LIFE HISTORY OF *ACROTHRIX PACIFICA* AND *SPHAEROTRICHIA DIVARICATA* IN LABORATORY CULTURES

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ABSTRACT

The life histories of *Acrothrix pacifica* Okamura & Yamada (Acrothricaceae) and *Sphaerotrichia divaricata* (Ag.) Kylin (Chordariaceae) in the Chordariales, Phaeophyceae were studied in the laboratory. Both species showed an alternation of macroscopic sporophyte (2n) and microscopic gametophyte (n).

In *A. pacifica,* unfused gametes developed into haploid sporophytes under cooler conditions or into gametophytes under warmer conditions. In *Sphaerotrichia pacifica,* unfused gametes developed into gametophytes under warmer conditions.

INTRODUCTION

The Chordariales in Japan contains four families and seventeen genera: Leathesiaceae (*Petrospongium, Cylindrocarpus, Leathesia*), Chordariaceae (*Chordaria, Cladosiphon, Eudesme, Haplogloia, Heterosaudersella, Myriogloia, Analipus, Papenfusiella, Pseudochorda, Sauvageaugloia, Saundersella, Sphaerotrichia, Tinocladia),* Spermatochnaceae (*Nemacystis*), and Acrothricaceae *(Acrothrix).* Most of the genera and species in this order are distributed in the northern part of Japan, except for *Cladosiphon okamuranus,* a locally edible species which grows in the subtropical waters of Okinawa and Amami Islands. Other edible species such as *Nemacystis decipiens, Tinocladia crassa, Sphaerotrichia divaricata,* and *Acrothrix pacifica* are distributed along the Pacific Ocean and Japan Sea coasts in Honshu and western and northern Kyushu. These five species have been used as "sea vegetables" in Japan.

The life histories of *N. decipiens* and *T. crassa* have been studied by Yotsui (1975, 1980), Yotsui and Migita (1974) and Yotsui (1978, 1979a, b), respectively. Shinmura (1974, 1975, 1976, 1977a, b) and Shinmura and Yamanaka (1974a, b) studied the life history and ecology of *C. okamuranus*. Based on these studies, *N. decipiens* and *C. okamuranus* are now artificially cultivated in the sea to increase production.

Since an understanding of the life history is fundamental to the cultivation of algal species in the sea, a study was conducted on the life history of *A. pacifica* and S. *divaricata* in the laboratory. In laboratory cultures, the life cycles of these two species were previously found to be heteromorphic, with an alternating macroscopic sporophyte and microscopic gametophyte (Ajisaka 1979; Ajisaka and Umezaki 1978). Furthermore, under given conditions, the sporophytic and gametophytic stages were alternately repeated without producing any further generations.

MATERIALS AND METHODS

The sporophytes of *A. pacifica* and *S. divaricata* were collected at Takahama in Wakasa Bay, which is in the middle part of Honshu facing Japan Sea. The fertile sporophytes bearing unilocular sporangia were collected during the early summer months of June and July. The isolation of zoospores discharged from the sporophyte was done by the micropipette method. The culture medium used in this study was Provasoli's ES medium. Cultures were illuminated with cool white flourescent lamps (ca. 1500-3000 lux) and were incubated under the following temperature-photoperiod regimes: 20°C: 18-6 hr (set l); 20°C: 10-14 hr (set 2); 15°C: 14-10 hr (set 3); 15°C: 10-14 hr (set 4); 10°C:14-10hr(set5). For the *A. pacifica* cultures, there were two additional regimes: 10°C: 10-14 hr (set 6); 5°C: 10-14 hr (set 7).

RESULTS AND DISCUSSION

Acrothrix pacifica

The unilocular sporangia of *A. pacifica* are elongated obovoid 44-66 x 25-41 (32 × 55 average) urn in size. Zoospores discharged from the unilocular sporangium are pear-shaped, with a single chromatophore and an eyespot, and laterally biflagellated. Soon after settling on the substratum (glass slide), zoospores became spherical with a diameter of 4.4-7.6 (5.6 average) urn.

The settled spores germinated and developed into creeping uniseriate filaments on which hyaline hairs were produced. Under warmer conditions (sets 1 and 2, 20C), the creeping filaments developed into prostrate and upright systems forming dense tufts. Under cooler conditions (sets 5 and 6, 10"C), the creeping filaments developed into comparatively smaller tufts and produced many larger erect filaments from the center. Each erect filament profusely branched off on the opposite side or on all sides. At 10°C, most of the cells of the branches and branchlets of erect filaments were transformed into uni- or biseriate plurilocular gametangia. The gametophytes mature within 2-3 months under set 5 (10°C: 14-10 hr) and within 3 months under set 6 (10°C: 10-14 hr).

The gametes from the gametangium are pear-shaped, measuring 5.7-10.2 x 3.0-5.3 μm. Under cooler conditions (sets 4-7, 10-15°C), gametic conjugations (usually isogamous, rarely anisogamous) were observed. The fused gametes settled on the substratum (glass slide) and soon became spherical. The settled zygote germinated to produce filament that later developed into a monosiphonous central axis. Each cell of the central axis divided giving rise to a primary assimilating filament. Then some basal cells of the primary assimilating filaments divided to produce a medullary layer which grew trichothallically into a cylindrical plant. Under cooler conditions (5-10°C), the cylindrical plant branched off laterally and after 3 months, it matured producing many unilocular sporangia which released zoospores. The branched fertile plants are similar in habit to sporophytes from the sea.

The gametophytes derived from zoospores of sporophytes carry 8-14 chromosomes while the sporophytes from zygotes of cultured gametophytes have 14-19 chromosomes (Table 1). This indicates that the sporophytes are diploid and the gametophytes are haploid. Unfused gametes germinated into sporophytes under cooler conditions (10°C). The sporophytes have 8-14 (of which 50% has 9) chromosomes and, therefore, seemed to be haploid. The haploid sporophytes bore unilocular sporangia and released zoospores. The zoospores directly germinated and gave rise to haploid gametophytes. On the other hand, under warmer conditions (sets 1 and 2, 20°C), most of the unfused gametes germinated into haploid gametophytes, repeating the same generation. Under moderate conditions (set 3 and 4, 15°C), the unfused gametes germinated into sporophytes (more in number than gametophytes). When one-celled germlings of unfused gametes were cultured under cooler conditions (10-15°C), they germinated into sporophytes which, however, grew smaller than normal diploid sporophytes and died within a month. On the other hand, when the unfused gametes under warmer conditions (10-15°C) were transferred into cooler conditions (5-10°C), they again germinated into gametophytes.

This study indicates that the life history of *Acrothrix pacifica* is morphologically an alternation of macroscopic sporophytes with microscopic gametophytes or, karyologically, a diploid sporophyte alternates with a haploid gametophyte. Furthermore, unfused gametes developed into haploid sporophytes under cooler conditions and into gametophytes under warmer condi-

Table 1. Chromosome number of *Acrothrix pacifica*

Karyology:	diploid	haploid	haploid
Plant:	sporophyte from zygote in culture	gametophyte from zoospore in culture	sporophyte from unfused gamete in culture
Chromosome number:	14-19	8-14	8-14 (50% is 9)

tions. This culture study also suggests that the sporophytes prefer the cooler waters of winter and spring. On the other hand, gametophytes, which have not yet been found in the sea, prefer the warmer waters of summer and autumn (Fig. 1).

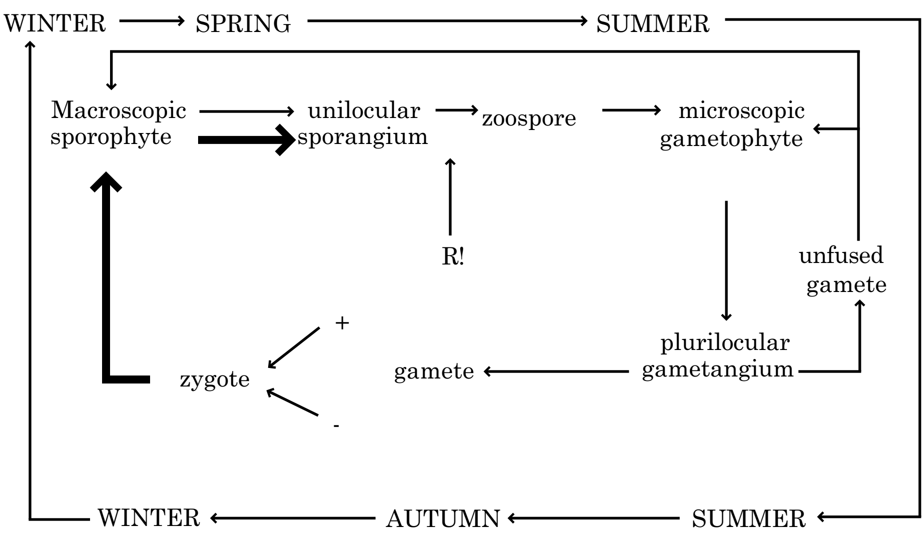

Fig. 1. Life history of *Acrothrix pacifica* (—haploid, ▬ diploid, R! meoisis).

Sphaerotrichia divaricate

S. divaricata grows epiphytic on *Sargassum confusum* and S. *pilulif-erum* or on rocks l-2 m below low tide mark. The fertile sporophytes have unilocular sporangia. The sporangia are elongated obovoid measuring 61-75 x 26-41 urn. Zoospores discharged from the sporangium are pear-shaped with a single chromatophore and an eyespot 4.7-6.6 x 2.8-3.8 µm in size, and laterally biflagellated. Soon after settling on the substratum (glass slide) they became spherical with a diameter of 2.8-4.8 µm. Later, they germinated and developed into creeping filaments. They branched laterally, forming dense tufts composed of prostrate and upright systems. Under warmer and long-day conditions (set 1, 12°C: 16-18 hr), the upper parts of some erect filament were transformed into uniseriate plurilocular gametangia within 13 days. However, under set 2 conditions of 20°C: 10-14 hr, the tufts were larger (over 1 cm dia.) and never bore plurilocular gametangia even after 2 months. Under cooler (10°C) and long-day (14-10 hr) conditions, 8-day old filaments which are usually provided with hairs, developed into simple tufts composed of small prostrate and sparsely branched erect systems. Within 20 days they matured and farmed plurilocular gametangia. Under moderate conditions (set 3, 15°C: 14-10hr; set 4, 15°C: 10-14 hr) two types of tufts were formed: dense tufts at 20°C (sets 1 and 2), and simple ones at 10°C (set 5). These two types were formed at nearly equal rates and matured and started liberating gametes within 18 days.

The gametes discharged from the gametangium were morphologically similar to zoospores, laterally biflagellated, and measured 4.7-6.2 x 3.3-5.2 urn in size. The discharge of gametes was induced when cultures were transferred from dark to light. The conjugation between gametes was isogamous or rarely anisogamous. While the rate of the gametic conjugation was 70-80% under cooler conditions (set 5, 10°C), the process scarcely occured under warmer conditions (sets 1 and 2, 20°C) and most of the gametes germinated without fusion and each developed into a gametophyte, repeating the same generation.

The zygotes became spherical and soon germinated to develop into cylindrical plants consisting of assimilating filaments, cortical and medullary layers. Under cooler conditions (set 5, 10°C: 14-10 hr), the erect cylindrical plants branched off and grew larger, with a habit similar to that of sporophytes in the sea.

Unfused gametes germinated and developed into gametophytes. The gametophytes grew faster and became fertile earlier under warmer and long-day conditions (set 1, 20°C: 16-18 hr) than under cooler and short-day conditions (set 6, 10°C:10-14 hr; set 7, 5°C: 10-14 hr.).

The chromosome number of sporophytes from the sea was 23-30 and that of gametophytes derived from zoospores was 7-17. Eighty percent of the gametophytes, however, had a chromosome number of 9-12 (Table 2). Although the sporophytes derived from conjugated gametes in laboratory culture had 15-27 chromosomes, 90% of them had 18-24.

This culture study has demonstrated that cooler conditions favor sporophyte growth while warmer temperatures induce sporophytes to produce unilocular sporangia earlier. Moreover, the study has confirmed that the life history of *S. divaricata* is heteromorphic, an alternation of diploid macro-

Table 2. Chromosome number of *Sphaerotrichia diuaricata*

Karyology:	diploid	haploid	diploid
Plant:	sporophyte from sea	gametophyte from zoospore in culture	sporophyte from conjugated gametes in culture
Chromosome number:	23-30	7-17 (80% is 9-12)	15-27 (90% is 18-24)

scopic sporophytes and haploid microscopic gametophytes. Meiosis may occur during the formation of zoospores in the unilocular sporangium on the diploid sporophyte (Fig. 2).

The results of culture experiments also suggest that although gametophytes have not yet been found in the sea, both gametophytes and sporophytes are present. In summer, gametophytes grow well and mature faster. Most of the gametes germinated without conjugation, repeating the same gametophytic generation. While the rate of sexual conjugation increases from autumn to winter when seawater temperature drops, the sporophytes derived from zygotes develop well into branched macroscopic fronds during

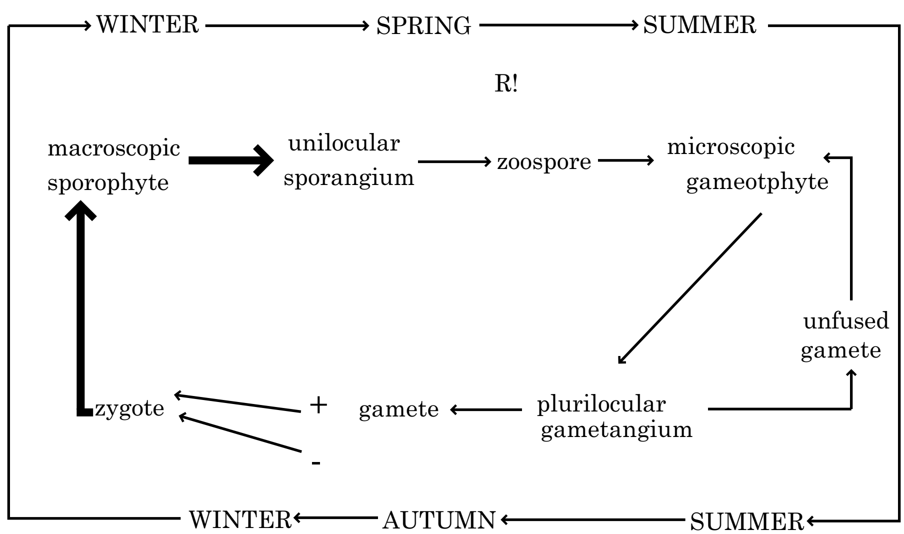

Fig. 2. Life history of *Sphaerotrichia divaricata* (— haploid, ▬ diploid, R! meiosis).

winter and spring. The macroscopic sporophytes bear unilocular sporangia in early summer when seawater temperature rises.

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