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Cytological studies on *Sirogonium* (Chlorophyceae) 2. Meiosis in *S. melanosporum* and *S. sticticum*

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In this study, meiosis in *S. melanosporum* and *S. sticticum* was observed. The adhesion and fusion of the conjugated gametangial nuclei, the pairing of chromosomes, the N. O. chromosomes, the parallel separation of chromatids, and the abortion of three daughter nuclei were studied throughout the meiotic cycle.

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

In the present paper, a detailed account of the meiotic division of *S. melanosporum* (RANDHAWA) TRANSEAU and *S. sticticum* (J. E. SMITH) KÜTZING are reported.

Materials and Methods

S. melanosporum was repeatedly collected from natural populations growing in the rice fields of Ikebe, Nara Prefecture, from September 1974 to August 1975. In the middle of August 1975, many young and mature zygospores were found in the collection. Fertile filaments of *S. sticticum* were also collected from rice fields in Ikaruga, Nara Prefecture, from December 1970 to April 1971, and in Higashiyama, Nara Prefecture, in August 1973.

Zygospores were used for observation of meiosis. Most of the zygospores were too young or too mature for good observation, so it was not possible to observe the meiotic process in all material.

Material was fixed with 1 : 3 acetic acid and ethyl alcohol and stored in the same solution. After fixation, the zygospres were stained with Wittmann's solution (Harada 1980). For staining, the zygospores were removed from conjugated filaments and put on slide glass. Then the contents of the zygospores were squashed and stained on the slide glass.

Observations

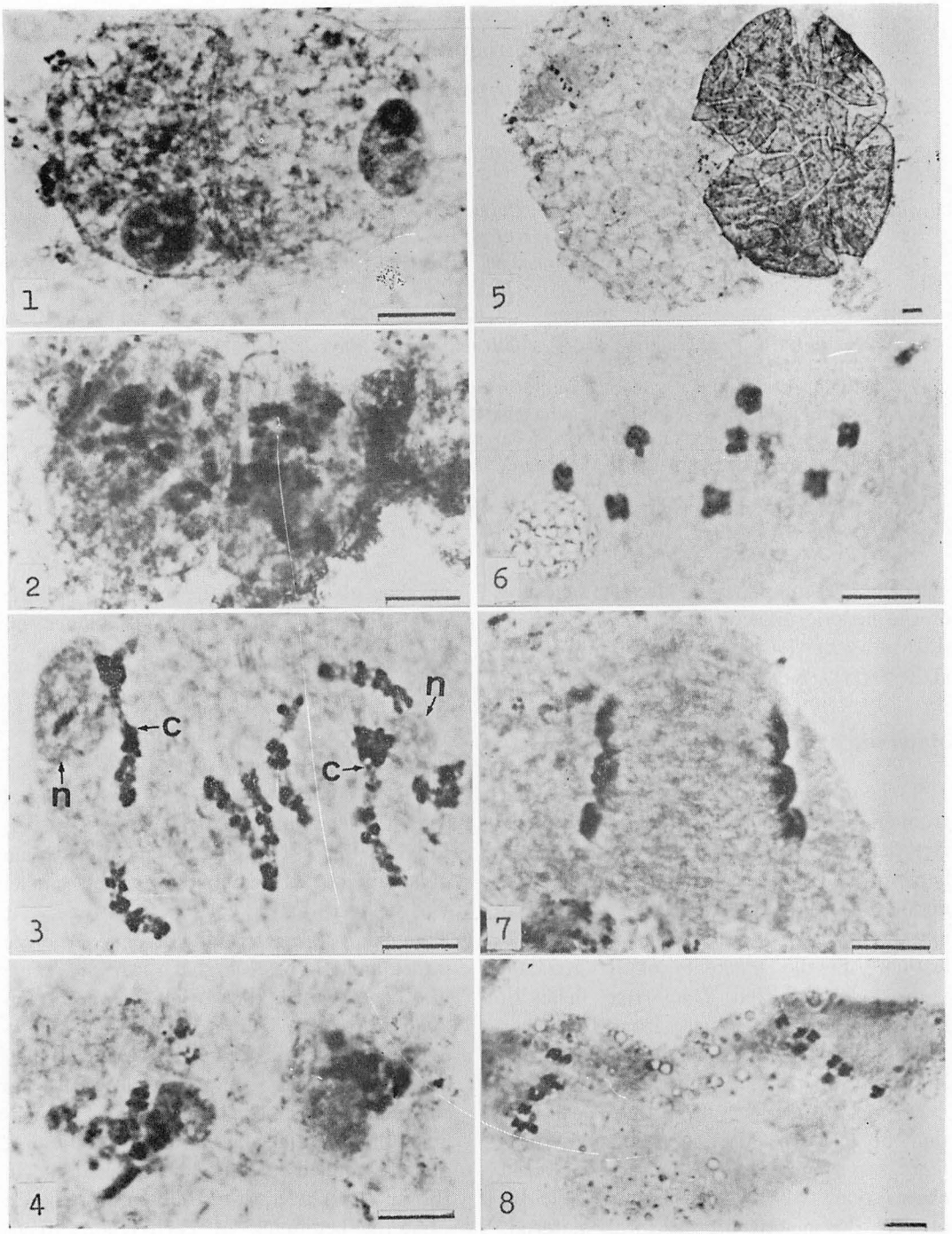
1 *Sirogonium melanosporum*

After conjugation of two gametangial cells, the contents of two cells (gametes) fused to form a zygospore in the female gametangial cell, but union of their nuclei was delayed for a considerable time. At leptotene, two nuclei, which remained quite intact and showed a sharp outline, came into contact with each other in the green zygospore.

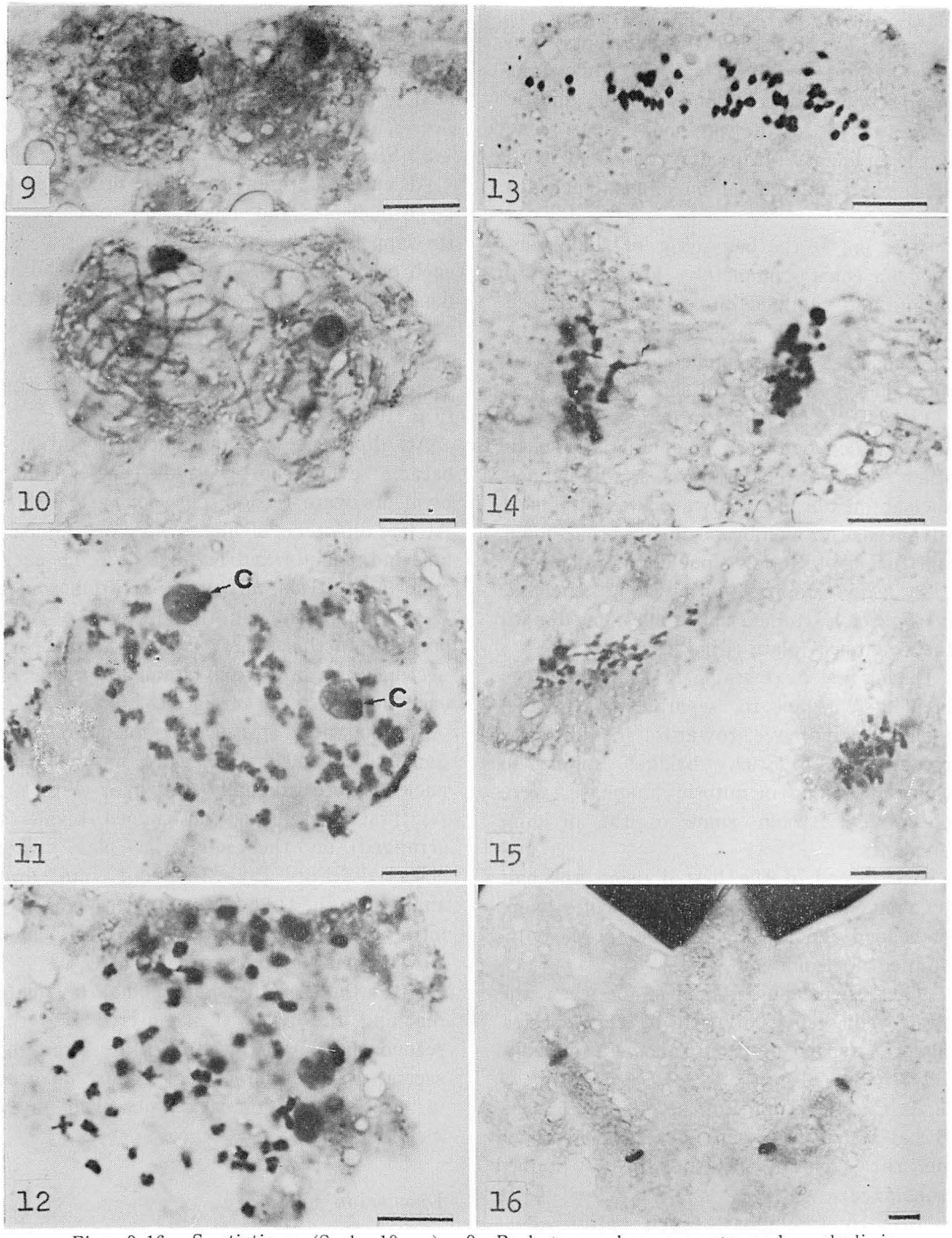
A large nucleolus was observed in each nucleus, and many thin chromatin threads scattered in the karyoplasm were distinguishable. In the nucleolus, two, or rarely one, densely stained regions, like those found in the mitotic nucleus, were observed (Fig. 1).

At the beginning of pachytene, thread-like chromonemata changed to string-shaped chromosomes. At this time, the two adhering nuclei remained unfused (Fig. 2).

At diplotene, bead-like chromatin linearly arranged along the chromosomes were



Figs. 1-8. *S. melanosporum* (Scale 10 μm). 1. Leptotene: two nuclei adhering to each other in a young zygospore; nucleolus and fine chromatins in each nucleus; 2. Diplotene: chromosomes in each nucleus; 3. Diplotene: four bivalents in each nucleus; nucleoli (n) and N.O. chromosomes (c); 4. Late diplotene; 5. First metaphase: cell contents squashed out from zygospore (left side) and chromosomes arranged on equatorial plate; 6. First metaphase: eight bivalents; 7. First anaphase: chromatids and sticky threads between separating chromatids; 8. Second anaphase.



Figs. 9-16. *S. sticticum* (Scale 10 μm). 9. Pachytene: chromonemata and nucleoli in two adhered nuclei; 10. Late pachytene: thread-like chromosomes; 11. Late diplotene: nucleoli and bivalents grouped in each nucleus (left and right parts separated by oblique line); two N.O. chromosomes (c) connecting with the nucleolus; 12. Late diakinesis: bivalents and two remained nucleoli fused karyoplasm; 13. First metaphase: bivalents arranged on equatorial plate; 14. First anaphase: sticky bridges between the separating half bivalents; 15. Second metaphase; 16. Second telophase: chromatids in squashed zygospore (under side).

observed. At this stage, four bivalents were clearly counted in each nucleus, while eight chromosomes were found in the mitotic cycle studied in the previous paper. One of the bivalents terminally connected with the densely stained region in each nucleolus (Fig. 3).

Later on, at the beginning of diakinesis, the nucleolus completely lost its sharp outline and changed into a heavily stained mass of nuclear substance. All of the chromosomes were embedded inside this substance at final diakinesis (Fig. 4).

Throughout the first prophase, the two nuclei which had been in contact with each other remained enclosed in a well-defined nuclear membrane. However, as prophase advanced, the membrane finally broke down and fusion of the two nuclei took place.

At first metaphase, 8 bivalents enclosed in the heavy-stained substance moved onto the equatorial plate (Figs. 5, 6).

During the next stage, first anaphase, the bivalents began to separate in parallel fashion and move towards the opposite poles (Fig. 7). Sticky bridges, similar to those appearing in mitotic anaphase, were observed between some of the dividing bivalents.

At the end of the first division, the half bivalents reaching towards the poles were embedded and arranged inside the heavily-stained nucleolar substance.

There was no interphase between the first and second division of this species, and the second division followed immediately (Fig. 8).

At the beginning of second metaphase, 8 half bivalents were observed arranged on the equator of the heavily-stained substance.

At second anaphase, each half bivalent divided into two chromatids and progressed into second telophase. Sticky bridges like those in mitosis were also observed between separating chromatids in this stage.

As second telophase proceeded, four daughter nuclei with membranes appeared. After the second division was complete the gradual abortion of three nuclei occurred, and only one nucleus developed into the next generation.

2 *Sirogonium sticticum*

At early leptotene of first prophase, a large nucleolus and much minute chromatin appeared in two nuclei attached to each other (Fig. 9). Each nucleolus had a densely-stained region like that found in *S. melanosporum*. During the next stage, pachytene, thread-like chromosomes were seen around the nucleolus in each nucleus (Fig. 10).

At diplotene the chromosome threads became even shorter and thicker, and bivalents were recognized soon after this. At late diplotene, bivalents were distinguishable individually and numbered 26 in each adhering nucleus, although 52 chromosomes were found in mitotic division of this species. In this stage, two bivalents connecting with a densely-stained portion of each nucleoli were observed (Fig. 11).

As diakinesis proceeded, fusion of two gametangial nuclei took place, and the two nucleoli were gradually destroyed (Fig. 12). At first metaphase, dot-shaped bivalents arranged on the equatorial plate were observed (Fig. 13). Entering into first anaphase, the bivalents divided parallely into two half bivalents (Fig. 14), which moved towards opposite poles.

After the first division, the half bivalents reaching the poles then entered into the second division (Figs. 15, 16). After the second division, three nuclei were aborted in the same manner as observed in *S. melanosporum*.

Discussion

During the meiotic cycle of *S. melanosporum* and *S. sticticum*, the most striking feature was the formation of bivalents. In young zygospores, two nuclei originating from gametangial cells remained connected with each other without fusion throughout first prophase.

Although certain meiotic stages—zygotene and pachytene in first prophase—could not be observed as distinctly as other stages in these two species, it may be said that the formation of bivalents occurred in each nucleus through first prophase.

Pairing of chromosomes was clearly seen in the diplotene stage of the two species (Figs. 3, 11). The nuclear membrane, however, broke down in later prophase, and fusion of two gametangial nuclei occurred (Figs. 4, 12).

KARSTEN (1908) and TRÖNDLE (1911) investigated meiosis in some species of *Spirogyra*, and found that the pairing of chromosomes occurred in each adhering nuclei during first prophase. This phenomenon, confirmed in the present study might be common in *Sirogonium* and *Spirogyra*, because adhering nuclei in young zygospores have been observed in other species of *Sirogonium* and *Spirogyra* (HARADA and YAMAGISHI, unpublished).

As far as we known, no reports have been published on the behaviour of the gametangial nuclei and the pairing of chromosomes in other haplonts. This is an interesting nuclear phenomenon and more research on other zygnemataceous algae should clarify this phenomenon.

The presence of nucleolar organizing chromosomes in mitosis was also confirmed in the diplotene stage of *S. melanosporum*, although these chromosomes were not so clearly observed in *S. sticuticum* due to their rather minute size.

In the leptotene nucleus of *S. sticticum*, these chromosomes were seen connecting with the heavily-stained structures in the nucleolus. This probably represented the nucleolar organising track as it appeared in mitotic prophase.

Although the nucleolar substance in mitotic division has already mentioned in some species of *Spirogyra* (GEITLER 1935, DORAISWAMI 1946, GODWARD 1950), and also in *Sirogonium* (WAER 1966, WELLS 1969, HARADA 1980), its formation during the meiotic cycle has not been clearly traced. However, a densely-stained mass of nuclear substance was usually seen around the chromosomes during the first and second metaphase of *S. melanosporum* and at second anaphase of *S. sticticum*. Furthermore, a remarkable parallel separation of chromatids was observed during the first and second anaphase (Figs. 7, 14), which suggested that the chromosomes were probably separated into two chromatids by bipartition of the mass enclosing the chromatids, such as occurred during mitosis in the two species previously investigated.

The wooly or sticky stainable substance covering the chromosomes (GEITLER 1930, GODWARD 1961), termed sticky bridges between the two separating chromatids in this study, was observed in some stage in the two species (Figs. 7, 14).

As soon as fusion of two gametes took place, a zygospore wall developed around the zygote while meiosis progressed. After meiosis was complete, one of the four daughter nuclei developed further, while the others gradually began to abort, and ripening of the zygospore proceeded.

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原田　彰*・山岸高旺：シロゴニウム属（緑藻類）の細胞学的研究 2. *S. melanosporum* と *S. sticticum* の減数分裂**

1報と同じく，奈良県下の水田から採集した2種のシロゴニウム属の2種を材料として，接合したあとで接合胞子内でおきる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合体融合することなく，接着したままの状態で第1分裂の前期が経過する。すなわち，接着した両核内で別々に染色体の対合，二価染色体の形成が進む。前期の終りに二価染色体が完成する頃に始めて両核が融合するのが観察された。これは誠に特異な現象で，このシロゴニウム属の生活史と核相との関連からみて興味のある点である。また，仁形成染色体，染色分体の平行分離が観察された。配偶子の接合直後から胞子膜は肥厚し始めるが，その中で減数分裂が進み，減数分裂完了のあと3核は退化して接合胞子は成熟する。（*581 大阪府八尾市千塚102 大阪府立清友高等学校 *154 東京都世田谷区下馬3　日本大学農獣医学部教養科生物学研究室）

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