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The life history of *Acrothrix pacifica* OKAMURA et YAMADA (Phaeophyta, Chordariales) in culture

Tetsuro AJISAKA

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The heteromorphic and haplo-diplontic life history of *Acrothrix pacifica* from Wakasa Bay, Japan Sea, has been completed in culture. Zoospores from unilocular sporangium of macroscopic sporophyte developed into microscopic haploid gametophytes (n=8-14). Under warmer conditions, they grew into dense tufts. However, characteristic erect filaments arose out of the smaller tuft under cooler conditions. Conjugation between gametes from uni- or bi-seriate plurilocular sporangia (gametangia) of the gametophyte was isogamous. Zygotes developed into macroscopic diploid sporophytes (2n=14-19). Unfused gametes germinated asexually and developed into gametophytes, repeating the same gametophytic generation under warmer conditions. Under cooler conditions, most of unfused gametes developed parthenogenetically into haploid sporophytes (n=8-14).

Tetsuro Ajisaka, Department of Fisheries, Faculty of Agriculture, Kyoto University, Kyoto, 606 Japan.

Acrothrix pacifica OKAMURA et YAMADA taxonomically belongs to the Acrotrichaceae of Chordariales, Phaeophyta (INAGAKI 1958). It is commonly distributed in Japan along both the coasts of Pacific and the Japan Sea. However, the southern region of the Pacific coast is an exception. This species which is used as tasteful food in Japan is epiphytic on *Chorda filum* (LINNAEUS) STACKHOUSE.

In ARASAKI's study (1948), based on the materials from Mikawa Bay facing the Pacific Ocean, the gametophytes had a dormant stage in hot summer months and arose 'bamboo-like' erect filaments out of them in autumn when sea-water temperature lowered.

In this paper, some observations on the life history, the karyology, the characteristic morphology of gametophyte and haploid sporophyte of *Acrothrix pacifica* from Wakasa Bay facing the Japan Sea are reported.

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Materials and Methods

The sporophytes of *Acrothrix pacifica* were collected at Takahama in Wakasa Bay facing the Japan Sea during the summer of 1976. The plants were found growing epiphytically on *Chorda filum* (LINNAEUS) STACKHOUSE which grew on rocks of one or two meters below the low tide mark.

Cultures were incubated in 1500-3000 lux light under the following temperature-photoperiod regimes. 20°C : 16-$\overline{8}$hr (Set 1); 20°C : 10-$\overline{14}$hr (Set 2); 15°C : 14-$\overline{10}$hr (Set 3); 15°C : 10-$\overline{14}$hr (Set 4); 10°C : 14-$\overline{10}$hr (Set 5); 10°C : 10-$\overline{14}$hr (Set 6); 5°C : 10-$\overline{14}$hr (Set 7).

Culture techniques and medium prescriptions as given by NAKAMURA and TATEWAKI (1975) were used.

For the karyological observations, aceto-iron-haematoxylin-chloral hydrate method (WITTMANN 1965) was employed.

Results

Cultures from zoospores of the sporophytic fronds in nature were started on June 1, 1976. The fertile fronds in nature bore only unilocular sporangia from June to July. Mature unilocular sporangia were usually elongated obovoid, measuring 44–66×25–41 (32×55 at an average) μm.

Zoospores (5.8–10.2×3.8–5.8 μm in size) from the unilocular sporangium were pear-

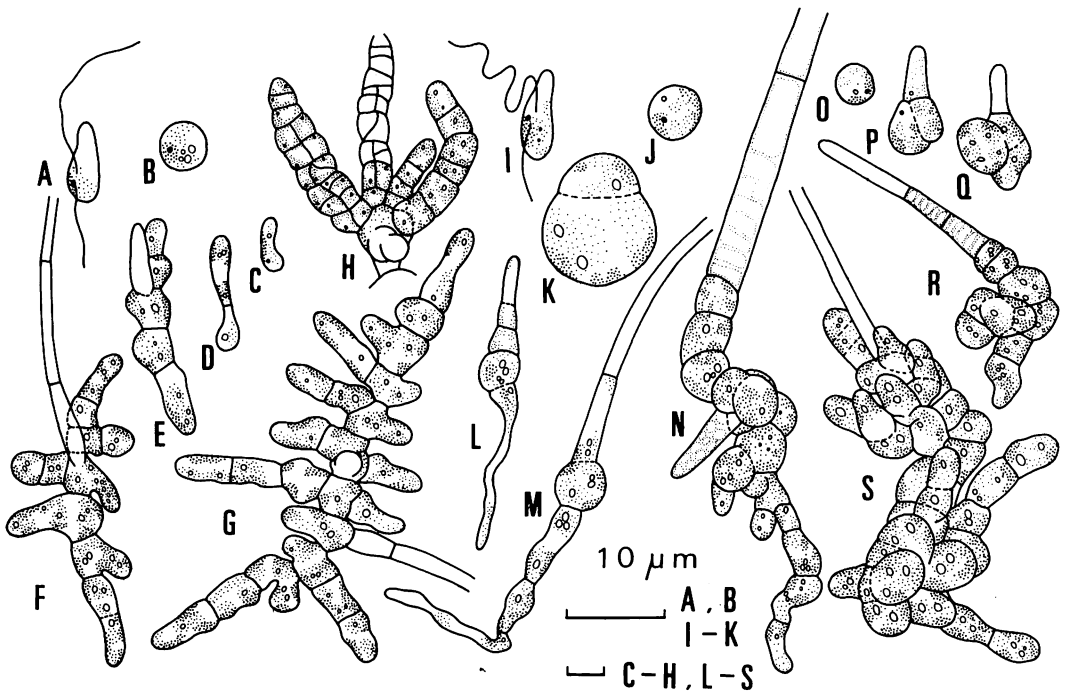

Fig. 1. *Acrothrix pacifica*. Developmental stages of zoospores, zygotes, and unfused gametes.

A: Zoospore. B: Settled zoospore. C, D: 2-day-old gametophytes in Set 1. E, F: 8(E)-, and 10(F)-day-old gametophytes in Set 6. G: 8-day-old gametophyte in Set 1. H: Gametangia in Set 7. I: Gamete. J: Settled gamete. K: Settled zygote. L–N: 5(L)-, 8(M)-, and 13(N)-day-old diploid sporophytes in Set 6. O–S: 2(O)-, 5(P, Q)-, 7(R)-, and 10(S)- day-old haploid sporophytes in Set 4.

Fig. 2. *Acrothrix pacifica*. Stages in development from gametophyte, through zygotic stage, to fertile sporophyte, and chromosome observations.

A: 28-day-old gametophyte (dense tuft) in Set 1. B: 25-day-old gametophyte (crust-like germling) in Set 2. C: 25-day-old gametophyte (small tuft with erect filaments) in Set 6. D: 80-day-old gametophyte in Set 7. E–G: Erect filament of 50-day-old gametophyte in Set 6. Upper portion (E), middle portion (F), and lower protion (G). H, I: Releasing gametes from gametangia of 80-day-old gametophyte in Set 7. J: Emptied gametangia with settled gametes and a zygote. K: 44-day-old sporophyte in Set 6. L: 34-day-old sporophyte in Set 4. M: 2-month-old cylindrical frond (sporophyte) in Set 7. N: Unilocular sporangia of 2-month-old sporophyte in Set 4. O–Q: Chromosome number in gametophyte and sporophytes. Haploid gametophyte, n=9 (O), diploid sporophyte, 2n=16 (P), and haploid sporophyte, n=9 (Q). R: Sporophyte with haploid chromosome (arrows).

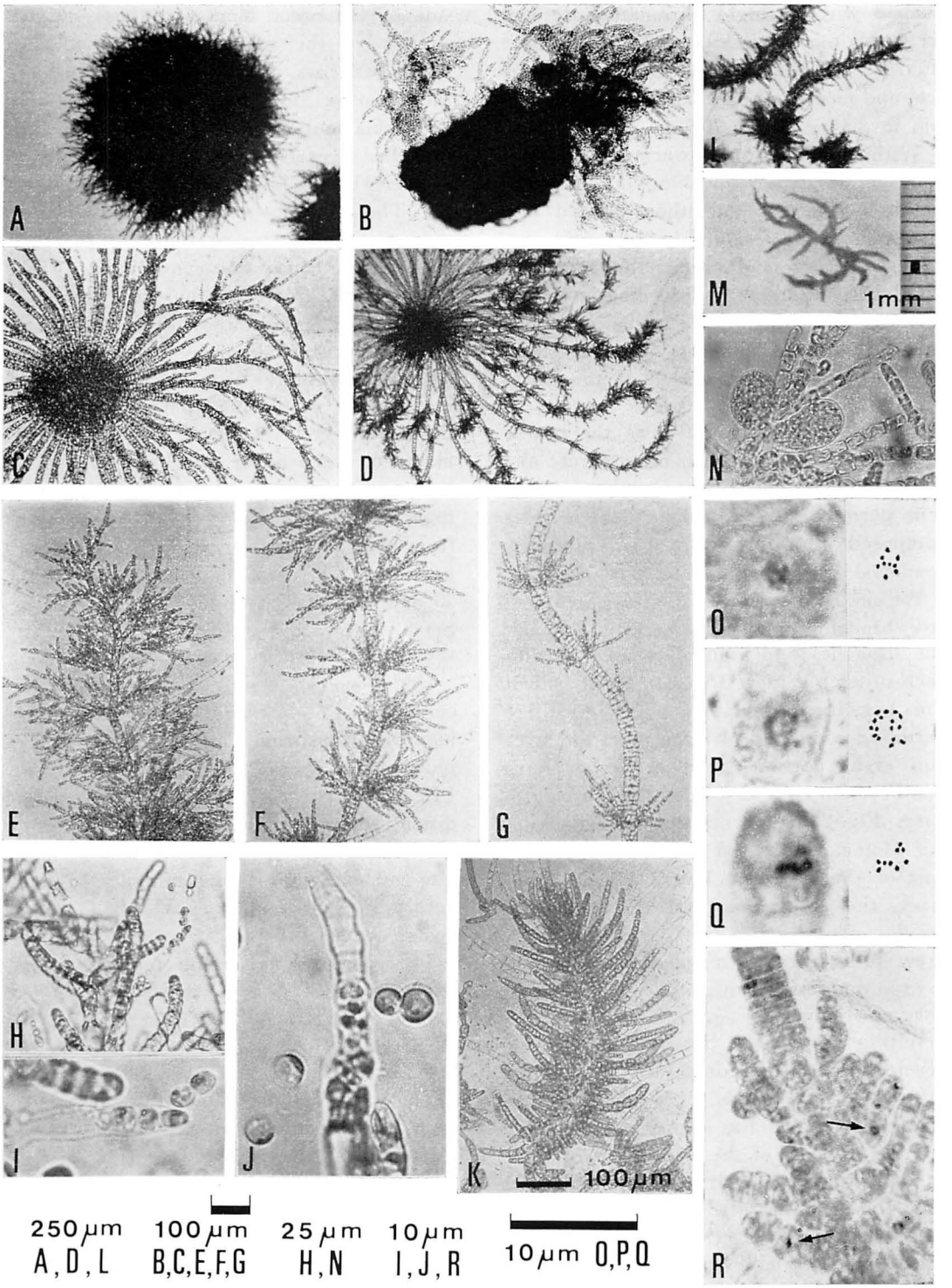
A
B
C
D
E
F
G
H
I
J
K
100μm
L
M
1mm
N
O
P
Q
R
250μm
A, D, L
100μm
B, C, E, F, G
25μm
H, N
10μm
I, J, R
10μm
O, P, Q

Fig. 2.

shaped with a single chromatophore and an eyespot, and were laterally biflagellated (Fig. 1 A). Settled zoospores became spherical and measured 4.4–7.6 (5.6 at an average) μm in diameter (Fig. 1 B).

Within 1–2 days under warmer conditions, they germinated by pushing out a protuberance (Fig. 1 C) and then divided into two cells transversely (Fig. 1 D). By successive transverse divisions, the germlings developed initially into a creeping uniseriate filament consisting of 5–10 cells, and then branched laterally. And in this stage, hyaline hairs were produced on the germlings (Fig. 1 E, F).

Within 2 weeks in Set 1, as the results of an extensive formation of prostrate and upright branching (Fig. 1 G), the filamentous germlings developed into dense tufts composed of large basal layer and profusely branched erect portion (Fig. 2 A).

Within 10 days in Set 2, after the extensive branching of the prostrate filament, the branches and branchlets cohered with each other. Within 3 weeks, about half of the germlings developed into crust-like germling composed of large prostrate layer and erect portion, cells of which were undifferentiated and formed cellular aggregates (Fig. 2 B). Several rhizoid-like creeping filaments gave off from the marginal part of the prostrate layer. On the contrary, the another half of the germlings developed into dense tufts, some of which grew into larger hemispherical ones (ca. 1 cm in diam.) without producing the reproductive organs.

Within 18 days in Set 6, the germling developed into a comparatively smaller tuft, and then arose many larger erect filaments out of the center of the tuft (Fig. 2 C). Cells of the erect filament were characterized by their larger dimension than those of the tuft. The erect filament was consisted of uniseriate cylindrical cells, which were variable in size, 20–30 μm in dimension. Cells of the upper portion of the erect filament were generally smaller than those of the lower one. As branching was at first continued to form on the one side of the erect filaments, these curved slightly in the upper portion (Fig. 2 C). Later, branches were profusely formed on the opposite side or on all sides of the erect filament (Fig. 2 D). The filament branched densely in its upper portion and sparsely in its lower portion (Fig. 2 E, F, G). The cells of the branch and branchlet were similar in size to those of the tuft containing dense chromatophores. The mode of the growth of the erect filaments was sympodial, and they grew 0.5–1.0 cm in height within 3 months in Sets 5 and 6. The cell-row of the erect filaments resembled to that of the medullary filaments of the sporophyte. However, since the erect filaments were never enveloped by gelatinous substances and did not cohered together, these filaments could be separated from each other just by applying some pressure. The erect filaments arose within 20 days in Sets 3 and 4, within 22 days in Set 5, and within 30 days in Set 7. But they never arose in Sets 1 and 2.

Under cooler conditions, most of the cells of the branch and branchlet of erect filaments transformed into uni- or bi-seriate plurilocular sporangia (gametangia) (Fig. 1 H, 2 H). However, under warmer conditions, only the upper parts of the profusely branched erect filaments transformed into gametangia, although these were superficially similar to those formed under cooler conditions.

The gametophyte became mature within one month in Sets 1 and 2, within 2 months in Sets 3 and 4, within 2–3 months in Sets 5 and 6, and within 3 months in Set 7.

The gametophytes (dense tufts in Set 1, crust-like germlings in Set 2, and erect filaments in Sets 3, 4, 5, 6 and 7) carried 8–14 chromosomes, indicating that they were in the haploid phase (Fig. 2 O).

The size of gametes (5.7–10.2$\times$3.0–5.2 μm in size) was quite similar to that of the zoospores (Fig. 1 I). Gametes were promptly released from gametangia when transferring from dark to light photoperiod regime, but their swimming was not active

(Fig. 2 I). Usually, the gametes did not fuse, and germinated directly. However, under cooler conditions (Sets 5, 6 and 7), sexual conjugation was sometimes observed between the gametes. Sexual plants bearing gametangia were all similar superficially. Two swimming gametes accidentally fused on their heads, and then settled on the substratum, becoming spherical. The conjugation was isogamous. However, a larger gamete sometimes fused with a smaller one (Fig. 2 J).

A naked zygote with two eyespots soon formed a cell wall, and increased its size within 2–3 days, becoming 7.8–15.0 (11.9 at an average) μm in diameter (Fig. 1 K).

Zygotes germinated usually by pushing out a protuberance on their one side and then issued a rhizoid-like cell on their another side (Fig. 1 L, M). In the later stage, the protuberance transferred into a hair of the plant and the rhizoid-like cells developed into creeping filaments which constituted the primary base of the plant. The original zygote divided into several cells to become an uniseriate filament, which later developed into a monosiphonous central axis (Fig. 1 N). Each cell of the central axis divided to give rise to primary assimilating filaments. And then, some basal cells of the primary assimilating filament formed a medullary layer (Fig. 2 K). The zygotic germling grew trichothallically, developing into a cyclindrical plant, which was filiform, somewhat cartilaginous, as in the plants growing in sea (Fig. 2 L).

Within 3 months in Set 6, the cylindrical plant branched laterally and grew 5–10 mm in height, and about 1 mm in thickness (Fig. 2 M).

Within one month in Sets 1 and 2 within 2 months in Sets 3 and 4, and within 3–4 months in Sets 5, 6 and 7, the cylindrical plant bore many ovoidal unilocular sporangia (40–55×25–42 μm in size) and released zoospores (Fig. 2 N). The zoospores germinated to develop into haploid gametophytes under all conditions, and their conjugation had never been observed, as mentioned above.

The sporophytes carried 14–19 chromosomes, indicating that they were in the diploid phase (Fig. 2 P).

Unfused gametes settled on the substratum and became spherical, measuring 3.0–7.6 (5.6 at an average) μm in diameter (Fig. 1 J). Under cooler conditions, most of the unfused gametes increased their size for 2–3 days before germination as in zygotes (Fig. 1 O). And they grew into sporophytes (Fig. 1 P, Q, R, S). Although their developmental modes had not been distinguished from those of diploid sporophytes, these sporophytes carried 8–14 (of which 50% was 9) chromosomes, indicating that they were in the haploid phase (Fig. 2 Q, R). On the other hand, under warmer conditions (Sets 1 and 2), most of unfused gametes directly germinated and developed into haploid gametophytes, repeating the same generation. Under moderate conditions (Sets 3 and 4), the unfused gametes developed into sporophytes more in number than gametophytes.

When the one-celled germlings of unfused gametes under cooler conditions (Sets 5, 6 and 7) were transferred into warmer conditions (Sets 1, 2, 3 and 4), they germinated to produce a hair and rhizoid-like cells, which developed later in haploid sporophytes. However, these sporophytes grew smaller than normal diploid sporophytes and decayed within one month. On the contrary, when those of unfused gametes under warmer conditions (Sets 1 and 2) were transferred into cooler conditions (Sets 5, 6 and 7), they germinated by pushing out a protuberance and developed into haploid gametophytes again.

The haploid sporophytes bore unilocular sporangia as in the case of diploid sporophytes and released many zoospores. Each of these zoospores developed into a haploid gametophyte.

Discussion

ARASAKI (1948) studied the life history of *Acrothrix pacifica* from Mikawa Bay

facing the Pacific Ocean. He reported that zoospores from unilocular sporangia developed into branched prostrate filamentous thalli (gametophytes) and that the gametophytes stopped their growth and had a dormant stage in hot summer months. When sea-water temperature lowered, they regained their growth and arose the characteristic small 'bamboo-like' thalli. Upper branches and all of the branchlets were transformed into the uni- or bi-seriate plurilocular sporangia (gametangia). The eyespot of the smaller gametes he had observed were absent. Sexual reproduction was observed: The zygote with 4 flagella and 2 chromatophores and one eyespot, grew into a sporophyte. However, the sporophyte he had observed had never matured under the culture conditions given by him. Development of unfused gametes had not been described in his report.

In this study, the knowledge on the sequence of the complete life history of *Acrothrix pacifica* from Wakasa Bay facing the Japan Sea has been established under culture conditions. The zoospores released from unilocular sporangia developed into filamentous germlings. Under warmer conditions, they grew into dense tufts. On the contrary, under cooler conditions, the characteristic erect filaments (described as 'bamboo-like' thalli by ARASAKI 1948) arose out of the small tuft. The mode of the growth of the erect filaments was sympodial and it developed polysiphonously. The occurrence of these erect filaments was obviously induced by lowering the temperature of water. The dense tufts under warmer conditions and the small tufts under cooler conditions were superficially similar to the gametophytic thalli in the members of Chordariaceae (e.g. *Sphaerotrichia divaricata*, AJISAKA and UMEZAKI 1978). However, the erect filament has not been found in any other species of Chordariales.

In this study, male and female plants with gametangia were both similar superficially. Sexual conjugation was isogamous and one eyespot was observed in both male and female gametes.

Under cooler conditions, zygote-like spores with or without eyespots were sometimes observed. These spores developed into thalli which were not distinguished superficially from diploid sporophytes. As these thalli possessed half of the chromosome number of diploid sporophytes, they were considered to be haploid sporophytes. When they attained maturity, unilocular sporangia were formed and released haploid zoospores without undergoing meiotic divisions. All of these zoospores developed into gametophytes.

A karyological study of haploid sporophyte in the Chordariales has not been reported, so far as the writer knows. However, parthenogenesis of swarmers or eggs released from gametophyte and their karyological studies were reported in Ectocarpales (*Ectocarpus siliculosus*, MÜLLER 1966, 1967), in Scytosiphonales (*Scytosiphon lomentaria*, and others, NAKAMURA and

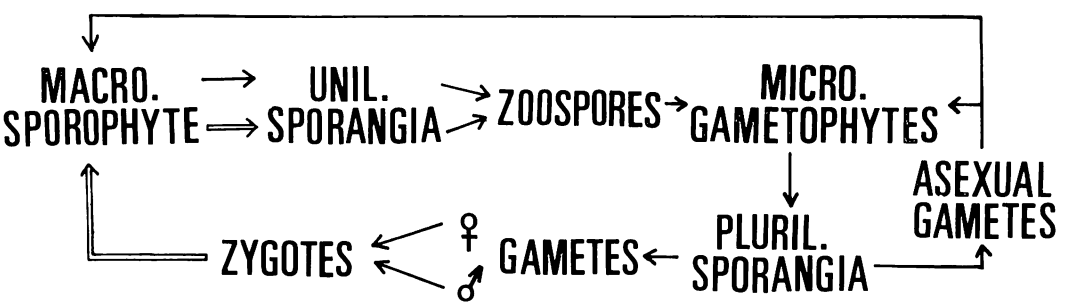

Fig. 3. A diagram of the life history of *Acrothrix pacifica*.
Black line: haploid White line: diploid

TATEWAKI 1975) and in Laminariales (*Alaria crassifolia*, NAKAHARA and NAKAMURA 1973). In *Ectocarpus siliculosus* (MÜLLER 1966), most of unfused gametes were degenerated within one day (2–3 cells stage) and a few gametes developed parthenogenetically into haploid sporophytes or gametophytes. MÜLLER has not mentioned as to whether parthenogenesis of gametes were induced by lowering of temperature or not. However, he has reported that some zoospores were released from unilocular sporangia and developed parthenogenetically into haploid sporophytes, when cultured in cooler condition (13±1°C).

From this study, most of the sporophytes under cooler conditions seemed to be haploid. And the occurrence of haploid sporophytes were induced at their one-cell stage by lowering the temperature of water (5–10°C).

Although the number of chromosome of this species showed a rather wide range (from 8 to 14 in haploid stage and from 14 to 19 in diploid stage), they seemed to fit the pattern for Chordariales (AJISAKA and UMEZAKI 1978).

The present results confirmed that the life history of this species consists of an alternation of heteromorphic generations (Fig. 3). Moreover, the development of haploid sporophytes from unfused gametes as induced by giving under cooler conditions has been clearly traced.

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鰺坂哲朗： 培養によるニセモヅク（褐藻類ナガマツモ目）の生活史の研究

日本海若狭湾産の褐藻ニセモヅクの生活史を室内培養によって完結した。自然に生育する胞子体の単子嚢から放出された遊走子は，そのまま発芽して顕微鏡的な単相（n＝8～14）の配偶体になる。この配偶体は高温では密に分枝した叢状発芽体になり，低温では小さな叢状発芽体から多くの特徴的な直立枝が発出する。配偶体の複子嚢（配偶子嚢）から放出された配偶子の間で，接合が行われる。その接合子は，発芽して肉眼的な複相（2n＝14～19）の胞子体になる。接合しなかった配偶子は，そのまま無性的に発芽して，高温では再び配偶体となり，その世代を繰り返す。一方，低温では，単相（n＝8～14）の胞子体になる。

（606 京都市左京区北白川追分町 京都大学農学部水産学教室）