Kaiichi OOSHIMA*: **The morphology and germination of resting spores of *Pediastrum simplex* (Chlorophyceae)****

大島海一*: 緑藻クンショウモ属一種の休眠胞子の形態と発芽

(Plates XII-XIII)

There are considerable reports about the morphology of *Pediastrum* (Chlorophyta, Hydrodictyaceae), but little work has been made on the formation and germination of their resting spores. In the course of the author's culture study of *Pediastrum simplex*, the orange-colored resting spores were often encountered in an old culture medium. Further observations revealed that when they were placed in a fresh medium, these resting spores were capable of producing initial colonies which were not plate-shaped but mass-like. Subsequently each cell of the initial colony formed the second colonies whose shapes were normal plate-like, characteristic of *P. simplex*. This paper presents the morphology of the resting spores, including morphogenesis during their germination and the formation of the initial colony observed for the first time in the laboratory.

Materials and methods Specimens of *Pediastrum simplex* (Meyen) Lemm. with resting spores were isolated from Shinobazunoike pond, Ueno Park, in Tokyo. The isolated colony was inoculated in an agar-water biphasic culture medium[1], which consisted of agar and sterile distilled water. The essential inorganic compounds and other trace elements were contained in the agar layer of the medium. The biphasic culture medium was prepared in a test tube by capping with aluminum foil. When this clonal culture was kept for over one year, all of the vegetative cells changed into orange-colored resting spores with thick walls.

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1) Devised by the author. The preparation of the medium will be published in detail later.

For the purpose of observing their germination, some of the resting spores were placed in Petri dishes containing modified Bold Basal Medium. These cultures were subjected to fluorescent lights of about 4000 lux intensity with a 12/12 hr light-dark cycle. The temperature was maintained at 25± 0.5°C.

Observations and results The resting spores found in the present culture were spherical in shape being surrounded by a thick wall and mostly measured about 30μ in diameter, ranging from 8μ to 50μ. Many of them were solitary or being arranged in the flat *Pediastrum* colonies (Fig. B), but some were aggregated in an irregular mass, a feature similar to *Coelastrum* (Fig. A). The thick wall which surrounded the resting spore was composed of two layers, an outerlayer and an inner one; the former being 1.2μ and the latter being 0.8μ in thickness (Fig. D). Observations using an ordinary light microscope revealed that the wall was furnished with a reticulate or loose mesh construction and a smaller horn or its trace on the surface of the outer layer (Fig. C). On the other hand, by means of a scanning electron microscope, it was disclosed that the outer layer possessed the surface being remarkedly verrucose but sometimes less verrucose (Fig. E).

The resting spores were orange in color and contained many small droplets (Fig. D). When a mass of dry or nearly dry spores was treated with concentrated sulfuric acid, the color of droplets changed promptly into light blue. When stained with Sudan IV, they turned red. Accordingly it should be safe to conclude that the small droplets are fatty and the occurrence of orange color is due to the presence of carotenoid pigment. The I_2-KI test also showed that there was a large amount of starch within the resting spores.

The resting spores inoculated in the fresh medium gradually changed color from orange to green and finally to dark green. After two weeks, they began to form zoospores under those conditions mentioned above. The formation of zoospores occurred within one hour after the beginning of the cyclic illumination. When a vesicle was released from the resting spore through a rupture formed in the spore wall (Fig. F), it started to expand at once and the already formed zoospores began to move about in it. The zoospores tightly crowded together and continued to wriggle about the middle position of the vesicle (Fig. H). They were mostly spherical or

nearly spherical in shape, measuring 10–12μ in diameter. However, their shape and size were sometimes variable even within the same vesicle. They contained many fat-droplets of irregular size as those observed in the resting spores. The zoospores seemed to have one or two parietal chloroplasts. Because of the existence of many droplets, it was difficult to confirm the numbers of chloroplasts. The zoospores also possessed two flagella which were equal in length at the anterior end. The flagella were not so long as the length of the zoospore (Fig. G).

The movement of zoospores usually continued for 10–15 min., but in some cases for 25 min. The zoospores did not move out from the central mass as the vesicle continued to expand, and so they never pushed the vesicle outwards (Figs. H–I). The vesicle was not lens-shaped but nearly spherical. These zoospores formed a mass-like initial colony when their movement had stopped. Each of the colonial cells then secreted cell-wall with granular surface, followed in most cases by the formation of a single or rarely two horns (Fig. J).

The number of zoospores produced in a resting spore was fairly variable depending on the size of the mother cell, ranging from 4 to 64 or more, in most cases 32 or 64. Each of the cells composing the initial colony became mature in a few days and produced zoospores, the components of a second colony. The second colonies were normal flat-shaped and consisted mostly of 16 cells, but sometimes of 8 or 32 cells. All of the subsequently formed daughter colonies were such flat forms as those of the second. These normal flat-shaped colonies usually again began to turn to the resting spores in the Petri dishes within about two months after the culture started.

Discussion Examining a unialgal culture of *Pediastrum boryanum*, Davis (1962) found thin-walled resting cells and described their formation and germination. The resting spores presented in this paper are quite similar to those cells described by Davis in such respects as having many fat-droplets, plenty of starch and carotenoid pigment, but differ from them in having thick walls composed of double layer. Taking this difference into consideration, the present resting spores should not be identical with the resting cells. The present one is a kind of resting spore which may be called akinete. It is interesting to note that these spores have good resemblance to the akinetes of *Tetraedron* reported by Davis (1966) in some characters,

such as having fat-droplets, starch grains and carotenoid pigment.

Although the process of the formation of irregular *Coelastrum*-like mass has not yet been observed in this study, the following assumptions may be drawn: 1) daughter cells changed directly into resting spores without becoming zoospores when placed in unsuitable conditions; 2) although zoospores were produced in a vesicle, they failed in forming a flat colony and became mass-like resting spores. The resting spores in the *Coelastrum*-like colonies reported here seem to be identical with the resting stage of *Coelastrum* which Strom (1921) observed in Wille's collection.

When found the akinetes of *P. simplex* in his algal material collected from natural waters, Yamagishi (1960) noticed a punctate ornamentation on the surface of the cell-wall. However, such a ornamentation has never been observed in this study even with the aid of both light and scanning electron microscope.

There are some differences between the formation of the initial colony presented in this paper and that of the flat colony described by Davis (1964). One is concerned with the shape of a vesicle containing zoospores. Davis observed a lens-shaped vesicle in his culture of *P. boryanum*. However, in the present culture with *P. simplex* the vesicles released from the resting spores were not lens-shaped but were all spherical. The spherical vesicle continued to expand while the zoospores wriggled about in it, resulting in the mass of zoospores being not in contact with the vesicle. Accordingly, these zoospores were not to be constrained to arrange as a plate by the vesicle during this process. Another is concerned with the period of zoospore movement. The zoospores within the initial colony usually continued to wriggle for a longer period than that in the flat colony.

According to the results obtained in the cultural experiments, the dark green resting spores usually began to germinate within one hour after the beginning of the cyclic illumination and, a few days later, many normal flat colonies were produced from the formed initial colony. This may suggest that the resting spores can produce a large number of colonies for a shorter period for widely distributing them in natural waters.

As to the life cycle of *P. simplex*, Davis (1967) offered many precise informations but did not make mention of the resting spores as described in this paper. By the finding of the new process through resting spore

formation, the knowledges concerning the life cycle of *Pediastrum* should partly be modified.

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References

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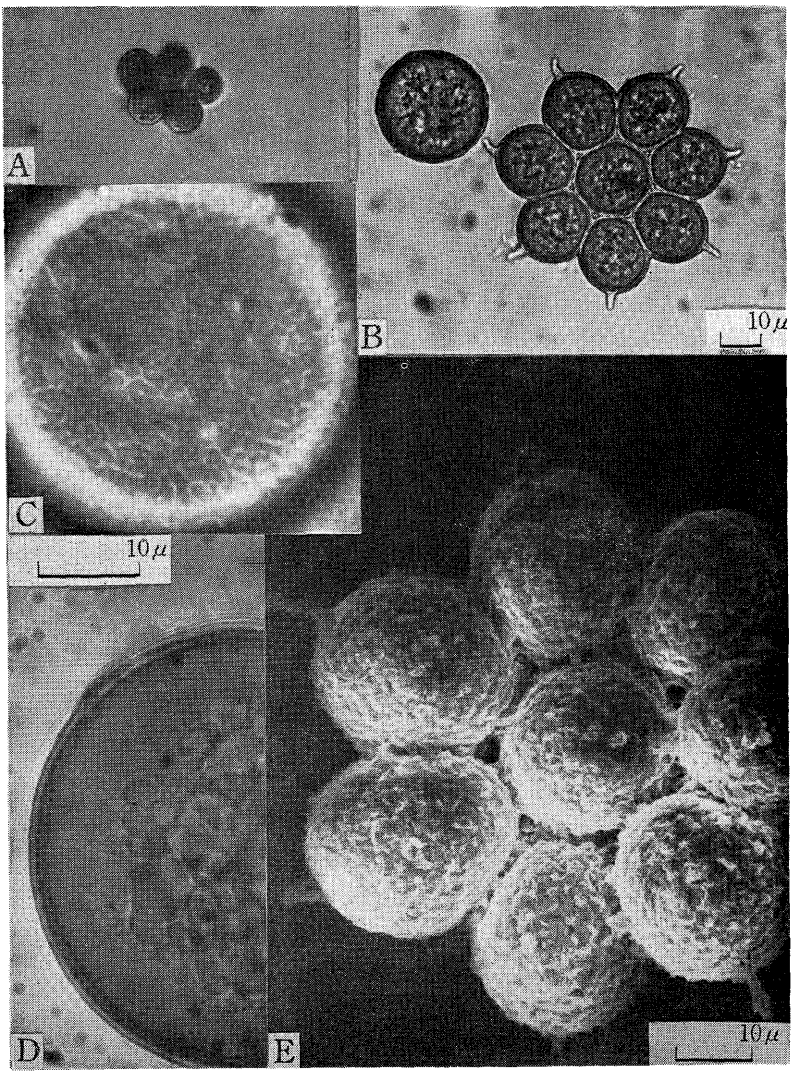
クンショウモ属の一種 *Pediastrum simplex* の生活環は，Davis(1967) によって詳細に記載されているが，その休眠胞子に関してはよく知られていない。日本産の本種の休眠胞子が培養中に得られたので，その諸形質と発芽及び一次群体形成について報告する。

休眠胞子は球形で直径は平均 30μ。二重の細胞壁をもち，内部には多数の油滴と多量のデンプンを含有していた。細胞壁は網目状構造でその表面には多くの突起が見られた。胞子の色は，細胞質内にカロチノイドを含有しているためオレンジ色を呈していた。この休眠胞子を新鮮な培養液中に移したところ，オレンジ色の胞子は次第に緑色に変わり，2 週間後には発芽を開始した。休眠胞子から遊離した小嚢 (vesicle) 内の遊走子は 10–15 分間運動を続け，静止した時塊状の一次群体を形成した。その小嚢は，Davis (1962) が別の種 (*P. boryanum*) で観察したようなレンズ状ではなく完全な球形で，遊走子の運動及びその配列とは無関係に膨張し続けた。一次群体から生じた 2 番目以後の娘群体は，特徴的な円板状をしたクンショウモの群体そのものであった。

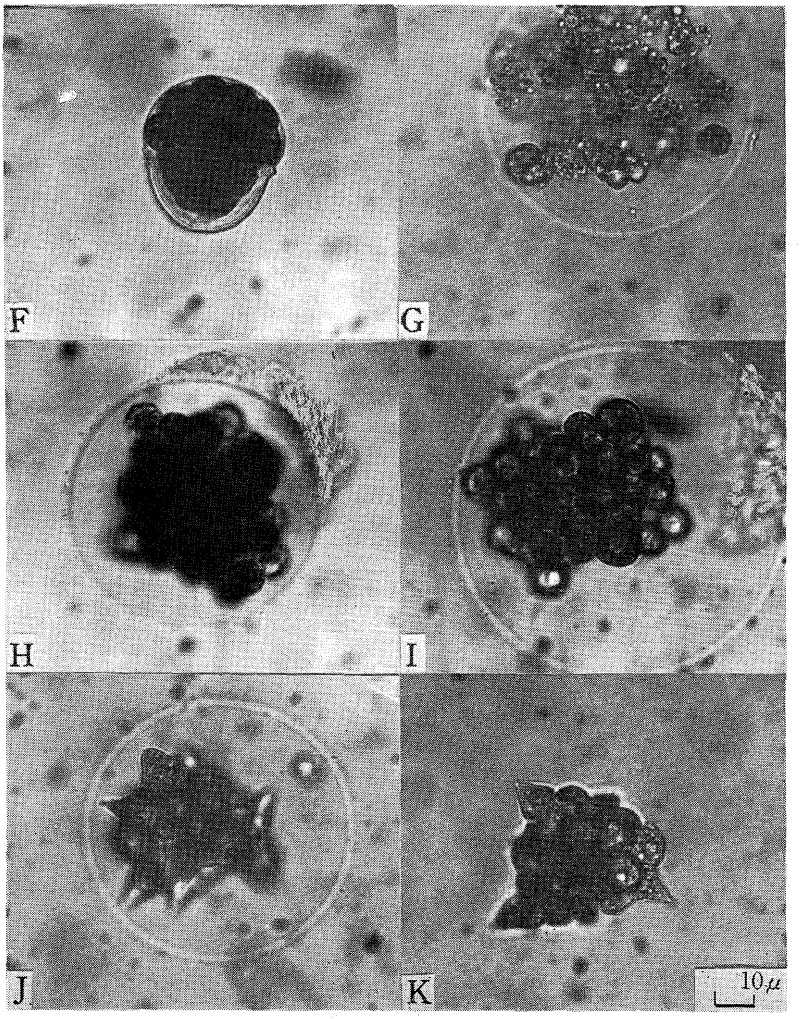
Explanation of Plates XII–XIII

Plate XII. A. Resting spores forming an irregular *Coelastrum*-like mass ×600. B. Solitary resting spore and colonial resting spores ×600. C. Reticulate construction on the wall of resting spore ×1500. D. Thick wall composed of double layer ×1500. E. Scanning electron microscopical view ×1000.

Plate XIII. F. Zoospores released from a resting spore through its rupture. G. Biflagellate zoospores with many droplets. H. Zoospores wriggling about in a vesicle. I. The expanded vesicle in 10 min. from Fig. H. J. Initial colony just formed in a vesicle. K. Initial colony freed from a vesicle. All figures ×600.



K. OOSHIMA: Resting spores of *Pediastrum simplex*



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