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Mixed phases reproduction of *Polysiphonia morrowii* Harvey (Rhodomelaceae, Rhodophyta) in culture*

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The life history of *Polysiphonia morrowii* Harvey isolated from Gyokpo, Korea, was investigated in culture. *P. morrowii* basically showed a *Polysiphonia* type of life history. However, monoecious and mixed phases reproduction was also exhibited. The procarps on these unusual plants were proved to be sterile, while spermatia were fertile. A few tetraspores from the mixed phases plant grew to both normal tetrasporophytes and sterile plants. These tetrasporophytes released tetraspores which grew to male, female, monoecious, mixed phases, and sterile plants. Monoecious and mixed phases plants repeatedly occurred twice in cycle via tetraspores during this culture.

Key Index Words: culture study—life history—mixed phases reproduction—monoecious—Polysiphonia morowwii.

Life history of the genus *Polysiphonia* has been regarded as the typical one, so called *Polysiphonia* type. However, a few species of *Polysiphonia* were reported to produce asexual propagules in addition to sexual reproduction (Kapraun 1977, Womersley 1979, Byun and Kang 1986, Kudo and Masuda 1986) and to have mixed phases plants in field (Yoon 1981).

Such unusual phenomenon as mixed phases reproduction has been reported frequently for a number of red algae (e.g. Knaggs 1969, West and Hommersand 1981). These studies, however, are mostly limited to descriptive observations of field collections except for a few laboratory cultures (West and Norris 1966, Rueness and Rueness 1973, 1978, 1985, van der Meer and Todd 1977, Lee and West 1979, Notoya and Yabu 1981, Boo and Lee 1983, Notoya 1983, Choi and Lee 1987, West and Calumpong 1988, Kim and Lee 1989). The only satisfactory genetical explanation for such phenomena was given to *Gracilaria tikvahiae* McLachlan by van der Meer and his co-workers (van der Meer and Todd 1977, van der Meer *et al.* 1984, van der Meer 1986).

In this study, we examined the life history and the fate of the spores produced by monoecious and/or mixed phases plants of *Polysiphonia morrowii* Harvey isolated in Korea.

Materials and methods

Polysiphonia morrowii Harvey was collected from the intertidal zone of Gyokpo in the western coast of Korea in April 1985. Unialgal culture was established with vegetative branch apices. All the isolates were precultured in 1/2 PES medium under cool white fluorescence light below 500 lux for 6–7 days. Subsequent cultures were obtained from tetraspores produced from these vegetative thalli after about three weeks. Cultures were maintained in PES-enriched seawater medium at 1,000 lux (photoperiod, 16 : $\overline{8}$ hr) and 15°C.

Results

Polysiphonia morrowii was originally described by Harvey (1856) on the basis of the

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specimens collected at Hakodate, Japan. This species is characterized by the tufts of axillary tetrasporangial branchlets. The followings have been described as characters to distinguish it from the related species, *P. senticulosa* Harvey and *P. urceolata* Greville (Segi 1951, 1960, Tokida 1954): 1) tufted tetrasporangial branchlets, 2) branchlets endogenously originated, 3) dark reddish thalli, and 4) relative length of segments of the main axis. However, these characters were regarded as variable with age and habitat (Kudo and Masuda 1981, Yoon 1986). Kudo and Masuda (1981) demonstrated that the alga called *P. senticulosa* in Japan was the same as *P. morrowii*. Yoon (1986) reduced *P. senticulosa* Harvey and *P. urceolata* sensu Yamada (1928), Okamura (1936), Segi (1951), and Kang (1966) to a synonym of *P. morrowii*. Kudo and Masuda (1988), however, mentioned that *P. morrowii* differed from genuine *P. senticulosa* in having thicker thalli and 7-8 axillary branchlets which bore tetrasporangia.

Descriptive characteristics of plants: The vegetative structure of *P. morrowii* was described and illustrated by Harvey (1856), Segi (1951), Kudo and Masuda (1981) and Yoon (1986). Our plants collected at Gyokpo accorded well with them.

The thallus consists of four siphons and adheres to rocky substrata with rhizoids. It is densely tufted, slender and elongate, becoming up to 25 cm high. Unicellular rhizoids irregularly arise as outgrowth of pericentral cells. They develop on the basal portion and sometimes on middle portion of the erect thallus.

The branches arise exogenously in every 3-7 segment. However, the prostrate branch is endogenous from the lower part of erect main axes, showing variable diameters (150-250 μm). The ratio of main axial segments (dia. 270-550 μm) in length to width is variable according to age and thallus (Table 1). Ultimate branch arises alternately in 3-8 segments interval and is sharply pointed. The axillary branchlets develop endogenously from a central axial cell.

A few colorless trichoblasts arise near the apex of branch and are 2-3 furcate and deciduous, leaving inconspicuous scar cell from which cicatrigenous branch sensu Hollenberg (1942) sometimes arises. Cultured thallus shows basically the same morphological characters as field collected one (Table 1).

When a tetrasporophytic plant becomes fertile, tetrasporangia develop on ultimate branches, axillary branchlets and sometimes on indeterminate branches of the thallus. Thus, 3-8 axillary branchlets bearing tetrasporangia congregate on an axil. Mature tetrasporophytes bear few thrichoblasts and rare scare cells. Tetrasporangia mature acropetally in a stichidium. Tetraspores released are 55-75 μm in diameter. A mature spermatangial branch is slightly incurved, 650-850 μm long and 45-85 μm broad. It provides with a long sterile tip con-

Table 1. Comparison of vegetative structure between field-collected and cultured plants.

		Field-collected*		Cultured**	
		January	March	1 month	2 months
Height (cm)		3-10	10-25	2-4	3-7
L/D of axis	Upper	0.3-0.5	0.3-0.5	0.5-1.0	0.5-1.0
	Middle	1.5-2.0	2.0-3.5	1.0-2.5	1.5-3.0
	Lower	2.0-4.0	2.5-4.0	1.2-2.0	1.0-2.0
Branch interval (segment)		3-7	3-5-(7)	3-15	3-10-(15)
Trichoblast		scarce	scarce	frequent/scarce	frequent/scarce
Length of determinate branch (segment)		10-20	13-20	15-35	15-35
Axillary tetrasporangial brachlets		non	1-8	non	non/1-5

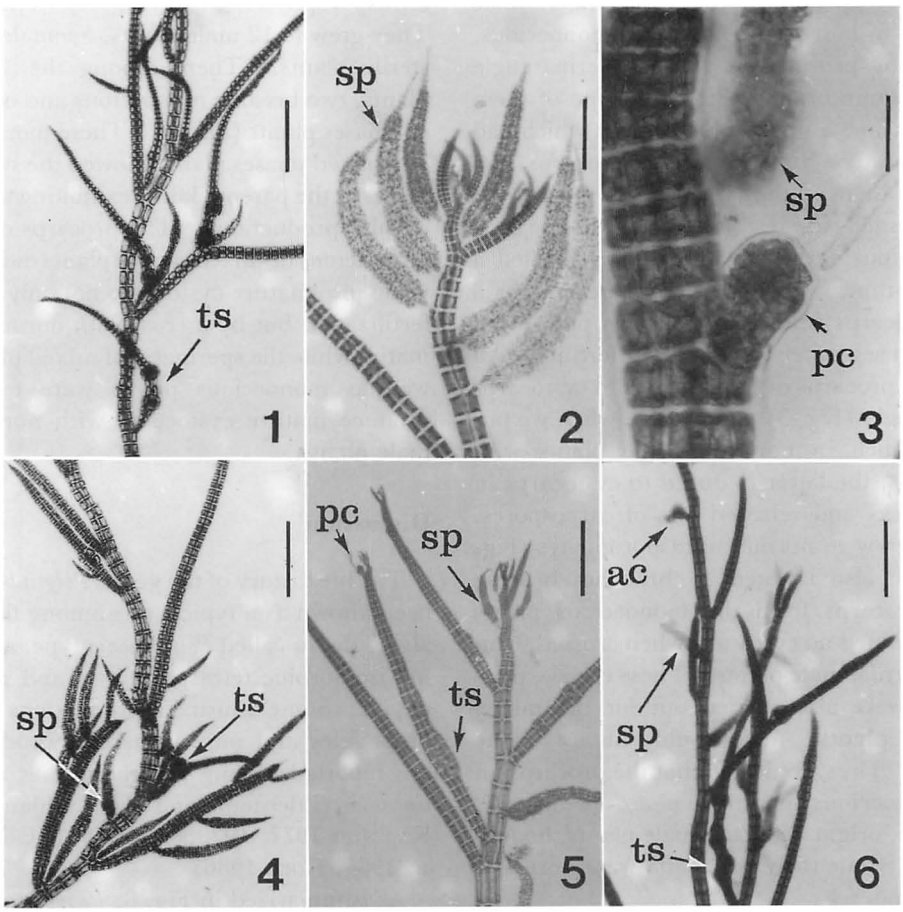
* Collected at Gyokpo.
** Cultured at 15°C and 1000 lux (18 : 6 LD).

sisting of a few elongate cells at the apex and is supported by an one-celled stalk. The branches bearing procarp arise alternately on the apical part of indeterminate branches. The mature cystocarp is urceolate, 350-450 μm long and 250-300 μm broad. The released carpospores are 50-65 μm in diameter. A fully mature pericarp is two-cell layered and has a wide ostiole at the top.

Life history in culture: Four vegetative thalli isolated from Gyokpo were proved to be tetrasporophytes in laboratory culture. All of them produced tetrasporangia in three weeks. A total of 76 tetraspores were isolated from the tetrasporophytes for further study. Among them, 33 spores grew to mature plants bearing the spermatangia in 6-7 weeks (Fig. 2), and 15 spores produced procarps one week later, while 28 spores remained vegetative (Fig. 7).

In order to examine crossability, a single female plant and two male plants were put together in a culture dish. Mature cystocarps appeared in two weeks after that. As a result, 37 carpospores were released from a cystocarp, of which 30 spores grew to tetrasporophytes in 7-8 weeks and 7 spores died in early stages of the growth.

Thus, *P. morrowii* at hand is demonstrated to show a typical *Polysiphonia* type of life histo-



Figs. 1-6. *Polysiphonia morrowii* Harvey in culture. Fig. 1. Tetrasporic plant. Fig. 2. Male plant. Fig. 3. Monoecious plant derived from male plant. Fig. 4. Mixed phases plant derived from male plant, bearing tetrasporangia and spermatangia on the same branch. Fig. 5, 6. Mixed phases plants derived from male plant, bearing procarps and tetrasporangia in addition to spermatangia on the same branch. ac, aborted cystocarp; pc, procarp; sp, spermatangial branch; ts, tetrasporangium. Scale bar: 1, 4-6, 300 μm; 2, 250 μm; 3, 130 μm.

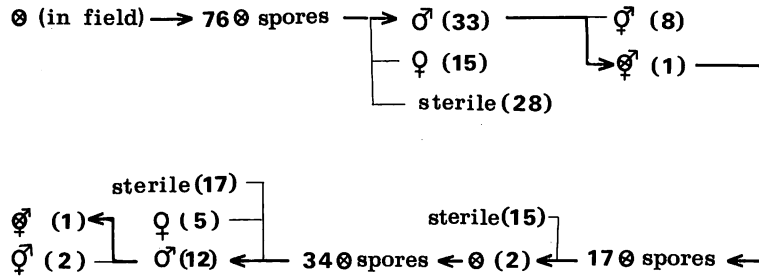


Fig. 7. The fate of tetraspores released from *Polysiphonia morrowii* Harvey in culture.

ry, and requires approximately 18-20 weeks for completion of the cycle in laboratory culture.

Unusual reproduction in culture: During the culture, some of the male plants exhibited unusual sexualities. Among 33 male plants, 8 individuals became monoecious, producing procarps as well as spermatangia on the same branch (Fig. 3). One of them later became a mixed phases plant which had tetrasporangia in addition to procarps and spermatangia on the same branch (Figs. 4-6).

In order to test the fertility of such monoecious sexual structures, we isolated a single branch which had both spermatangia and procarps from a monoecious plant, and cultured separately to check self-fertility. All of these procarps did not mature to cystocarps for 6 weeks (Fig. 3). However, when we put this branch in normal female plants with procarps, the latter matured to cystocarps in two weeks and released lots of carpospores, which grew to normal tetrasporophytes (Fig. 1). We also isolated 13 branches bearing only procarps from the monoecious plants and obtained no cystocarp when crossed them with normal male plants. These cross experiments were also carried out for the mixed phases plants, and could obtain same results. Thus, we found that the procarps on the monoecious and mixed phases plants of *P. morrowii*, originated from male plants, had no fertility, while their spermatia were normally functional.

On the other hand, tetrasporangia of the mixed phases plant released tetraspores after maturation. We could isolate 17 tetraspores among them, and traced the fate individually by separate culture. Two of them grew to produce tetrasporangia on the whole branches in 7 weeks, and other 15 spores remained as sterile thalli for 13 weeks. In addition, among the tetrasporangia obtained from two tetrasporophytes, 34 tetraspores were viable. They grew to 12 male plants, 5 female and 17 sterile plants. Then, among the 12 male plants, two became monoecious and one mixed phases plants (Fig. 7). These monoecious and mixed phases plants showed the same fertilities as the parent plants, exhibiting such unusual reproductions. The procarps on these monoecious or mixed phases plants did not develop into mature cystocarps not only by self-fertilization but by a cross with normal spermatia, while the spermatia of mixed phases as well as monoecious plants were fertile to produce mature cystocarps with normal female plants.

Discussion

The life history of the genus *Polysiphonia* has been known as a typical one among floridean algae, the so-called *Polysiphonia* type, alternating isomorphic tetrasporophyte and gametophytes. Some unusual reproductions such as propagules and mixed phases reproductions are reported among several species of *Polysiphonia* (Edelstein and McLachlan 1967, Kapraun 1977, 1978, Yoon 1981, Cheung *et al.* 1984, Koch 1986).

As summarized in Fig. 8, *P. morrowii* from Gyokpo showed a very interesting unusual life history although based on a typical *Polysiphonia* type. Especially it is remarkable that the monoecious procarps originated from

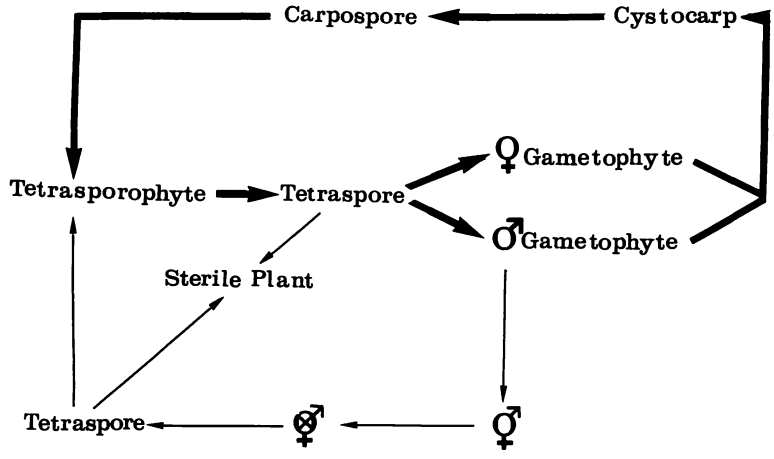


Fig. 8. Life history of *Polysiphonia morrowii* Harvey in culture.

male thallus show no fertility while spermatia are functional, and the tetraspores on the mixed phases plant exhibit viability. Moreover, this unusual life history is repeated through mixed phases tetraspores.

There have been lots of reports about such unusual sexuality during the life history, especially in Ceramiaceae (West and Hommersand 1981). For instance, Whittick and West (1979) demonstrated that monoecious plant of *Callithamnion baileyi* Harvey produced the carpospores by self-fertilization, and the spores developed into tetrasporophytes as seen in dioecious plants. Boo and Lee (1983) reported that the monoecism of *Antithamnion sparsum* Tokida showed a self-fertility and carpospores released from this plants developed into male plant, missing tetrasporophytes in culture.

On the tetraspore of mixed phases plant, West and Norris (1966) reported that the tetraspores on the gametophyte of *Antithamnion pygmaeum* Gardner developed into the same gametophytes as parent in sexuality. Rueness and Rueness (1973) demonstrated that male/tetra mixed phases plants of *Antithamnion tenuissimum* (Hauck) Schiffner were haploid and the spermatia produced by such plants were functional. Moreover, tetraspores derived from the mixed phases plants grew to nonsporangiate normal male and female plants. They demonstrated that spores produced on the male/tetra mixed phases plants were formed apomeiotically. Notoya and Yabu (1981) reported that male/tetra mixed phases plants of *Platythamnion yezoense* Inagaki were always derived from carpospores, while the mixed phases plants bearing tetrasporangia, spermatia and carpogonial branches were derived from tetraspores in culture.

Rueness and Rueness (1985) demonstrated that tetraspores of *Callithamnion tetragonum* (With.) Gray from the mixed phase plant bearing both non-functional spermatia and procarps in addition to tetrasporangia developed into similar mixed phases plants as parent, where the spermatia and procarps were also non-functional and the tetraspores were inviable. The fate of tetraspores on the mixed phases plant of *C. tetragonum* is similar to that of our study, although they did not observe the sterile plants in addition. L'Hardy-Hales (1986) reported that tetraspores on the male gametophyte of *Antithamnionella spirographidis* (Schiffner) Wollaston developed into male and female plants. Hassinger-Huizinga (1952) in *Callithamnion corymbosum* (Sm.) Lyngb., West and Norris (1966) in *Callithamnion* sp. and L'Hardy-Halos (1986) in *Antithamnionella sarniensis* Lyle reported that tetraspores on the tetrasporophyte developed into tetrasporophytes repeatedly, missing the gametophytic phases.

As a result, these unusual sexualities generally seem to exhibit their own peculiar tendency according to species. *P. morowii* at hand

also shows lack of the gametophytic phase in the unusual life history, but the result is not equivalent to those reported previously (Hassinger-Huizinga 1952, West and Norris 1966, L'Hardy-Halos 1986).

van der Meer and Todd (1977) demonstrated that the formation of gametangia on the tetrasporophyte of *Gracilaria tikvahiae* resulted from a mitotic recombination of the gene determining sexuality. But this was in case of diploid tetrasporophytes. They did not explain the mixed phases reproduction in the gametophytes observed by such as West and Norris (1966), Rueness and Rueness (1973, 1985) and in this study.

According to our culture study, *P. morrowii* demonstrates that the mixed phases reproduction occurs during the life history via tetraspores. It seems to be that the mixed phases reproduction once induced in course of the life history can be succeeded stably generation to generation, even though the frequency of occurrence is variable according to environmental conditions (Kim and Lee 1989).

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Wook Jae Lee·In Kyu Lee：モロイトグサ（紅藻フジマツモ科）の培養における混合相生殖

韓国 Gyokpo で単離したモロイトグサ (*Polysiphonia morrowii* Harvey) を培養し，その生活史を調べた。本種は基本的にはイトグサ型の生活史を示したが，雌雄同株ならびに混合相の生殖がみられた。これら正常でない藻体のプロカルプは不稔性であったが，不動精子は稔性であった。混合相の藻体に由来する若干の四分胞子は正常な四分胞子体ならびに不稔性の藻体に発達した。これら四分胞子体は四分胞子を放出し，この四分胞子からは雄性，雌性，雌雄同株，混合相，不稔性の藻体が生じた。本培養実験で，四分胞子経由のサイクルでは雌雄同株ならびに混合相の藻体は引き続いて 2 回生じた。(Department of Botany, Seoul National University, Seoul, 151–742 Korea)