this study, however, meiosis was observed in young zygospores, and finished before the maturation of the zygospores. Only one nucleus was usually observed in the matured zygospores laying dormant.

References

GEITLER, L. 1930. Über die Kernteilung von *Spirogyra*. Arch, Protistenk. **85**: 10-19.

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

GODWARD, M. B. E. 1961. Meiosis in *Spirogyra crassa*. Heredity **16**: 53-62.

HARADA, A. and YAMAGISHI, T. 1981. Cytological studies on *Sirogonium* 2. Meiosis in *S. melanosporum* and *S. sticticum*. Jap. J. Phycol. **29**: 157-162.

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

KARSTEN, G. 1908. Die Entwicklung der Zygoten von *Spirogyra jugalis* Kütz. Flora **99**: 1-11.

KUSANAGI, A. 1967. On the behaviour of the nucleoleolus in cell division, with special reference to the relationship between the nucleolus and cytological characteristics in *Luzula*. Jap. Journ. Bot. **19**: 387-418.

TRÖNDLE, A. 1911. Über die Reduktionteilung in den Zygoten von *Spirogyra*. Zeitschr. Bot. **3**: 593-619.

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

原田　彰*・山岸高旺：　アオミドロ属（緑藻類）の減数分裂**

奈良県下の水田，池から採集したアオミドロ属4種を材料として，接合胞子内での減数分裂の経過を調べた。接合した雌雄両配偶子の核は若い接合胞子内では接着したままの状態で第1分裂前期を経過する。すなわち接着した両核内で別々に相同染色体が対合して二価染色体を形成する。このことはそれぞれの核内の2個の仁形成体が対合し，仁に接している部分が離れていることでも確かめられた。減数分裂の経過からアオミドロ属の糸状体細胞の核相は n ではなく 2n と見なされるのではないかと考えられる。分裂終了後3核は退化する。この時期には接合胞子膜は完熟していることからアオミドロ属の減数分裂は接合直後にはじまり，接合胞子の完熟時には終っているといえる。(*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)