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The life history of *Griffithsia japonica* OKAMURA (Rhodophyceae, Ceramiales) in laboratory culture*

Masafumi IIMA and Seiji MIGITA

Laboratory of Algal Culture, Faculty of Fisheries, Nagasaki University, Nagasaki, 852 Japan

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The life history of the marine red alga *Griffithsia japonica* OKAMURA, collected from Saikai-bashi, Nagasaki Pref., Kyushu, Japan was studied in laboratory culture. Tetraspores released from a field-collected plant developed into uniseriate dichotomously branched male or female plants within two months. After fertilization, female plants formed cystocarps surrounded by one-celled incurved involucres. Carpospores released from these culture plants developed into tetrasporophytes which discharged tetraspores after two months. Thus, in laboratory culture, the life history of *G. japonica* was completed within four months. This species can also easily fragment and each detached fragment can regenerate into a new plant as reported in other *Griffithsia* species.

Key Index Words: *Ceramiaceae—Ceramiales*—Griffithsia japonica—*life history*—*Rhodophyceae.*

Griffithsia japonica OKAMURA is an epilithic or epiphytic dichotomously branched filamentous red alga which grows in the intertidal and subtidal zones on the Pacific and East China Sea coasts of Japan. Plants of the genus *Griffithsia* (named after the British phycologist, Amelia W. GRIFFITHS) have uniseriate uncorticated axes of characteristically large vegetative cells which are visible to the unaided eye (ca. 500–700 μm diam. in *G. japonica*). Moreover each cell has the ability to regenerate into a new plant. Because of these features, *Griffithsia* has been widely used in cytological (MYERS *et al.* 1956, PRILOU 1962, RAMUS 1971) and morphogenetic studies (DUFFIELD *et al.* 1972, WAALAND and CLELAND 1972, 1974, WAALAND *et al.* 1972, WAALAND and WAALAND 1975, WAALAND 1978).

In spite of its characteristic morphology, this group (tribe Griffithsieae) is a taxonomically complicated group (ITONO 1981). Various taxonomic criterions in this tribe have been proposed by many workers (KYLIN 1956, HOMMERSAND 1963, BALDOCK 1976).

On the other hand, little information has been published on the reproduction and life history of *Griffithsia*. Only LEWIS (1909) reported the reproduction and subsequent development of *G. bornetiana*.

YENDO (1909) first reported *Griffithsia japonica* from Japan as *G. schousboei* MONTAGNE, a species found on Atlantic and Mediterranean coasts. Subsequently OKAMURA (1930) described the Japanese taxon as a new species, *G. japonica*, on the basis of its differences in morphology and distribution from *G. schousboei*. Since then, the distribution of *G. japonica* has been confirmed as southern parts of Japan (cf. SEGAWA 1956) and China (TSENG 1942). However there has been no report on the life history of this species, or of the genus *Griffithsia*, in Japan. Hence an attempt has been made in the present study to follow the life history of *G. japonica* in laboratory culture.

* Dedicated to the memory of the late Dr. Munenao KUROGI (1921–1988), Professor Emeritus of Hokkaido University.

Materials and methods

Culture studies were initially started from tetrasporophyte as this was the predominant phase in the field populations of *Griffithsia japonica*.

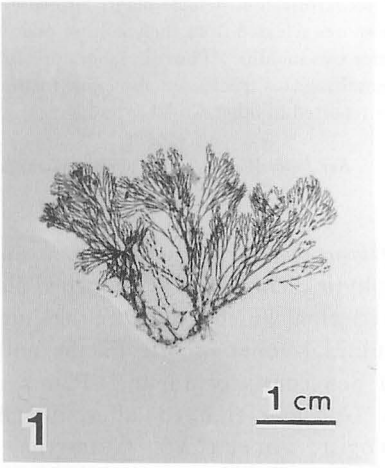
Fertile tetrasporic plants were collected at Saikai-bashi, Nagasaki Pref. and brought to the laboratory on May 29, 1987, June 4 and June 15, 1988. Plants were rinsed in filtered seawater and placed in Petri dishes (6 × 2 cm) containing 20-30 m*l* sterile seawater for few hours to induce spore liberation. The released tetraspores were rinsed several times with filtered seawater from a capillary pipette and inoculated into culture vessels (7 × 2 cm) containing 40 m*l* of PES medium (PROVASOLI 1966). The dishes were then maintained at 18-20°C under cool-white 40W fluorescent lamps at 2000-3000 lux, and a 12 : $\overline{12}$ photoperiod. The medium in the culture vessels was renewed weekly. When germlings grew up to about 5 mm in height, they were transferred to aeration cultures.

Chromosome counts were made using plants cultured from carpospores. Mature tetrasporophytes were fixed in ethanol : acetic acid (3 : 1 v/v) and stained with an aceto-iron-haematoxylin-chloral hydrate solution (WITTMANN 1965).

Results

Griffithsia japonica is a dichotomously branched filamentous alga which grows up to 2-5 cm in height (Fig. 1). Most of the plants found in the field were tetrasporophytes, with very few female gametophytes bearing cystocarps and no male plants found. Tetrasporangial fascicles appear in whorls around the second joint from the apex and each fascicle bears a single inflated involucral cell recurved to enclose the tetrasporangial clusters (Fig. 2). The tetraspores are spherical (35-40 μm in diam.) and dark red in color (Fig. 3-A). In culture, released tetraspores attached to the substratum within a few hours and germinated by forming an elongated hyaline rhizoidal cell, and an apical cell which later divided to form the thallus. Within 3 days, germlings developed to a 3-celled stage about 150 μm in length and 50 μm in width (Fig. 3-B). Within one week, cell number increased to more than 10 cells and new lateral branches were produced (Fig. 3-C). After 21 days, germlings were about 2-3 mm in length (Fig. 3-D) and became detached from the substratum (glass surface). At this stage, the free-living germlings were transferred into aeration flasks. The germlings grew up to 1-2 cm in length after 40 days in aeration culture (Fig. 3-E).

Spermatangia (Fig. 3-F) formed in whorls



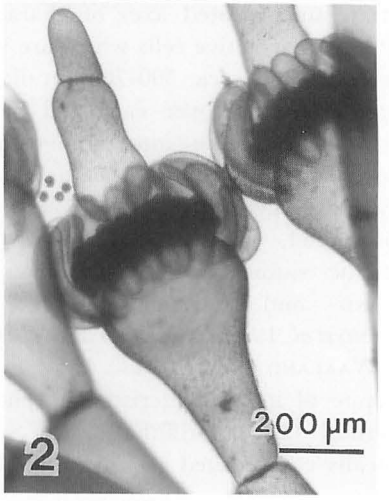


Fig. 1. Field-collected plant of *Griffithsia japonica*.

Fig. 2. Branches with tetrasporangia releasing tetraspores.

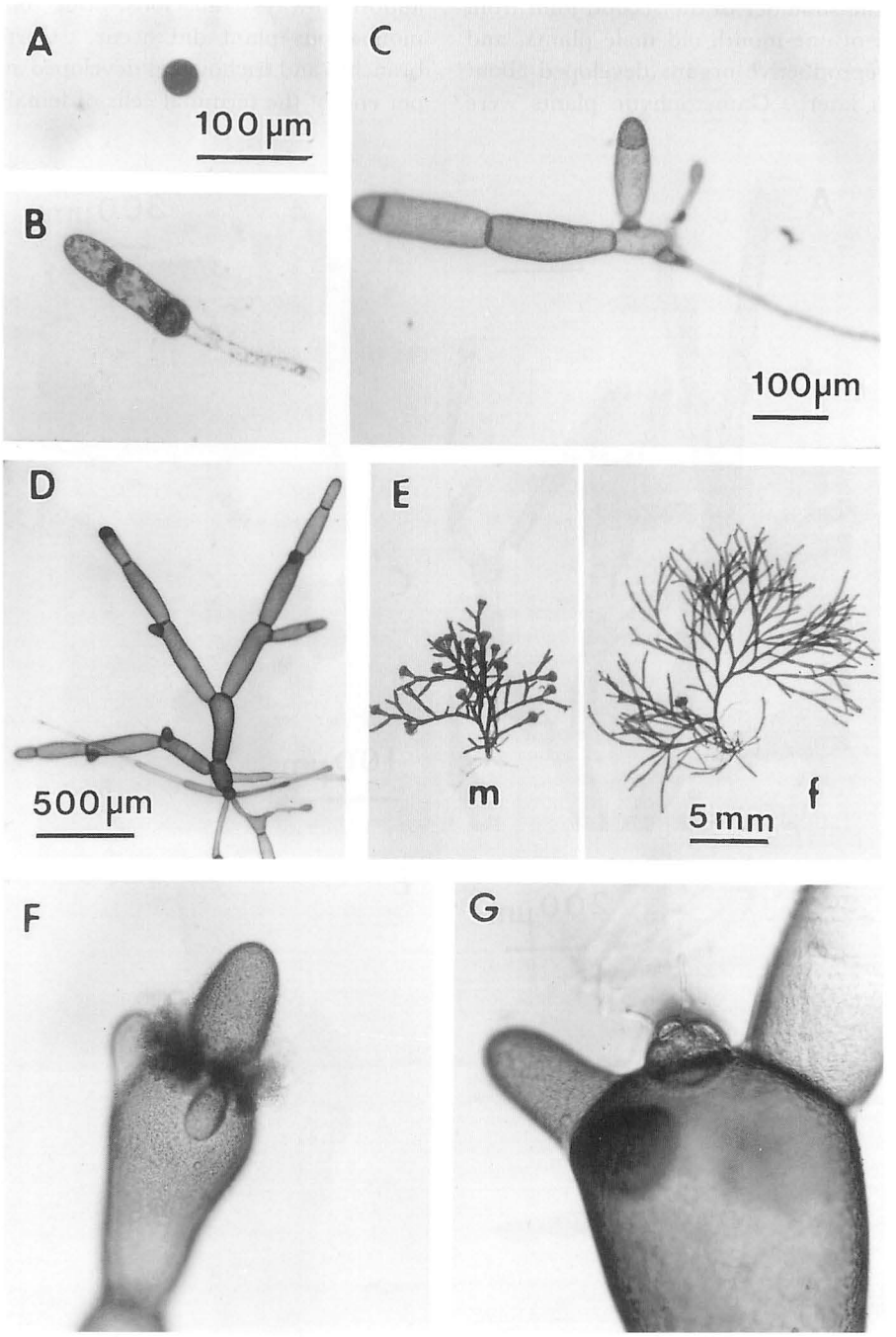


Fig. 3. Development of tetraspore. A. Tetraspore released from tetrasporangium. B. Three-day-old germling with hyaline rhizoidal cell. C. Seven-day-old plant with a branch. D. Twenty-one-day-old plant with pseudodichotomous branches. E. Forty-day-old plants with reproductive organs (m, male plant; f, female plant). F. Spermatangia of male plant releasing spermatia. G. Procarps of female plant with two trichogynes. Scale in A applies also to B, F and G.

around the shoulder of the second joint from the apex of one-month-old male plants, and female reproductive organs developed about ten days later. Gametophytic plants were almost always dioecious, but only one monoecious plant did occur. Carpogonial branches and trichogynes developed at the upper end of the terminal cells of female plants

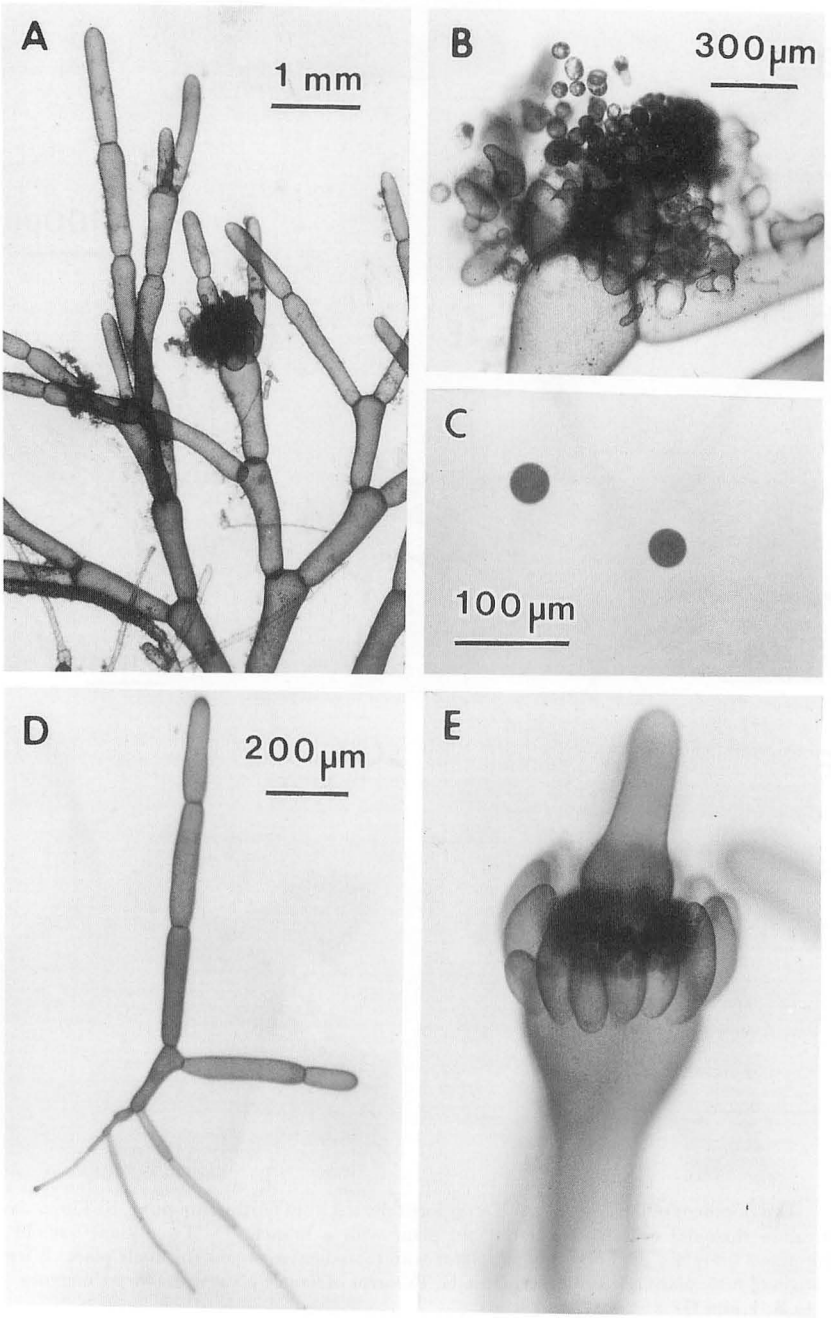


Fig. 4. Development of carpospore released from cultured plant. A. Branch with mature cystocarp. B. Mature cystocarp releasing carpospores. C. Carpospores from cultured plant. D. Fourteen-day-old germling from carpospore. E. Mature tetrasporangial clusters of cultured plant. Scale in C applies also to E.

(Fig. 3-G).

The spermatia released from spermatangia attached to trichogynes and ultimately resulted in the formation of cystocarps (Fig. 4-A). Carpospores were released (Fig. 4-B) within 2 months of germination. The released carpospores were 35–40 μm in diameter (Fig. 4-C). The germination pattern and development of the carpospores were identical to those of the tetraspores (Fig. 4-D).

After two months in culture, germlings derived from carpospores formed tetrasporangial fascicles with 14–16 one-celled incurved involucres (Fig. 4-E). The morphology of the tetrasporangia was the same as in field-collected plants.

The chromosome number was about 20 (n) in tetrasporangia (Fig. 5). Unfortunately chromosomes in the diploid stage (2n) or during meiosis were not observed in the present study.

Some mature vegetative filaments were fragmented into single cells and maintained in culture under the same conditions. Most of the fragments regenerated into normal plants as reported earlier for other species of *Griffithsia* (DUFFIELD *et al.* 1972).

Discussion

All phases in the life history of *Griffithsia japonica* were observed over 4 months in laboratory culture. Germlings derived from tetraspores developed into dioecious male and female gametophytes and, after fertilization, cystocarps were formed. The carpospores released from cystocarps subsequently developed into tetrasporophytes. *G. japonica*, therefore, has a triphasic *Polysiphonia*-type life history, having dioecious gametophytes, an isomorphic tetrasporophyte, and a carposporophyte which remains attached to the female gametophyte. Such a life history is reported to be common in other members of the Ceramiales (cf. WEST and HOMMERSAND 1981).

EDWARDS (1968, 1969, 1973) reported other types of life history, in addition to the *Polysiphonia*-type, in some members of the Ceramiales, but *G. japonica* showed only the *Polysiphonia*-type of life history in the present study.

In the field, *G. japonica* is abundant during spring (April to June), then disappears in summer (July or August). At the beginning of this study, tetraspores from *G. japonica* were

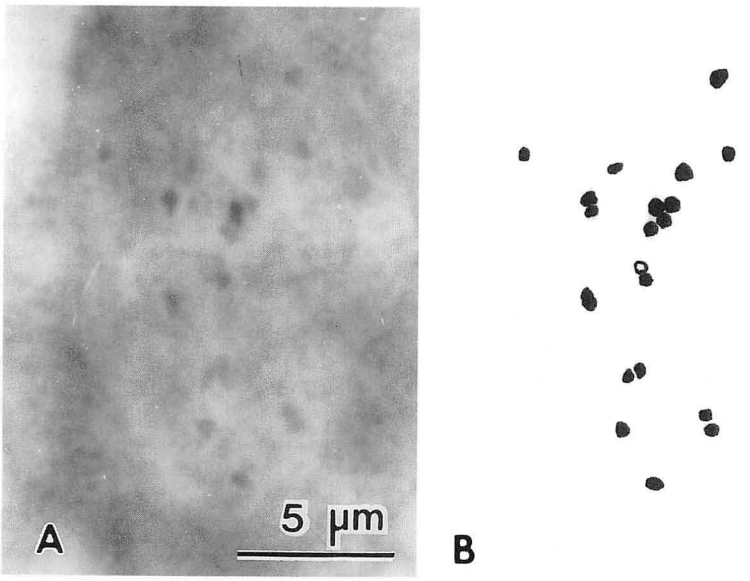


Fig. 5. A. Chromosomes in the tetrasporangium (about 20). B. Drawing of A.

cultured at 18-20°C and, simultaneously, at room temperature (above 25°C). While spores cultured at the former temperature could germinate, those of the latter did not and gradually degenerated. This may indicate the inhibition of germination at higher temperatures, perhaps together with other factors, which may inhibit growth of *G. japonica* in the field in summer.

The early development of this species appears to be similar to that of *G. bornetiana* (LEWIS 1909). LEWIS (1909) observed that the 3-cell stage was reached about twelve hours after the spore was shed. However, in the present study, *G. japonica* did not reach the 3-cell stage until the second day after settlement.

Morphological features typical of plants in the field were also observed in plants grown in culture; the filamentous plants being dichotomously branched with giant vegetative cells and tetrasporangial fascicles surrounded by one-celled incurved involucres around the second joint from the apex. Such features are characteristics of the tribe Griffithsieae (ITONO 1981).

However, as NORRIS and MOLLOY (1988) recently reported in the culture experiments of *Griffithsia schousboei*, many rhizoids were produced in cultured plants though they appeared to less common in the original field-collected plants.

The chromosome number of *Griffithsia japonica* (n=ca. 20) corresponds with that reported for *G. corallina* (KYLIN 1916; n=20, 2n=40), but differs from that of *G. bornetiana* (LEWIS 1909; n=7, 2n=11-14).

In the present study, *Griffithsia japonica* from Japan was found to be easily cultured in the laboratory. The species therefore has potential for use in cytological and morphogenetic studies as performed with other *Griffithsia* species.

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飯間雅文・右田清治：室内培養における紅藻カザシグサ *Griffithsia japonica* の生活史

長崎産紅藻カザシグサ *Griffithsia japonica* OKAMURA（イギス目，イギス科）の発生・生活史が，室内単藻培養で調べられた。天然藻体から放出された四分胞子の発芽体は培養2ヵ月後には体長約1 cmとなり成熟し，雌雄異株で頂端部にそれぞれ受精毛と精子器が形成された。雌性配偶体では受精後，嚢果が形成され，果胞子が放出された。放出された果胞子は四分胞子と同様の発生を行い，発芽後約2ヵ月で成熟し，四分胞子を放出した。培養藻体の四分胞子嚢が十数本の輪生枝に囲まれて形成されるなどの形態的特徴は，天然藻体と同様であった。カザシグサは，四分胞子体と雌雄配偶体が同形のイトグサ型生活史を行っていることが明らかとなった。染色体数は，四分胞子嚢の細胞分裂時でn＝ca. 20と観察された。また，この種は他のカザシグサ属の種で報告されているように，切断された藻体の枝は容易に新個体に再生した。（852 長崎市文教町1–14　長崎大学水産学部藻類増殖学研究室）