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Karyogamy in *Spirogyra verruculosa* Jao (Chlorophyceae)

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The karyogamy in *Spirogyra verruculosa* Jao was investigated by light and electron microscopy. In 10-day-old zygotes the two pronuclei from both the male and female gametes were connected to each other by internuclear bridges, which were various in width, ranging from 0.5 to 1.5 μm. About 12 days after plasmogamy the nucleoplasms of the two pronuclei commenced to intermingle. The fused nucleus first contained two nucleoli, which sometimes lay close to each other, but one nucleolus later, suggesting the union of the two nucleoli into a single one. The formation of synkaryon was completed by 14 days after plasmogamy in the present species.

Key Index Words: karyogamy—nucleolus—nucleus—pronucleus—Spirogyra—*synkaryon—zygote formation.*

The karyogamy is one of the most important phases in fertilization. This process in *Spirogyra* (Zygnematales, Chlorophyceae) has been repeatedly observed with the light microscope (Overton 1888, Tröndle 1907, 1911, Karsten 1908, etc.), but ultrastructural studies are few. The difficulty in embedding of the zygote which develops a thick wall to withstand various kinds of environmental stress renders the electron microscopic observations difficult (Fowke and Pickett-Heaps 1971, Jordan 1974). An improved embedding method of thick-walled zygotes (Ogawa 1982) made it possible to demonstrate the process of karyogamy in *S. verruculosa* ultrastructurally, at least to some extent (Ogawa 1981). But, the behavior of nuclear membranes and nucleoli in the fusing two pronuclei was observed only partially. This is mainly due to the lack of the information on the timing of the pronuclear fusion in this species.

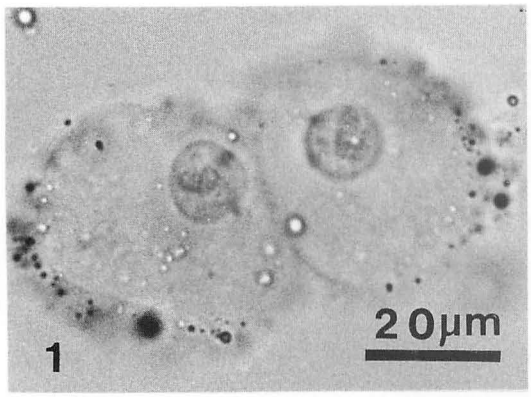
Generally in *Spirogyra*, karyogamy is known to precede the meiosis, however, some species are not the case, in which the pairing of homologous chromosomes takes place in each of the two adjacent pronuclei (Harada and Yamagishi 1984). Although the previous electron microscopic observation indicated the formation of synkaryon (Ogawa 1981), it is necessary to reinvestigate the later stage of karyogamy. The present study concentrates mainly upon clarifying the accurate time requisite for the completion of pronuclear union in *S. verruculosa* with the object of demonstrating the behavior of nuclear contents and nuclear membranes in fusing pronuclei.

Materials and methods

The filaments of *Spirogyra verruculosa* Jao, which were forming conjugation tubes, were collected from a pond in Sendai City, Miyagi Prefecture. The zygotes were allowed to mature in Erlenmeyer flasks each half-filled with the culture medium of Reichart (1967) under a 12 hr light: 12 hr dark cycle (ca. 2,000 lux, white fluorescent lamps) at 25°C.

For light microscopy, some of the zygotes were fixed every day in acetic acid-alcohol (1 : 3) fixative for 2–3 hr at 25°C. They were then gently squashed on slide glass, stained with acetic orcein, and observed with either a Zeiss light microscope or an Olympus light microscope equipped with the Nomarski differential-interference apparatus.

The embedding method of thick-walled zygotes for electron microscope observation was almost the same as that described else-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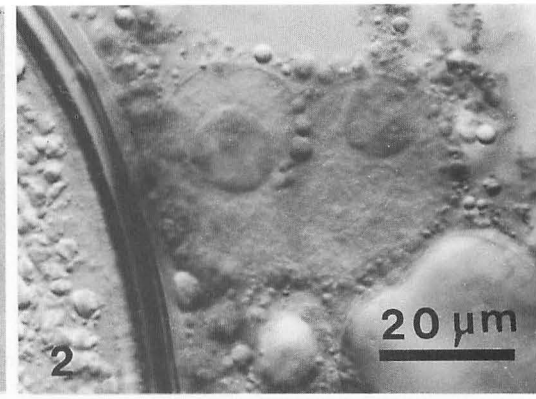
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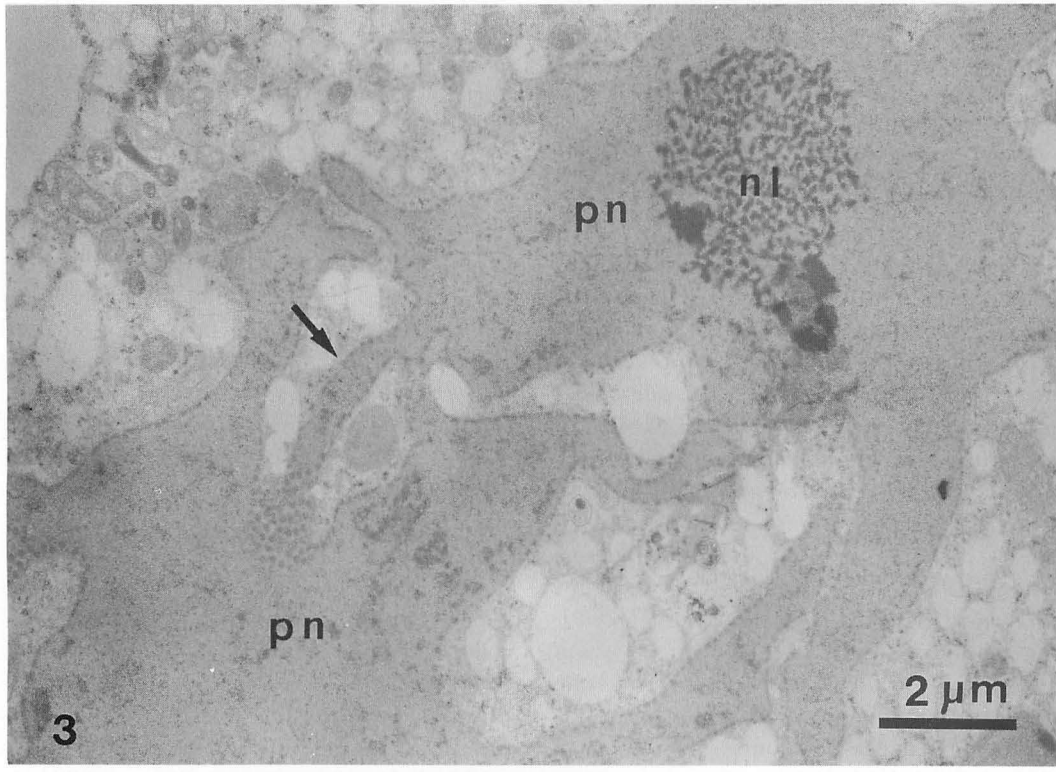
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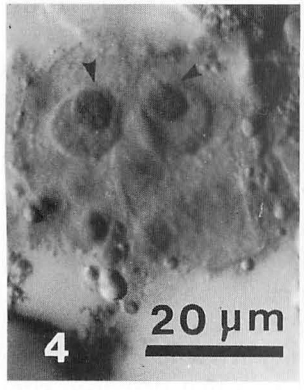
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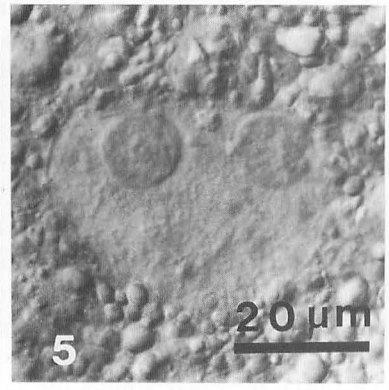
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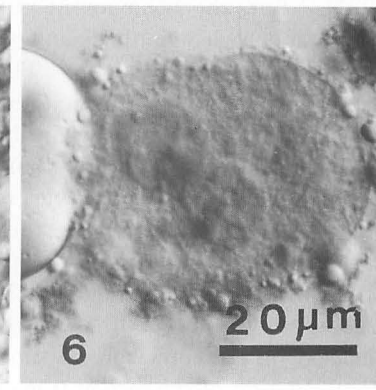
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where (Ogawa 1982). The zygotes were fixed first with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) and then with phosphate-buffered 1% osmium tetroxide. After slight rinse in the buffer, one of the two ends of each of the ellipsoid zygotes was cut with a razor blade under a binocular dissecting microscope to facilitate the penetration of the epoxy resin embedding medium of Spurr (1969) into the zygotes. They were dehydrated in an ethanol series and embedded in the epoxy resin. Thin sections were stained with uranyl acetate and lead acetate and examined with a JEOL 2000EX electron microscope, operating at 80 kV.

Results

In individual young zygotes of several days old, the two pronuclei from both gametes were juxtaposed. Each of them included one nucleolus, and their adjacent surfaces came in contact with each other (Fig. 1). The area of contiguous surfaces of the two pronuclei seemed to extend over a wide range with time, and they assumed an ellipsoidal form on the whole. Cytoplasmic contents, probably lipid droplets, were sometimes squeezed between the two pronuclei (Fig. 2). The adjacent surfaces of the two pronuclei in 10-day-old zygotes irregularly undulated, and the two pronuclei were connected to each other by internuclear bridges (Fig. 3), which were various in width, ranging from 0.5 to 1.5 μm.

The mixture of nucleoplasms of the two pronuclei could frequently be seen at about 12 days after plasmogamy. The nuclear membranes that disturbed the complete interminglement of both nucleoplasms (Fig. 4) became obscure (Fig. 5), leaving two nucleoli present simultaneously within the same nucleus (Fig. 6). The two nucleoli sometimes lay close to each other (Fig. 6). The fused nucleus, or synkaryon, contained one nucleolus (Fig. 7). Electron microscopy revealed the presence of neither chromosomes nor synaptonemal complex within the nucleoplasm of the synkaryon (Fig. 8). As typically seen in Fig. 4, each nucleolus of pronuclei usually had an area stainable with acetic orcein somewhat densely, while the nucleolus of synkaryon in zygotes about 12 days old sometimes contained two orcein-stainable areas (Fig. 9, arrowheads).

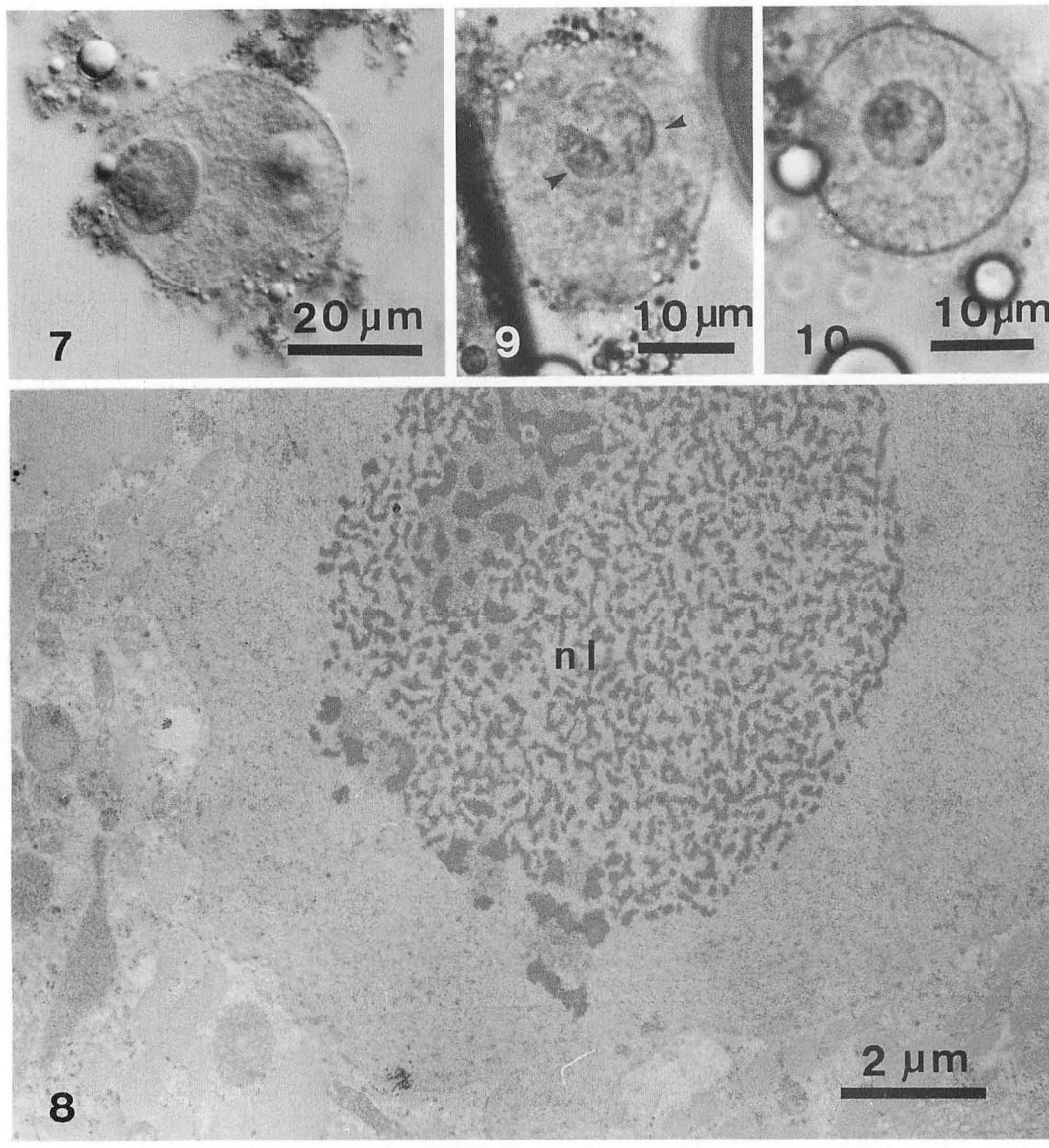
In zygotes 14 or more days old, the nucleus contained only one nucleolus with one orcein-stainable area (Fig. 10). So far as examined, meiotic division could not be observed in the present species at least within 20 days after gametic union.

Discussion

The present investigation demonstrated that in *Spirogyra verruculosa* the male and female pronuclei united together, as hitherto known in other *Spirogyra* species. The behavior of the two pronuclei during zygote maturation has been observed three times in this species. In any case examined, the two pronuclei completed their union by 14 days after plasmogamy. Accordingly, the previous description that in *S. verruculosa* karyogamy finished within 30 days after plasmogamy (Ogawa 1981) is incorrect. About three weeks elapsed until the completion of karyogamy in *S. communis* (Trödle 1907), and the two pronuclei fused together shortly after plasmogamy in *S. crassa* (Godward 1961). The timing of karyogamy may largely vary depending on the species of *Spirogyra*.

In the present species, the nucleus with two nucleoli was observed mostly at about 12 days after gametic union (Fig. 6), but the frequen-

Figs. 1–6. Light and electron micrographs of nuclei in zygotes of *Spirogyra verruculosa*. 1. Two pronuclei in a 7-day-old zygote. Each pronucleus contains one nucleolus. 2. Differential-interference-contrast micrograph of two pronuclei in a 10-day-old zygote. 3. Electron micrograph of part of two pronuclei (pn). They are connected together by internuclear bridges (arrow). Nucleolus (nl). 4. Two pronuclei in a zygote of 12 days old. Each nucleolus has an orcein-stainable area (arrow head). 5. Fusing pronuclei of a 12-day-old zygote. Nuclear membranes separating them is obscure. 6. Fused nucleus in a zygote of 12 days old. Two neucleoli lie close to each other.



Figs. 7–10. Light and electron micrographs of synkaryon of *Spirogyra verruculosa*. 7. Synkaryon in a 12-day-old zygote. It includes one nucleolus. 8. Electron micrograph of a part of a synkaryon in a zygote of 12 days old. Nucleolus (nl). 9. Synkaryon in a 12-day-old zygote. It has a single nucleolus with two orcein-stainable areas (arrowheads). 10. Synkaryon in a 14-day-old zygote.

cy of its appearance was relatively low. These results suggest that the mixture of nucleoplasms of the two pronuclei proceeds not gradually but rather quickly. This is probably a major cause for the present inadequate demonstration on the behavior of nucleoli and nuclear membranes during the mixture of nucleoplasms at the ultrastructural level.

The two pronuclei in seven-day-old zygotes were connected by internuclear bridges, each of which had a fairly regular width of about 0.17 μm (Ogawa 1981). By contrast, the internuclear bridges joining the two pronuclei of 10-day-old zygotes were various in width, ranging from 0.5 to 1.5 μm (Fig. 3), and

wider than those of seven-day-old zygotes. Though their developmental process is obscure, one of the possibilities is that the union of the internuclear bridges, each about 0.17 μm in width, results in the formation of the wider ones.

The fused nucleus first contained two nucleoli from both the male and female pronuclei (Fig. 6), but only one nucleolus later (Figs. 7 and 10). The two nucleoli in the fused nucleus were sometimes close together (Fig. 6). The nucleolus of each of the two adjacent pronuclei usually possessed one orcein-stainable area (Fig. 4), whereas that of the fused nucleus of zygotes about 12 days old sometimes included two orcein-stainable areas (Fig. 9). These results suggest that the two nucleoli unite together into a single one in *S. verruculosa* like in other species of *Spirogyra* (Tröndle 1907, 1911, Karsten 1908).

It is generally described that in *Spirogyra* the two pronuclei from both gametes fuse together into a synkaryon (Overton 1888, Tröndle 1907, 1911, Karsten 1908). But, exceptions are also known. According to Harada and Yamagishi (1984), in *S. crassa, S. hunanensis*, and *S. lacustris* homologous chromosomes begin to pair within each of the two pronuclei which merely come into contact with each other and meiotic division takes place without the formation of synkaryon. So far as examined in *S. verruculosa*, neither chromosome nor synaptonemal complex, an important criterion of meiosis, could be demonstrated throughout the karyogamy (Figs. 3-8). The appearance of pairing of chromosomes in two adjacent pronuclei may be dependent on species. The occurrence of chromosome synapsis in two adjacent pronuclei was first discovered in *S. neglecta*, in which, however, this phenomenon was not observed in all zygotes (Tröndle 1911). In *S. crassa*, the chromosome pairing was observed by Harada and Yamagishi (1984), but not by Godward (1961). The biological significance of this remarkable phenomenon remains unknown. To understand its nature, ultrastructural reinvestigations, together with the extensive observations using various species, would be necessary.

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小川　茂：緑藻アオミドロ (*Spirogyra verruculosa* Jao) の核融合

アオミドロ (*Spirogyra verruculosa* Jao) の核融合を光学顕微鏡と電子顕微鏡で観察した。雌雄両配偶子の融合後10日経過した接合子では，両配偶子に由来する二つの前核は，互いに連結されていた。その連結部の幅は様々で，0.5 μm から 1.5 μm であった。約12日目になると，両前核の核質は混合を始めた。融合した核は，最初は二つ，しかし，後には一つの核小体を有していた。融合核内の二つの核小体は，時に，互いに接近して存在していた。これらの観察結果は，二つの核小体が融合して一つになることを示唆した。本種では，融合核の形成は，雌雄両配偶子の融合後14日目までに終了した。(943 新潟県上越市山屋敷町　上越教育大学自然系生物)