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<td>著者(s)</td>
<td>Migita, Seiji</td>
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<td>引用</td>
<td>長崎大学水産学部研究報告 v.24, pp.55-64; 1967</td>
</tr>
<tr>
<td>発行日</td>
<td>1967-12</td>
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<tr>
<td>URL</td>
<td><a href="http://hdl.handle.net/10069/31358">http://hdl.handle.net/10069/31358</a></td>
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Cytological Studies on *Porphyra yezoensis* UEDA

Seiji Migita

Abstract

In recent years, morphological details of the life-cycle of *Porphyra* had been presented by many investigators. However, the cytological evidence of *Porphyra* at various stages of the life-cycle had not been clearly established, especially that of *Conchocelis*-thalli is entirely obscure.

In this paper, the author deals with the cytology of *P. yezoensis* UEDA by means of Godward's iron-alum acetocarmine squash technique. The results obtained are summarized as follows:

At the nuclear division of vegetative and spermatangial cells in leafy thalli of *P. yezoensis*, three chromosomes were counted. Monospores from young leafy thalli and conchospores from *Conchocelis*-thalli also have three chromosomes each at their germination. On the other hand, in fertilized carpogonia and *Conchocelis* filaments, the nuclei have six chromosomes as diploid. The meiosis of this alga seems to take place at the time of conchospore-formation in the mature conchosporangium.

Introduction

*Porphyra yezoensis* UEDA is widely distributed in Japan and is one of the important species of cultivated lavers like *P. tenera* Kjellman. Cytological studies of this alga have already been reported by YABU and TOKIDA. They verified that vegetative and spermatangial cells have three chromosomes, and carpospores are formed without meiosis and have six chromosomes.

As to other species of *Porphyra*, cytological studies were reported by ISHIKAWA, FUJIYAMA and others, and TSENG and CHANG for *P. tenera*. ISHIKAWA, and TSENG and CHANG thought that the meiosis of *P. tenera* takes place after fertilization of carpogonia. DANGEARD also described the same opinion for *P. umbilicalis f. linearis*. On the other hand, FUJIIYAMA and others reported that fertilized carpogonia of *P. tenera* produced carpospores without meiosis like the case of *P. linearis* observed by MAGNE.

Cytological observations on *Conchocelis*-thalli of *Porphyra* have been less studied than on leafy thalli. FUJIYAMA and others suggested that cells of *Conchocelis*-thalli usually have diploid nuclei. However, they did not show the text-figures or photomicrographs to verify them.

In summary, the cytological evidence through all stages of the life-cycle still awaits further studies.

From January 1966 to September 1967, the author carried out cytological studies on various stages of leafy thalli and *Conchocelis*-thalli in *P. yezoensis*.

Before going further, the author wishes to express his sincere thanks to Prof.
Yoshio Kô, Nagasaki University Faculty of Fisheries, for his kind guidance throughout the course of this work.

**Material and Method**

Leafy thalli of *P. yezoensis* were collected on the cultural laver-nets at Kashima, Saga Prefecture, during the winter of 1966 and 1967. *Conchoecis*-thalli which developed from carpospores of these thalli were cultured in vitro.

These natural and cultured materials, growing in different phases and stages, were fixed at various times of night and day throughout the year.

The schedule of acetocarmine squash technique employed in this work is as follows:

Fix the material with alcohol acetic acid (3:1), for 1~12 hr., change fixative after the first hour.
Wash it in running water for about 10 min.
Transfer it to 10% aqueous iron alum for 1 or 2 min.
Wash it in running water for 10 min.
Place it on a slide and add a few drops of acetocarmine.
Heat it up boiling temperature, put a coverslip and repeat boiling twice or thrice.
If overstained, add a drop of 45% acetic acid and heat it gradually.

These temporary preparation were observed after ringing with paraffin. As this method is a simple and rapid one, it was very useful to examine many materials.

**Results**

**Leafy thallus**

*Vegetative cell* The dividing nuclei of vegetative cells are abundant in the dark period, especially in the midnight, and rare in the daytime. However, even in the daytime, the nuclear division frequently occurred in leafy thalli by half to one hour’s immersion in sea water after several hours exposure.

The resting nuclei of vegetative cells are globular in shape and about 1.5-2μ in diameter (Fig.1, A). At the beginning of nuclear division, the nuclei slightly decrease in size. During the prophase the chromatin threads appear in the nuclear cavity (Fig.1, B) and then they become chromosomes themselves (Fig.1, C). In the early metaphase, three chromosomes were clearly observed in each cell (Fig.1, D).

*Formation of spermatium and carpospore* The spermatia are formed in the marginal area of leafy thalli by successive divisions of male cells. In formation of spermatia, the nuclear division seems to occur through the night and daytime.

In the prophase the nucleus of spermatium showed a slight enlargement with differentiation of chromosomes. In the successive process, the metaphase figures showed to have three chromosomes as haploid (Fig.1, E-H).

These cytological phenomena that vegetative and male cells have haploid nuclei had been observed by previous investigators on many species of *Porphyra*.

In this alga, a fertilized carpogonium undergoes successive vertical and transverse divisions to form 8 or 16 carpospores. During the prophase of this process, chromatin threads deep stained by acetocarmine appeared and they grew shorter to become
Fig. 1. Photomicrographs of nuclear divisions in vegetative and spermatangial cells of *P. yezoensis.*
A-D, vegetative cells: A, resting nuclei; B, prophase; C, late prophase; D, metaphase. E-H, spermatangial cells: E, F, four-cell stage, showing some nuclei in metaphase; G, some parts in thirty-two cell stage; H, sixty-four cell stage.

(All figs, × ca 1400)

chromosomes (Fig.2, A). The figures of late prophase and metaphase showed to have six chromosomes in each cell (Fig.2, B, C).

**Germination of monospore** The entire contents of marginal cells of the leafy thalli may be liberated as monospore (neutral spore) in some species of *Porphyra.* In this alga, such spores are formed on young thalli in the autumn, and directly germinate into leafy thalli.

At the germination of these monospores, the nuclei grew larger and became to have many chromatin threads (Fig.3, A, B). Then three chromosomes were counted clearly in the first or second division of the germling (Fig.3, C-F).

**Conchocelis-thallus**

**Germination of carpospore** When carpospores liberated from mature leafy thalli are placed on glass slides, they immediately begin to germinate with elongation of germ-tubes and grow up to filamentous thalli of Conchocelis-phase. Early germlings of the Conchocelis were fixed during the night and stained by acetocarmine squash method.

The amoeboid or spherical carpospores have resting nuclei which are globular in shape and about 1.5-2μ in diameter. After the germ-tube elongated to 3 or more times as long as the diameter of the original spore, the first nuclear division took place in the
Fig. 2. Photomicrographs of nuclear divisions in formation of carpospores in *P. yezoensis*, and the appended sketches.
A prophase of first division; B metaphase in two-cell stage; C side view of cystocarp, showing one nucleus in metaphase; D, surface view of cystocarp, nuclei in resting stage; E-H. appended sketches of the nuclei shown in A-D.
(All figs, × ca 1500)

Fig. 3. Photomicrographs of nuclear divisions in germination of monospores in *P. yezoensis*, and the appended sketches.
A-B, prophase; C, late prophase; D, metaphase, showing three chromosomes; E-F, two-cell germling, one nucleus in metaphase.
(All figs, × ca 1200)
germling. In this nuclear division, the prophase nucleus is usually situated near the base of germ-tube in the original spore (Fig. 4, A). Before the late prophase, chromatin threads appear at the periphery of nucleus, and then the nuclear membrane shrinks away (Fig. 4, B, C). In the metaphase chromosomes were contracted to be shorter and were counted clearly to be six in number (Fig. 4, D, E). In the anaphase two groups of chromosomes tend to be separated from each other and chromosomes in each group are very closely packed together. However, occasionally six chromosomes moving toward the pole were counted (Fig. 4, H, I). In the telophase, while one daughter nucleus remains in the original spore, the other nucleus moves toward the center of germ-tube (Fig. 4, J), and finally the segment is formed near the root of the germ-tube.

Branch of Conchocelis Narrow branches of the Conchocelis have very small nuclei which are spherical or cylindrical in shape, measuring about 1 μ in diameter (Fig. 5, A, B). In these narrow branches, the details of nuclear division could not be observed distinctly. However, broader branches of the Conchocelis have somewhat larger nuclei. The observation of nuclear division in these branches showed that vegetative growth of Conchocelis-filaments is made by mitotic division. In this case, six chromosomes were also observed in the metaphase (Fig. 5, D-E).
Photomicrographs of nuclear divisions in branches of *Conchocelis*-thalli in *P. yeozensis*, and the appended sketch. A-B, resting nuclei of narrow cells; C, prophase in a broader cell; D-E, metaphase, showing six chromosomes. (All figs. × ca 1400)

**Immature conchosporangium**   As to sexual reproduction of the *Conchocelis* thallus, **Drew** has shown "fertile cell-rows" and "plantlets" in the shell-living *Conchocelis*. Thereafter, **Migita** demonstrated that the "fertile cell-rows" and "plantlets" are essentially the same reproductive organs which are conchosporangial branches in the *Conchocelis*-thallus of *Porphyra*. These conchosporangial branches are also formed on the free-living *Conchocelis* grown *in vitro*.

The first attempts regarding the nuclear division of conchosporangial cells were made using the free-living *Conchocelis* with immature sporangia cultured at higher temperatures of 26-27°C. At such high temperatures, the *Conchocelis* of *P. yeozensis* can produce new sporangial cells, but cannot discharge conchospores from them.

The conchosporangial cells each have a single spherical nucleus measuring 1.5-2μ in diameter, and they are connected together by strands of cytoplasm (pit-connection) which are well stained with acetocarmine (Fig.6, A-C). In the prophase of nuclear division, thin chromatin threads and a nucleolus appeared in the nuclear cavity (Fig.6, D, E). The threads gradually become shorter and thicker and then they give rise to chromosomes. In the metaphase, the nuclear membrane and the nucleolus disappeared and six chromosomes could be counted (Fig.6, F-I). The same number of chromosomes was also counted in favourable figures of the anaphase (Fig.6, J-M).

**Mature conchosporangium**   Another experiments were carried out using the mature *Conchocelis* cultured at the temperature of about 20°C. According to the observation of **Migita** and **Abe**, at such temperature, conchospores are formed by division of cell-contents in each sporangium and then they are liberated within the following few days.

The nuclear division of mature sporangium may be distinguished from that of other stages in the life-cycle of this alga. In the beginning of this nuclear division, six chromonemata appeared in pairs and two chromonemata of each pair became very inti-
mately associated. This phase may be considered as the diploten stage. At the diakinesis stage three bivalent chromosomes could be seen (Fig. 7, A, F), and then they separated into two sets which began to move apart toward the opposite poles of the cell (Fig. 7, B-D, G-I). After the second division following the first one, four nuclei are usually formed in each sporangium (Fig. 7, E, J). However, frequently two conchospores are formed in a single sporangium. In this case, the meiosis seems to occur without the second division.

*Germination of conchospore* Conchospores liberated from the Conchocelis show ameboid shape at first, then after adhered to the substratum they become spherical and directly germinate to leafy thalli. In this process, the liberated conchospore has one nucleus and one nucleolus (Fig. 8, A). At the germination of the conchospore, the nucleus grows larger and becomes to contain many chromatin threads in its cavity (Fig. 8, B). The

Fig. 6. Photomicrographs of nuclear divisions in immature conchosporangial cells of *Porphyra yezoensis*, and the appended sketches,
A-B, conchosporangial branch, showing strands of cytoplasm; C, resting nuclei; D-E, prophase, showing chromatin threads and a nucleolus; F-I, metaphase, showing six chromosomes; J-M, anaphase.
(A-B, × ca 800; C-M, × ca 1500)
metaphase figures in the first cell division of the germling showed the appearance of three chromosomes in each cell (Fig.8, C-E).

**Discussion**

The cytological evidence that the vegetative cells of leafy thalli have haploid nuclei had been presented by many previous investigators; ISHIKAWA for *P. tenera*, DANGEARD for *P. umbilicalis*, MAGNE for *P. linearis*, TSENG and CHANG for *P. tenera*, FUJIYAMA and others for *P. tenera*, KRISHNAMURTHY for *P. umbilicalis*, etc. In these works, ISHIKAWA, DANGEARD, and TSENG and CHANG have supposed that meiosis of Porphyra takes place in carpogonia after fusion of male and female nuclei. Recently, YABU and TOKIDA, and KITO have demonstrated that the carpospores are produced without meiosis and have diploid chromosomes, but they did not describe the occurrence of meiosis.

From the observations in this study, it is clear that leafy thalli of *P. yezoensis* have three chromosomes in vegetative and spermatangial cells, and have six chromosomes in fertilized carpogonia. Accordingly the haploid chromosome number of this alga is three. This result is closely similar to that obtained by YABE and TOKIDA.
Fig. 8. Photomicrographs of nuclear divisions in germination of conchospores in *P. yeoensis*, and the appended sketches. A, a conchospore, have a resting nucleus; B, prophase; C, metaphase, showing three chromosomes; D-G, early anaphase; H, telophase; I-J, two-cell germling, a nucleus in anaphase.

(All figs, × ca 1600)

Fig. 9. Diagram showing the nuclear phases in the life-cycle of *P. yeoensis*. 
On the other hand, in germination of carpospores and in branching of the Conchocelis, the nuclear divisions of this alga occur to be mitotic and to have six chromosomes in the metaphase. The nuclear divisions in the formation of conchosporangium are also mitotic. However, the observations suggest that meiosis takes place at the time of spore-formation in the conchosporangium. Therefore, the conchospores somewhat resemble to tetraspores of Florideae in spore-formation.

As to the formation of conchospore, Migita and Abe observed that the contents of a sporangium were divided to 2, 4 or more spores, which were discharged within one or two days after their division. These morphological findings appear to be favorable for the cytological evidence of this study.

Furthermore, it is clear that the germling developed from the conchospore has three chromosomes before the first cell division.

Juding from these observations, the alternation of nuclear phases in the life-cycle of P. yeoensis may be shown as Fig. 9; the vegetative cell, monospore, spermatangium and conchospore have haploid nuclei, whilst the fertilized carpogonium, carpospore, Conchocelis-branch and conchosporangial cell have diploid nuclei; the meiosis takes place in the cell division of conchospore-formation.

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