Alternation of Generations of some Brown Algae in Unialgal and Axenic Cultures*

By

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Introduction

Many phycologists have observed that life cycles of seaweeds are closely related to the seasons. A number of seaweeds appear at a certain period of the year and disappear at another period. To understand how they exist when the conspicuous forms disappear is an important long-standing problem. In some seaweeds, it is clear that the alternation of morphologically different forms or generations is associated with the change of some environmental conditions (KUROGI, 1958; KORNMANN, 1961; MÜLLER, 1962). Culturing experiments under artificially controlled conditions are necessary to reveal the life cycles of seaweeds and to study the environmental factors relating to the periodic expression of life forms or the alternation of generations.

Since the end of the 19th century, the life history of seaweeds has been studied by many investigators. REINKE (1878) observed separate gametophytic and sporophytic plants in *Zanardia collaris* and thus furnished the first evidence for the alternation of generations in brown algae. In 1915, SAUVAGEAU found that *Sacchorhiza bulbosa* has a life cycle involving the alternation of a macroscopic sporophyte and a microscopic gametophyte. Since then many investigations on the life cycles of brown algae have been published. From observations in culture and field, it is inferred that most members of brown algae have a life cycle with alternation of generations which may be divided broadly into three types; 1) alternation of isomorphic generations, 2) alternation of heteromorphic generations with the sexual generation dominant and 3) alternation of heteromorphic generations with the asexual generation dominant.

In cultures, however, many workers have observed diverse deviations from the normal life cycle of brown algae, i. e., the occurrence of filamentous asexual generations (plethysmothalli) in the life histories of the species with a macroscopic asexual generation. The following three types of parthenogenetic development are found; unfused gametes develop

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into 1) sexual generation (KORNMANN, 1957; AJISAKA and UMEZAKI, 1978), 2) asexual generation (SCHREIBER, 1930; KORNMANN, 1956; YABU, 1964b; MÜLLER, 1966; NAKAHARA and NAKAMURA, 1973), and 3) both generations or intermediate forms between the two (CHURCH, 1898; VAN DEN HOEK and FLINTERMAN, 1968). Apogamy of brown algae, the formation of a sporophyte from a vegetative cell of a gametophyte without the intervention of sexual organs, was observed by several workers (SAUVAGEAU, 1926, 1931; SEGI and KIDA, 1957; NAKAHARA and NAKAMURA, 1973). Apospory of brown algae, the formation of a gametophyte from a vegetative cell of a sporophyte, was reported by KORNMANN (1956) and NAKAHARA and NAKAMURA (1973).

In the past, difficulties in techniques of culture and of cytological observation have hampered the elucidation of the entire life cycle in culture and the alternation of nuclear phases. Therefore, major observations on the life cycles of brown algae have been made in the early stages of development of zoospores and zygotes. In the last thirty years, however, cultural techniques and equipment have improved and several species have been cultured from generation to generation in the laboratory. The favorable conditions for the growth and maturation have been revealed to some extent.

Furthermore, morphological differences between sexual and asexual generations may reflect differences in metabolism, but our knowledge of this relationship is still insufficient. According to KNIGHT (1931), the alternation between the macroscopic and microscopic phases in *Ectocarpus* may be related to the availability of nitrates and phosphates. HSIAO (1969), studying *Petalonia*, reported that the four asexual forms in its life cycle have different iodide requirements for growth and development. Recently, several seaweeds have been cultured axenically by some investigators to study their nutritional requirements. However, these studies have dealt only with one of the two generations.

With the object of clarifying the role of the environmental factors in the periodic expression of life forms and the alternation of generations in the brown algae, I carried out cultural studies of selected species of Ralfsiales, Desmarestiales and Laminariales under the guidance of Prof. Y. NAKAMURA at the Institute of Algological Research, Faculty of Science, Hokkaido University at Muroran. The present work deals with observations on the entire life cycle, the alternation of sexual and asexual generations, parthenogenesis, apogamy, and apospory in culture. Nuclear phases were confirmed cytologically. The favorable conditions for growth and maturation of sexual and asexual generations and the nutritional requirements of both generations of the species studied were revealed to a certain degree.

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I Alternation of Generations in Culture

Materials and Methods

The present investigation was carried out on the following seven species of brown algae; *Analipus japonicus* (HARVEY) WYNNE, *Desmarestia viridis* (MÜLLER) LAMOUROUX, *Desmarestia ligulata* (LIGHTFOOT) LAMOUROUX, *Desmarestia tabacoides* OKAMURA, *Laminaria japonica* ARESCHOUG, *Agarum cribrosum* BORY and *Alaria crassifolia* KJELLMAN.

The collection data of fertile materials of these species are as follows:

Species	Locality	Date
Analipus japonicus	Muroran, Hokkaido	Mar. 26, 1966
		Jan. 31, 1967
Desmarestia viridis	Muroran, Hokkaido	June 7, 1966
		June 9, 1967
Desmarestia ligulata	Muroran, Hokkaido	June 15, 1966
		July 1, 1968
Desmarestia tabacoides	Shimoda, Shizuoka	Apr. 23, 1969
Laminaria japonica	Muroran, Hokkaido	Nov. 2, 1966
		Nov. 15, 1967
Agarum cribrosum	Muroran, Hokkaido	Oct. 20, 1969
		Mar. 26, 1970
Alaria crassifolia	Muroran, Hokkaido	Nov. 3, 1966
		Nov. 15, 1967

For unialgal cultures, fertile fragments of the desired alga were rinsed with filtered seawater and placed separately in Petri dishes containing culture medium. Newly liberated swarmers swimming vigorously, were washed 2–3 times in culture medium using finely-drawn glass capillary pipettes. After washing, zoospores and gametes were pipetted on to a glass slide with several drops of medium and kept moist while swimming, usually for one hour. Then this slide was transferred to a glass culture vessel (6.5 cm×8.0 cm) containing 200 m*l* of medium. Several 10–15-day-old germlings attached to the glass slide were isolated with a micropipette and transferred to a test tube (1.8 cm×13.0 cm) with a screw cap containing 10 m*l* of medium or to a culture vessel.

Sexual reproduction was determined by placing a drop of culture medium containing swarmers from different individuals on a glass slide, mixing and then examining for fusion under a microscope. Zygotes were easily detected by two eyespots which persisted for a few days after conjugation. The culture of the zygotes was arranged in the following way: Male and female gametes were mixed on a scored glass slide and kept moist for about 30 minutes. Zygotes and unfused gametes were recognizable on the glass slide. The zygotes settled on or near the score were left and the other zygotes and unfused gametes were removed with a fine needle under a dissecting microscope. Then this slide was transferred to the glass culture vessel and was re-examined microscopically every two days. Several 10-15-day-old germlings attached were isolated from the glass slide with a micropipette and transferred singly to a test tube or a culture vessel.

The culture medium adopted was a modification (PESI medium) of PROVASOLI's ES medium (TATEWAKI, 1966). Twenty m*l* of the PESI stock solution sterilized by autoclaving for 20 minutes at 120℃ and 1 kg/cm^2 was added to one litre of autoclaved seawater. The medium was usually changed at 30-40-day intervals.

Cultures were kept in freezer-incubators illuminated with cool white fluorescent lamps and with controlled the temperature and photoperiod. During the course of these studies five sets of conditions were usually used and they will be referred to simply as Set 1, Set 2, Set 3, Set 4 or Set 5 conditions. The variables are summarized as follows:

	Temperature (℃)	Photoperiod (light hr : dark hr)	Illumination (lux)
Set 1	5	10 : 14	2000
Set 2	12	10 : 14	2000
Set 3	10	14 : 10	2700
Set 4	14	14 : 10	2700
Set 5	18	14 : 10	2700

Other culture conditions were obtained by changing the temperature and photoperiod of the incubators.

To observe chromosomes, the material obtained from the cultures was fixed with a aceto-alcohol solution (1 : 3) during the dark period. WITTMANN's (1965) aceto-iron-hematoxylin-chloral hydrate solution was used for staining (YABU and TOKIDA 1966). A small quantity of the material, stored in the fixative, was put on a glass slide and a drop of the stain added. When necessary, the material was squashed on the glass slide before placing the cover glass. The preparation was then carefully heated until a slight color change occurred. The chromosomes were found to stained dark immediately.

Observations

Ralfsiales, Ralfsiaceae

Analipus japonicus (HARVEY) WYNNE

The alternation of nuclear phases and early development of *Analipus japonicus* (*Heterochordaria abietina*) were studied by ABE (1935a, 1936). He discovered that *A. japonicus* possessed isomorphic alternation of generations and zygotes and zoospores developed into discoidal germlings. NAKAMURA (1972) established new order Ralfsiales and placed *Analipus* in the Ralfsiaceae, because of its discoidal early development, anatomy and isomorphic life cycle. This treatment was followed by WYNNE (1972), BOLD and WYNNE (1978) and TANAKA and CHIHARA (1980). However, several authors did not recognize the use of the order Ralfsiales. ABBOTT and HOLLENBERG (1976) referred this genus to Chordariaceae, Chordariales, and NELSON (1982a) to Heterochordariaceae, Ectocarpales.

Analipus japonicus is one of the most common brown algae, growing on rocks in the littoral zone along the Pacific coast of northern Japan. In the vicinity of Muroran, this alga thrives most luxuriantly from winter to spring and during these seasons the plant consists of an erect part with lateral branches and a small prostrate base. In summer the erect part disappears and the base remains, and develops into a branched prostrated horizontal thallus. Reproductive organs are formed on the erect fronds. There are three kinds of erect plants; female, male and asexual, all of which are isomorphic. Sexual plants bear plurilocular sporangia and asexual plants bear unilocular sporangia. Plurilocular sporangia terminate in a 2–3-celled sterile clavate tip. After liberation of gametes, surface cells of the frond are stripped off and the inner tissues are exposed. Unilocular sporangia are sessile on the basal cell of the cortical filaments and obovoid in shape, measuring about 49–72 μm×25–35 μm. Each sporangium contains about 64–128 zoospores.

1) Zoospores and their development

Zoospores are pyriform, measuring about 8.8 μm×4.8 μm. They contain a single chromatophore and one eyespot and are laterally biflagellate (Fig. 1, A). They display a negative phototaxis, not any sexual behaviour. After short period of motility, they become sluggish and settle on the substratum. Settled zoospores become spherical and measure 6.0–7.0 μm diam. (Fig. 1, B). Within 24 hours they send out a germination tube, into which the protoplasm migrates either completely or partially (Fig. 1, C) forming a new cell by cross-wall production. In a few days the new cell gradually increases in size and divides both transversally and longitudinally to form a minute parenchymatous disc (Fig. 1, D–F). When the protoplasm remains partially in the original cell, this cell often issues a hyaline hair (Fig. 1, E). The discoid germlings grew better in longer photoperiod and at higher temperatures.

Under Set 3 conditions, the germlings consisted of about 16 cells in 10-day-old cultures. In 35–40 days, protuberances were formed in the centre of some discs. These were the erect frond initial (Pl. I, A & C). At the same time, the discs became thicker, growing

horizontally and branching along their margin, thus developing into a branched prostrate thallus. Later, apices of some branches of the prostrate thallus produced the erect fronds. The erect frond formed lateral branches which were morphologically identical to those produced in nature.

In 80 days after the formation of the erect frond (Pl. I, D), the cortical filaments of the branches and the upper part of the main axis of the frond became plurilocular sporangia, terminating in a 2–3-celled sterile clavate tip. Within 4 months all erect fronds produced from zoospores were covered with plurilocular sporangia. The chromosome number of these plants was about 20 (Pl. I, L).

Under Set 1 and Set 2 conditions, the discs formed the erect fronds, the basal part of which did not develop into the prostrate thalli. Whereas all discs cultured under Set 4 and Set 5 conditions branched horizontally and developed into the prostrate thalli (Pl. I, B & E), which were morphologically identical with the plants found in nature during summer.

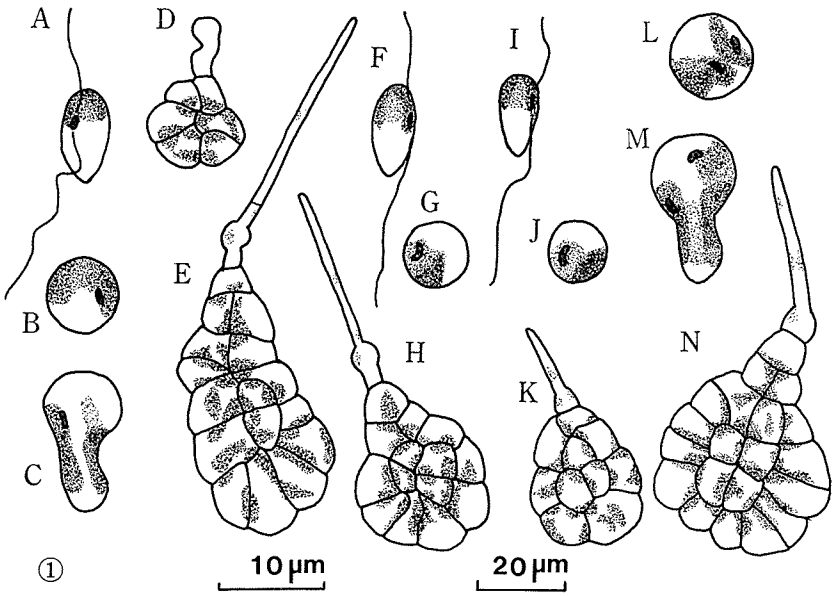

Fig. 1 *Analipus japonicus*. A. Zoospore. B. Settled zoospore. C. Germination of zoospore. D. 6-day-old germling from a zoospore grown under Set 3 conditions. E. 10-day-old germling from a zoospore grown under Set 3 conditions. F. Female gamete. G. Settled female gamete. H. 10-day-old germling from a female gamete grown under Set 3 conditions. I. Male gamete. J. Settled male gamete. K. 10-day-old germling from a male gamete grown under Set 3 conditions. L. Zygote. M. Germination of zygote. N. 10-day-old germling from a zygote grown under Set 3 conditions. (A–C, F–G, I–J and L–M drawn to 10 μm scale. D–E, H, K and N drawn to 20 μm scale.)

An apical portion of horizontal branches of a prostrate thalli were excised and transferred to nine different culture conditions: 5℃, 10 : 14 LD (light-dark cycle); 5℃, 14 : 10 LD; 10℃, 10 : 14 LD; 10℃, 14 : 10 LD; 14℃, 10 : 14 LD; 14℃ 14 : 10 LD; 18℃, 10 : 14 LD; 18℃, 14 : 10 LD; and 22℃, 14 : 10 LD. Within one month, all the apical fragments of the horizontal branches cultured at 5℃ and 10℃ in a 10-hr photoperiod produced erect fronds. Within 2 months, the formation of erect fronds was about 80% at 14℃ in a 10-hr photoperiod and at 5℃ and 10℃ in a 14-hr photoperiod, and about 30% at 18℃ in a 10-hr photoperiod. At 14℃, 18℃ and 22℃ in a 14-hr photoperiod, only prostrate thalli were produced.

Small erect fronds just produced under Set 1 conditions were transferred to the nine different conditions mentioned above. They grew well under the 5℃, 10℃, and 14℃ conditions and at 18℃ in a 10-hr photoperiod, exhibiting the best growth at 10℃ in a 14-hr photoperiod. Under these conditions they became fertile and formed plurilocular sporangia. At 18℃ and 22℃ in a 14-hr photoperiod, they grew slightly for about 20 days and then became firm. These firm erect fronds did not become fertile.

2) Gametes and their movement

Gametes are pyriform and laterally biflagellate, containing a single chromatophore and one eyespot. They are slightly anisogamous; female gametes measure about 8.7 μm×4.5 μm (Fig. 1, F) and male ones about 7.0 μm×4.0 μm (Fig. 1, I). The gametes of both sexes swim for a while and display negative phototaxis. They soon become sluggish and settle. The period of motility of the female gametes is longer than that of the zoospores, but shorter than that of the male gametes. By mixing both kinds of gametes, conjugation occurs and zygotes are formed. Unfused gametes can develop parthenogenetically.

3) Development of zygotes

After sexual fusion, zygotes soon settle on the substratum and become spherical, measuring 6.8-7.7 μm diam. (Fig. 1, L). Within 24 hours, settled zygotes send out a germination tube, into which the protoplasm migrates. The germlings invariably developed into parenchymatous discs (Fig. 1, N).

Under Set 3 conditions, the discs consisted of about 20 cells in 10-day-old cultures. In 35 days, an erect frond arose from the central part of the disc. At the same time, the basal part of the erect frond developed into a prostrate thallus with horizontal branches.

In 80 days after formation of the erect frond, sessile unilocular sporangia were formed laterally on the basal cell of the cortical filaments (Fig. 2, A; Pl. I, F). Fertile unilocular sporangia were of the same size as those observed on plants collected in nature, measuring about 53-66μm×26-36μm. The chromosome number of the plants with unilocular sporangia was about 40 (Pl. I, K) and meiosis occurred in the sporangia.

Under Set 1 and Set 2 conditions, the discs produced the erect fronds whose basal part did not develop. Under Set 4 and Set 5 conditions, the discs developed into branched prostrate thalli only.

An apical portion of horizontal branches of the prostrate thalli was excised and transferred to the nine different conditions mentioned above. Within one month, all the apical fragments of the horizontal branches cultured at 5℃ and 10℃ in a 10-hr photoperiod produced erect fronds. Within two months, the formation of erect fronds was about 80% at 14℃ in a 10-hr photoperiod and at 5℃ and 10℃ in a 14-hr photoperiod. At 18℃ in a 10-hr photoperiod, a few fragments produced the erect fronds. At 14℃, 18℃ and 22℃ in a 14-hr photoperiod, they never produced erect fronds but developed into prostrate thalli.

Small erect fronds grown under Set 1 conditions were transferred to the nine different conditions. The erect fronds grew well under the 5℃, 10℃ and 14℃ conditions and at 18℃ in a 10-hr photoperiod, exhibiting the best growth at 10℃ in a 14-hr photoperiod. Under these conditions the erect fronds became fertile and formed unilocular sporangia. At 18℃ and 22℃ in a 14-hr photoperiod, they grew slightly, soon becoming firm but never matured.

4) Parthenogenesis

Unfused female and male gametes commonly develop parthenogenetically. After swimming, they settle on the substratum and become spherical. Settled female gametes measure about 5.4–6.4 μm diam. (Fig. 1, G) and male ones about 4.7–5.4 μm diam. (Fig. 1, J). They develop into the same parenchymatous discs (Fig. 1, H & K) as those of the zoospores and zygotes. In 10 days, such discs from female gametes consisted of about 14 cells and those from female gametes about 11 cells.

In about 40 days under Set 3 conditions, some discs from the female and male gametes began to produce erect fronds, though the formation of the erect fronds in the former usually

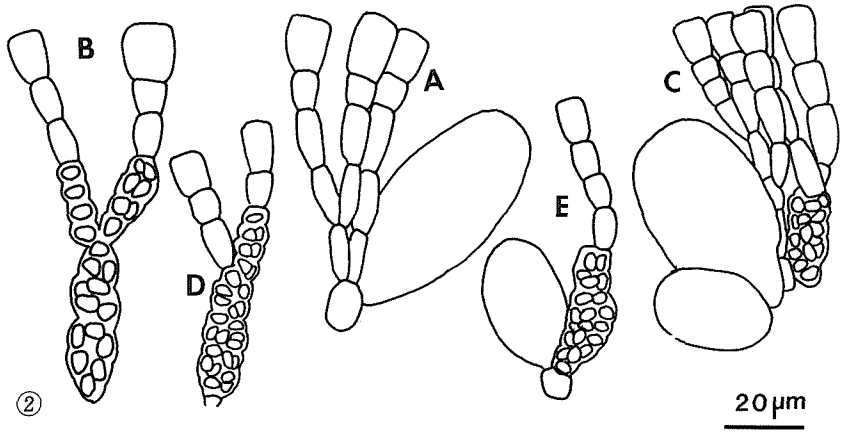

Fig. 2 *Analipus japonicus*. A. Unilocular sporangium formed on an erect frond derived from a zygote. B. Plurilocular sporangium formed on an erect frond derived from a female gamete. C. Unilocular and plurilocular sporangia formed on the same. D. Plurilocular sporangium formed on an erect frond from a male gamete. E. Unilocular and plurilocular spornagia formed on the same.

commenced 4–5 days earlier than in the latter. The basal parts of the erect fronds developed into the branched prostrate thalli. Within 3 months, all of discs derived from the female and the male gametes under Set 1 conditions, about 75% of them under Set 2 conditions and about 60% of them under Set 3 conditions produced the erect fronds. All the discs grown under Set 4 and Set 5 conditions developed into the branched prostrate thalli only. The formation of the sporangia began to occur 80 days after the formation of the erect fronds.

Parthenogenetic plants from the female gametes

Among 90 fertile individuals cultured in 1966, 85 individuals bore plurilocular sporangia only (Fig. 2, B; Pl. I, I) and 5 individuals bore both plurilocular and unilocular sporangia on the same frond (Fig. 2, C; Pl. I, G). These fertile unilocular sporangia were obovoid, measuring 37–63 μm×14–38 μm, and were slightly shorter than those derived from the zygotes. In 160 fertile individuals cultured in 1967, however, all bore plurilocular sporangia only. The chromosome number of these parthenogenetic plants was about 20, being haploid. Meiosis did not occur in the unilocular sporangia.

Parthenogenetic plants from the male gametes

Of 33 plants cultured in 1966, 6 individuals bore plurilocular sporangia only (Fig. 2, D; Pl. I, J) and 27 individuals bore unilocular and plurilocular sporangia on the same frond (Fig. 2, E; Pl. I, H). In the cultures of 1967, 160 fertile individuals were examined. Of them, 3 individuals bore plurilocular sporangia only and 157 individuals bore both kinds of sporangia on the same frond. The fertile unilocular sporangia were obovoid, measuring 34–66 μm×20–42 μm, slightly shorter than those derived from the zygotes. The chromosome number of these parthenogenetic plants was about 20, and meiosis did not occur in the unilocular sporangia.

Effects of temperature and photoperiod on the development of parthenogenetic plants

The basal prostrate portion of an erect frond bearing both kinds of sporangia grown under Set 3 conditions was excised into fragments and cultured under Set 1, Set 2 and Set 3

Table 1 Development of isolated apical fragments from a prostrate thallus which possessed erect fronds bearing plurilocular and unilocular sporangia.

	Female			Male		
Culture conditions	Set 1	Set 2	Set 3	Set 1	Set 2	Set 3
Number of isolated fragments of a prostrate thallus	6	2	6	7	6	13
Erect fronds	6	2	6	7	5	11
Fertile erect fronds with plu. spo. only	1	0	3	0	1	4
Fertile erect fronds with uni. spo. only	2	0	0	1	0	1
Fertile erect fronds with both sporangia	2	0	0	4	2	1
Unfertile erect fronds	1	2	0	2	2	5
Prostrate thalli	0	0	0	0	1	2

conditions. Within 2 months all of the fragments kept under Set 1 conditions, about 65% under Set 2 conditions and about 50% under Set 3 conditions produced erect fronds. Within 6 months, all of the isolated prostrate fragments derived from a female gamete produced erect fronds under Set 1, Set 2 and 3 conditions. However, a few derived from a male gamete did not produce the erect fronds but developed into the prostrate thalli under Set 2 and Set 3 conditions. In 3–4 months after the formation of the erect fronds, the plants became fertile. As shown in Table 1, among the erect fronds derived from the same prostrate thalli, some of them formed plurilocular sporangia only and others formed either unilocular sporangia only or both kinds of sporangia. The formation of the plurilocular sporangia was promoted by Set 3 conditions and that of the unilocular sporangia by Set 1 conditions.

5) The second generation from parthenogenetic plants

Four kinds of parthenogenetic plants occurred in culture, plant *1* which was derived from female gametes and bore plurilocular sporangia only, plant *2* which was derived from female gametes and bore both plurilocular and unilocular sporangia, plant *3* which was derived from male gametes and bore plurilocular sporangia only, and plant *4* which was derived from male gametes and bore both plurilocular and unilocular sporangia. Swarmers liberated from the plant *1* and the plant *3* conjugated. The settled gametes from the plant *1* measured about 6.4 μm diam. and those from the plant *3* measured about 5.1 μm diam. Consequently, the plant *1* is female and the plant *3* is male.

In order to investigate the second generation from the parthenogenetic plant, cultures were made (a) from the zygotes derived from plant *1* and *3*, (b) from the unfused female and male gametes liberated from the plant *1* and *3*, and (c) from the isolated plurilocular and unilocular sporangia borne on the plant *2* and *4*. Discussion of the various F_2 plants (a–c) follows.

(a) The zygotes soon settled on the substratum and became spherical. Their development was the same as that of zygotes of the first generation mentioned above. Erect fronds were produced under Set 1, Set 2 and Set 3 conditions and formed unilocular sporangia only. The chromosome number of these plants was about 40, being diploid. Meiosis occurred in the unilocular sporangia.

(b) The unfused female and male gametes were cultured separately under Set 1 and Set 3 conditions. They developed normally and produced the erect fronds. Eighty days after the formation of the erect frond, the plants from the female gametes formed plurilocular sporangia only, whereas those from the male gametes formed both unilocular and plurilocular sporangia on the same frond (Table 2).

(c) The plurilocular and unilocular sporangia formed on the same frond of the plant *2* and *4* were isolated singly into test tubes and kept under Set 3 conditions. Within 5 days, some of them liberated swarmers, but some swarmers germinated in the sporangia without being liberated. Swarmers liberated from the plurilocular and the unilocular sporangia

Table 2 Second generation of parthenogenetic plants. Development of female and male gametes derived from parthenogenetic plants.

	Female		Male	
Culture conditions	Set 1	Set 3	Set 1	Set 3
Number of isolated germlings	30	90	30	120
Erect fronds	30	82	30	32
Fertile erect fronds with plu. spo. only	30	82	0	0
Fertile erect fronds with uni. spo. only	0	0	0	0
Fertile erect fronds with both sporangia	0	0	30	32
Prostrate thalli	0	0	0	38

Table 3 Second generation of parthenogenetic plants descended from female and male gametes. Development of swarmers from plurilocular sporangia of the parthenogenetic plant bearing both plurilocular and unilocular sporangia.

	Female			Male		
Culture conditions	Set 1	Set 2	Set 3	Set 1	Set 2	Set 3
Number of isolated germlings	8	6	8	9	4	19
Erect fronds	8	6	0	9	4	7
Fertile erect fronds with plu. spo. only	0	0	/	1	2	4
Fertile erect fronds with uni. spo. only	5	0	/	3	0	1
Fertile erect fronds with both sporangia	0	0	/	5	2	2
Unfertile erect fronds	3	6	/	0	0	0
Prostrate thalli	0	0	8	0	0	12

developed into minute parenchymatous discs. After one month, these discs were isolated singly and cultured under Set 1, Set 2 and Set 3 conditions.

All the discs derived from plurilocular sporangia swarmers of plant *2* (descended from a female gamete) produced erect fronds under Set 1 and Set 2 conditions. Under Set 3 conditions, however, the discs did not produce the erect fronds but developed into the prostrate thalli. Under Set 1 conditions, some erect fronds formed unilocular sporangia only but others did not form any reproductive organs. Under Set 2 conditions they never matured (Table 3).

All the discs derived from the plurilocular sporangia swarmers of the plant *4* (descended from a male gamete) under Set 1 and Set 2 conditions, and about 65% of them under Set 3 conditions produced the erect fronds. Among these fertile fronds, some bore plurilocular or unilocular sporangia only and others formed both kinds of sporangia. The formation of plurilocular sporangia was promoted by Set 3 conditions and unilocular sporangia by Set 1 conditions (Table 3).

A half of the discs derived from unilocular sporangia swarmers of plant *2* produced erect

Table 4 Second generation of parthenogenetic plants descended from female and male gametes. Development of swarmers from unilocular sporangia of the parthenogenetic plant bearing both plurilocular and unilocular sporangia.

	Female			Male		
Culture conditions	Set 1	Set 2	Set 3	Set 1	Set 2	Set 3
Number of isolated germlings	11	8	16	4	4	21
Erect fronds	5	6	0	4	4	14
Fertile erect fronds with plu. spo. only	0	1	/	2	4	11
Fertile erect fronds with uni. spo. only	2	0	/	2	0	0
Fertile erect fronds with both sporangia	0	0	/	0	0	0
Unfertile erect fronds	3	5	/	0	0	3
Prostrate thalli	6	2	16	0	0	7

fronds under Set 1 conditions. Some of the plants became fertile and formed unilocular sporangia only. Under Set 2 conditions, about 75% of the discs produced erect fronds, one of which became fertile and formed plurilocular only. Under Set 3 conditions the discs did not produce erect fronds (Table 4).

All the discs derived from the unilocular sporangia swarmers of plant *4* under Set 1 and Set 2 conditions and about 65% of the discs under Set 3 conditions produced erect fronds. Some fertile fronds bore both plurilocular and unilocular sporangia, but some produced only the former under Set 1 conditions. However, all the fertile fronds under Set 2 and Set 3 conditions formed only plurilocular sporangia (Table 4). The fertile plants were all haploid.

6) The third generation from parthenogenetic plants

As shown in Table 2, 3 and 4, ten kinds of fertile plants were obtained from the second generation of parthenogenetic plants. The cultures of the third generation of parthenogenetic plants were started from the sporangia of these ten kinds of the fertile plants in the same way as those of the second generation. The isolated sporangia were cultured under Set 3 conditions and liberated swarmers germinated, developing into the minute parenchymatous discs. These discs were isolated singly and transferred to Set 1, Set 2 and Set 3 conditions. Under these culture conditions almost all of the discs produced the erect fronds, as shown in Table 5–6. Under Set 3 conditions several discs developed into prostrate thalli only. Within one year after the isolation of the discs, most of the erect fronds became fertile.

In the third generation, as shown in Tables 5–6 and Fig. 3, all the erect fronds from the swarmers liberated from unilocular sporangia formed plurilocular sporangia only. The erect fronds from plurilocular sporangia descended from the female gametes, bore either plurilocular sporangia or unilocular sporangia. The plants from plurilocular sporangia descended from the male gametes, formed either plurilocular sporangia only or both kinds of

Table 5 Third generation from parthenogenetic plants descended from female gametes.

Group of third generations from a sporangium	3-1			3-2			3-3			3-4		
Kind of isolated sporangia	plu.			uni.			plu.			uni.		
Culture condition	Set 1	Set 2	Set 3	Set 1	Set 2	Set 3	Set 1	Set 2	Set 3	Set 1	Set 2	Set 3
Number of isolated germlings	5	5	11	10	10	11	10	10	10	9	9	10
Number of erect fronds	5	5	10	10	10	6	10	10	7	9	9	4
Fertile erect fronds with plu. spo. only	5	5	10	10	10	6	0	0	0	9	9	2
Fertile erect fronds with uni. spo. only	0	0	0	0	0	0	10	10	7	0	0	0
Fertile erect fronds with the both sporangia	0	0	0	0	0	0	0	0	0	0	0	0
Unfertile erect fronds	0	0	0	0	0	0	0	0	0	0	0	2
Prostrate thalli	0	0	1	0	0	5	0	0	3	0	0	6

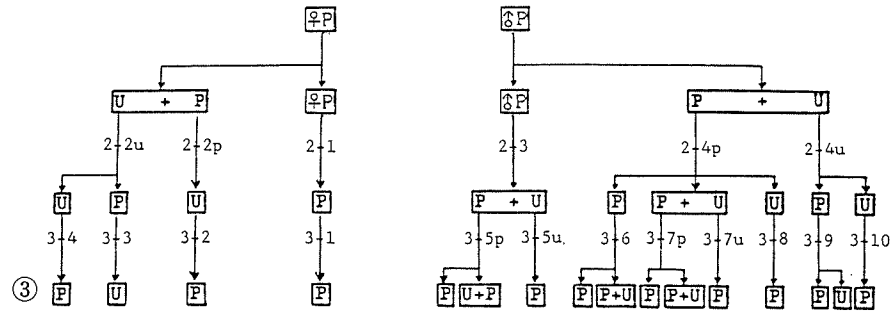

Fig. 3 *Analipus japonicus*. Succession of generations in cultures. U, Erect frond with unilocular sporangia; P, Erect frond with plurilocular sporangia; 2-1～3-10, Group of germlings isolated from a sporangium.

sporangia. The formation of unilocular sporangia was promoted by Set 1 conditions. The plants of this generation were all haploid.

Discussion of the life history of *Analipus japonicus*

ABE (1935a, 1935b, 1936) studied *A. japonicus* from several localities of Japan and reported that the early stages of development of zygotes, unfused gametes and zoospores were quite similar. He also observed that the chromosome number of the plants with plurilocular sporangia was 20 and plants with unilocular sporangia was 40 and that meiosis

Table 6 Third generation from parthenogenetic plants descended from male gametes.

Group of third generations from a sporangium	3-5p			3-5u			3-6			3-7p		
Kind of isolated sporangia	plu			uni			plu			plu		
Culture conditions	Set 1	Set 2	Set 3	Set 1	Set 2	Set 3	Set 1	Set 2	Set 3	Set 1	Set 2	Set 3
Number of isolated germlings	3	3	6	11	8	14	12	12	14	7	7	7
Number of erect fronds	3	2	4	11	8	13	12	12	11	7	7	6
Fertile erect fronds with plu. spo. only	1	1	2	11	8	12	0	4	4	0	3	0
Fertile erect fronds with uni. spo. only	0	0	0	0	0	0	0	0	0	0	0	0
Fertile erect fronds with the both sporangia	2	1	1	0	0	0	12	8	6	7	4	6
Unfertile erect fronds	0	0	1	0	0	1	0	0	1	0	0	0
Prostrate thalli	0	1	2	0	0	1	0	0	3	0	0	1
Group of third generations from a sporangium	3-7u			3-8			3-9			3-10		
Kind of isolated sporangia	uni			uni			plu			uni		
Culture conditions	Set 1	Set 2	Set 3	Set 1	Set 2	Set 3	Set 1	Set 2	Set 3	Set 1	Set 2	Set 3
Number of islated germlings	10	10	10	2	2	2	4	4	7	13	13	13
Number of erect fronds	10	10	10	2	2	2	4	4	4	13	13	11
Fertile erect fronds with plu. spo. only	10	10	10	2	2	2	0	1	2	13	13	11
Fertile erect fronds with uni. spo. only	0	0	0	0	0	0	3	0	1	0	0	0
Fertile erect fronds with the both sporangia	0	0	0	0	0	0	0	0	0	0	0	0
Unfertile erect fronds	0	0	0	0	0	0	1	3	1	0	0	0
Prostrate thalli	0	0	0	0	0	0	0	0	3	0	0	2

occurred in the unilocular sporangia. Furthermore, he reported that conjugation occurred not only between female and male gametes from plurilocular sporangia but also among the swarmers from unilocular sporangia of different but rarely of the same individuals. Recently, NELSON and COLE (1981) measured the relative DNA content of nuclei and NELSON (1982b) followed the growth of plants to reproductive maturity started from swarmers in

culture. They concluded that plants bearing plurilocular sporangia were haploid and plants bearing unilocular sporangia were diploid.

The results obtained from the present study agree with those of ABE and NELSON, excepting the conjugation of the swarmers from unilocular sporangia. The germlings from zoospores, zygotes and unfused gametes first send out a germination tube and the protoplasm migrates into this tube, forming a new cell. Then the new cell is divided transversally and longitudinally, developing into a minute disc. Such a developmental type of *A. japonicus* quite differs from that of the members of the Ectocarpales and Chordariales, in which germlings form creeping filaments and then develop into pseudoparenchymatous discs (NAKAMURA, 1972).

Analipus japonicus is perennial and is found two life forms in nature ; winter-form which is an erect pseudoparenchymatous frond with lateral branches and summer-form which is a prostrate thallus with horizontal branches. The occurrence of these two forms in culture has been experimentally demonstrated in the present study. Production of erect fronds were favored by cool and short-day conditions (5-10℃ and 10-hr photoperiod). The prostrate thalli developed exclusively under warmer and long-day conditions (14-22℃ and 14-hr photoperiod).

Reproductive organs were formed only on the erect fronds. The plants from zoospores formed plurilocular sporangia and were haploid, while the plants from zygotes bore unilocular sporangia and were diploid. No exception in the regular alternation of diploid asexual plants with haploid sexual plant was observed.

On the other hand, the plants derived from unfused gametes formed either plurilocular sporangia or both kinds of sporangia on the same individual. They were all haploid. Most of the parthenogenetic plants from the female gametes formed only plurilocular sporangia which were female gametangia, and a few plants bore both plurilocular and unilocular sporangia on the same individual. Most of the parthenogenetic plants from the male gametes formed both plurilocular and unilocular sporangia and a few plants bore only plurilocular sporangia which were male gametangia. It is necessary to carry out the conjugation tests among swarmers from the plants with both plurilocular and unilocular sporangia to determine their sex. Unfortunately, I was unable to do this as simultaneous liberation of swarmers from different plants in culture has not been established. In *Ectocarpus siliculosus*, haploid plants with unilocular sporangia were asexual and haploid sexual plants never formed unilocular sporangia (MÜLLER, 1967). If the same is true for *A. japonicus*, the haploid plant with unilocular sporangia is asexual.

From the observation of the second and third parthenogenetic generations, it seems that *A. japonicus* exhibits the following parthenogenetic succession: 1) The plant repeats the generations with plurilocular sporangia only ; 2) The plant has an alternation between the generation with plurilocular sporangia and that with either unilocular sporangia or both kinds of sporangia ; 3) The plant repeats the generation with both kinds of sporangia. The

parthenogenetic succession derived from female gametes shows type 1) or 2) succession and that derived from male gametes shows type 2) or 3) succession.

On the plants with both unilocular and plurilocular sporangia, it depends on environmental conditions which sporangia are formed. It has been reported that in *Ectocarpus siliculosus* and *E. confervoides* unilocular sporangia are formed under lower temperature conditions (MÜLLER, 1962). Also SAKAI and SAGA (1981) have found that macrothalli of *Pogotrichum yezoense* form unilocular sporangia under lower temperature conditions and form plurilocular sporangia under higher temperature conditions. In *A. japonicus*, unilocular sporangia are formed more abundantly under lower temperature conditions on the erect fronds derived from unfused gametes.

Desmarestiales, Desmarestiaceae

The life history of *Desmarestia* was first studied by SCHREIBER (1932), who discovered that *D. aculeata* possesses a heteromorphic alternation of generations identical to that of the Laminariales. Gametophytes consist of luxuriant filamentous thalli and are dioecious (*D. aculeata*, SCHREIBER, 1932; CHAPMAN and BURROWS, 1971) or monoecious (*D. viridis*, KORNMANN, 1962; *D. tabacoides*, NAKAHARA and NAKAMURA, 1971). In the early stages of development, sporophytes consist of unbranched erect filaments which later branch. The axial series becomes surrounded by a cortical envelope. The sporophytes form unilocular sporangia in which meiosis occurs (ABE, 1938).

The effects of environmental conditions on growth and maturation have been reported by some workers. According to KORNMANN (1962), the gametophytes of *D. viridis* become fertile at lower temperature (3–5℃). The maturation of gametophytes of *D. aculeata* is governed by the total quantity of light energy received per day at 8.5℃ (CHAPMAN and BURROWS, 1970). The gametophytes of *D. tabacoides* become fertile under cool and short-day conditions (NAKAHARA and NAKAMURA, 1971).

Desmarestia viridis (MÜLLER) LAMOUROUX

D. viridis is one of the most common brown algae, growing on rocks in the sublittoral zone in the middle and northern Japan, Shikoku, Honshu and Hokkaido. In the vicinity of Muroran, *D. viridis* is found in the upper sublittoral zone from November to August. Fertile plants are found from May to August. Unilocular sporangia are formed lying embedded in the superficial layer of the cortical envelope (Fig. 4, A–B) and discharge zoospores.

1) Gametophytes

Zoospores and their development

Zoospores are pyriform and laterally biflagellate (Fig. 5, A), measuring about 7.6 μm × 4.8 μm. They contain a single chromatophore and eyespot. They swim for about 2 hours at room temperature (15–20℃) and display negative phototaxis. Settled zoospores

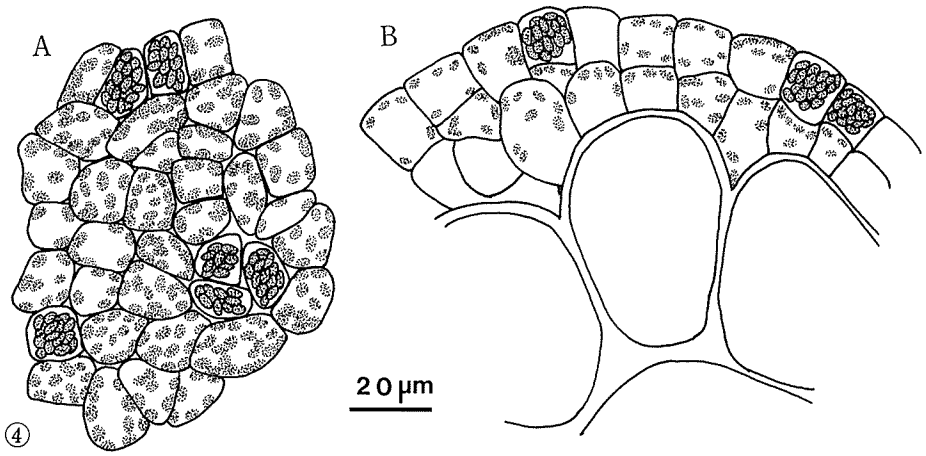

Fig. 4 *Desmarestia viridis*. A. Surface view of thallus with unilocular sporangia collected from nature. B. Cross section of the same.

become spherical and measure 5.5–6.0 μm diam. (Fig. 5, B). Within 24 hours, these send out a germination tube, into which most of the protoplasm migrate (Fig. 5, C) and then form a cross-wall to separate this tube from the original spore. By successive transversal cell divisions and branching, germlings develop into minute thalli (gametophytes) composed of branched uniseriate filaments (Fig. 5, D).

In 18-days cultures under Set 2 conditions, gametophytes began to form antheridia and oogonia. They were monoecious (Fig. 5, E; Pl. II, B), but fertile gametophytes composed of less than about 30 cells, formed either oogonia or antheridia (Fig. 5, F–G).

The antheridia arose laterally from cells of branches and formed clusters. The oogonia developed from the terminal cells of branches. In many cases, the eggs were only partially extruded from the oogonium. Such eggs began to germinate *in situ*, developing into sporophytes (Pl. II, D–F). Some oogonia discharged an egg through a narrow apical opening and the egg usually remained attached to the opening. When the egg was extruded completely from the oogonium, the wall of the empty oogonium could be detected for some time. Later, the adjacent cell to the empty oogonium developed into an oogonium (Pl. II, C–F).

The gametophytes grown under Set 1 conditions became fertile within 25 days. Whereas gametophytes grown under Set 3, Set 4 and Set 5 conditions (long-day conditions) developed only vegetatively into copiously branched filamentous thalli (Pl. II, A). When the culture medium was changed, however, maturation of gametophytes commenced in Set 3 conditions but soon after these gametophytes grew only vegetatively, not producing gametangia. Under Set 4 and Set 5 conditions, the fragments or single cells cut off from the gametophytes developed again into profusely branched immature filamentous thalli. The cell diameter and length adjacent to the terminal cell of branches were 7.8 μm$\pm$1.0$\times$48.1

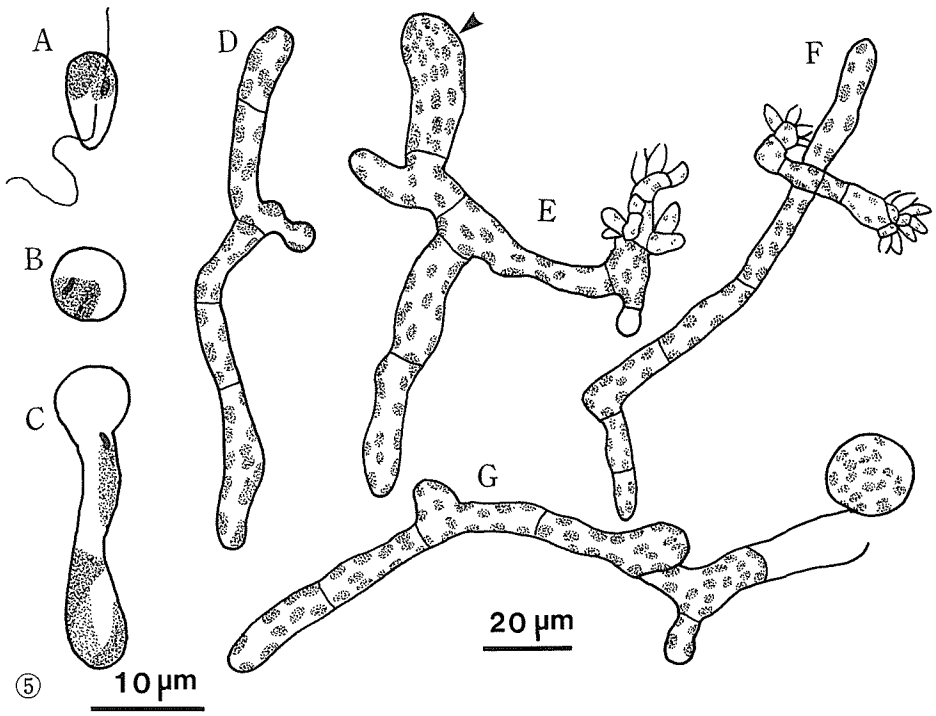

Fig. 5 *Desmarestia viridis*. A. Zoospore. B. Settled zoospore. C. Germination of zoospore. D. 10-day-old germling grown under Set 2 conditions. E–G. 18-day-old fertile gametophytes grown under Set 2 conditions: E, Fertile gametophyte bearing an oogonium (arrowhead) and antheridia; F, Fertile gametophyte with antheridia; G, Fertile gametophyte with an egg on the oogonium. (A–C drawn to 10 μm scale. D–G drawn to 20 μm scale.)

μm$\pm$7.1. The chromosome number of the gametophytes was about 22 (Pl. II, L).

Effect of temperature and photoperiod on growth and maturation

Experiment 1: Zoospores were cultured at 10℃ and 18℃ in four different photoperiods; 8, 10, 12 and 14 hrs. The growth of gametophytes was promoted at the higher temperature and in longer photoperiod (Fig. 6). Maturation occurred in shorter photoperiod and lower temperature (Table 7). In the 8- or 10-hr photoperiod all apical cells of branches of the gametophytes formed gametangia, showing no further vegetative growth. In a 12-hr photoperiod, they formed gametangia and grew vegetatively.

Experiment 2: A large gametophyte grown under Set 4 conditions was cut into small pieces composed of 100–150 cells. These were cultured at 5℃ in a 10- or 14-hr photoperiod (Fig. 7), at 10℃ in a 10- or 14-hr photoperiod (Fig. 8), at 14℃ in a 10- or 14-hr photoperiod (Fig. 9), at 18℃ in a 10- or 14-hr photoperiod (Fig. 9) and at 22℃ in a 14-hr photoperiod. The maturation of gametophytes was determined by the percentage of fertile branches to the

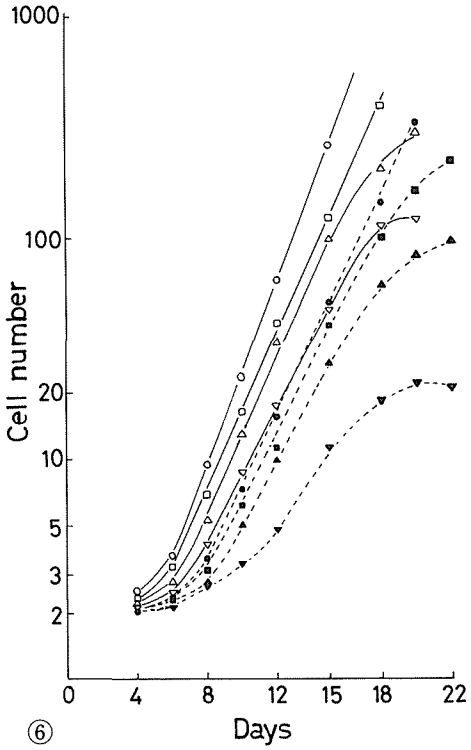

Fig. 6 Growth of *Desmarestia viridis* gametophytes in four photoperiods at 18℃, ○—○, 14 : $\overline{10}$; □—□, 12 : $\overline{12}$; △—△, 10 : $\overline{14}$; ▽—▽, 8 : $\overline{16}$, and in four photoperiods at 10℃, ●—●, 14 : $\overline{10}$; ■—■, 12 : $\overline{12}$; ▲—▲, 10 : $\overline{14}$; ▼—▼, 8 : $\overline{16}$.

Table 7 Effect of photoperiod on maturaion of *D. viridis* gametophytes.

Temperature (℃)	Photoperiod (hrs daily)	First appearance of gametangia	
		Days	Cell number
10	8	12	7
	10	17	48
	12	24	about 600
	14	—	—
18	8	14	28
	10	20	about 400
	12	27	about 800
	14	—	—

total number of branches. Complete maturation occurred after 7 days in a 10-hr photoperiod at 10℃, 14℃ or 18℃ and after 9 days in a 10-hr photoperiod at 5℃. In fully matured gametophytes, the percentage of antheridial branches was 30-40 and that of oogonial branches 50-60 after 14 days in a 10-hr photoperiod at 5℃, 10℃, 14℃ or 18℃ (Table 8). In 14-hr photoperiod at 5℃ and 10℃, the percentage of both sexual organs was 15-27. The gametophytes never matured in the 14-hr photoperiod at 14℃, 18℃ or 22℃.

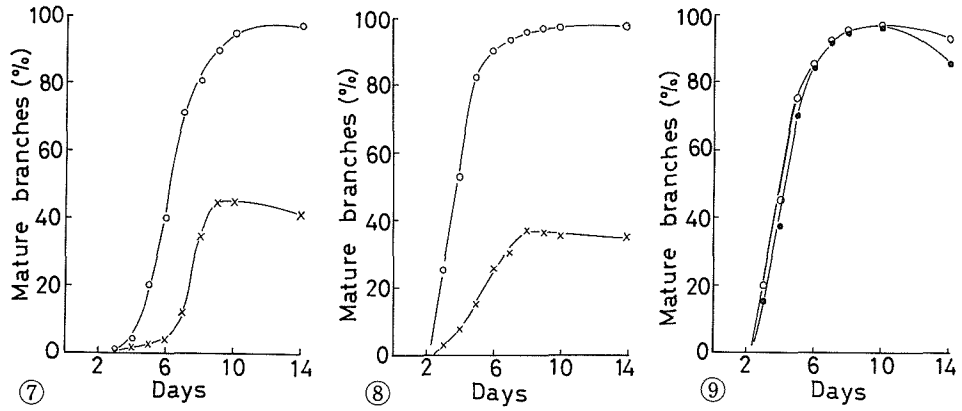

Fig. 7 Maturation of *Desmarestia viridis* gametophytes in two photoperiods at 5℃, ○—○, 10 : $\overline{14}$; ×—×, 14 : $\overline{10}$.

Fig. 8 Maturation of *Desmarestia viridis* gametophytes in two photoperiods at 10℃, ○—○, 10 : $\overline{14}$; ×—×, 14 : $\overline{10}$.

Fig. 9 Maturation of *Desmarestia viridis* gametophytes in a 14-hr photoperiod at 14℃, ○—○ and 18℃, ●—●.

Table 8 Percentage of mature branches formed on *D. viridis* gametophytes after 14 days at four temperatures in 10- and 14-hr photoperiods.

Photoperiod (hours)	10				14			
Temperature (℃)	5	10	14	18	5	10	14	18
% of antheridia branches	36.9	37.0	40.0	31.9	26.9	15.5	0	0
% of oogonial branches	59.5	59.3	52.6	52.2	14.5	18.7	0	0
% of immature branches	3.6	3.7	7.2	15.8	58.5	65.8	100	100

Each value is the average of six samples from three cultures.

2) Sporophytes

Development of fertilized eggs germinated inside oogonia

As mentioned above, most eggs germinated inside the oogonia. Under Set 2 conditions, the fertilized eggs elongated to twice the length of the oogonium and divided transversally into two cells. By successive transverse divisions and enlargement of cells, germlings developed into unbranched erect filaments which constituted the main axis of young sporophytes. The lowest cell of the erect filament, which is covered by the oogonium wall did not enlarge and the oogonial wall could be detected for a long time (Fig. 10, A–C). Within 6 days after germination, the erect filaments consisted of up to 30 cells and began to give off opposite branches in the middle and upper parts of the main axis (Fig. 10, D). At the same time, a part of the main axis developed into a intercalary meristem. Then the primary rhizoid was produced from the lower part of the cell adjacent to the lowest cell of the main

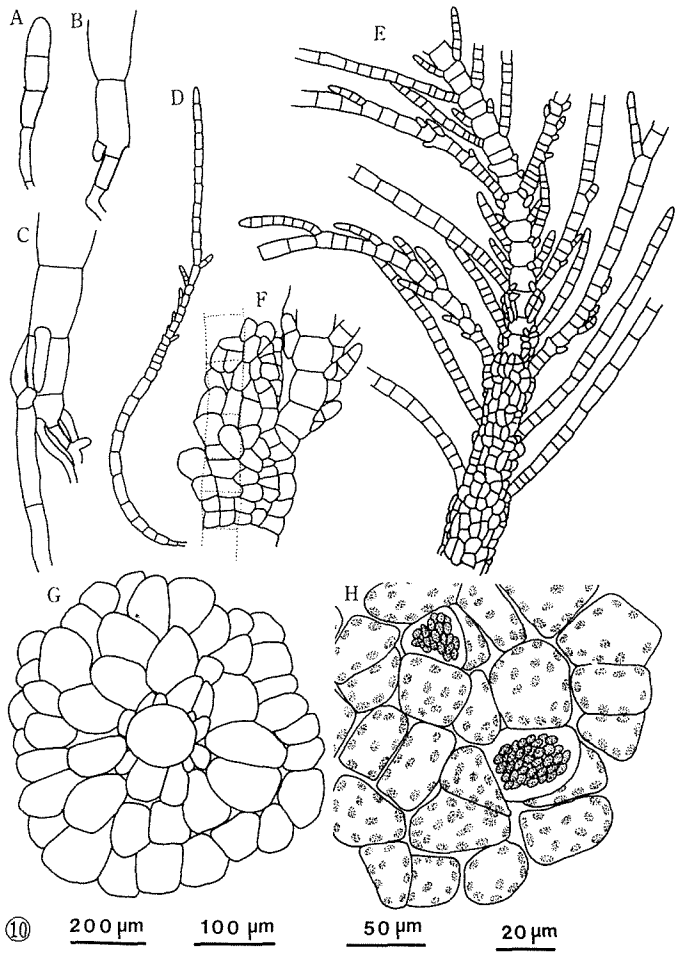

Fig. 10 *Desmarestia viridis.* A. 3-celled sporophyte germinated in an oogonium. B. Basal part of 35-celled sporophyte with a rhizoid initial. C. Basal part of 50-celled sporophyte with several rhizoids. D. Young monosiphonous sporophyte. E. Middle part of young sporophyte, showing development of the cortex. F. Part of corticated main axis. G. Cross section of the upper part of 20-day-old sporophyte. H. Surface view of thallus with unilocular sporangia from 3-month-old culture grown under Set 4 conditions. (A–C and F–G drawn to 50 μm scale. D drawn to 200 μm scale. E drawn to 100 μm scale. H drawn to 20 μm scale.)

axis, which was uncovered with the original oogonial wall (Fig. 10, B) and later from the lower axial cells (Fig. 10, C). The rhizoidal cells were distinguishable by their poor chromatophores which is located at their distal end. The elongation and cell divisions of the rhizoids took place in the apical cells only. Numerous rhizoids were gradually covered the

gametophytes.

When the main axis was about 100 cells, corticating filaments were produced from the basal cells of branches in the lower part and later in the upper part of the meristem. The corticating filaments developed in close apposition to the cells of the main axis below. By successive elongation, cell divisions and branching, the corticating filaments entirely enveloped the axial cells (Fig. 10, E; Pl. II, G). The one-layered cortex gradually increased in thickness by branchings of corticating filaments and by the production of hyphae from the cortical cells. Finally, the thallus became terete (Pl. II, H & J).

In about 110-day cultures, some sporophytes became fertile (Pl. II, J). The unilocular sporangia were formed in the superficial layer of the cortical envelope (Fig. 10, H). The chromosome number of the sporophytes was about 44 (Pl. II, M) and meiosis occurred in the unilocular sporangia. The zoospores liberated from these sporophytes developed into monoecious gametophytes in the same way as those from sporophytes collected in nature.

Development of fertilized eggs germinated outside oogonia

Sixty-five eggs discharged from oogonia (free eggs) were introduced singly into test tubes or glass depression-slides. Within 2-6 days, 28 eggs germinated among them. No differences in the germination rate of the free eggs were observed between the five sets of growth conditions. However, various types of germlings occurred as follows: (1) The egg first issued a rhizoidal cell and then gave rise to an erect filament. (2) The eggs produced a protuberance and divided into two cells; one or often both of these produced erect filament. (3) The egg formed a minute constriction and both the original egg cell and the constriction formed the erect filament. In the latter two types of germlings, the primary rhizoid was issued only from the original egg cell after the erect filaments grew to more than 20 cells (Fig. 11, A-B). The erect filaments of 10-20 cells began to give off mainly opposite branches. Sometimes, the original egg cell produced two or more erect filaments in opposite directions (Fig. 11, B-C). Furthermore, the branchings of the main axis took place in an inverse direction at the distal part of some germlings (Fig. 11, B). The chromosome number of these plants was about 44, showing diploid.

Effect of temperature and photoperiod on growth and maturation

Growth and development: Some 10-20-celled sporophytes under Set 2 conditions were separated from the gametophytes and introduced into test tubes. They were cultured at 5℃, 10℃, 14℃ or 18℃ in a 10- or 14-hr photoperiod and at 22℃ in a 14-hr photoperiod. No marked differences were recognized in the early stages of development of sporophytes under 5-18℃ conditions and the cortical envelope was formed in 10 days. As shown in Fig. 12, the best growth was obtained under the 5-10℃ conditions and the growth was strongly affected by temperature rather than photoperiod. Under 14℃ and 18℃ conditions, the elongation of the main axis of the sporophytes was arrested within 30 days and the cortical filaments grew separately around the main axis. Consequently, the sporophytes became mass tangled with cortical filaments (Pl. II, I). The sporophytes cultured at 22℃ did not form the cortex and

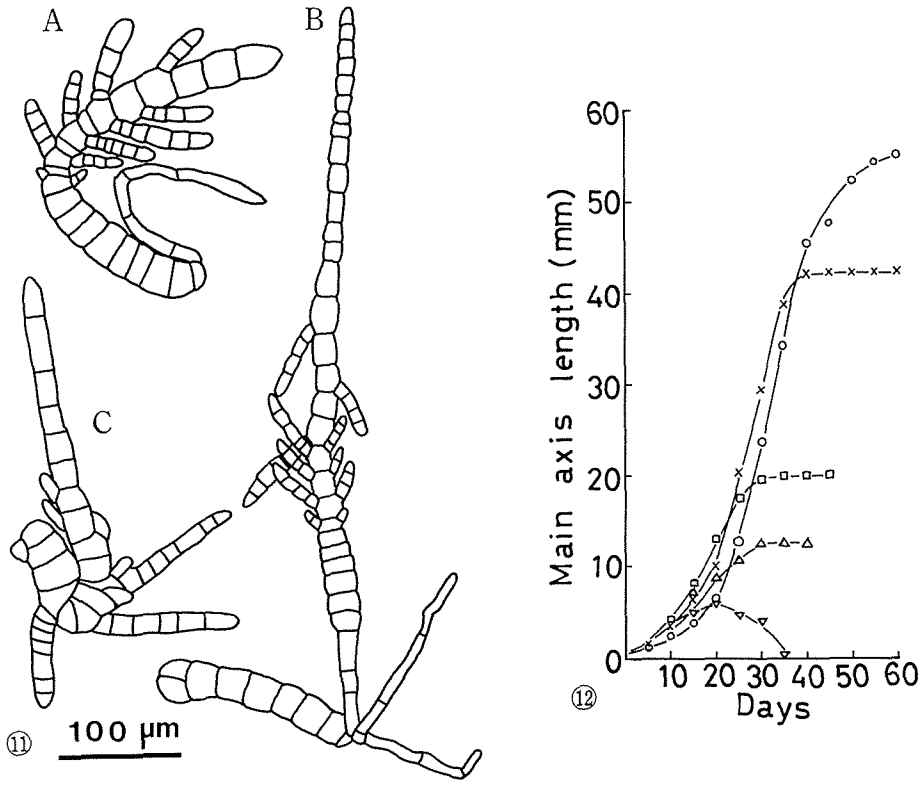

Fig. 11 *Desmarestia viridis*. Sporophytes derived from free zygotes.
Fig. 12 Growth of *Desmarestia viridis* sporophytes at five temperatures in a 14-hr photoperiod, ○—○, 5℃ ; ×—×, 10℃ ; □—□, 14℃ ; △—△, 18℃ ; ▽—▽, 22℃.

bleached within 20 days.

Maturation : The sporophytes were singly cultured for 180 days under Set 1, Set 2, Set 3, Set 4 and Set 5 conditions. In 70 days after the isolation, some sporophytes became fertile under Set 4 and Set 5 conditions. In process of time, numerous sporophytes became fertile under Set 3 and Set 2 conditions (Table 9). Under Set 1 conditions, however, the sporophytes formed no sporangia.

3) Parthenogenesis

It is not easy to obtain unfertilized eggs in culture, because the gametophytes are monoecious. For this purpose, cultures of gametophytes were established in the following way. Immature gametophytes grown under Set 4 conditions were transferred to Set 2 conditions. After 3 days, the distal 4-5-cell-branches which had slightly thickened were cut off with a fine glass needle. These fragments were transferred to a glass depression-slide and cultured under Set 2 conditions (Pl. III, A). They formed oogonia only for 20-38 days

Table 9 Number of fertile sporophytes of *D. viridis* within 6 months under various culture conditions.

Culture conditions	Number of isolated sporophytes	Number of fertile sporophytes				
		Months				
		2	3	4	5	Total
Set 1	30					0/30
Set 2	49		3	18	20	41/49
Set 3	63		15	28	12	55/63
Set 4	69	19	29	19		67/69
Set 5	65	20	22	23		65/65

Table 10 Number of *D. viridis* sporophytes germinated from unfertilized eggs.

Isolated apical cells from gametophytes	Days for cells producing oogonia only	Number of eggs produced	Number of sporophytes
Series 1-1	38	12	1
2	20	5	0
3	38	13	3*
4	20	6	1
Series 2-1	22	3	0
2	24	11	4
3	22	8	3
Series 3-1	50	4	4
2	50	31	14
3	50	39	12

* One sporophyte was diploid

1-1～1-4 and 2-1～2-3 were isolated from a gametophyte germinated from single zoospore of the diploid sporophyte.

3-1～3-3 were isolated from a gametophyte germinated from single swarmer of the haploid sporophyte.

and extruded several eggs (Pl. III, B). These free eggs were transferred to glass depression-slides or test tubes by a micropipette and cultured under Set 2 conditions. Within 6 days of transfer, a small constriction was produced from some unfertilized eggs (Pl. III, C-D). This constriction, as well as the original egg cell, developed into unbranched erect filaments and formed the main axis of young sporophytes (Pl. III, E-F). The germlings developed into sporophytes quite similar to those derived from the free fertilized eggs (Pl. III, G). The number of isolated eggs and the resultant germlings are shown in Table 10. In 5-month cultures, these sporophytes from unfertilized eggs became fertile (Pl. II, K) and formed unilocular sporangia in the superficial layer of the cortical envelope (Fig. 13).

The sporophytes obtained in this culture experiment were all confirmed by cytological

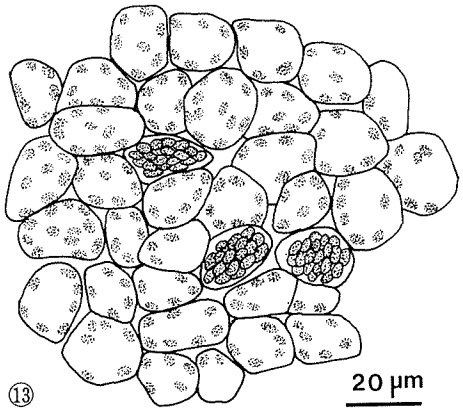

Fig. 13 *Desmarestia viridis*. Surface view of fruiting haploid sporophyte derived from an unfertilized egg.

observations. Excepting only one sporophyte which was diploid, the chromosome number of the sporophytes was all counted about 22–24, being haploid (Pl. II, N). Meiosis did not occur in the unilocular sporangia.

Swarmers liberated from unilocular sporangia of the haploid sporophytes, developed into the normal monoecious gametophytes in the same way as zoospores (Pl. III, H–I).

Apical fragments of branches from the immature gametophytes derived from swarmers of a haploid sporophyte were cultured to obtain unfertilized eggs in the same way as described above. As shown in series 3 of Table 10, these fragments continued fo form oogonia only for 50 days. Some of these discharged eggs developed into haploid sporophytes.

4) Regeneration of a single cell isolated from sporophytes

Cell divisions take place in the meristem at the upper part of the main axis and in the basal cells of branches. In rhizoids, however, it occurs in apical cells. The meristematic cells, the basal cells of branches and the apical cells of rhizoids were isolated singly from young sporophytes by excising with a fine glass needle. The isolated cells were transferred to glass depression-slides and cultured under Set 2 conditions. The isolated cells from the thallus did not regenerate and died within 5 days, whereas those from the rhizoid regenerated. The latter cells contained at first very poorly developed chromatophores at their distal end (Pl. IV, A), Within 2–3 days after isolation, these cells began to elongate at the distal end and septated transversally into two cells (Pl. IV, B). The distal cell developed into a uniseriate filament consisting of rhizoidal cells (Pl. IV, C–D). The cells of the proximal part of this filament, including the original cell, gradually increased in number of chromatophores and became normal vegetative cell, forming the erect main axis (Pl. IV, E–F). Later the cells of an erect part of the filament differentiated into an intercalary meristem. The erect main axis issued branches, forming the cortical envelope and finally developed into normal sporophytes (Pl. IV, G).

***Desmarestia ligulata* (LIGHTFOOT) LAMOUROUX**

D. ligulata is one of the common brown algae, growing on rocks in the sublittoral zone in northern Japan. In the vicinity of Muroran, *D. ligulata* is found growing on rocks in the upper sublittoral zone from November to August. Fertile plants are found from May to August. Unilocular sporangia are formed in the superficial layer of the cortical envelope (Fig. 14, A–B) and produce zoospores.

1) Gametophytes

Zoospores and their development

Zoospores are pyriform, and laterally biflagellated measuring about 7.7 μm×4.7 μm. They contain a single chromatophore and one eyespot (Fig. 15, A). They swim for about 2 hours at room temperature (15–20℃) and display negative phototaxis. Settled zoospores become spherical and measure 5.5–6.0 μm diam. (Fig. 15, B). Within 48 hours, they usually increase in size, becoming slightly ovoid (Fig. 15, C–D) and divide transversally into two cells. Infrequently, they send out a germination tube, into which the protoplasm migrates completely or partially. By successive transversal cell divisions and branching, germlings develop into minute thalli (gametophytes) composed of branched uniseriate filaments (Fig. 15, E).

In 18-day cultures under Set 1 conditions, the gametophytes were composed of five cells or less but they began to form gametangia. Most of them formed antheridia only at this time (Fig. 15, G) and a few formed oogonia only (Fig. 15, H). Later, however, they formed both antheridia and oogoina (Fig. 15, F). Under these culture conditions, a few settled zoospores did not divide. These single cells produced antheridia.

The antheridia arose laterally from cells of branches and formed clusters. The oogonia

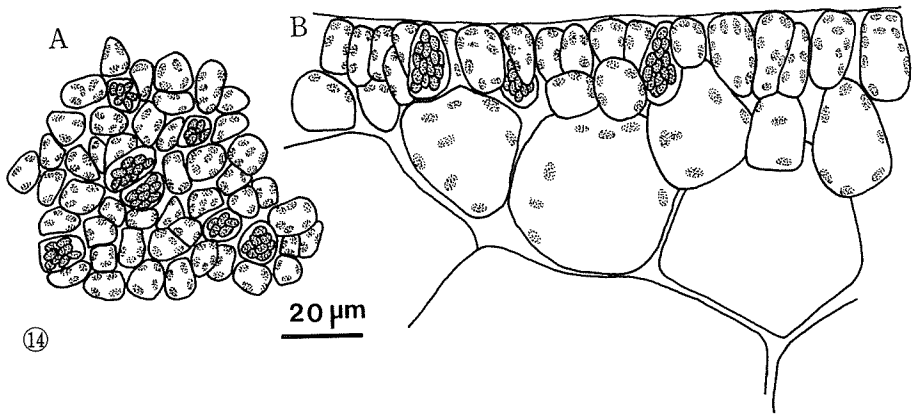

Fig. 14 *Desmarestia ligulata.* A. Surface view of thallus with unilocular sporangia collected from nature. B. Cross section of the same.

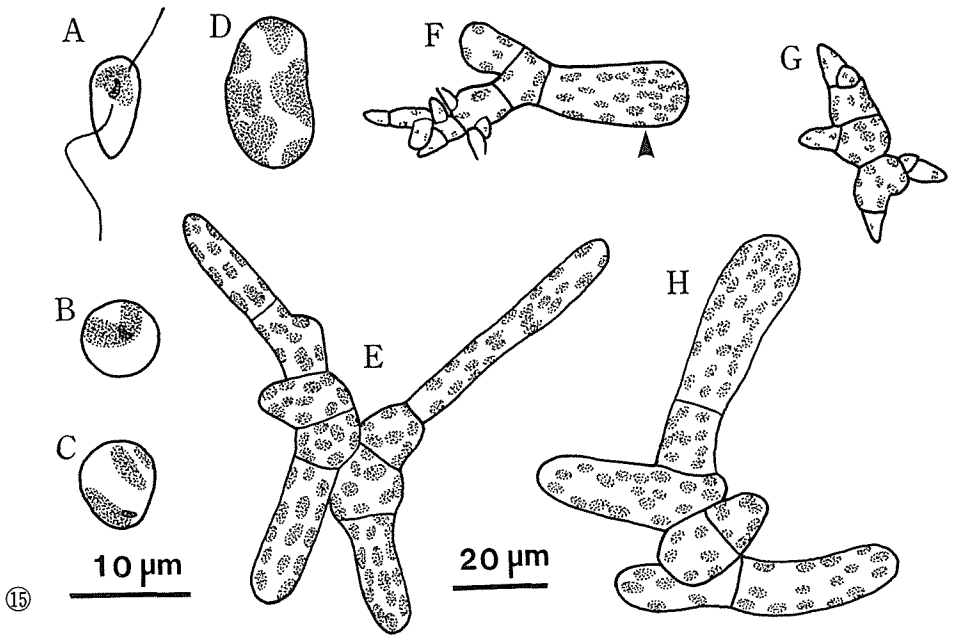

Fig. 15 *Desmarestia ligulata.* A. Zoospore. B. Settled zoospore. C. Germination of zoospore. D. 8-day-old germling grown under Set 2 conditions. E. 8-day-old germling grown under Set 4 conditions. F–G. 18-day-old fertile gametophytes grown under Set 2 conditions: F, Fertile gametophyte bearing an oogonium (arrowhead) and antheridia; G, Fertile gametophyte bearing antheridia only; H, Fertile gametophyte bearing an oogonium only. (A–D drawn to 10 μm scale. E–H drawn to 20 μm scale.)

developed from the terminal cells of branches. In most cases the eggs were only partially extruded from the oogonium (Pl. V, A). Such eggs began to germinate *in situ*, developing into sporophytes (Pl. V, B). A few oogonia discharged an egg through a narrow apical opening and the egg usually remained attached to the opening. When the egg was extruded completely from the oogonium, the wall of the empty oogonium could be detected for a while at the apex of branches.

The gametophytes grown under Set 2 conditions became fertile within 23 days. Whereas, gametophytes grown under Set 3, Set 4 and Set 5 conditions (long-day conditions) developed only vegetatively into copiously branched filamentous thalli (Pl. V, C). When the culture medium was changed, however, maturation of gametophytes was induced under Set 3 conditions but soon after these gametophytes grew only vegetatively without formation of gametangia. When the gametophytes grown under Set 4 and Set 5 conditions were cut into small fragments, sometimes into single cells, the fragments developed again into profusely branched filamentous thalli. The cell diameter and length adjacent to the terminal cell of branches were 10.2 μm$\pm$1.6$\times$28.7 μm$\pm$7.1. The chromosome number of the gameto-

phytes was about 26–28 (Pl. V. G).

Effect of temperature and photoperiod on growth and maturation

A well grown gametophyte from Set 4 conditions was cut into fragments of 100–150 cells. These were cultured at 5℃ in a 10- or 14-hr photoperiod (Fig. 16), at 10℃ in a 10- or 14-hr photoperiod (Fig. 17), at 14℃ in a 10- or 14-hr photoperiod (Fig. 18), at 18℃ in a 10- or 14-hr photoperiod (Fig. 18) and at 22℃ in a 14-hr photoperiod. Maturation occurred after 6 days in 10-hr photoperiod at 5–10℃ and after 7 days in a 10-hr photoperiod at 14℃. In fully matured gametophytes the percentage of antheridial branches was 50–60% and that of oogonial branches was 30–40% after 14 days in 10-hr photoperiod at 5–14℃ (Table 11). Nearly complete maturation occurred in a 10-hr photoperiod at 18℃ and in a 14-hr photoperiod at 5℃. The gametophytes never matured in 14-hr photoperiods at 14℃, 18℃ and 22℃.

2) Sporophytes

Development of fertilized eggs germinated inside oogonia

As mentioned above, most eggs germinated inside the oogonia. Under Set 2 conditions, the fertilized eggs developed into unbranched erect filaments. Within 6 days of germination, the erect filaments were up to 15-celled and had begun to produce opposite branches in their middle and upper parts (Fig. 19, D). A part of the erect filament differentiated into an intercalary meristem. At the same time, the primary rhizoid was produced from the lowest cell of the erect main axis, which was covered with the original oogonium wall (Fig. 19, B) and then from the lower axial cells (Fig. 19, C). The elongation and cell divisions of the rhizoids took place in the apical cells only.

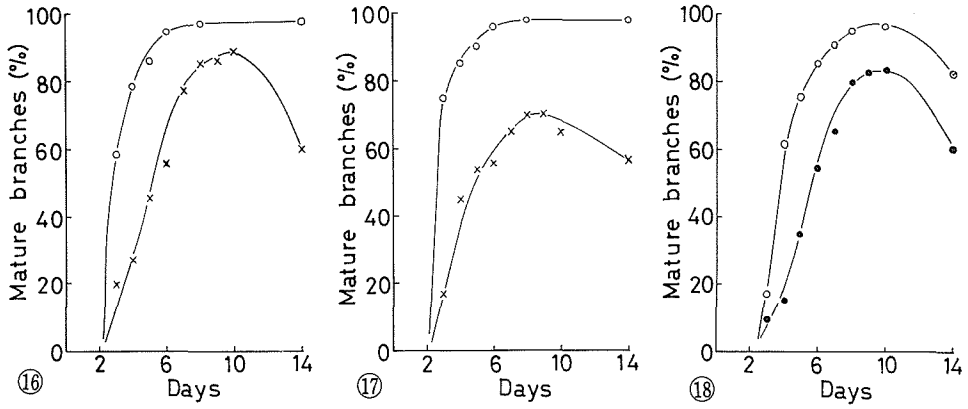

Fig. 16 Maturation of *Desmarestia ligulata* gametophytes in two photoperiods at 5℃, ○—○, 10 : $\overline{14}$; ×—×, 14 : $\overline{10}$.

Fig. 17 Maturation of *Desmarestia ligulata* gametophytes in two photoperiods at 10℃, ○—○, 10 : $\overline{14}$, ×—×, 14 : $\overline{10}$.

Fig. 18 Maturation of *Desmarestia ligulata* gametophytes in a 10-hr photoperiod at 14℃, ○—○, and 18℃, ●—●.

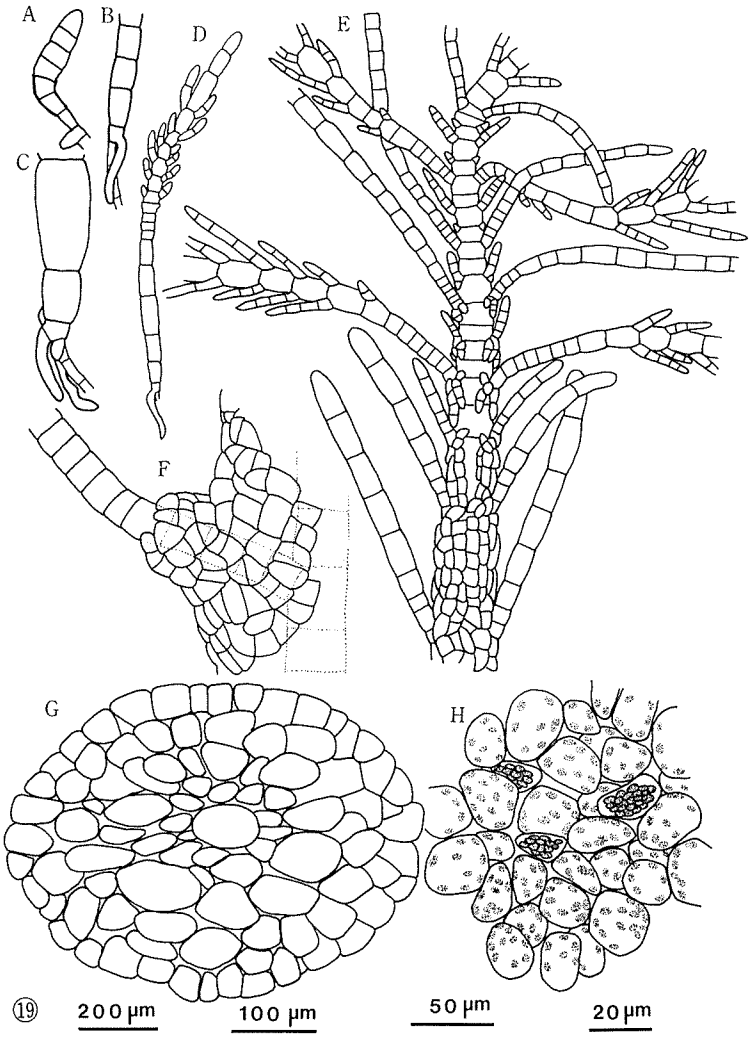

Fig. 19 *Desmarestia ligulata.* A. 6-celled sporophyte germinated in an oogonium. B. Basal part of 17-celled sporophyte with a rhizoid initial. C. Basal part of 30-celled sporophyte. D. Young monosiphonous sporophyte. E. Middle part of young sporophyte, showing development of the cortex. F. Part of corticated main axis. G. Cross section of the upper part of 20-day-old sporophyte. H. Surface view of thallus with unilocular sporngia from 3-month-old culture grown under Set 4 conditions. (A–C and F–G drawn to 50 μm scale. D drawn to 200 μm scale. E drawn to 100 μm scale. H drawn to 20 μm scale.)

When the main axis consisted of about 60 cells, corticating filaments arose initially from the basal cell of the older branches and later from the younger branches. By successive elongation, cell divisions and branching, they ensheathed first the main axis (Fig. 19, E; Pl.

Table 11 Percentage of mature branches formed on *D. ligulata* gametophytes after 14 days at four temperatures in 10- and 14-hr photoperiods.

Photoperiod (hours)	10				14			
Temperature (℃)	5	10	14	18	5	10	14	18
% of antheridia branches	59.4	51.6	53.9	55.1	36.4	37.5	0	0
% of oogonial branches	38.2	40.2	17.4	5.4	23.1	20.0	0	0
% of immature branches	2.4	8.2	28.7	39.5	40.5	43.5	100	100

Each value is the average of six samples from three cultures.

V, D) and later the branches (Fig. 19, F). The elongation and branchings of the cortical filaments occurred for more abundantly across the main axis horizontally than below. Accordingly, the thallus gradually became ligulate. When the sporophytes matured, new sporophytes arose from the basal part.

In about 100-day cultures, some sporophytes became fertile (Pl. V. F) and formed unilocular sporangia in the superficial layer of the cortical envelope (Fig. 19, H). The chromosome number of these sporophytes was about 52-56 (Pl. V, H) and meiosis occurred in the unilocular sporangia.

The zoospores liberated from these sporophytes usually sent out germination tube and developed into monoecious gametophytes.

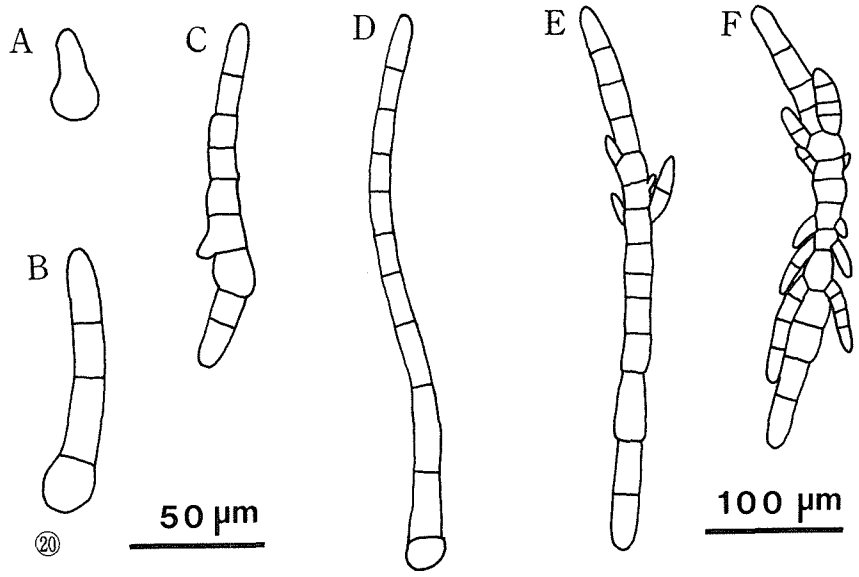

Fig. 20 *Desmarestia ligulata.* Sporophytes derived from free zygotes. (A-C drawn to 50 μm scale. D-F drawn to 100 μm scale)

Development of fertilized eggs germinated outside oogonia

Forty-six eggs released from oogonia were introduced singly into test tubes and cultured. Among them, 24 eggs germinated. The germlings usually pushed out a protuberance (Fig. 20, A) and divided into two cells. One (Fig. 20, B & D) and often both of these (Fig. 20, C, E & F) developed into the erect filaments. The primary rhizoid arose from the original egg cell when the erect filaments attained 15 cells size. Sometimes one or more new erect filaments were produced from the origianl egg cell.

Effect of temperature and photoperiod on growth and maturation

Some 10–20-celled sporophytes germinated under Set 2 conditions were separated from the gametophytes and introduced into test tubes. They were cultured at 5℃, 10℃, 14℃ and 18℃ in a 10- or 14-hr photoperiod and at 22℃ in a 14-hr photoperiod. No marked difference was recognized in the early stages of development under the 5–18℃ conditions and the corti-

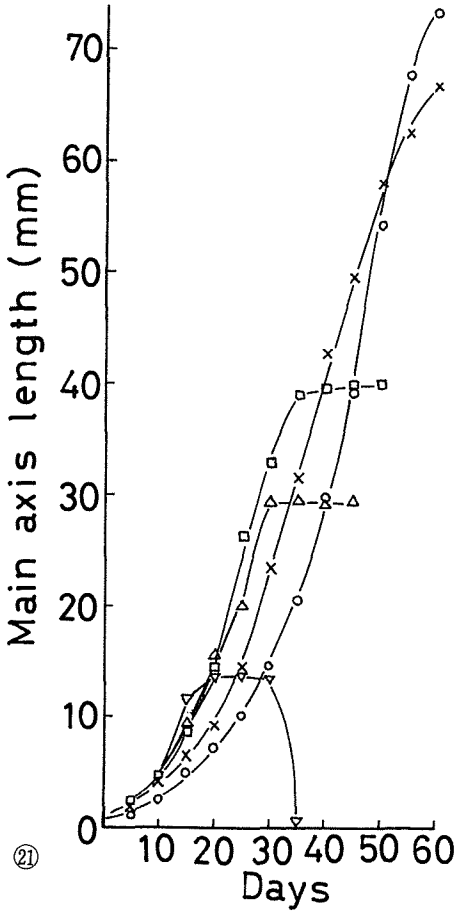

Fig. 21 Growth of *Desmarestia ligulata* sporophytes at five temperatures in a 14-hr photoperiod, ○—○, 5℃; ×—×, 10℃; □—□, 14℃; △—△, 18℃; ▽—▽, 22℃.

Table 12 Number of fertile sporophytes of *D. ligulata* within 6 months under various culture conditions.

Culture conditions	Number of isolated sporophytes	Number of fertile sporophytes				
		Months				
		2	3	4	5	Total
Set 1	22			3	5	8/22
Set 2	31		3	25		28/31
Set 3	56		4	15	14	33/56
Set 4	55	15	18	18		51/55
Set 5	54	13	18	10		41/54

cal envelope was formed 10 days after isolation. The growth was affected by temperature rather than photoperiod. The best growth was obtained at 5–10℃ (Fig. 21). At 14℃ and 18℃, elongation of the main axis of the sporophytes was arrested within 25–30 days and then some cortical filaments grew separately around the main axis (Pl. V, E). Consequently, the sporophytes became a mass tangled with the cortical filaments. The sporophytes cultured at 22℃ did not form the cortical filaments and breached within 20 days.

The isolated sporophytes were cultured for 180 days under Set 1, Set 2, Set 3, Set 4 and Set 5 conditions (Table 12). Sixty-five days after isolation, some sporophytes became fertile under Set 4 and Set 5 conditions and later under other culture conditions.

3) Parthenogenesis

Unfertilized eggs were obtained by the same method as used for *D. viridis*. All the eggs discharged from the oogonia soon degenerated and the parthenogenetic development of unfertilized eggs was not observed.

4) Regeneration of a single isolated cell from sporophytes

Among the cells isolated singly from the meristem, the basal cell of branches and the rhizoid of young sporophytes, only the apical cells from the rhizoid survived and regenerated. Within 5–6 days after isolation, the cells gradually increased their number of chromatophores (Pl. IV, H–I) and divided into several daughter cells (Pl. IV, J). These cells constituted the erect part of young sporophytes and acted as the new meristem, producing branches (Pl. IV, K–L). The primary rhizoid arose from the distal end of the original cell (excised from the rhizoid). These plants developed into the normal corticated sporophytes.

***Desmarestia tabacoides* OKAMURA**

D. tabacoides grows on rocks in the sublittoral zone on the Pacific coast of middle and southern Japan. Fertile plants formed unilocular sporangia embedded in the superficial layer of the cortical envelope. The fertile material was collected by Mr. H. UEDA at Shimoda by means of Scuba diving. Cultures were started on April 23 th, 1969 at the Shimoda Marine Biological Station, Faculty of Science, Tsukuba University (Tokyo Kyoiku University) and within a week they were transferred to incubatores at the Institute

of Algological Research, Muroran. The new findings of the life cycle of *D. tabacoides* was reported previously (NAKAHARA and NAKAMURA, 1971).

1) Gametophytes

Zoospores and their development

Zoospores are pyriform and laterally biflagellated, measuring about 11.0 μm×5.0 μm. They contain a single chromatophore and eyespot. They swim for about one hour at room temperature (20℃), displaying negative phototaxis. Settled zoospores become spherical and measured about 6.7 μm diam. Within 24 hours, they send out a germination tube, into which most of the protoplasm generally migrate. By successive transversal cell-divisions and branching, germlings develop into minute thalli (gametophytes) composed of branched uniseriate filaments. The cell diameter and length adjacent to the terminal cell of branches

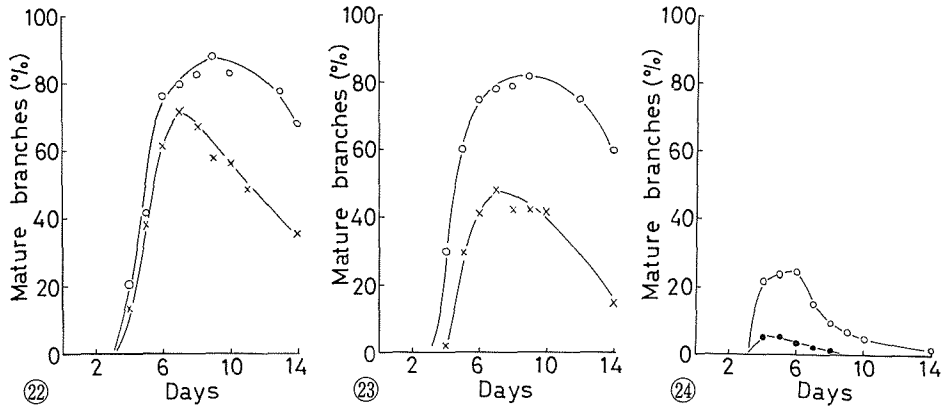

Fig. 22 Maturation of *Desmarestia tabacoides* gametophytes in two photoperiods at 5℃, ○—○, 10 : $\overline{14}$; ×—×, 14 : $\overline{10}$.

Fig. 23 Maturation of *Desmarestia tabacoides* gametophytes in two photoperiods at 10℃, ○—○, 10 : $\overline{14}$; ×—×, 14 : $\overline{10}$.

Fig. 24 Maturation of *Desmarestia tabacoides* gametophytes in a 10-hr photoperiod at 14℃, ○—○, and 18℃, ●—●.

Table 13 Percentage of mature branches formed on *D. tabacoides* gametophytes after 8 days at four temperatures in 10- and 14-hr photoperiods.

Photoperiod (hours)	10				14			
Temperature (℃)	5	10	14	18	5	10	14	18
% of antheridia branches	40.8	30.3	3.2	2.1	25.0	7.5	0	0
% of oogonial branches	48.0	51.0	6.4	0	32.2	26.4	0	0
% of immature branches	11.2	18.7	90.4	97.9	42.7	66.1	100	100

Each value is the average of six samples from three cultures.

were 12.5 μm±1.3×56.5 μm±10.7. The chromosome number of the gametophytes was about 34–38.

In 18-day cultures after transfer at 10℃ in a 10-hr photoperiod, the gametophytes began to form antheridia and oogonia monoeciously. The antheridia arose laterally from some cells of branches and formed clusters. The oogonia developed from the terminal branch cells. In most cases, the eggs were only partially extruded from oogonium. Such eggs began to germinate *in situ,* developing into sporophytes. A few oogonia discharged an egg through a narrow apical opening, and the egg usually remained attached to the opening. When the egg was extruded completely from the oogonium, the wall of the empty oogonium could be detected for a while. Sometimes a new oogonium developed inside the empty oogonium from the adjacent cell. The eggs discharged completely from the oogonium did not germinate and degenerated within 3 days.

Effect of temperature and photoperiod on growth and maturation

A well grown gametophyte from Set 4 conditions was cut into small fragments composed of 100–150 cells. These were cultured at 5℃ in a 10- or 14-hr photoperiod (Fig. 22), at 10℃ in a 10- or 14-hr photoperiod (Fig. 23), at 14℃ in a 10- or 14-hr photoperiod (Fig. 24), at 18℃ in a 10- or 14-hr photoperiod (Fig. 24) and at 22℃ in a 14-hr photoperiod. Complete maturation of the gametophytes occurred after 8 days in 10-hr photoperiods at 5–10℃ and the percentage of antheridial branches was 30–45% and that of oogonial branches was 45–55% (Table 13). Nearly complete maturation occurred in 14-hr photoperiod at 5℃. In 14-hr photoperiod at 10℃, about a half of branches formed gametangia and in a 10-hr photoperiod at 14℃ or 18℃ a few branches matured. The gametophytes never matured in 14-hr photoperiod at 14–22℃.

2) Sporophytes

Development of fertilized eggs

At 14℃ in a 10-hr photoperiod, the fertilized eggs germinated in the oogonia developed into unbranched erect filaments. Within 6 days of germination, the erect filaments grew up to about 15 cells and began to form mainly opposite branches in the middle and upper parts of the main axis. A part of the main axis differentiated into an intercalary meristem. At the same time, the primary rhizoids were issued at first from the cell adjacent to the oogonium wall and later from the lower axial cell.

When the main axis attained up to about 50 cells, corticating filaments arose from the basal cell of branches in close apposition to the surrounding cells. By successive elongation, cell divisions and branching, the corticating filaments developed into a multilayered cortex, ensheathing the axial cells. Soon after the cortical filaments extended to the branches all together and the thallus became flattened. When the sporophytes matured, new sporophytes were issued from the basal portion.

In about 120-day cultures some sporophytes became fertile and formed unilocular sporangia in the superficial layer of the cortical envelope. The chromosome number of these

sporophytes was about 70–76 and meiosis occurred in the unilocular sporangia. The zoospores liberated from these sporophytes developed into normal monoecious gametophytes.

Effect of temperature and photoperiod on growth and maturation

Some 10–20-celled sporophytes, germinated at 10℃ in a 10-hr photoperiod, were separated from the gametophyte and introduced into test tubes. They were cultured at 5℃, 10℃, 14℃ and 18℃ in a 10- or 14-hr photoperiod and at 22℃ in a 14-hr photoperiod. No marked difference was recognized in the early stages of development and the cortical envelope was formed 5–7 days after isolation. The best growth was obtained at 14–18℃ (Fig. 25) and the growth was not affected by photoperiods. At 22℃ in a 14-hr photoperiod, growth of sporophytes was inhibited, and the main axis and branches gradually degenerated in the upper part.

The isolated sporophytes were cultured for 200 days under the nine different conditions mentioned above. In 100-day cultures after isolation, some sporophytes became fertile at 18℃ conditions and then at 14℃ and 22℃. Whereas at 5℃ and 10℃ conditions, the sporophytes did not form unilocular sporangia.

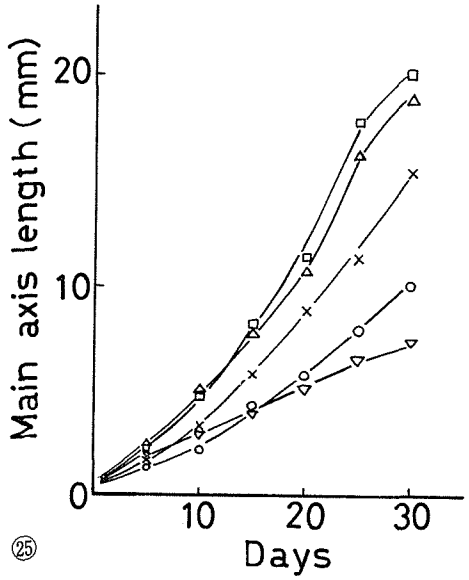

Fig. 25 Growth of *Desmarestia tabacoides* sporophytes at five temperatures in a 14-hr photoperiod, ○—○, 5℃; ×—×, 10℃; □—□, 14℃; △—△, 18℃; ▽—▽, 22℃.

3) **Parthenogenesis**

Unfertilized eggs were obtained by the same method as used for *D. viridis*. All eggs discharged from oogonia soon degenerated and the parthenogenetic development of unfertilized eggs was not observed.

4) **Regeneration of a single isolated sporophyte cell**

The meristimatic cells, the basal cells of branches and apical cells of rhizoids were

isolated singly from young sporophytes. The isolated cells from the thallus did not regenerate and died within 5 days. Isolated apical cells from the rhizoid gradually increased their number of chromatophores and divided into several cells. However, further development did not occur.

Discussion of the life history of Desmarestiales

In the present study, I have observed the life cycles of three species of *Desmarestia* in culture from generation to generation. These three species all showed quite the same type of life cycle.

Gametophytes

It has been reported that the gametophytes of *D. aculeata* (SCHREIBER, 1932; CHAPMAN and BURROWS, 1970; MÜLLER and LÜTHE, 1981) are dioecious. According to KORNMANN (1962), however, the gametophytes of *D. viridis* are monoecious. CHAPMAN and BURROWS (1971) suggested that the alternately branched species are dioecious and the oppositely branched species monoecious. Three Japanese species, *D. viridis*, *D. ligulata* and *D. tabacoides* were oppositely branched species and monoecious. Recently, however, ANDERSON (1982) showed that *D. firma* was oppositely branched and dioecious. It was not found any relationships between the sporophyte structure and the gametophyte sexuality. MÜLLER and MEEL (1982) found that the gametophytes of *Arthrocladia villosa* were monoecious.

A difference in the mode of zoospore germination was observed between the three species. In *D. viridis* and *D. tabacoides*, the settled zoospores send out a germination tube into which most of the protoplasm generally migrate, and then form a cross-wall to separate this tube from the original spore as in *D. aculeata* (SCHREIBER, 1932), in *D. viridis* (from Asamushi, ABE, 1938; from Helgoland, KORNMANN, 1962), in *D. firma* (ANDERSON, 1982) and in *Arthrocladia villosa* (MÜLLER and MEEL, 1982). In *D. ligulata*, however, most settled zoospores increase in size, becoming slightly ovoid and divide into two cells, only a few send out a germination tube.

Regarding the gametophyte structure, the cells of the thallus were slightly different in length and breadth. The cells adjacent to the terminal cell of immature branches of *D. viridis* were the narrowest and those of *D. tabacoides* were the largest. The percentage of the number of antheridial branches was a little higher than that of oogonial branches in *D. viridis* and *D. tabacoides*, and *vice versa* in *D. ligulata* under every culture condition adopted in the present experiment.

As to the effect of temperature and photoperiod on growth and maturation of the gametophytes, CHAPMAN and BURROWS (1970, 1971) reported in *D. aculeata* from Isle of Man that the gametophyte became fertile at 8.5–9℃ in a 18-hr photoperiod with a mean daily illuminance of 188–1540 lux. MÜLLER and LÜTHE (1981) also found in *D. aculeata* from Helgoland that formation of oogonia and antheridia required low temperatures and a

minimum amount of light energy. In addition, they found that sexual differentiation in *D. aculeata* was a blue-light mediated response as found in *Laminaria* by LÜNING and DRING (1975). KORNMANN found in *D. viridis* from Helgoland that the gametophytes never matured at 15℃ and matured fully at 3–5℃, but soon after grew only vegetatively. The development of gametangia of *A. villosa* was predominantly found in a short-day condition at 10℃ (MÜLLER and MEEL, 1982). These experimental data suggest that temperature and light conditions affect the maturation of gametophytes of the members of Desmarestiales.

In the present experiment, the growth of the gametophytes of *D. viridis, D. ligulata* and *D. tabacoides* was promoted by higher temperatures and longer photoperiods. The gametophytes of the three species became fertile under cool and short-day conditions. Gametogenesis was best promoted by 5–18℃ and 10-hr photoperiod for *D. viridis* and *D. ligulata,* and the best conditions for *D. tabacoides* were 5–10℃ and 10-hr photoperiod. Namely, gametogenesis of the species growing in cool regions was controlled by photoperiods but that of the warm region species was controlled by photoperiods and temperatures.

Sporophytes

When the eggs were discharged completely from the oogonia, a half of them germinated in *D. viridis* and *D. ligulata* but never germinated in *D. tabacoides.* Some of them at first sent out the primary rhizoid as described by SCHREIBER (1932) in *D. aculeata.* Some pushed out a protuberance and divided into two cells, one of them or both of them developed into an erect filament as reported by KORNMANN (1962) in *D. viridis.* Some eggs produced a small constriction and both the original cell and this constriction developed into erect filaments. However, most germlings from free eggs were irregular in growth direction of the main axis. The germination of eggs dislodged from oogonia was not observed in *D. firma* (ANDERSON, 1982) and in *A. villosa* (MÜLLER and MEEL, 1982).

In the present study of the three species of *Desmarestia,* the fertilized eggs usually germinated in the oogonia. The same observations were made for *D. viridis* by KORNMANN, *D. firma* by ANDERSON and *A. villosa* by MÜLLER and MEEL. KORNMANN (1962) suggested that this situation may not be as common in nature. However, I think this situation is as common in natural population and is more profitable than free-egg germination by two reasons. First, most free-egg germlings are abnormal in culture. Second, when eggs germinate in the oogonia, the place for development is maintained and they are not necessary to seek for new naked places for development in the sea.

When the erect filaments attained up to about 30 cells in *D. viridis* and 15–20 cells in *D. ligulata* and *D. tabacoides,* they began to form opposite branches. The latter two species formed branches more densely than the former. The primary rhizoid was at first given off from the lowest cell of the main axis which is surrounded by the original oogonium wall in *D. ligulata,* but in *D. viridis* and *D. tabacoides,* it arose from the lower part of the cell adjacent to the lowest cell of the main axis. ANDERSON (1982) found in *D. firma* that the primary rhizoid arosed from the same position with *D. viridis* and *D. tabacoides.* When the

main axis attained up to about 100 cells in *D. viridis*, about 60 cells in *D. ligulata* and about 50 cells in *D. tabacoides*, the corticating filaments arose from the basal branch cells. The main difference in the sporophyte structure is the shape of the thallus, which is terete in *D. viridis*, ligulate in *D. ligulata* and flattened in *D. tabacoides*. In *D. viridis* the corticating filaments grew in close apposition to the axial cells, surrounding the main axis and consequently, the thallus became terete. In *D. ligulata* the corticating filaments grew in close apposition to not only the axial cells but also the cells of the branches issued from the axial cells. Consequently, the thallus became ligulate. In *D. tabacoides*, the corticating filaments grew in close apposition to the axial cells and the cells of branches, mainly crossing the main axis. Therefore, the thallus became flattened.

In *D. viridis* and *D. ligulata*, younger sporophytes grew well at 5–18℃ and later best at 5–10℃. Sporophytes grown at 22℃ bleached within 15 days. In *D. tabacoides*, sporophytes grew at 5℃ to 22℃ and best at 14–18℃. At 22℃ the growth was slightly inhibited. Fertile sporophytes were obtained at 10–18℃ in *D. viridis*, at 5–18℃ in *D. ligulata* and at 14–22℃ in *D. tabacoides*.

Parthenogenesis

MÜLLER and MEEL (1982) studied the life history of *Arthrocladia villosa* and found that sporophytes and gametophytes have the same chromosome number. This sexuality seemed to be suppressed and replaced by apomeiosis and parthenogenesis. In the present study, parthenogenetic development of eggs was observed in *D. viridis* only. Most unfertilized eggs developed into haploid sporophytes which were quite similar to the diploid. The haploid sporophytes formed unilocular sporangia and produced swarmers without meiosis. The swarmers developed into monoecious gametophytes. In this experiment of parthenogenetic development of the eggs, only one of forty-two sporophytes was diploid.

Regeneration

Single isolated cells of the gametophytes of three species of *Desmarestia* germinated and developed into profusely branched thalli again. Whereas, the regeneration of a single isolated sporophyte cell took place only in the apical cell of the rhizoids of *D. viridis* and *D. ligulata*. When an apical cell of the rhizoid was isolated, it showed rhizoidal growth and then developed into normal sporophytes in *D. viridis*. In *D. ligulata*, an isolated apical cell of the rhizoid changed into the meristem and developed into a normal sporophyte. An isolated apical cell of *D. tabacoides* increased in number of chromatophores and divided into several cells but further development did not occur.

Nuclear phases

Cytological elucidation in *Desmarestia* has been given in *D. viridis* from Asamushi by ABE (1938) and *A. villosa* by MÜLLER and MEEL (1982). According to ABE, the haploid chromosome number of *D. viridis* is 22 in meiotic metaphase in the sporangia. In the present study, haploid counts were obtained from dividing cells of the gametophytes and from the sporophytes derived from unfertilized eggs in *D. viridis*. The haploid chromosome number

of *D. viridis* was about 22 which corresponds with ABE's observation. The haploid chromosome number of *D. ligulata* was about 26–28 and that of *D. tabacoides* was 34–38. Diploid counts were obtained from the young sporophytes from zygotes and the diploid chromosome number of *D. viridis* was about 44, that of *D. ligulata* was 52–56 and that of *D. tabacoides* was 70–76.

Laminariales

Since the discovery of microscopic gametophytes in *Saccorhiza bulbosa* by SAUVAGEAU (1915), a number of culture studies on members of Laminariales have been published. According to them, all members of the Laminariales have life cycles involving the alternation of a large diploid sporophyte and a microscopic haploid gametophyte with oogamous reproduction. The sex of gametophytes is determined genetically (SCHREIBER, 1930). Parthenogenetic development of eggs was reported by SCHREIBER (1930), KEMP and COLE (1961), YABU (1964 b) and NAKAHARA and NAKAMURA (1973). SEGI and KIDA (1957) observed the development of vegetative cells of female gametophytes into sporophytes (apogamy). Apospory, the development of vegetative cells of sporophytes into gametophytes, was reported by NAKAHARA and NAKAMURA (1973).

The effect of environmental conditions on growth and maturation of gametophytes have been reported by many workers. According to them, the maturation of gametophytes takes place at a narrower temperature range than that tolerated by vegetative gametophytes and lower temperatures are favorable for maturation in many species. Other factors, viz. light intensity and quality, salinity and nutrient have been studied by various investigators (HARRIS, 1932; SAITO, 1956 a, 1956 b; SEGI and KIDA, 1957, 1958; YABU, 1964 b; KAIN, 1964; DRUEHL, 1967; HSIAO and DRUEHL, 1971, 1973 a, 1973 b; LÜNING and DRING, 1972, 1975; LÜNING, 1980). However, there have been few culture experiments on the growth and maturation of sporophytes (SANBONSUGA and HASEGAWA, 1967, 1969). KAIN (1979) reviewed the life history and its physiological aspects of *Laminaria* in detail.

Laminariaceae

Laminaria japonica ARESCHOUG

L. japonica is one of the most common algae on the coast of Muroran, growing on rocks in the lower littoral and upper sublittoral zones. Young plants appear in the middle of February and attain full maturity from early September to January of the next year. Fertile plants become detached from their substratum during fertile season. They bear unilocular sporangia forming sori on both sides of the blade-surface and produce zoospores. KANDA (1936) has already given a detailed description of the gametophytes and the early stages of the sporophyte development of this species from Muroran. Cytological observations have been made by ABE (1939) and YABU (1973).

1) Gametophytes

Within 24 hours, settled zoospores sent out a germination tube into which the whole protoplasm usually migrated. In 5-day-old cultures, two types of germlings are distinguishable, the female and the male. The distal end of the germination tube of the female gametophytes gradually increased in size until it became much larger than the original spore. The distal end of the germination tube of the male ones continued to grow, dividing transversally and branching. In 10 days the female gametophytes consisted of 2–4 cells, average 2.7, under Set 3 conditions and 4–7 cells, average 5.7, under Set 5 conditions. The male gametophytes consisted of 6–15 cells, average 9.8 and 8–18 cells, average 10.2 in Set 3 and 5 conditions, respectively.

In 11-day cultures under Set 3 conditions, both types of gametophytes became fertile, producing oogonia and antheridia (Pl. VI, A). Within 30 days, vegetative cells of the gametophytes were all converted into gametangia. Under Set 1 conditions the gametophytes became fertile in 20 days. Under Set 2 conditions, the formation of antheridia occurred after 20 days and oogonia after 30 days. Under Set 4 conditions the maturation of the gametophytes occurred in 15 days, but the number of mature gametophytes was low. Under Set 5 conditions, the gametophytes never matured but developed into large filamentous thalli (Pl. VI, B).

The gametophytes grown under Set 5 conditions were isolated and introduced into test tubes, one gametophyte per tube. Under Set 4 and Set 5 conditions the isolated gametophytes remained sterile, growing vegetatively and became profusely branched and took a more or less spherical form. Cells of the female gametophytes cultured under Set 4 conditions were 11.8 μm$\pm$0.6 diam. in the cells adjacent to the terminal cells and those of the male 5.2 μm$\pm$0.5 diam. Two strains of the female gametophytes (L. j. F-1 and L. j. F-2) and those of the male gametophytes (L. j. M-1 and L. j. M-2) derived from two sporophytes of

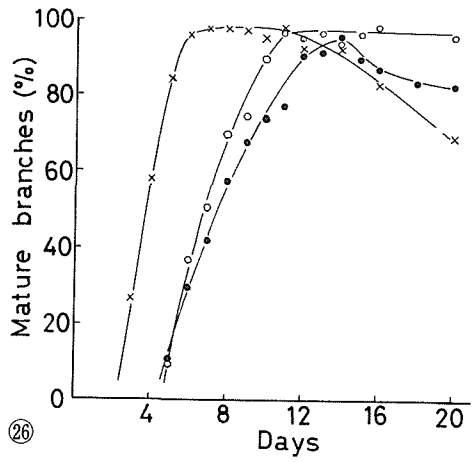

Fig. 26 Maturation of *Laminaria japonica* gametophytes at 10℃ in a 14-hr photoperiod, ×—×, male gametophytes; ●—●, female gametophytes cultured alone; ○—○, female gametophytes cultured with males.

different years were cultured further.

When fragments excised from the gametophytes grown under Set 4 conditions were introduced into fresh medium under Set 3 conditions either separately or with female and male gametophytes together, they became fertile (Fig. 26). The formation of antheridia began 3 days after transfer and complete maturation was attained after 6 days. The maturity of the male gametophytes was not promoted when cultured with the female gametophytes. The formation of oogonia began 5 days after transfer. When the female gametophytes were cultured with male gametophytes, they matured fully after 10 days and each cell of the gametophytes produced short branches which developed into the oogonia. When cultured alone, complete maturation occurred after 12 days. Then some branches grew vegetatively only and the number of immature branches gradually increased. The chromosome number of the immature female and male gametophytes was about 28–35 (Pl. VIII, A–B).

2) Sporophytes

Most eggs, extruded from oogonia, adhered to the opening of the oogonia tightly and germinated *in situ*. They could not be dislodged by shaking of glass slides or vigorous

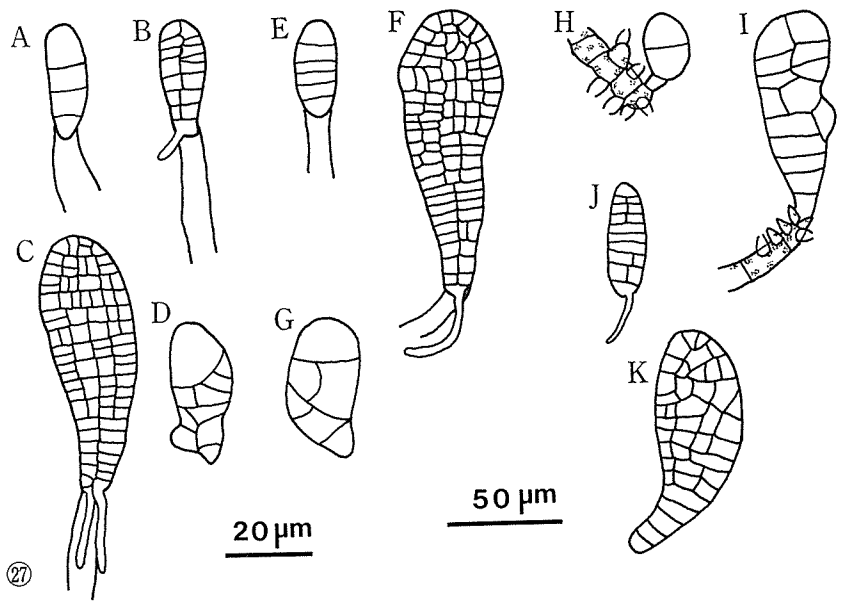

Fig. 27 *Laminaria japonica.* A–D. Sporophytes derived from zygotes. E–G. Sporophytes derived from unfertilized eggs. H. Large elliptical cell (sporophyte initial) on a male gametophyte. I–K. Sporophytes derived from vegetative cells of male gametophytes. (A–G and J–K drawn to 50 μm scale. H–I drawn to 20 μm scale.)

aeration of medium. Within 24 hours after liberation, the eggs became elliptical with a long axis directed in the plane of the empty oogonia and divided transversally into two cells. Initially, only transverse cell-divisions took place in the young sporophytes (Fig. 27, A). Soon after longitudinal divisions occurred and the sporophytes became flat expanded plantlets. The primary rhizoid was produced from the basal cell of the plantlets (Fig. 27, B-C). A few eggs developed into abnormal sporophytes consisting of an irregular globular mass of cells (Fig. 27, D). These abnormal sporophytes degenerated within one month. Ten days after the discharge of eggs, under Set 3 conditions, the eggs developed into sporophytes

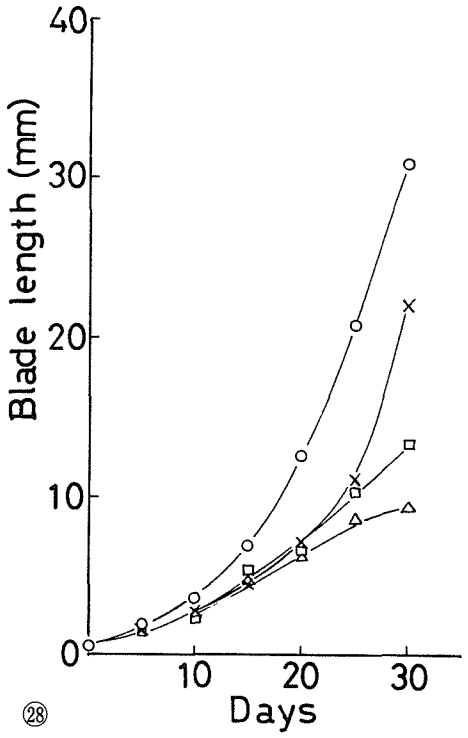

Fig. 28 Growth of *Laminaria japonica* sporophytes at four temperatures in a 14-hr photoperiod, ×—×, 5℃ ; ○—○ , 10℃ ; □—□ , 14℃ ; △—△ , 18℃.

Table 14 Growth of *L. japonica* sporophytes after 15- and 30-day at four temperatures in 10- and 14-hr photoperiods.

Temperature (℃)		5		10		14		18	
Photoperiod (hours)		10	14	10	14	10	14	10	14
Length of blades of sporophytes (mm)	15 days	4.6	4.7	5.1	6.9	4.6	5.3	4.2	5.1
	30 days	19.2	22.0	20.2	30.8	9.3	13.2	7.1	9.4

of about 470 μm long and consisting of about 1570 cells. When they were about 2 mm long, the basal part developed into a disc-shaped expansion, covering the primary rhizoids. In one month, they differentiated into a blade, stipe and holdfast (Pl. VII, A).

When the sporophytes were about 0.3–0.5 mm long(in 20 days from a mixed culture of female and male gametophytes under Set 3 conditions), they were separated from the gametophytes and were transferred to the culture vessels containing 200 m*l* of medium and maintained under the following eight conditions; 5℃, 10℃, 14℃ and 18℃ in a 10- or 14-hr photoperiod (Fig. 28; Table 14). Within 5 days under these conditions, the sporophytes grew to about 2 mm long and differentiated into a disc-shaped holdfast, a short stipe and a blade. The sporophytes grew well under cool and long-day conditions. In one-month cultures the best growth of the sporophytes was obtained at 10℃ in a 14-hr photoperiod (Set 3 conditions). Growth was suppressed under warm conditions. In 3–4 months under the eight growth conditions, the upper part of the blade of all the sporophytes gradually became bleached.

When three-month-old sporophytes grown under Set 3 conditions were 15 cm long and 3 cm broad, they were transferred to culture vessels containing 1400 m*l* of medium. Three months after transfer, they grew up to 1 m long and 8 cm broad. These sporophytes were cultured under 10℃ or 14℃ in a 10- or 14-hr photoperiod, but they did not form unilocular sporangia.

The chromosomes of the normal sporophytes were observed at the monostromatic stage and were about 58–62, being diploid (Pl. VIII, F). Abnormal plantlets' chromosome number were irregular, up to 60 and having several nuclei per cell.

3) Parthenogenesis

As mentioned above, the female gametophytes cultured alone under Set 3 conditions began to form oogonia in 5-day cultures and were fully matured in 12 days. Within 2 days after discharge of eggs, some eggs became detached from an opening of the oogonia, some became enlarged and became either spherical or elliptical. All of the spherical eggs and some of the elliptical eggs developed into abnormal sporophytes consisting of an irregular mass of cells (Fig. 27, G), but soon died. Some of the elliptical eggs attaching to the opening of the oogonia developed into normal sporophytes (Fig. 27, E–F; Pl. VI, D). The germination rate of the normal sporophytes from unfertilized eggs was 8.9% (Table 15).

Within one month the sporophytes differentiated into a blade, stipe and holdfast (Pl. VII, B). In three months they grew to 15 cm long under Set 3 conditions. In 3–4 months, the upper part of the blade gradually degenerated and bleached. The formation of sporangia did not occur in these sporophytes.

The chromosomes of the sporophytes derived from unfertilized eggs were observed at the monostromatic stage. Most of the normal sporophytes were haploid, having about 30–32 chromosomes (Pl. VIII, D) and some of them were diploid, having 60–66 chromosomes (Pl, VIII, E). The abnormal sporophytes had irregular numbers of chromosomes (36–42, 58–64

and 90+). Parthenogenesis did not occur in the male gametophytes.

4) Apogamy

Under Set 3 conditions, the isolated male gametophytes continued to grow vegetatively, forming antheridia and developed into well branched bushy thalli. In these thalli, some cells of branches, usually the terminal apical cells increased in size and became elliptical. Some of these elliptical cells divided transversally and longitudinally (Fig. 27, H–K; Pl. VI, E). By successive cell-divisions they developed into flat expanded plantlets which were identical to young sporophytes from eggs (Pl. VI, F). Primary rhizoids issued from the basal cells of these plantlets.

When the apogamous sporophytes attained a length of about 1–2 mm, the basal portion developed into a disc-shaped expansion covering the primary rhizoids. After one month the sporophytes differentiated into a blade, stipe and holdfast. When they attained a height of about 1–3 cm, they became stunted and the blade twisted (Pl. VII, C). The chromosome number of the apogamous sporophytes was about 28–34, being haploid (Pl. VIII, C). Under the other culture conditions, the apogamy by male gametophytes was not observed. Apogamy by female gametophytes did not occur in the present culture experiments.

5) Apospory

The distal end of sporophyte blades gradually degenerated under the present culture conditions. Under Set 4 conditions, however, a few epidermal cells and outer cortical cells survived and became richly pigmented and spherical in shape. These spherical cells divided

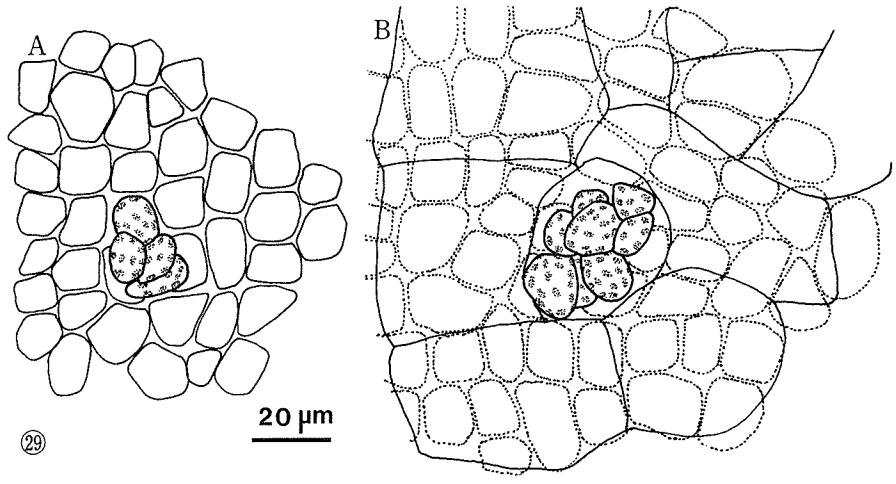

Fig. 29 *Laminaria japonica.* Apospory of sporophytes derived from zygotes. A. Germination of diploid gametophyte from an epidermal cell of sporophyte. B. Germination of diploid gametophyte from an outer cortical cell of sporophyte . Dotted lines show surface epidermal cells.

into several daughter cells *in situ* and each cell germinated (Fig. 29, A–B). These germlings developed into small thalli composed of uniseriate branched filaments, attaching to the bleached area of the sporophytes (Pl. VI, G–H ; VII, F).

A filamentous thallus derived from a epidermal cell or outer cortical cell was separated from a sporophyte and cultured under Set 4 conditions. The thickness of the cells adjacent to the apical cells was 6.6 μm$\pm$0.5 diam., a size intermediate to the male and female gametophytes.

When some fragments were excised from these thalli and transferred to Set 3 conditions, they became fertile. At first they formed antheridia only and some branches continued to grew vegetatively. In 15–20 days after the initiation of the formation of antheridia, some wider branches were produced. These wider branches formed oogonia and discharged eggs (Fig. 30, A ; Pl. VI, I). Almost of all eggs germinated at the opening of the oogonia and developed into the normal sporophyte (Fig. 30, B–C ; Pl. VI, J ; VII, E). Within 3 months the sporophytes attained up to 20 cm long under Set 3 conditions.

In this experiment, 12 aposporous gametophytes (L. j. D–1～D–12) were isolated from four different sporophytes. All of the thalli were the same cell width and they formed antheridia and oogonia on the same individuals under Set 3 conditions.

The chromosome number of these monoecious gametophytes about 58–64, being diploid

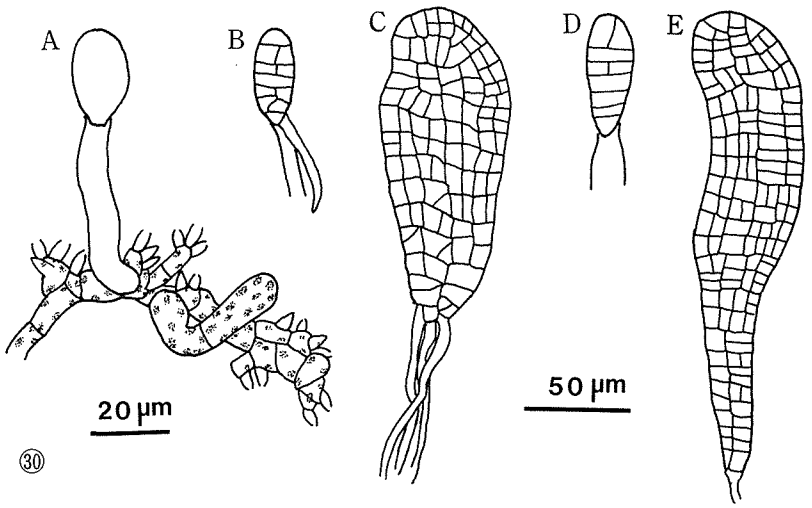

Fig. 30 *Laminaria japonica*. A. Diploid monoecious gametophyte bearing clusters of antheridia and an egg on the oogonium. B–C. Tetraploid sporophytes formed on a diploid monoecious gametophyte. D–E. Triploid sporophytes formed on a female gametophyte cultured with a diploid monoecious gametophyte. (A drawn to 20 μm scale. B–E drawn to 50μm scale.)

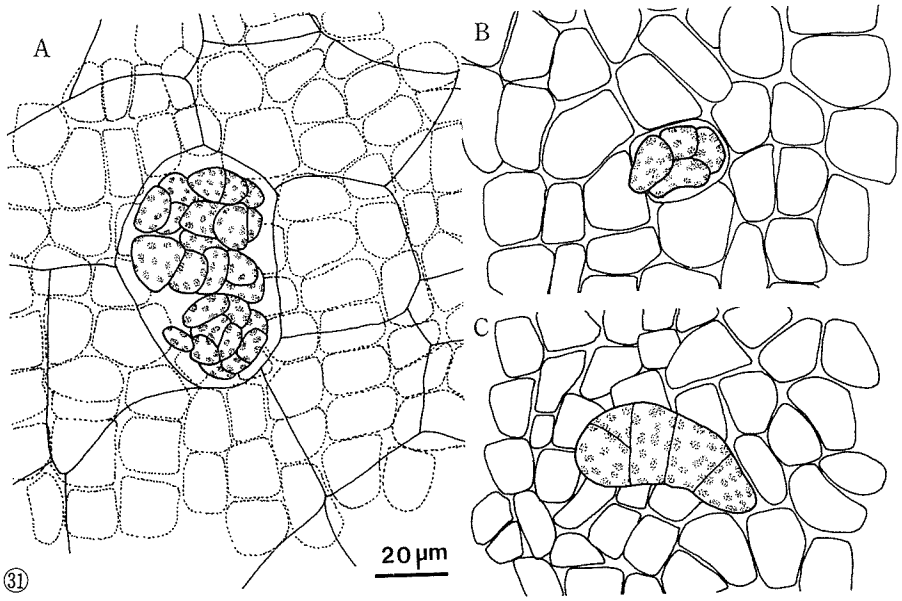

Fig. 31 *Laminaria japonica*. A–B. Apospory of sporophytes derived from an unfertilized egg. A. Germination of outer cortical cell on a sporophyte. Dotted lines show surface epidermal cells. B. Germination of epidermal cell on a sporophyte. C. New sporophyte from an epidermal cell of sporophyte derived from an unfertilized egg.

(Pl. VIII, G). The chromosome number of the sporophytes derived from the zygotes of these monoecious gametophytes was about 110–130, being tetraploid (Pl. VIII, I).

Apospory of the sporophytes derived from unfertilized eggs was observed in the same way as that of the sporophytes from zygotes (Fig. 31, A–B; Pl. VI, L; VII, G). The width of the cells adjacent to the apical cells of the filamentous thallus was about 10.9 μm± 0.6 which was nearly the same with that of the female gametophytes. Under Set 3 conditions some branches of the thallus formed oogonia. However, the thallus bore few oogonial branches compared with the usual female gametophyte, and only 2.0% of the eggs discharged from them developed into the normal sporophytes parthenogenetically (Table 15). In this experiment, one individual was isolated from each of the five different sporophytes (L. j. fH –1~fH–5). No difference was recognized between them. The chromosome number of these aposporous female gametophytes was about 29–32, being haploid. The apospory of the sporophytes from the male gametophytes was not observed in the present culture experiments.

6) Vegetative reproduction of the sporophytes from unfertilized eggs

As mentioned above, some epidermal cells and outer cortical cells survived independ-

Table 15 Development of eggs of *L. japonica* from cultures of parthenogenetic and matings among three strains of gametophytes with oogonia and two strains of gametophytes with antheridia. Cultures were transferred to Set 3 conditions from Set 4 conditions with fresh media and sampled after 20 days.

Gametophytes with oogonia	×	Gametophytes with antheridia	E	ab-S	n-S	n-S%	Chromosome numbers of normal sporophytes
L. j. F-1			196	246	43	8.9	30-32 (a few was 58-64)
L. j. F-1	×	L. j. M-1	157	57	685	76.2	58-62
L. j. F-1	×	L. j. D-1	142	90	452	66.1	88-96
L. j. fH-1			84	12	2	2.0	30-32
L. j. fH-1	×	L. j. M-1	190	82	64	19.0	58-62
L. j. D-1	×	L. j. D-1	22	24	292	86.4	110-130

E; Number of eggs and degenerated eggs. ab-S; Number of abnormal sporophytes. n-S; Number of normal sporophytes. n-S%; Percentage of normal sporophytes (n-S/E+ab-S+n-S).

ently in the bleached area of the sporophytes. Under Set 3 and Set 4 conditions, a few of these epidermal cells of the sporophytes from unfertilized eggs began to germinate and developed into flat expanded plantlets (Fig. 31, C; Pl. VII, G). The primary rhizoids issued from the cells which were attached to the original sporophyte. Finally, the plantlet differentiated into a blade, stipe and holdfast. Some of the epidermal cells developed into an irregular mass of undifferentiated sporophytes (Pl. VI, M). Vegetative reproduction was not observed in the sporophytes from the zygotes and the apogamous sporophytes.

7) Crossing experiments between the haploid and diploid gametophytes

Crossing experiments were undertaken with 2 strains of the female gametophytes (L. j. F-1 and L. j. fH-1), one strain of the male gametophyte (L. j. M-1) and one strain of the diploid monoecious gametophytes (L. j. D-1) which were maintained under Set 4 conditions. Crossing were carried out by transferring a few sterile fragments of the gametophytes to a new culture vessel with fresh medium and kept under Set 3 conditions. In 20-day cultures, the number of the normal sporophytes (n-S), abnormal sporophytes (ab-S) and ungerminated eggs (E) produced on the gametophytes were counted (Table 15).

The eggs from the female gametophytes which were cultured with the diploid gametophyte mostly developed into the normal sporophytes (Fig. 30, D-E; Pl. VI, K; VII, E). The germination percentage of the normal sporophytes in these cultures was nearly the same with that of the female gametophytes cultured with male gametopaytes (Pl. VI, C) and higher than that in the cultures of the female ones alone (Pl. VI, D). The chromosome number of these normal sporophytes was about 88-97, being triploid (Pl. VIII, H). Thus fertilization occurred between the haploid eggs and the diploid sperms.

Agarum cribrosum BORY

Ag. cribrosum is a common alga on the coast of Muroran, growing on rocks in the sublittoral zone. Fruiting plants are found from the end of October to May of the next year. The blade gradually degenerates during the fertile season and regenerates in early January, bearing unilocular sporangia on the new blade. A detailed description of the gametophytes and young stages of the sporophytes of this species from Muroran has already been given by Kanda (1941). Cytological observations have been reported by Yabu (1964 a).

1) Gametophytes

Within 24 hours, settled zoospores sent out a germination tube into which the whole protoplasm generally migrated. At first the germlings were all alike. In 10-day-old cultures, they consisted of 3–6 cells, average 3.8 cells, under Set 3 and Set 5 conditions. In 15 days they were distinguishable as female and male gametophytes by the thickness of the cells (Pl. IX, A).

In 30 days under Set 3 and Set 2 conditions, both gametophytes became fertile (Pl. IX, B). In the male gametophytes, the terminal or intercalary cells of branches formed numerous bulges which developed into antheridia. Finally, the male gametophytes were surrounded by the numerous antheridia. In the female gametophytes the terminal cells elongated and enlarged up to twice as thick as the vegetative cells. These cells produced thicker branches which developed into oogonia.

Under Set 1 conditions, the formation of antheridia was observed in 35-day cultures but that of oogonia in 40-day ones. Under Set 4 conditions the maturation occurred in 25-day-old cultures but the number of mature gametophytes was few. Under Set 5 conditions the gametophytes never matured but developed into large filamentous thalli.

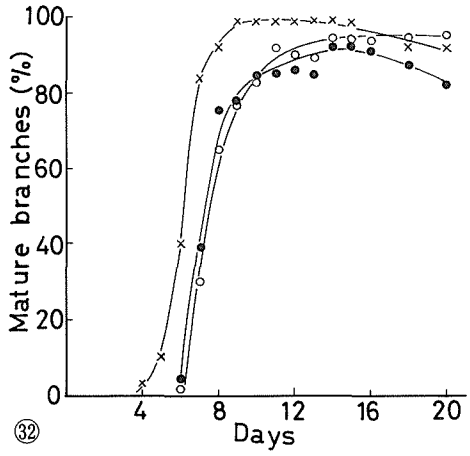

Fig. 32 Maturation of *Agarum cribrosum* gametophytes at 10℃ in a 14-hr photoperiod, ×——×, male gametophytes; ●——●, female gametophytes cultured alone; ○——○, female gametophytes cultured with males.

Gametophytes grown under Set 5 conditions were isolated and placed into test tubes, one gametophyte per tube. Under Set 4 and Set 5 conditions, the isolated gametophytes remained sterile and continued to grow vegetatively. They became profusely branched and took a more or less spherical form. The thickness of the female gametophyte cells cultured under Set 4 conditions was about 8.5 μm$\pm$1.0 diam. in the cells adjacent to the terminal cells. The male gametophyte cells were about 5.9 μm$\pm$0.5 diam. Two strains of the female gametophytes (Ag. c. F-1 and Ag. c. F-2) and two strains of the male gametophytes (Ag. c. M-1 and Ag. c. M-2) from two sporophytes of different year were continued in culture.

When fragments excised from the gametophytes were introduced into test tubes with fresh media under Set 3 conditions, they became fertile (Fig. 32). The male gametophytes began to mature in 4-day cultures and reached full maturity in 8 days. The female gametophytes began to mature in 6 days and reached full maturity in 11 days. The time required for maturity was not altered when male and female gametophytes were cultured together. The chromosomes of the female and male gametophytes were about 22 (Pl. XI, A-B).

2) Sporophytes

Within 24 hours, fertilized eggs attaching to the opening of the oogonia became elliptical

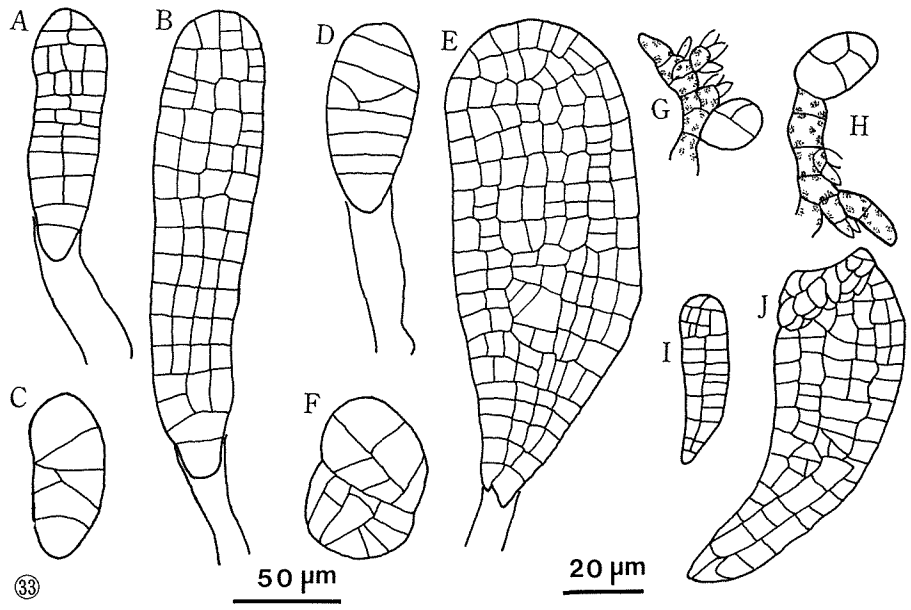

Fig. 33 *Agarum cribrosum*. A-C. Sporophytes derived from zygotes. D-F. Sporophytes derived from unfertilized eggs. G-H. Large elliptical cells (sporophyte initials) on male gametophytes. I-J. Sporophytes derived from vegetative cells of male gametophytes. (A-F and I-J drawn to 50 μm scale. G-H drawn to 20 μm scale.)

and then divided transversally into two cells. At the youngest stage of development, the sporophytes divided only transversally. Soon after longitudinal cell-division occurred and the sporophytes developed into flat expanded plantlets (Fig. 33, A–B). The primary rhizoids were given off from the basal cell of the plantlets. A few eggs developed into abnormal sporophytes consisting of an irregular mass of cells (Fig. 33, C), which degenerated within one month.

In 10 days after the discharge of eggs, the sporophytes attained a length of about 500 μm and consisted of about 410 cells under Set 3 conditions. When the sporophytes attained a length of about 2 mm, the basal part developed into a disc-shaped expansion, covering the primary rhizoids. In one month under Set 3 conditions the sporophytes differentiated into a blade, stipe and holdfast (Pl. X, A). The formation of the midrib occurred in three months and soon after became perforated.

When the sporophytes were 0.3–0.5 mm long (in 20 days from a mixed culture of female

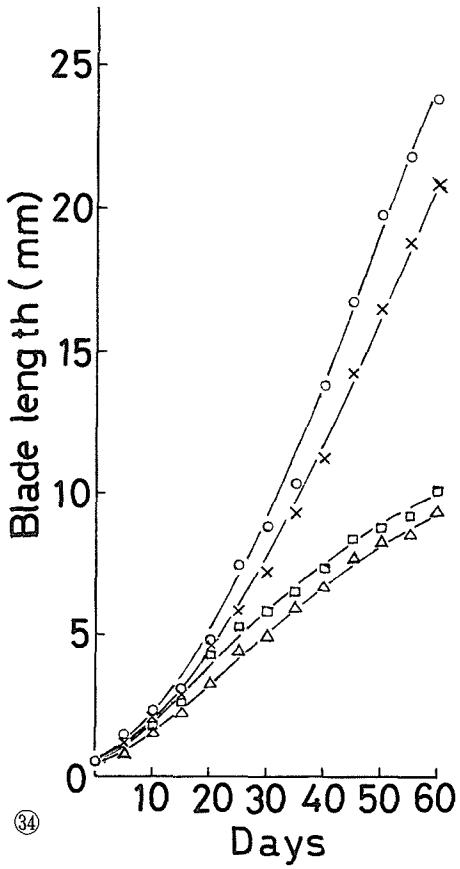

Fig. 34 Growth of *Agarum cribrosum* sporophytes at four temperatures in a 14-hr photoperiod, ×—×, 5℃; ○—○, 10℃; □—□, 14℃; △—△, 18℃.

Table 16 Growth of *Ag. cribrosum* sporophytes after 30- and 60-day at four temperatures in 10- and 14-hr photoperiods.

Temperature (℃)		5		10		14		18	
Photoperiod (hours)		10	14	10	14	10	14	10	14
Length × breadth of blades of sporophytes (mm)	30 days	4.7 × 2.3	7.2 × 3.3	5.8 × 3.4	8.4 × 3.9	4.6 × 2.7	5.8 × 3.1	4.1 × 2.1	5.0 × 3.3
	60 days	14.4 × 6.1	20.8 × 11.3	18.0 × 11.3	23.8 × 13.4	10.0 × 5.0	10.2 × 5.2	6.7 × 3.3	9.5 × 6.1

and male gametophytes under Set 3 conditions), they were separated from the gametophytes and transferred to culture vessels containing 200 m*l* of medium. They were maintained in the following eight conditions ; 5℃, 10℃, 14℃ and 18℃ in a 10- or 14-hr photoperiod(Fig. 34 ; Table 16). Within 5 days under these growth conditions, the sporophytes were up to about 1-2 mm length and differentiated a disc-shaped holdfast, a short stipe and a blade. The sporophytes grew well under cool and long-day conditions and the best growth was obtained at 10℃ in a 14-hr photoperiod (Set 3 conditions). The formation of the midrib occurred in 90 days at 10℃ and in 110 days at 5℃. Soon after the blades perforated. Under warm conditions the growth of sporophytes was suppressed, and the formation of the midrib and the perforation of the blades did not occur. In 4-5 months the distal end of the blades gradually became bleached.

When 3-month-old sporophytes grown under Set 3 conditions were about 5 cm long and 3.5 cm broad, they were transferred to culture vessels containing 1400 m*l* of medium and put back in Set 3 conditions. Four months after transfer to large vessels the sporophytes were about 13 cm long and 6.5 cm broad and had formed sporangial sori (Pl. X, F & H). The zoospores liberated from these sporophytes developed into normal male and female gametophytes.

The chromosome number of the normal sporophytes determined for cells of the blade at the monostromatic stage was about 44-48, being diploid (Pl. XI, F). On the other hand, the chromosome number of the abnormal sporophytes was irregular in number and usually over 60. They often contained two or three nuclei per cell.

3) Parthenogenesis

As mentioned above, the female gametophytes cultured alone under Set 3 conditions began to form oogonia in 6 days and fully matured in 11 days. Most eggs detached from the opening of the oogonia. Within 2 days some eggs degenerated, some became enlarged and spherical or elliptical. All of the spherical and elliptical eggs detached from the oogonia, developed into the abnormal sporophytes (Fig. 33, F) which died at the early stage (Pl. IX, D). Some elliptical eggs attached to the opening of the oogonia and developed into the normal sporophytes (Fig. 33, D-E ; Pl. IX, E). The germination rate of the normal

sporophytes from unfertilized eggs was 0.7% (Table 17).

Within one month under Set 3 conditions, the sporophytes derived from unfertilized eggs differentiated into a blade, stipe and holdfast (Pl. X, B). The formation of the midrib occurred in 3-month cultures and then the blade perforated. In 4–5 months, the distal part of the blades gradually degenerated and became bleached.

In 8-month cultures, one sporophyte kept in a culture vessel containing 200 m*l* of medium under Set 3 conditions, attained up to 4 cm in length and 2 cm in breadth, forming sori (Pl. X, G & I). The zoospores liberated from this sporophyte developed into normal female gametophytes.

The chromosomes of the sporophytes derived from unfertilized eggs were observed in the blade cells. Some were haploid 22–26 chromosomes (Pl. XI, D) and these plants were normal. However, some normal sporophytes had a diploid number of chromosomes (Pl. XI, E). Chromosome numbers of the abnormal sporophytes were irregular and usually over 60. They contained two or three nuclei per cell. Parthenogenetic development of the male gametes (sperms) did not occur.

4) Apogamy

Under Set 3 conditions, the isolated male gametophytes continued to form antheridia and grew vegetatively, developing into branched bushy thalli. Some apical cells and small bulges in the cells of branches of these thalli increased in size and became elliptical. Some of these elliptical cells divided transversally and longitudinally (Fig. 33, G–H ; Pl. IX, F). They either remained or detached from the branches. By successive cell-divisions, they developed into flat expanded plantlets which were identical with those of young sporophytes developed from the fertilized eggs (Fig. 33, I–J ; Pl. IX, G). When the plantlets attained a length of about 1–2 mm, the basal part developed into a disc-shaped expansion covering the primary rhizoid. In one-month cultures, the plantlets differentiated into a blade, stipe and holdfast (Pl. X, C). When these apogamous sporophytes attained a length of about 1–3 cm,

Table 17 Development of eggs of *Ag. cribrosum* from cultures of parthenogenetic and matings among two strains of gametophytes with oogonia and two strains with antheridia. Cultures were transferred to Set 3 conditions from Set 4 conditions with fresh media and sampled after 20 days.

Gametophytes with oogonia	×	gametophytes with antherida	E	ab-S	n-S	n-S%	Chromosome numbers of normal sporophytes
Ag. c. F-1			546	36	4	0.7	22-26 or 44-50
Ag. c. F-1	×	Ag. c. M-1	20	1	413	95.2	44-48
Ag. c. F-1	×	Ag. c. D-1	46	14	328	86.8	64-70
Ag. c. D-1	×	Ag. c. D-1	3	0	72	96.0	80-90

E ; Number of eggs and degenerated eggs. ab-S ; Number of abnormal sporophytes. n-S ; Number of normal sporophytes. n-S% ; Percentage of normal sporophytes (n-S/E+ab-S+n-S)

they became stunted and twisted. The formation of the midrib and perforations did not occur. Apogamy from the male gametophytes was not observed under other culture conditions. The chromosome number of these apogamous sporophytes was about 22–24, being haploid (Pl. XI, C). Apogamy from the female gametophytes was not observed.

5) Apospory

When the distal part of the blades of the sporophytes derived from zygotes degenerated and became bleached, some scattered epidermal cells survived. Most of them gradually degenerated. Under Set 4 conditions, however, a few epidermal cells became richly pigmented and became spherical. These cells began to germinate (Fig. 35, A) and divided transversally. They developed into small thalli composed of uniseriate branched filaments, which were attached to the bleached area of the sporophytes (Pl. IX, H ; X, J). These filamentous thalli were removed from the sporophytes and cultured under Set 4 conditions. The thickness of the thalli was about 6.1 μm$\pm$0.5 in the cells adjacent to the terminal cells, and was intermediate to that of the male and the female gametophytes.

When fragments excised from these thalli were introduced into test tubes with fresh

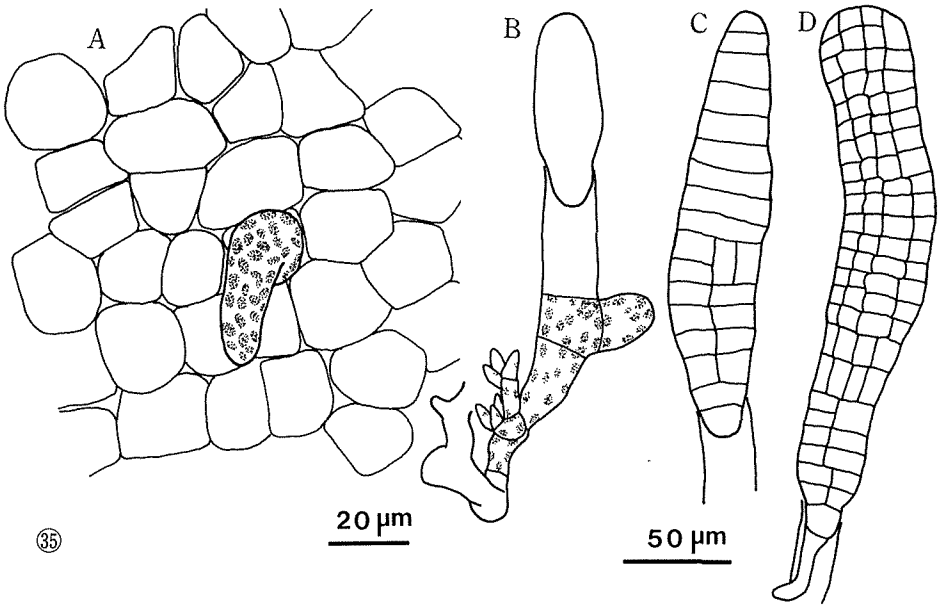

Fig. 35 *Agarum cribrosum*. A. Apospory of sporophyte derived from a zygote, showing germination of diploid gametophyte from an epidermal cell of sporophyte. B. Diploid monoecious gametophyte bearing a cluster of antheridia and an oogonium. C. Tetraploid sporophyte formed on a diploid monoecious gametophyte. D. Triploid sporophyte formed on a female gametophyte cultured with a diploid monoecious gametophyte. (A–B drawn to 20 μm scale. C–D drawn to 50 μm scale.)

medium under Set 3 conditions, they became fertile. They usually formed only antheridia and some branches developed vegetatively. In 15–20 days after the initiation of the antheridia, branches wider than usual were produced. These wider branches bore oogonia (Fig. 35, B; Pl. IX, I). Thus, the thallus derived from the epidermal cell of the sporophyte was the monoecious gametophyte. Almost all of the eggs germinated at the opening of the oogonia and developed into the normal sporophytes (Fig. 35, G; Pl. IX, J). In this experiment, 12 aposporous gametophytes (Ag. c. D-1～D-12) were isolated from 4 sporophytes. All of them were the same cell width and they formed oogonia and antheridia on the same individuals.

The chromosome number of these monoecious gametophytes was about 44–48, being diploid (Pl, XI, G), and that of the sporophytes germinated on these gametophytes was about 80–90, being tetraploid (Pl. XI, I).

6) Crossing experiments between the haploid and diploid gametophytes

Crossing experiments were undertaken between female gametophytes (Ag. c. F-1), male gametophytes (Ag. c. M-1) and diploid monoecious gametophytes (Ag. c. D-1), which were maintained under Set 4 conditions. Crossings were conducted by transferring a couple of sterile fragments of gametophytes to a new culture vessel with fresh medium and culturing under Set 3 conditions. In 20 days, a number of normal sporophytes (n-S), abnormal sporophytes (ab-S) and ungerminated eggs (E) produced were counted (Table 17).

Most of the eggs from female gametophytes cultures with diploid monoecious gametophytes and those from diploid monoecious gametophytes, developed into normal sporophytes (Fig. 35, D; Pl. X, D-E). The germination of the normal sporophytes in the above-mentioned cultures (Pl. IX, K) was nearly the same with that of the female gametophytes cultured with males (Pl. IX, C) and higher than that for females alone (Pl. IX, D). The chromosome number of the sporophytes obtained from the cultures of the haploid female and the diploid monoecious gametophytes, was about 64–70, being triploid (Pl. XI, H). This shows that the fertilization occurred between the haploid eggs and the diploid sperms.

Alariaceae

Alaria crassifolia KJELLMAN

A. crassifolia is found commonly in the vicinity of Muroran, growing on rocks in the lower littoral and upper sublittoral zone. Young plants appear in the middle of February and attain full maturity from early September to January of the next year. Most ot the plants become detached from substratum during the fertile season and some survive to the next season. Fruiting plants produce sporophylls from the stipe, on which unilocular sporangia are formed. KANDA (1936) has given a detailed description of the gametophytes and young

stages of the sporophytes of this species from Muroran. Cytological observations were reported by YABU (1957).

1) Gametophytes

Within 24 hours, the settled zoospores sent out a germination tube into which the whole protoplasm generally migrated. In 5 days, the female gametophytes are distinguishable from the males. In 10-day cultures under Set 3 conditions, both types gametophytes became fertile and the female gametophytes were composed of 2-3 cells, average 2.1 and the males 15-26 cells, average 22.6. Under Set 5 conditions, the female gametophytes were composed of 8-12 cells, average 9.9 and the males 18-30 cells, average 25.0, but they never matured. Under Set 1 conditions the gametophytes became fertile in 30 days. Under Set 4 and Set 2 conditions, slight maturation occurred in 30 days.

The gametophytes grown under Set 5 conditions were isolated and introduced into test tubes, one gametophyte per tube. Under Set 4 and Set 5 conditions, the isolated gametophytes remained sterile and continued to grow vegetatively. They became profusely branched, assuming a more or less spherical form. The thickness of the female gametophyte cultured under Set 4 conditions was 11.8 μm$\pm$0.7 in the cells adjacent to the terminal cells and that of the male ones 5.6 μm$\pm$0.5. Two strains of the female

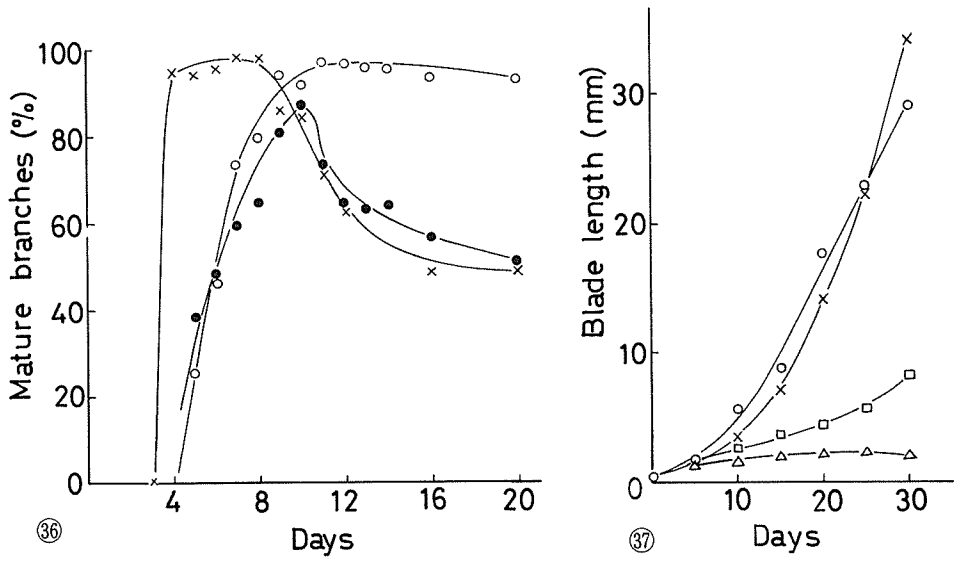

Fig. 36 Maturation of *Alaria crassifolia* gametophytes at 10℃ in a 14-hr photoperiod, ×—×, male gametophytes; ●—●, female gametophytes cultured alone; ○—○, female gametophytes cultured with males.

Fig. 37 Growth of *Alaria crassifolia* sporophytes at four temperatures in a 14-hr photoperiod, ×—×, 5℃; ○—○, 10℃; □—□, 14℃; △—△, 18℃.

Table 18 Growth of *A. crassifolia* sporophytes after 15- and 30-day at four temperatures in 10- and 14-hr photoperiods.

Temperature (℃)		5		10		14		18	
Photoperiod (hours)		10	14	10	14	10	14	10	14
Length of blades of sporophytes (mm)	15 days	5.0	7.0	6.4	8.5	3.9	3.6	2.0	2.0
	30 days	18.0	34.2	23.4	29.0	4.6	8.2	1.4	2.0

gametophytes (A. c. F-1 and A. c. F-2) and two strains of the male gametophytes (A. c. M-1 and A. c. M-2) from two sporophytes of the different year were cultured further.

When fragments excised from the gametophytes grown under Set 4 conditions were introduced into fresh medium and cultured under Set 3 conditions, they formed gametangia (Fig. 36). In 4-day cultures, maturation of the male gametophytes was completed and after 4 days vegetative branches were produced. Maturation of the male gametophytes was not promoted when they cultured with the females. Maturation of the female gametophytes occurred in 5 days. When cultured with the males, the female gametophytes matured fully in 9 days. When the female gametophytes were cultured alone, complete maturation occurred in 10 days and then vegetative branches were produced. The chromosome number of the female and male gametophytes was about 22-28.

2) Sporophytes

Within 24 hours, fertilized eggs (attaching to the opening of the oogonia) became elliptical and then divided transversally into two cells. Initially, only transverse cell-divisions occurred and then the sporophytes developed into flat expanded plantlets. A few eggs developed into abnormal sporophytes consisting of an irregular, globular mass of cells, which degenerated within one month. In 10 days after the discharge, the eggs developed into sporophytes of about 495 μm length and consisting of about 1400 cells under Set 4 conditions. When they were about 2 mm length, the basal part developed into a disc-shaped expansion, covering the primary rhizoids. In one month under Set 3 condition, the sporophytes differentiated into a holdfast, stipe and blade with a midrib.

When the sporophytes were about 0.3-0.8 mm length (in 20 days from a mixed culture of female and male gametophytes under Set 3 conditions), they were removed from the gametophytes and transferred to culture vessels containing 200 m*l* of medium. They were maintained in the following eight culture conditions; 5℃, 10℃, 14℃ and 18℃ in a 10- or 14-hr photoperiod (Fig. 37; Table 18). Within 5 days under these conditions, the sporophytes were up to 1-2 mm long and differentiated into a disc-shaped holdfast, a short stipe and a blade. The sporophytes grew well under cool and long-day conditions and the best growth was obtained at 5℃ in a 14-hr photoperiod. The formation of the midrib occurred within one month at 5℃ in a 14-hr photoperiod and 10℃ in a 10- or 14-hr photoperiod, and within

50 days at 5℃ in a 10-hr photoperiod. On the other hand, under warm conditions the growth of sporophytes was suppressed and the midrib was not produced. Under 18℃ conditions the sporophytes did not grow longer than 2 mm.

Three months after transfer to Set 3 conditions, the sporophytes were up to 12 cm in length and 1.5 cm in breadth. These sporophytes were transferred to culture vessels containing 1400 m*l* of medium. They were 80 cm long and 5 cm broad in 3 months. Some of these well grown sporophytes were transferred to eight different conditions mentioned above. Among them, the sporophytes cultured at 10℃ in a 10-hr photoperiod formed sporangial sori 4 months after transfer. The fertile sporophytes did not form the sporophylls and the sporangial sori were formed on the lower marginal part of the blade (Pl. XII, A). The zoospores liberated from these sporophytes developed normally into the male and female gametophytes.

The chromosome number of the normal sporophytes was determined from the cells of the blade at the monostromatic stage and was about 44–48, being diploid. The chromosome number of abnormal sporophytes was irregular and usually over 60. These contained two or three nuclei per cell.

3) Parthenogenesis

As above-mentioned, the female gametophytes cultured alone under Set 3 conditions began to form oogonia in 5 days and fully matured in 10 days. Most eggs detached from the opening of the oogonia. Within 2 days some eggs degenerated, some became enlarged and spherical, and some became elliptical. All of the spherical and some of the elliptical eggs detached from the oogonia, developed into abnormal sporophytes consisting of an irregular, globular mass of cells and died at an early stage. Some elliptical eggs developed into normal sporophytes, attached to the opening of the oogonia. The germination percentage of the normal sporophytes from unfertilized eggs was 13.4% (Table 19).

Within one month under Set 3 conditions, the sporophytes differentiated into a holdfast, stipe and blade with a midrib. These sporophytes in 5-month cultures were transferred to 10℃ in a 10-hr photoperiod. Four months after transfer the sporophytes formed sporangial sori in the lower marginal part of the blade without the formation of sporophylls (Pl. XII, C). The zoospores liberated from these sporophytes, developed into the normal female gametophytes.

The chromosome number of the sporophytes derived from unfertilized eggs was observed in the cells of the blade at an early stage. Most of the normal sporophytes had the haploid number of chromosomes, about 22–26. However, a few had 44–50 chromosomes, being diploid. Most of the abnormal sporophytes were irregular in the number of chromosomes and contained two or four nuclei per cell. The parthenogenesis of the male gametes did not occur.

4) Apogamy

Under Set 3 conditions, isolated male gametophytes continued to form antheridia and at

the same time grew vegetatively and developed into well branched bushy thalli. In these thalli, some apical cells and small bulges of the branch-cells increased in size and became elliptical. Some of these elliptical cells divided transversally and then longitudinally, some remained to the branches, some became detached. By successive cell-divisions they developed into flat expanded plantlets which were identical to those of young sporophytes from eggs. The primary rhizoids were issued from the basal cell of the plantlets. When the plantlets were about 1–2 mm long, the basal part developed into a disc-shaped expansion, covering the primary rhizoid. In two months, they differentiated into a blade, stipe and holdfast, but the stipe was very short and the blade was narrow without formation of the midrib. In other culture conditions apogamy from the male gametophytes was not observed. The chromosome number of the apogamous sporophytes was 22, being haploid. Apogamy from the female gametophytes was not observed.

5) Apospory

When the distal part of sporophyte blades derived from zygotes degenerated and became bleached, some epidermal cells survived. These were scattered in the bleached area. Most of them gradually degenerated. Under Set 4 conditions, however, a few of them became richly pigmented and spherical in shape. These cells began to divide transversally. They developed into small thalli composed of uniseriate filaments, attached to the bleached area of the mother plant (Pl. XII, E). Four filamentous individuals were isolated from each of these four sporophytes and maintained under Set 4 conditions. Among them, 15 individuals (A. c. D-1~D-15) were 6.4 μm$\pm$0.5 wide in the cells adjacent to the terminal cells and were intermediate between the male and female gametophytes in width. Only one individual (A. c. D-16) was wider than the female gametophytes, measuring 14.7 μm$\pm$1.5 wide in the cells adjacent to the terminal cells.

When fragments excised from these thalli were introduced into test tubes with fresh medium under Set 3 conditions, they became fertile. The narrower individuals (A. c. D-1 ~D-15) usually formed antheridia and grew vegetatively, issuing branches. In 15–20 days after the initiation of the antheridia, they gave off branches wider than usual. These wider branches formed oogonia. Almost all of the eggs germinated at the opening of the oogonia and developed into normal sporophytes. The wider individuals (A. c. D-16) formed oogonia only and discharged eggs. About 1.4% of these eggs developed into the normal sporophytes (Table 19).

The chromosome number of the narrower monoecious gametophytes was about 44–48, being diploid and that of the sporophytes developed from these diploid gametophytes was about 80–90, being tetraploid. The chromosome number of the wider female gametophytes was about 44 and that of the sporophytes developed parthenogenetically from these diploid female gametophytes was about 44–48.

The tetraploid sporophytes which were produced by autogamy of the diploid monoecious gametophytes developed normally and differentiated into a holdfast, stipe and blade with a

Table 19 Development of eggs of *A. crassifolia* from cultures of parthenogenetic and matings among five strains of gametophytes with oogonia and four strains with antheridia. Cultures were transferred to Set 3 conditions from Set 4 conditions with fresh media and sampled after 20 days.

Gametophytes with oogonia	×	Gametophytes with antheridia	E	ab-S	n-S	n-S%	Chromosome numbers of normal sporophytes
A. c. F-1			154	26	28	13.4	22-26 or 44-50
A. c. F-1	×	A. c. M-1	145	28	342	66.4	44-48
A. c. F-1	×	A. c. mH-1	55	20	181	70.7	44-48
A. c. F-1	×	A. c. D-1	89	18	327	75.3	66-74
A. c. F-1	×	A. c. Te-1	204	9	5	2.2	22-26
A. c. fH-1			61	20	1	1.2	22-26
A. c. fH-1	×	A. c. M-1	55	20	181	70.7	44-48
A. c. fH-1	×	A. c. mH-1	11	1	37	75.5	44-48
A. c. fH-1	×	A. c. Te-1	96	15	3	2.6	22-26
A. c. D-16			349	68	6	1.4	44-48
A. c. D-16	×	A. c. M-1	184	94	668	70.7	66-76
A. c. D-16	×	A. c. mH-1	42	12	122	69.3	66-74
A. c. D-16	×	A. c. D-1	227	49	247	47.2	80-96
A. c. D-16	×	A. c. Te-1	147	9	0	0	
A. c. D-1	×	A. c. D-1	7	5	42	77.8	80-94
A. c. Te-1	×	A. c. M-1	26	42	78	53.4	100-110
A. c. Te-1	×	A. c. Te-1	34	4	3	7.3	

midrib within one month. However, the diploid sporophytes produced from unfertilized eggs of wider diploid aposporous gametophytes differentiated into a blade, stipe and holdfast, but their stipes were short and their blades were narrow without formation of the midrib. They were quite similar to the apogamous sporophytes derived from the male gametophytes.

A part of a sporophyte blade collected at Muroran on August 11, 1967, was washed carefully and cut off into small pieces. When cultured under Set 4 conditions, these pieces did not grow but survived for a long time. After 5 months some epidermal cells of the blade germinated and developed into filamentous thalli. The width of the thallus was the same that of the diploid monoecious gametophytes. These thalli usually formed antheridia and sometimes oogonia. The chromosome number of the thalli was about 44-48, being diploid.

Apospory of the sporophytes derived from unfertilized eggs was observed in the same way as that of the sporophytes from zygotes under Set 4 conditions (Pl. XII, F). The width of these filamentous thalli (A. c. fH-1~fH-3, from each of three sporophytes) was 11.7 μm$\pm$0.7 in the cells adjacent to the apical cells being nearly the same as that of the female gametophytes. Under Set 3 conditions, they formed oogonia but bore far fewer oogonia than normal female gametophytes. Only 1.2% of the eggs produced from them developed parthenogenetically into normal sporophytes (Table 19). The chromosome number of these

aposporous female gametophytes was about 22–26, being haploid and that of the sporophytes developed parthenogenetically from the above-mentioned gametophytes was also 22–26.

Apospory of agogamous sporophytes was observed under Set 3 and Set 4 conditions (Pl. XII, G) in the same way as that of sporophytes from zygotes. When these filamentous thalli (A. c. mH-1～mH-3, from each of the three sporophytes) were cultured under Set 4 conditions, their width was 5.6 μm±0.5 in the cells adjacent to the apical cells of branches being the same as that of the male gametophytes. Under Set 4 conditions, they formed antheridia only. The chromosome number of these male gametophytes was about 22–24, being haploid.

Apospory of the tetraploid sporophytes which were produced by autogamy of the diploid monoecious gametophytes occurred in the same way as that of the diploid or haploid sporophytes mentioned above. The thallus of the aposporous gametophyte from the tetraploid sporophytes (A. c. Te-1～Te-3) was 8.9 μm±1.2 wide in the cells adjacent to the terminal cells of branches grown under Set 4 conditions. It was wider than the diploid

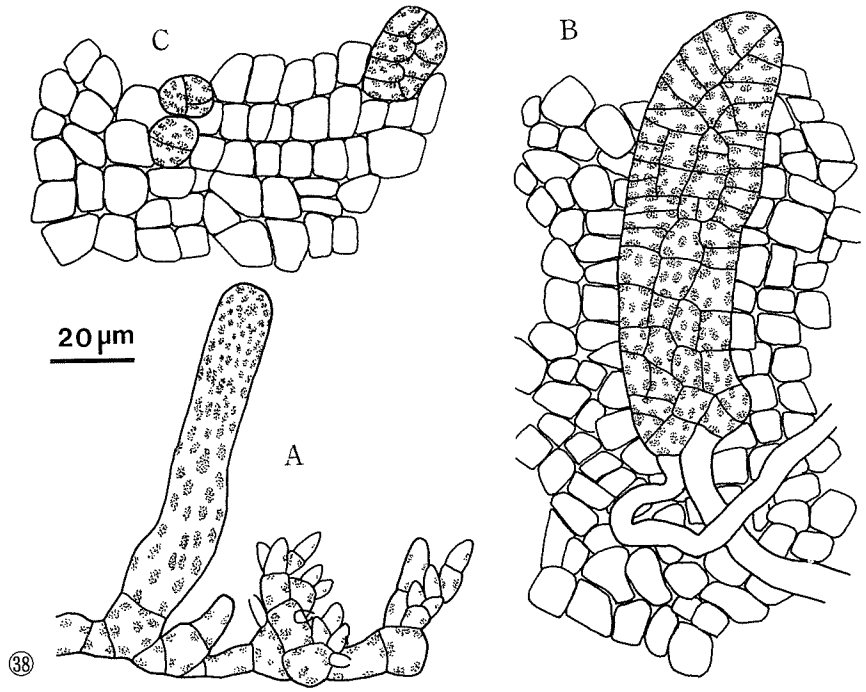

Fig. 38 *Alaria crassifolia*. A. Part of tetraploid monoecious gametophyte bearing and oogonium and clusters of antheridia formed on a tetraploid sporophyte. B. New sporophyte from an epidermal cell of sporophyte derived from an unfertilized egg. C. New sporophytes from epidermal cells of sporophyte derived from a vegetative cell of male gametophyte.

monoecious gametophyte and narrower than the female gametophyte. Thalli cultured under Set 3 conditions, usually formed antheridia and often formed oogonia (Fig. 38, A). Only 7.3% of the eggs developed into the normal sporophytes (Table 19). This percentage was nearly the same as that of the normal sporophytes from unfertilized eggs. The chromosome number of these aposporous gametophytes was about 80–90, being tetraploid.

6) Vegetative reproduction of sporophytes

As mentioned above, some epidermal cells in the bleached area of sporophytes developed into the gametophytes under Set 4 conditions. Under Set 3 conditions, however, some epidermal cells of the bleached area of the haploid sporophytes from either unfertilized eggs or vegetative cells of the male gametophytes, divided transversally and longitudinally. These developed into flat expanded plantlets (Fig. 38, B–C). The plantlets grew into the sporophytes which were identical with the mother plants (Pl. XII, G). Vegetative reproduction of the sporophytes from zygotes was not observed.

7) Crossing experiments among various gametophytes

Crossing experiments were undertaken with two strains of the female gametophytes (A. c. F-1 and A. c. fH-1), two strains of the males (A. c. M-1 and A. c. mH-1), one strain of the diploid monoecious gametophyte (A. c. D-1), a diploid female (A. c. D-16) and a tetraploid monoecious gametophyte (A. c. Te-1), which were maintained under Set 4 conditions. Crossings were carried out by transferring a few sterile gametophytes fragments to a new vessel with fresh medium under Set 3 conditions. In 20-day cultures, a number of the normal sporophytes (n-S), abnormal sporophytes (ab-S) and ungerminated eggs (E) produced on the gametophytes, were calculated (Table 19).

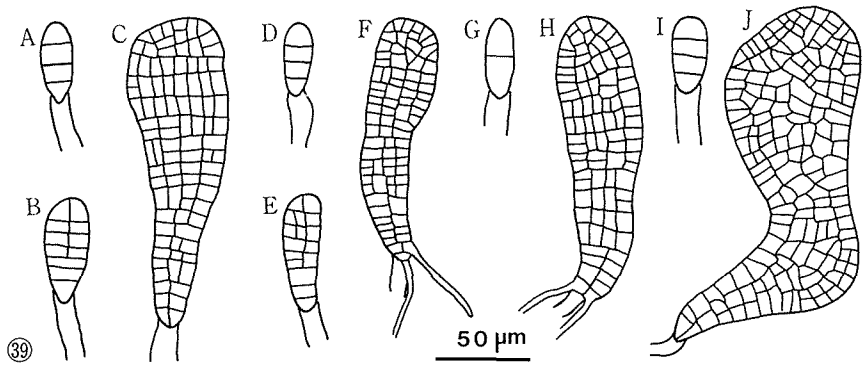

Fig. 39 *Alaria crassifolia.* A–C. Triploid sporophytes formed on a haploid female gametophyte cultured with a diploid monoecious gametophyte. D–F. Triploid sporophytes formed on a diploid female gametophyte cultured with a haploid male gametophyte. G–H. Tetraploid sporophytes formed on a diploid female gametophyte with a diploid monoecious gametophyte. I–J. Pentaploid sporophytes formed on a tetraploid monoecious gametophyte cultured with a haploid male gametophyte.

Most eggs from the haploid female gametophytes (A. c. F-1) cultured with the haploid apogamous male gametophyte (A. c. mH-1) or the diploid monoecious gametophytes (A. c. D-1) developed into normal sporophytes (Fig. 39, A-C). The germination percentage of these normal sporophytes was nearly the same as that of the haploid female gametophyte cultured with the haploid male gametophyte and higher than that in cultures of the haploid female gametophytes alone. The chromosome number of these sporophytes was about 44-48 or 66-74 respectively.

Most eggs from the diploid aposporous female gametophyte (A. c. D-16) cultured with the haploid male gametophyte (A. c. M-1) or the diploid monoecious gametophyte (A. c. D-1) developed into the normal sporophytes (Fig. 39, D-H). The germination percentage of these normal sporophytes was higher than that of the diploid female gametophytes alone. The chromosome number of the normal sporophytes concerned was about 66-74 or 80-96 respectively.

Only a few eggs from the three kinds of the female gametophytes cultured with the tetraploid monoecious gametophyte, developed into the normal sporophytes, having nearly the same germination percentage as normal sporophytes from female gametophytes cultured alone. A half of the eggs from the tetraploid monoecious gametophyte (A. c. Te-1) cultured with the haploid male gametophyte (A. c. M-1) developed into the normal sporophytes (Fig. 39, I-J), exhibiting a higher germination percentage than the tetraploid monoecious gametophyte alone. The chromosome number of these normal sporophytes was about 100-110, being pentaploid. From these results, it is inferred that the fertilization occurred between the haploid or diploid or tetraploid eggs and the haploid or diploid sperms. It seems, however, that the sperm from the tetraploid gametophyte was nonfunctional.

Discussion of the life history of Laminariales

Gametophytes

The gametophytes of the members of Laminariales show a similar course of development. The female gametophytes are usually composed of only one to several cells and the males are always multicellular. Juvenile sporophytes of many species of Laminariales appear in early spring. It shows that their gametophytes mature in winter. Various investigators have observed in cultures that in many species the gametophytes became fertile at room temperature in winter (KANDA, 1936). Immature profusely branched gametophytes have been observed at room temperature in summer (high temperature) but when the temperature falls to about 10℃ and below, they become fertile (IKARI, 1921; SCHREIBER, 1930; KEMP and COLE, 1961; YABU, 1964b).

The germlings of *Laminaria japonica, Agarum cribrosum* and *Alaria crassifolia* developed into fertile gametophytes at lower temperatures (5-12℃). However, the development of germlings was slightly different among these three species. The zoospores of *A. crassifolia* developed into fertile gametophytes in 10 days at 10℃ in a 14-hr photoperiod,

and those of *L. japonica* in 11 days. In *Ag. cribrosum* it required 30 days at 10℃ in a 14-hr photoperiod that the zoospores developed into fertile gametophytes. The gametophytes of the three species grew well vegetatively and became profusely branched thalli under warm conditions (14–18℃). When immature gametophytes were transferred to 10℃, the males matured completely after 4–8 days and the females after 9–11 days. The 10-hr and 14-hr photoperiods did not affect the growth and maturation of the gametophytes. However, HSIAO and DRUEHL (1971) showed that photoperiod did effect maturation in *Laminaria saccharina*.

LÜNING (1980) found that in three *Laminaria* species at higher temperature an appreciably higher photon flux density is needed to induce fertility in the same percentage of gametophytes than at lower temperature. In the present study, the higher temperature inhibition for fertility observed in *L. japonica, Ag. cribrosum* and *A. crassifolia* may be caused by the lack of light necessary for induction of fertility.

Sporophytes

The sporophytes of the members of Laminariales show a similar cource of early development. It is known that they grow well under the culture conditions where the maturation of gametophytes occurs. However, there have been only a few experiments on the growth of sporophytes under different culture conditions. SEGI and KIDA (1958) reported that in *Undaria undarioides* the sporophytes grew best at 1500–2000 lux. SUNDENE (1962) observed that the sporophytes of *Alaria esculenta* grew well at 14℃ under 500 or 1000 lux of light in 25 or 30‰ of salinity and they grew slightly at 17℃ under 500 lux in 25 or 30‰. KAIN (1965) found that the sporophytes of *Laminaria hyperborea* grew almost equally well at 10–20℃. However, she (1969) reported that the growth rate at 5℃ was lower than at 10℃. PÉREZ (1971) found slightly faster growth at 11℃, 13℃ and 15℃ than 7℃ and 9℃, with a marked inhibition at 20℃ in *L. digitata*. These earlier experiments on the growth of sporophytes, however, were conducted on young sporophytes which are less than 1 cm long and not differentiated into a holdfast, stipe and blade.

In the present experiment, juvenile sporophytes of *Laminaria japonica, Agarum cribrosum* and *Alaria crassifolia* grew well at 5℃ to 18℃ up to about 1–2 mm long and differentiated into a disc-shaped holdfast, a short stipe and a blade within 5 days after transfer of 0.5 mm undifferentiated sporophytes grown at 10℃. The young differentiated sporophytes of *L. japonica* grew well at 5–10℃ in 10-hr or 14-hr photoperiods. At 14℃ and 18℃ growth was very slow. *Ag. cribrosum* grew well at 5–10℃ in a 14-hr photoperiod and best at 10℃. The formation of the midrid occurred after 90 days at 10℃ and after 110 days at 5℃. Under these conditions the blades perforated soon after the formation of the midrib. At 14℃ and 18℃ growth was very slow, and the formation of the midrib and the perforations of the blade did not occur. The young differentiated sporophytes of *A. crassifolia* grew well at 5–10℃ in 10-hr or 14-hr photoperiods. The formation of the midrib occurred after 30 days at 10℃ in a 14-hr photoperiod. At 14℃ growth was slow. At 18℃ the sporophytes did not

grow more than 2 mm and the midrib did not differentiate. In three months at 10℃ in a 14-hr photoperiod, the sporophytes of *L. japonica* reached 15 cm long and 1.8 cm broad, those of *Ag. cribrosum* reached 5 cm long and 2.5 cm broad and those of *A. crassifolia* reached 12 cm long and 1.5 cm broad.

In the laboratory rearing of laminariaceous plants, it is comparatively easy to produce large blades but it is difficult for the blade to mature. In cultured *Undaria pinnatifida* and *Costaria costata,* further development of sporophytes, the formation of sporangia and the liberation of zoospores from the latter species have been observed in the laboratory culture tank containing 60 liters of medium by SANBONSUGA and HASEGAWA (1967). In the present study, the formation of unilocular sporangia and the liberation of zoospores of *Ag. cribrosum* occurred in 7-month cultures at 10℃ in a 14-hr photoperiod in culture vessels containing 200 m*l* of medium. Those of *A. crassifolia* occurred in one-year cultures at 10℃ in a 10-hr photoperiod in vessels containing 1400 m*l* of medium. In cultures, sporangial sori of *A. crassifolia* were formed on the lower and marginal parts of the blade. The formation of sporangial sori on the lamina of *Alaria* was found in field materials (KAWASHIMA, 1977). Sporangium formation of *L. japonica* was not observed.

Parthenogenesis

SCHREIBER (1930) studied parthenogenesis in three species of *Laminaria*, KEMP and COLE (1961) in *Nereocystis* and YABU (1964b) in nine species of Laminariales. According to them, the resulting sporophytes are irregular in shape compared with the diploid form and their development is restricted. FANG *et al.* (1978) found that many parthenosporophytes of *Laminaria japonica* grew normally and formed clusters of sporangia after about half a year's cultivation in the sea. The produced zoospores grew into female gametophytes.

In the present experiment, about 8.9% of unfertilized eggs in *L. japonica,* about 0.7% in *Ag. cribrosum* and 13.4% in *A. crassifolia* developed into the normal sporophytes. Most of these normal sporophytes were haploid and a few were diploid. No difference was recognized between the sporophytes from zygotes and those from unfertilized eggs. Most of the unfertilized eggs developed into the abnormal sporophytes. These contained two to four nuclei per cell and had an irregular chromosome number. The formation of unilocular sporangia and the liberation of swarmers from parthenosporophytes were observed in *Ag. cribrosum* and *A. crassifolia.* These swarmers developed into the female gametophytes only.

Apogamy

Sporophyte formation from vegetative cells of the gametophytes has been observed in *Undaria undarioides* by SEGI and KIDA (1957). According to them, the terminal cell of immature female gametophytes undergoes repeated transversal and longitudinal divisions resulting in an embryonic plantlet of abnormal shape. They interpreted such abnormal plantlets as having developed parthenogenetically. However, this is apogamy as parthenogenesis refers to development from a sexual cell.

In the present experiment, apogamy was observed on male gametophytes of *L. japonica, Ag. cribrosum* and *A. crassifolia.* When the male gametophytes of these species were cultured alone at 10℃ in a 14-hr photoperiod, sporophytes arose from the terminal cell or small bulges of mature branches. Apogamous sporophytes were haploid and developed into a slightly abnormal shape. In *L. japonica* and *Ag. cribrosum,* the blades became stunted and twisted abnormally. In *A. crassifolia* the stipe was short and the blade was narrow without a midrib. Apogamous sporophytes from the female gametophytes were not observed.

Apospory

In the Laminariales, the meristem is localized at the base of the blade and the distal part of the blade gradually degenerates and bleaches. In this bleached area some scattered epidermal cells survive. Some of these epidermal cells in *L. japonica, Ag. cribrosum* and *A. crassifolia* germinated and developed into gametophytes. Apospory was observed in the sporophytes grown at 14℃ in a 14-hr photoperiod.

Aposporous gametophytes from the sporophytes developed from zygotes were diploid. In these diploid gametophytes of *L. japonica, Ag. cribrosum, and A. crassifolia,* the width of the thallus was intermediate between that of the male gametophytes and the female gametophytes. These gametophytes were monoecious, forming antheridia more abundantly than the oogonia on the same individuals. In *A. crassifolia*, however, some of these diploid gametophytes produced oogonia only, they were wider than that haploid female gametophytes. Aposporous germlings from the sporophytes derived from unfertilized eggs grew into the haploid female gametophytes and those from the apogamous sporophytes derived from the male gametophytes grew into haploid male gametophytes.

Vegetative reproduction of sporophytes

The regeneration of sporophytes from growing regions occurred in perennial species. SAGA and SAKAI (1977) observed that in *Laminaria japonica* fragments which comprised the lower part of the blade and the stipe regenerated the complete individuals. New sporophyte formation from vegetative cells of blades was scarce in Laminariales. SAGA *et al.* (1978) found single isolated cells of *L. angustata* which separated from callus-like masses developed into new sporophytes.

New sporophytes were formed from cells of blades of the haploid sporophytes which were parthenogenetic (in *L. japonica* and *A. crassifolia*) or apogamous (in *A. crassifolia*). Several epidermal cells which survived in the bleached area developed into new sporophytes. However, this was not observed in the diploid sporophytes derived from zygotes.

Nuclear phases

Cytological elucidation has been given for various members of the Laminariales (COLE, 1967, 1968). Most haploid counts are obtained from dividing cells of the female gametophytes, including oogonia and meiotic metaphases in the sporangia. Diploid counts are obtained from two or four celled sporophytes. The haploid chromosome number of sporo-

phytes from unfertilized eggs has been reported in *Nereocystis luetkeana* (KEMP and COLE, 1961). However, polyploides have not been cytologically determined in the Laminariales.

In the present experiment, haploid counts were obtained from dividing cells of the female and the male gametophytes, and of the sporophytes from unfertilized eggs and vegetative cells of the male gametophytes. The haploid chromosome number of *L. japonica* is about 31, different from ABE'S (1939) and YABU'S (1973) observations, 22. That of *Ag. cribrosum* is about 22 and that of *A. crassifolia* is about 22, which correspond with YABU'S observation (1957, 1964a). Diploid counts were obtained from young monostromatic sporophytes from zygotes and rarely from unfertilized eggs. The diploid chromosome number of *L. japonica* is about 62, that of *Ag. cribrosum* is about 44 and that of *A. crassifolia* is about 44. The diploid counts were obtained also from the aposporous gametophytes from the sporophytes derived from zygotes.

Triploid counts were obtained from young sporophytes of *L. japonica, Ag. cribrosum* and *A. crassifolia* from haploid female gametophytes and diploid monoecious gametophytes. The counts also were obtained form aposporous gametophytes from the triploid sporophytes in *A. crassifolia.* The triploid gametophytes were monoecious. Tetraploid counts were obtained from young sporophytes from diploid monoecious gametophytes of the three species and also in the cultures of the diploid female gametophytes with the diploid monoecious gametophytes in *A. crassifolia.* The counts also were obtained from the aposporous gametophytes from the tetraploid sporophytes in *A. crassifolia.* The tetraploid gametophytes were monoecious, but the sperm apparently was not functional. Pentaploid counts were obtained from young sporophytes of *A. crassifolia* from tetraploid monoecious gametophytes with the haploid male gametophytes.

II Growth and Nutrition in Axenic Culture

In the preceding chapter, I described the morphological and nuclear alternation of generations of several brown algae in the unialgal culture. In this study attempts were made to obtain axenic cultures of some oogamous brown algae (*Desmarestia viridis, D. ligulata, Laminaria japonica* and *Alaria crassifolia*) and then were investigated nutritional differences between gametophytes and sporophytes. For reliable algal nutrition and metabolic studies axenic cultures under defined media should be used.

The extensive culture work of PROVASOLI, MCLAUGHLIN and DROOP (1957) has established the growth requirements for several species of planktonic algae and the ASP series of artificial sea-water medium has been used in subsequent culture work by various investigators. Since 1957, certain seaweeds have been cultured axenically and numerous studies have reported the requirements of trace metals, other micronutrients, vitamins and plant growth regulators (O'KELLEY, 1974; PROVASOLI and CARLUCCI, 1974; DEBOER, 1982). However, all these studies were concerned only with one generation of the life cycle. No

work has been reported on the growth and nutrition of both generations in the axenic culture.

Materials and Methods

The following four species were used in this study; *Desmarestia viridis, D. ligulata, Laminaria japonica* and *Alaria crassifolia*. They were collected from Muroran in 1966.

Purification

A piece of the fertile thallus was cut off and rinsed several times with sterilized sea water. It was then placed in a sterilized Petri-dish with sterilized medium. Newly liberated zoospores were pipetted with a capillary pipette and purified by washing successively in sterile media. They were inoculated into test tubes, one spore per tube, and cultured under Set 4 conditions. Each culture was tested for sterility by inoculating in liquid and agarized medium ST3 (TATEWAKI and PROVASOLI, 1964) and finally the grown plants were examined microscopically with oil immersion (Table 20). All culture transfers were conducted in an inoculation chamber. This chamber was sterilized by an ultraviolet lamp.

Cultural conditions and media

Small fragments of the gametophytes of *D. viridis, D. ligulata, L. japonica* and *A. crassifolia* were cultured in seven artificial media, (ASP_1, ASP_2, ASP_2NTA, ASP_6, ASP_7, ASP_{12}, and $ASP_{12}NTA$) (PROVASOLI, 1963) at 16℃ in a 16-hr photoperiod illuminated with cool-white fluorescent lamps at about 3000 lux (Set 7 conditions), at 14℃ in a 14-hr photoperiod (Set 4 conditions), at 10℃ in a 14-hr photoperiod (Set 3 conditions) and at 10℃ in a 10-hr photoperiod (Set 6 conditions). Under Set 7 conditions the gametophytes of *D. viridis* and *D. ligulata* grew well vegetatively in ASP_{12} and $ASP_{12}NTA$. Under Set 3 and Set 4 conditions, they also grew well in ASP_{12} and $ASP_{12}NTA$, but sometimes they became fertile in ASP_{12}. Under Set 6 conditions they matured fully in ASP_{12} and $ASP_{12}NTA$ but the number of mature branches of plants grown in $ASP_{12}NTA$ was fewer than that in ASP_{12}. The sporophytes of both species grew well in ASP_{12} and $ASP_{12}NTA$. The gametophytes cultured in $ASP_{12}NTA$ medium under Set 4 conditions were maintained as the stock cultures and they were transplanted every 4 months.

The female and male gametophytes of *L. japonica* and *A. crassifolia* were cultured separately. They grew well under Set 7 and Set 4 conditions in ASP_{12} and $ASP_{12}NTA$.

Table 20 Results of purification with the capillary pipette method (repeated micropipette washing of zoospores).

Species	Swarmers	Phototaxis	Times of washing in sterile media	Degree of success
Desmarestia viridis	zoospores	negative	3	8/10
D. ligulata	zoospores	negative	3	8/10
Laminaria japonica	zoospores	none	2	9/10
Alaria crassifolia	zoospores	none	2	8/10

They become fertile under Set 3 and 6 conditions. When both female and male gametophytes were mixed and cultured under Set 3 and 6 conditions many normal sporophytes were formed. The sporophytes grew up to 40–50-celled plantlets, but then they bleached in the seven media.

In the following experiments, gametophytes and sporophytes of *D. viridis* and *D. ligulata* were used. ASP_{12}NTA with silicate lowered was the base medium used in nutrition studies on growth of gametophytes, and ASP_{12} with silicalte lowered was used for studies on maturation of gametophytes and growth of sporophytes (Table 21). The concentration

Table 21 Media composition.

	ST_3	PESI	ASP_{12} (NTA)	Inoculation medium
Sea water	70 m*l*	98 m*l*		
Distilled water	25 m*l*	2 m*l*	100 m*l*	100 m*l*
NaCl			2.8 g	2.4 g
$MgSO_4 \cdot 7H_2O$			0.7 g	
$MgCl_2 \cdot 6H_2O$			0.4 g	0.4 g
KCl			70 mg	50 mg
Ca(as Cl^-)			40 mg	20 mg
$NaNO_3$	5 mg	7 mg	10 mg	
K_3PO_4			1 mg	
Na_2 glycerophosphate	1 mg	1 mg	1 mg	
$Na_2SiO_3 \cdot 9H_2O$			5 mg	
Vitamin B_{12}	10 mμg		2 mμg	
Biotin			0.1 mg	
Thiamin			10 μg	
Vitamin mix 8A	0.1 m*l*			
P II metals		0.5 m*l*	1 m*l*	
S II matals			1 m*l*	
Fe(as EDTA)		0.05 mg		
I(as KI)		2 μg		
Tris buffer		10 mg	0.1 g	
Nitrilotriacetic acid			(10 mg)	
Soil extract	5 m*l*			
Hy-case	2 mg			
Yeast extract	1 mg			
Liver oxoid L25	2 mg			
C-source mix II	2 m*l*			
Glycylglycine	40 mg			
Agar	(1.2 g)			
pH	7.9	(7.8)*	7.8–8.0	

* pH of PESI enrichment

of each component of experimental media was varied. The water used was passed through an ionexchange cartrige and then was distilled in an all glass still. Media pH was adjusted by adding NaOH or HCl. Ten m*l* of media was used per culture tube (1.8 cm×13 cm with screw cap). Culture tubes with media were sterilized by autoclaving for 20 min. The glassware was washed autoclaved with deionized water and baked for 3 hours at 300℃.

Inoculation of cultures

Growth of gametophytes; The gametophytes grown under Set 4 conditions were washed three times with inoculation medium (Table 21) and then fragmentated into small pieces composed of 5–20 cells with a capillary pipette. One drop (about 0.05 m*l*) of inoculation medium containing about 30–60 fragments was introduced into each new test tube. Five replicates were employed for each experimental situation.

Maturation of gametophytes; After washing the gametophytes with inoculating medium, they were cut into small pieces of 100–150 cells. Two pieces was inoculated into each culture. Duplicates were employed in each experimental situation.

Growth of sporophytes; Sporophytes about 400 μm long (15–20 cells) grown in ASP_{12} medium under Set 6 conditions were separated from the gametophytes by a capillary pipette under a stereo-microscope. One sporophyte was inoculated into each of four tubes for each experimental situation.

Estimation of growth

Gametophytes; In 150 days the gametophytes had usually reached maximum size. At this time the gametophytes were removed from the test tubes by scraping and then filtering through Toyo No.1 filter paper. The gametophytes on the filter paper were rapidly washed three times with distilled water. They were removed from the filter paper, dried for 4 hours at 105℃ and weighted. Then their nitrogen content was determined by the micro-kjeldahl method.

Sporophytes; The growth of the sporophytes was determined every 10 days by measuring the length of the main axis. The sporophytes of *D. viridis* usually reached the maximal size in 50 days and then gradually degenerated. The fronds were removed from the wall of the test tubes in 50-day cultures and the dry weight was measured in the same manner as described for the gametophytes. However, the weight was less than 1 mg and proved to be variable and unreliable. The development of *D. ligulata* sporophytes was the same as those grown at 18℃ in unialgal cultures as described in the preceding chapter. In 90 days the cultures reached maximum size. These sporophytes were removed from the test tubes and washed four times in distilled water. They were weighted after drying 4 hours at 105℃.

Estimation of maturation of gametophytes

As above-mentioned, the gametophytes of *D. viridis* and *D. ligulata* are monoecious and form oogonia and antheridia on the same individuals. The oogonia are formed from the apical cells and the antheridia arise laterally from the cells of branches, forming a cluster. The sporophytes germinate while attached to the oogonia. The visible expression of

maturation was the production of clusters of antheridia (A) and of oogonia emptied or with an egg (O) or with a sporophyte (S). The number of these mature branches and immature branches (I) on a gametophyte were counted. The maturation was expressed as the percentage of the mature branches (A+O+S) to the total number of branches (A+O+S+I).

Results

1. Growth and maturation

Growth of gametophytes

D. viridis; Growth curves derived from the average of three experiments of cultures grown in $ASP_{12}NTA$ and PESI media under Set 4 conditions are illustrated in Fig. 40 and Fig. 41. Cell-division rarely occurred within 1–3 days after inoculation (lag phase). The second phase of growth (logarithmic phase) continued for about 15 days. The phase of declining growth was very long and continued over 100 days. After 120 days in PESI and in 150 days in $ASP_{12}NTA$, there was no further increase in nitrogen content. However, the increase of dry weight continued. The relative growth constant k was calculated from a line

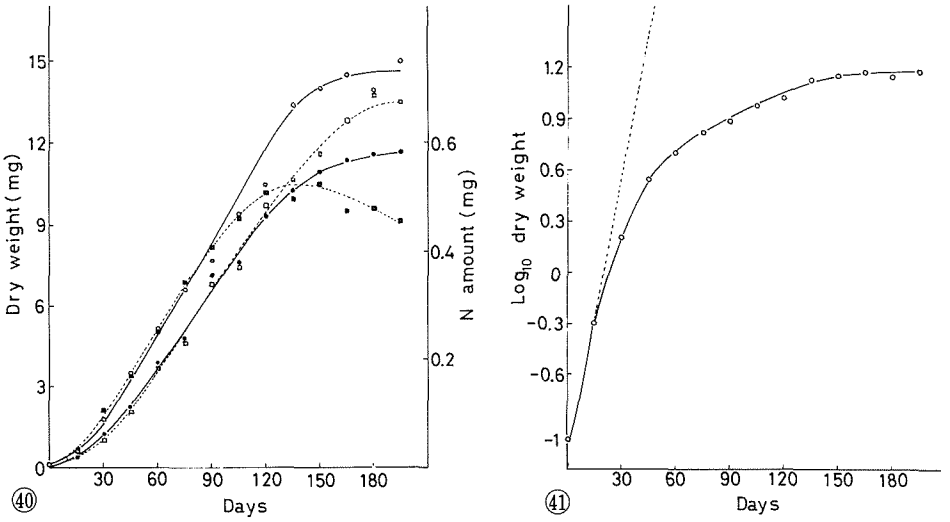

Fig. 40 *Desmarestia viridis*. Growth curves of gametophyte at 14℃ in a 14-hr photoperiod, ○—○, increase in dry weight in $ASP_{12}NTA$; ●—●, increase in nitrogen content in $ASP_{12}NTA$; □—□, increase in dry weight in PESI; ■—■, increase in nitrogen content in PESI.

Fig. 41 *Desmarestia viridis*. Logarithmic growth curve of gametophyte grown in $ASP_{12}NTA$.

projecting from the log phase curve (Fig. 41 dotted line). During logarithmic phase growth was expressed by the following equation;

$$\log N_1 = \log N_0 + k(t_1 - t_0)$$

where N is dry weight or length of fronds in the culture and t is time. The relative growth constant k was expressed as $\log_{10}$ units/day. The value of k of the gametophytes was about 0.057.

D. ligulata; Growth curves of the cultures grown in $ASP_{12}NTA$ under Set 4 conditions are illustrated in Fig. 42 and Fig. 43. They were similar to that of *D. viridis*. The k value was about 0.061.

Growth of sporophytes

D. viridis; Growth curves from the average of three experiments employing cultures grown in ASP_{12} and PESI media under Set 3 conditions are illustrated in Fig. 44 and Fig. 45. Elongation occurred within 1 day after inoculation. The growth of the main axis continued vigorously for 10 days, then the sporophytes grew gradually for 20 days while the corticating filaments were formed. Within 50 days the growth of the sporophytes ceased and the plants gradually degenerated. Growth was better in ASP_{12} than in PESI. The k value of the sporophytes during log phase in ASP_{12} was about 0.10.

D. ligulata; Growth curves of cultures grown in ASP_{12} and PESI media under Set 3

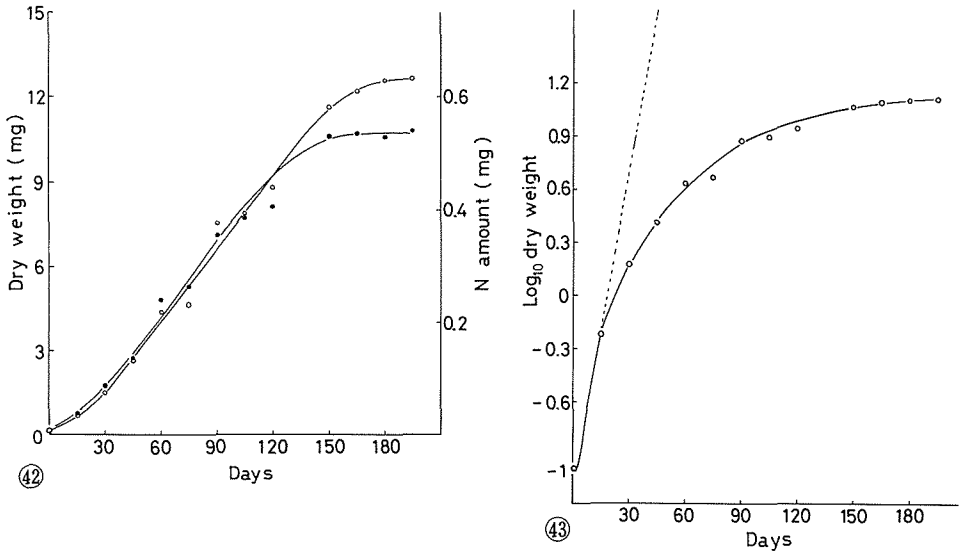

Fig. 42 *Desmarestia ligulata*. Growth curves of gametophyte grown in $ASP_{12}NTA$ at 14℃ in a 14-hr photoperiod, ○—○, increase in dry weight; ●—●, increase in nitrogen content.

Fig. 43 *Desmarestia ligulata*. Logarithmic growth curve of gametophyte grown in ASP_{12} NTA.

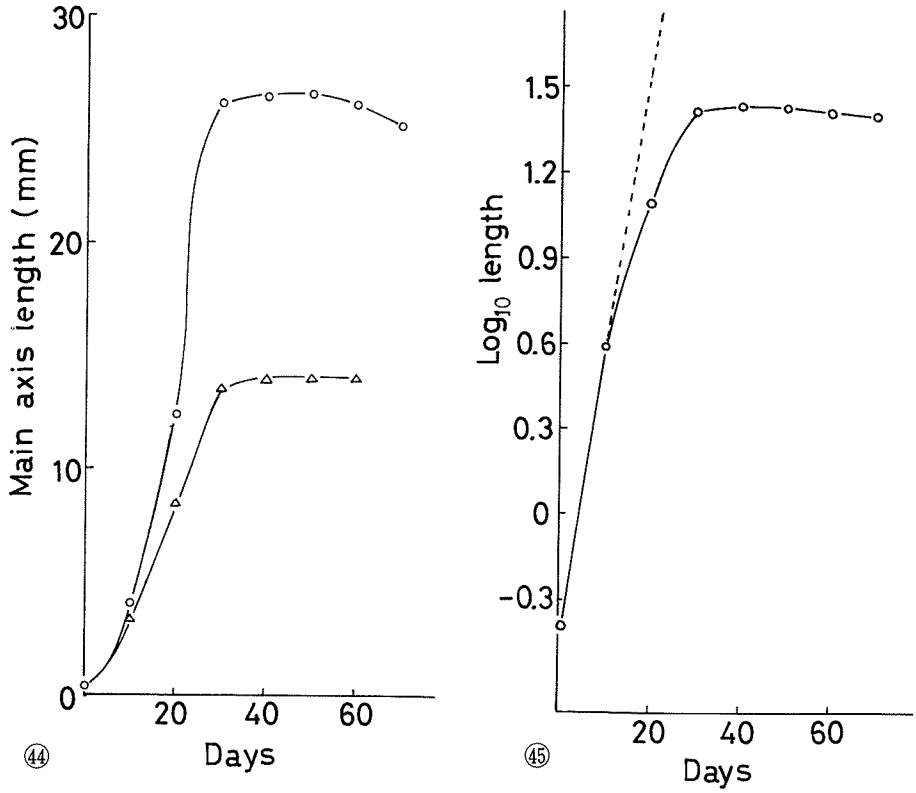

Fig. 44 *Desmarestia viridis*. Growth curves of sporophyte at 10℃ in a 14-hr photoperiod, ○—○, in ASP_{12}; △—△, in PESI.

Fig. 45 *Desmarestia viridis*. Logarithmic growth curve of sporophyte grown in ASP_{12}.

conditions are illustrated in Fig. 46 and Fig. 47. The growth of the main axis continued vigorously for 10 days. Within 20 days, however, the growth of the main axis ceased and corticating filaments were formed. In ASP_{12} the corticating filaments elongated from the main axis and the sporophytes developed into a mass tangled with branches and cortical filaments. The growth of the mass continued for 70 days and the final yeild was about 4.2 mg in dry weight. In PESI the growth of the main axis ceased within 30 days and the corticating filaments were formed in a close apposition to the main axis and the sporophytes developed normally. In 90-day cultures the dry weight was less than 1 mg. The k value of the sporophytes during log phase was about 0.11.

Maturation of gametophytes

The gametophytes of *D. viridis* and *D. ligulata* became fertile in short day conditions and

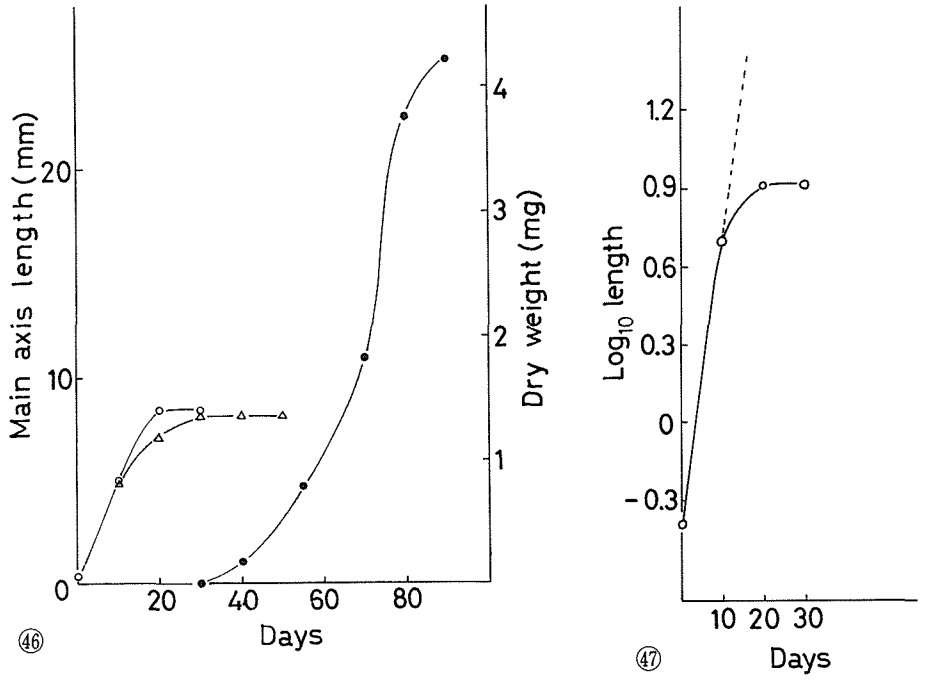

Fig. 46 *Desmarestia ligulata.* Growth curves of sporophyte at 10℃ in a 14-hr photoperiod, ○——○, increase in length of main axis in ASP_{12}; ●——●, increase in dry weight in ASP_{12}; △——△, increase in length of main axis in PESI.

Fig. 47 *Desmarestia ligulata.* Logarithmic growth curve of sporophyte grown in ASP_{12}.

at relatively low temperatures. Effects of photoperiod and temperature on maturation of the gametophytes were re-examined in the axenic culture.

Photoperiods; In the first experiment, the gametophytes of both species were cultured at 10℃ and in seven different photoperiods of 24-hour cycles (light : dark regimes of 8 : $\overline{16}$ hr, 10 : $\overline{14}$ hr, 12 : $\overline{12}$ hr, 14 : $\overline{10}$ hr, 16 : $\overline{8}$ hr, 18 : $\overline{6}$ hr and 24 : $\overline{0}$ hr. Fig. 48 A, B ; Tables 22-23). Complete maturation of the gametophytes of *D. viridis* occurred in 8 days in 8-hr and 10-hr photoperiods. After 14 days in 8-hr and 10-hr photoperiods, the percentage of the branches with gametangia was over 80%, those with antheridia 20-40%, those with oogonia (O+S) 50−65%. The percentage of the mature branches in 12-hr photoperiod was 55-70%, those with antheridia was 20-35% and those with oogonia 30-40%. In 14-18-hr photoperiods the percentages of the mature branches were below 15%. The gametophytes never matured in continuous light.

Complete maturation of the gametophytes of *D. ligulata* occurred in 7 days in 8-hr and 10-hr photoperiods and in 12 days in a 12-hr photoperiod. After 14 days in 8-12-hr

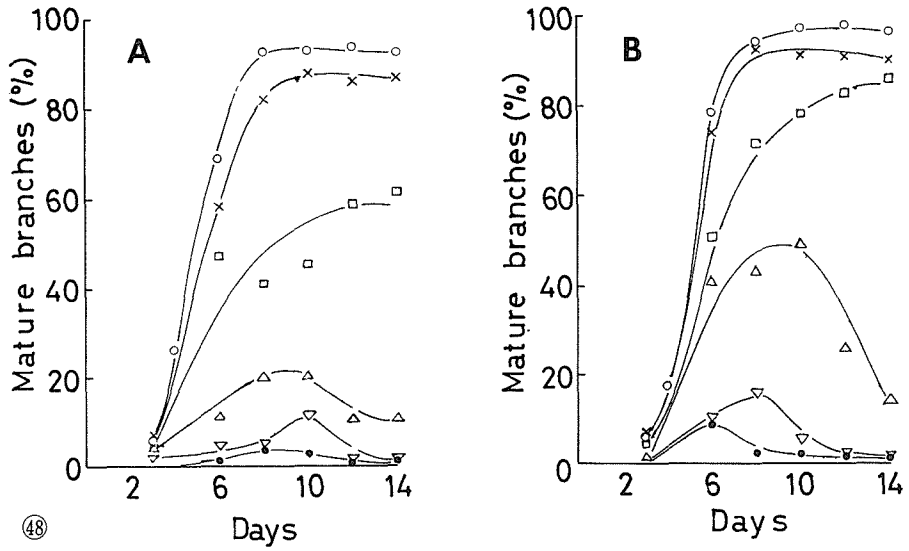

Fig. 48 Maturation of *Desmarestia* gametophytes in six photoperiods at 10℃, ×—×, 8 : $\overline{16}$; ○—○, 10 : $\overline{14}$; □—□, 12 : $\overline{12}$; △—△, 14 : $\overline{10}$; ▽—▽, 16 : $\overline{10}$; ●—●, 18 : $\overline{6}$. A. *D. viridis*. B. *D. ligulata*.

Table 22 Percentage of mature branches formed on *D. viridis* gametophytes after 8- and 10-day in seven photoperiods at 10℃.

Days of treatment	8							14						
Photoperiod (hours)	8	10	12	14	16	18	24	8	10	12	14	16	18	24
% of antheridia branches	30.6	35.4	21.7	15.0	3.9	3.0	0.4	22.8	29.4	28.2	6.3	2.1	0.5	0
% of oogonial branches	51.6	57.1	19.0	5.0	1.3	0.1	0	63.5	63.1	33.5	4.6	0	0.7	0
% of immature branches	17.9	7.5	59.3	80.0	94.8	96.9	99.6	13.7	7.3	37.3	89.1	97.9	98.8	100

Each value is the average of six samples from three cultures.

photoperiods, the percentage of the mature branches was over 80%, those with antheridia was 50–65% and those with oogonia was 30–45%. The percentage of mature branches in 14–18-hr photoperiods was below 15%. The gametophytes never matured in continuous light.

In a second experiment, cultures were exposed to 10-hr photoperiod (10 : $\overline{14}$ hours) or to 10-hr photoperiod with a further hour of light in the middle of the dark period (i. e. 10 : $\overline{6\frac{1}{2}}$: 1 : $\overline{6\frac{1}{2}}$) (Table 24–25). After 14 days, the percentage of the mature branches of *D. viridis* was 10.4%, which was the same value as in a 14-hr photoperiod. That of *D. ligulata*

Table 23 Percentage of mature branches formed on *D. ligulata* gametophytes after 8- and 14-day in seven photoperiods at 10℃.

Days of treatment	8							14						
Photoperiod (hours)	8	10	12	14	16	18	24	8	10	12	14	16	18	24
% of antheridia branches	60.7	57.9	58.5	26.6	16.0	2.1	1.2	57.9	52.5	57.9	8.6	1.4	1.2	0
% of oognial branches	32.8	36.1	13.0	16.0	0..9	0	0	32.5	44.3	28.9	5.7	0.4	0.8	0
% of immature branches	6.5	6.0	28.5	57.4	83.1	97.9	98.8	9.6	3.2	13.2	85.3	98.2	98.0	100

Each value is the average of six samples from three cultures.

Table 24 Effect of an additional hour of light in the middle of the dark period on maturation of *D. viridis* gametophytes at 10℃ for 14 days.

Photoperiods (hours)	$10 : \overline{14}$	$10 : \overline{6½} : 1 : \overline{6½}$	$14 : \overline{10}$
% of antheridia branches	35.4	7.0	6.3
% of oogonial branches	57.1	3.4	4.6
% of immature branches	7.5	89.6	89.1

Each value is the average of six samples of three cultures

Table 25 Effect of an additional hour of light in the middle of the dark period on maturation of *D. ligulata* gametophytes at 10℃ for 14 days.

Photoperiods (hours)	$10 : \overline{14}$	$10 : \overline{6½} : 1 : \overline{6½}$	$12 : \overline{12}$	$14 : \overline{10}$
% of antheridia branches	52.5	47.7	57.9	8.6
% of oogonial branches	44.3	26.2	28.9	5.7
% of immature branches	3.2	26.1	13.2	85.3

Each value is the average of six samples of three cultures.

was 73.9%, which was less than in 10-hr and 12-hr photoperiods, but more than in a 14-hr photoperiod.

In a third experiment, cultures were transferred to continuous light at 10℃ after 1 to 6 days treatment in a 10-hr photoperiod. After 8 days from the start of this experiment, the percentages of the mature branches were estimated (Fig. 49 A, B). In *D. viridis* the

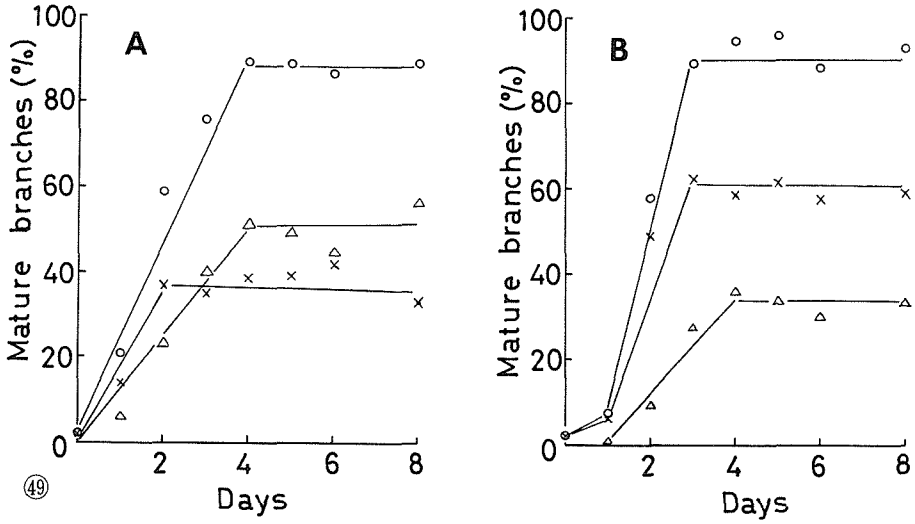

Fig. 49 Gametangium formation of *Desmarestia* gametophytes in response to numbers of days in a 10-hr photoperiod at 10℃. Cultures were transferred to continuous light at 10℃ after 1-6 days in a 10-hr photoperiod and sampled after 8 days, ○—○, mature branches; ×—×, antheridial branches; △—△, oogonial branches. A. *D. viridis*. B. *D. ligulata*.

percentage of the mature branches with oogonia increased steadily with the addition of days in a 10-hr photoperiod up to 4 days. However, the percentage of antheridia branches reached a maximum in 2 days treatment in 10-hr photoperiod. In *D. ligulata* maturation rarely occurred in 1 day in 10-hr photoperiod, but complete maturation occurred in 3 days in 10-hr photoperiod.

In a fourth experiment, cultures were exposed to different hour cycles (light : dark regimes of 8 : $\overline{16}$ hr, 4 : $\overline{8}$ hr, $2\frac{2}{3}$: $\overline{5\frac{1}{3}}$ hr and 2 : $\overline{4}$ hr) at 10℃ (Tables 26-27). After 14

Table 26 Percentage of mature branches formed on *D. viridis* gametophytes exposed to various cycles of light and dark at 10℃ for 14 days.

light : dark regim (hours)	8 : $\overline{16}$	4 : $\overline{8}$	$2\frac{2}{3}$: $\overline{5\frac{1}{3}}$	2 : $\overline{4}$
% of antheridia branches	20.4	25.1	14.4	9.4
% of oogonial branches	65.9	59.4	17.3	8.5
% of immature branches	13.7	15.5	68.4	82.1

Table 27 Percentage of mature branches formed on *D. ligulata* gametophytes exposed to various cycles of light and dark at 10℃ for 14 days.

light : dark regime (hours)	8 : $\overline{16}$	4 : $\overline{8}$	$2\frac{2}{3}$: $\overline{5\frac{1}{3}}$	2 : $\overline{4}$
% of antheridia branches	57.9	50.7	52.7	29.2
% of oogonial branches	32.5	42.5	36.8	5.0
% of immature branches	9.6	6.9	10.5	65.8

Table 28 Percentage of mature branches formed on *D. viridis* gametophytes after 14 days at four temperatures in a 10-hr photoperiod.

Temperature (℃)	5	10	14	18
% of antheridia branches	34.5	29.2	33.5	25.5
% of oogonial branches	57.3	55.8	49.8	57.1
% of immature branches	8.2	15.0	16.7	17.4

Table 29 Percentage of mature branches formed on *D. ligulata* gametophytes after 14 days at four temperatures in a 10-hr photoperiod.

Temperature (℃)	5	10	14	18
% of antheridia branches	51.7	50.4	40.7	42.8
% of oogonial branches	43.8	43.3	26.9	15.2
% of immature branches	4.5	6.3	32.4	42.0

days complete maturation had occurred in 8 : $\overline{16}$ hr and 4 : $\overline{8}$ hr treatment in *D. viridis* and 8 : $\overline{16}$ hr, 4 : $\overline{8}$ hr and $2\frac{2}{3}$: $\overline{5\frac{1}{3}}$ hr treatments in *D. ligulata*.

Temperature ; Gametophytes of the both species were cultured in a 10-hr photoperiod and at 5℃, 10℃, 14℃ and 18℃, The percentage of the mature branches was calculated after 14 days (Table 28-29). The gametophytes of *D. viridis* matured fully at all these temperatures. The gametophytes of *D. ligulata* matured fully at 5℃ and 10℃. At 14℃ and 18℃ the percentage of the mature branches decreased and the formation of oogonia was inhibited at these higher temperatures.

2. Nutrition

Major elements

Most of the chemical elements in sea water are in constant proportions. The concentration of the major elements is an important ecological factor for seaweeds inhabiting on the coast. The tolerance and requirement of NaCl, Mg, Ca, and K for growth and maturation of *D. viridis* and *D. ligulata*, were investigated.

1) Sodium chloride

D. viridis (Fig. 50 A) ; The gametophytes grew well at concentrations ranging from 28 to 32 g/*l*. Concentrations higher than 38 g/*l* inhibited growth. Complete maturation of the gametophytes occurred at concentrations ranging from 12 to 28 g/*l*. Concentrations higher than 28 g/*l* inhibited maturation. At concentrations lower than 12 g/*l* the eggs did not germinate. The sporophytes grew in narrow range of concentrations. No growth occurred at concentrations lower than 12 g/*l*. The best growth was obtained at 28 g/*l* and concen-

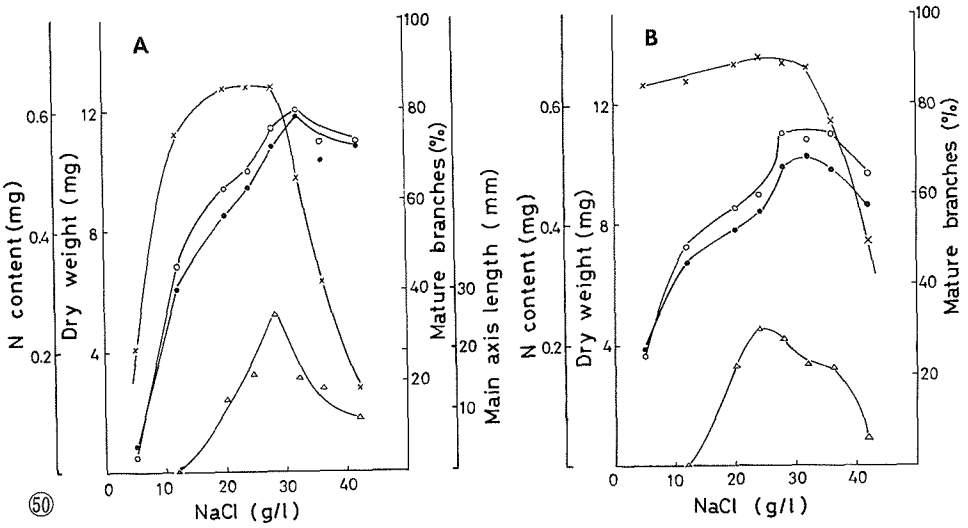

Fig. 50 Effects of sodium chloride concentraions on growth and maturation of gametophytes and growth of sporophytes in *Desmarestia*, such as nitrogen content, dry weight, maturation rate of gametphyte and length of main axis or dry weight of sporophytes. A. *D. viridis* : ●—●, nitrogen content of gametophyte (after 150 days) ; ○—○, dry weight of gametophyte (after 150 days) ; ×—×, percentage of mature branches of gametophyte (after 14 days) ; △—△, length of main axis of sporophyte (after 50 days). B. *D. ligulata* : ●—●, nitrogen content of gametophyte (after 150 days) ; ○—○, dry weight of gametophyte (after 150 days) ; ×—×, Percentage of mature branches of gametophyte (after 14 days) ; △—△, dry weight of sporophyte (after 90 days). In the following Figures 53–64, experimental procedures and symbols are the same as those mentioned in Fig. 50 A (for *D. viridis*) and Fig. 50 B (for *D. ligulata*).

trations higher than 32 g/*l* inhibited growth.

D. ligulata (Fig. 50 B) ; The gametophytes grew well at concentrations ranging from 28 to 38 g/*l*. Concentrations higher than 38 g/*l* inhibited growth. Complete maturation of the gametophytes occurred at concentrations ranging from 5 to 32 g/*l*. Concentrations higher than 32 g/*l* inhibited maturation. At concentrations lower than 12 g/*l*, the eggs did not germinate. The sporophytes grew well at 24 to 28 g/*l*. No growth occurred at concentrations lower than 12 g/*l* and concentrations higher than 28 g/*l* inhibited growth.

2) Magnesium

For the experiments on magnesium concentrations, $MgSO_4 \cdot 7H_2O$ and $MgCl_2 \cdot 6H_2O$ were either omitted or added in the desired amount and the SO_4^{--}level in the medium was kept constant by addition of an appropriate amount of $Na_2SO_4 \cdot 10H_2O$.

D. viridis (Fig. 51 A) ; The gametophytes grew well at concentrations higher than 1.17 g/*l* and dry weight increased considerably up to 5 g/*l*. The nitrogen content of the gametophytes was constant from 1.17 to 5 g/*l*. Complete maturation of the gametophytes occurred at 0.25 to 1.17 g/*l*. Concentrations higher than 1.17 g/*l* inhibited maturation. The sporophytes grew well at 1.17 to 2.5 g/*l*. No growth occurred at concentrations lower than 50 mg/*l* and concentrations higher than 2.5 g/*l* inhibited growth.

D. ligulata (Fig. 51 B) ; The gametophytes grew well at concentrations higher than 1.17 g/*l*. Their dry weight increased considerably and the nitrogen content slightly up to 5 g/*l*. No growth occurred in magnesium-deficient cultures. Complete maturation of the gametophytes occurred at 0.05 to 1.17 g/*l*. Concentrations higher than 2.5 g/*l* inhibited maturation. The sporophytes grew well at 1.17 to 2.5 g/*l*. No growth occurred in magnesium-free media and concentrations higher than 2.5 g/*l* inhibited growth.

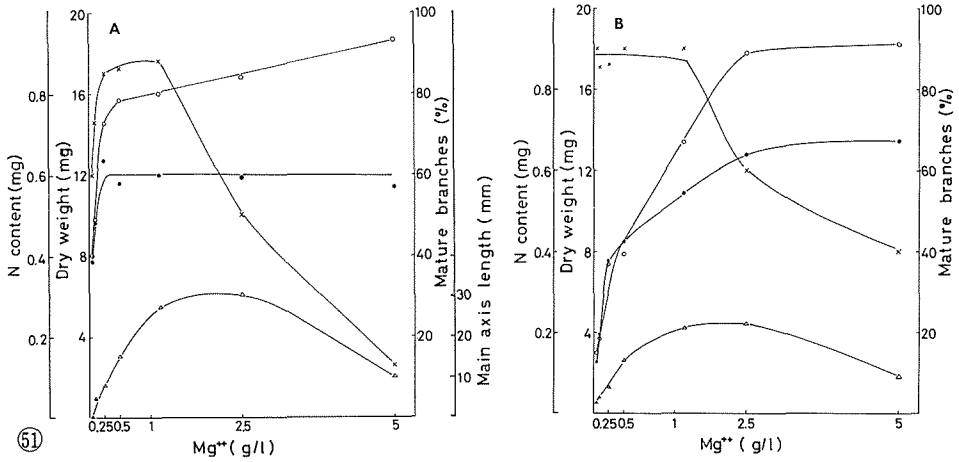

Fig. 51 Effects of magnesium concentrations on growth and maturation in *Desmarestia*. A. *D. viridis*. B. *D. ligulata*.

3) Calcium

For the experiment on calcium concentrations, $CaCl_2 \cdot 2H_2O$ was used at levels of 0.05–1.5 g/*l* calcium.

D. viridis (Fig. 52 A); The gametophytes grew well at 0.2 to 0.4 g/*l* and the best growth was obtained at 0.4 g/*l*. No growth occurred calcium-free media, and concentrations higher than 0.4 g/*l* inhibited growth. Complete maturation of the gametophytes

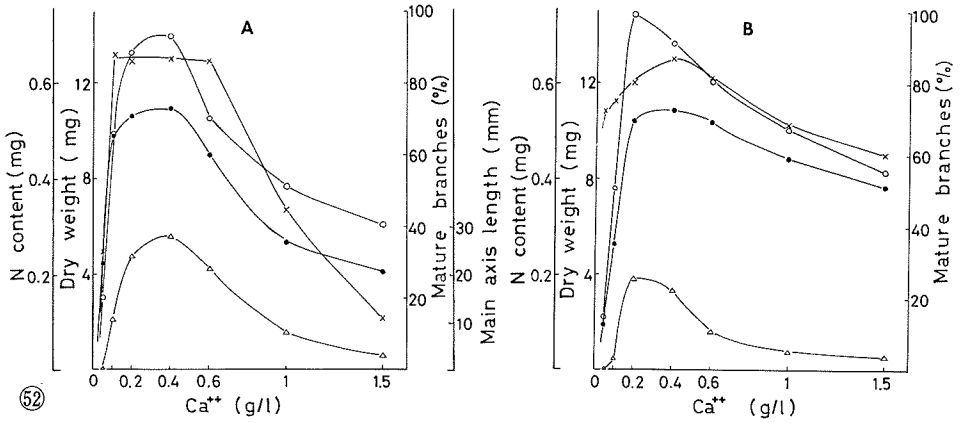

Fig. 52 Effects of calcium concentrations on growth and maturaion in *Desmarestia*. A. *D. viridis*. B. *D. ligulata*.

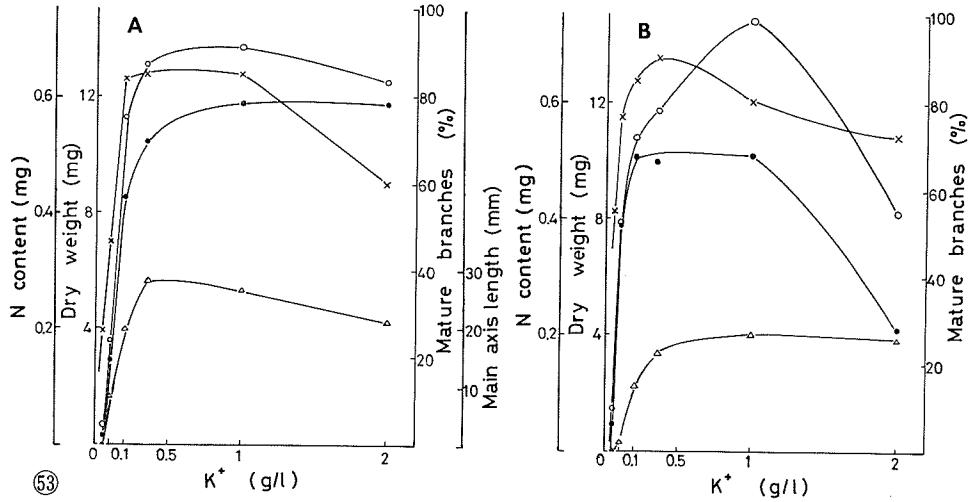

Fig. 53 Effects of potassium concentrations on growth and maturation in *Desmarestia*. A. *D. viridis*. B. *D. ligulata*.

occurred at 0.1 to 0.6 g/*l*. Concentrations higher than 0.6 g/*l* inhibited maturation. The sporophytes grew well at 0.2 to 0.4 g/*l* and best growth was obtained at 0.4 g/*l*. No growth occurred at concentrations lower than 0.05 g/*l* and concentrations higher than 0.4 g/*l* inhibited growth.

D. ligulata (Fig. 52 B) ; The gametophytes grew well at 0.2 to 0.6 g/*l* and the best growth obtained at 0.2 g/*l* on the base of dry weight and at 0.4 g/*l* on the base of nitrogen content. No growth occurred in calcium-free media and concentrations higher than 0.6 g/*l* inhibited growth. Complete maturation of the gametophytes occurred at 0.2 to 0.6 g/*l*. Concentrations higher than 0.6 g/*l* inhibited maturation. The sporophytes grew well at 0.2 to 0.4 g/*l* and best growth was obtained at 0.2 g/*l*. No growth occurred at concentrations lower than 0.05 g/*l*. Concentrations higher than 0.4 g/*l* inhibited growth.

4) Potassium

Potassium was added as a freshly prepared solution of potassium chloride at levels of 0.025–2 g/*l* potassium.

D. viridis (Fig. 53 A) ; The gametophytes grew well at 0.37 to 2 g/*l*. No growth occurred in potassium-free media. Complete maturation of the gametophytes occurred at 0.2 to 1 g/*l*. Concentrations higher than 1 g/*l* inhibited maturation. At concentrations of 0.05 g/*l* and higher than 1 g/*l*, the eggs did not germinate. The sporophytes grew well at concentrations higher than 0.37 g/*l*. At concentrations of 1 to 2 g/*l* the length of the main axis was slightly shorter than that at 0.37 g/*l* but the thallus was thicker. No growth occurred at concentrations lower than 0.05 g/*l*.

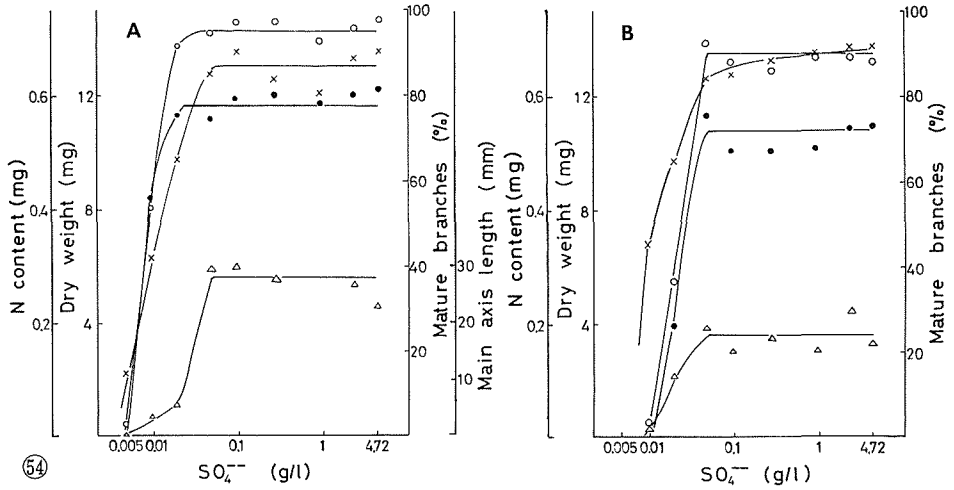

Fig. 54 Effects of sulfate concentrations on growth and maturation in *Desmarestia*. A. *D. viridis*. B. *D. ligulata*.

D. ligulata (Fig. 53 B) ; The gametophytes grew well at 0.2 to 1 g/*l* and the best growth was obtained at 1 g/*l* on the basis of dry weight. Concentrations higher than 1 g/*l* inhibited growth. No growth occurred in potassium-free media. Complete maturation of the gametophytes occurred at 0.2 to 0.37 g/*l*. Concentrations higher than 1 g/*l* inhibited maturation slightly and the eggs did not germinate. The sporophytes grew well at concentrations higher than 0.37 g/*l*. No growth occurred at concentrations lower than 0.05 g/*l*.

Sulfate

In this experiment, sulfate concentrations were varied by altering the concentrations of $MgSO_4 \cdot 7H_2O$. The Mg level was kept constant by adding $MgCl_2 \cdot 6H_2O$.

D. viridis (Fig. 54 A) ; The gametophytes grew well at concentrations ranging from 0.02 to 4.72 g/*l*. No growth occurred in sulfate-free media. Complete maturation of the gametophytes occurred at 0.05 to 4.72 g/*l* and the sporophytes grew well at 0.05 to 4.72 g/*l*.

D. ligulata (Fig. 54 B) ; The gametophytes grew well at concentrations ranging from 0.05 to 4.72 g/*l*. No growth occurred in sulfate-free media. Complete maturation of the gametophytes occurred at 0.1 to 4.72 g/*l*. The sporophytes grew well at 0.02 to 4.72 g/*l*.

Trace metals

The requirements for the trace metals contained in PII and SII metals mixtures of ASP_{12} and ASP_{12} NTA were investigated. The basal medium was prepared by omitting one element at a time and then adding it at different concentrations. Trace element starved cells were produced by culturing in media lacking the trace metals for 2 days. All glassware was soaked in 1% EDTA solution and then by rinsed with deionized water to remove trace EDTA.

1) Boron

Boron was added as a freshly prepared solution of boric acid at levels of 0.1–100 mg/*l*.

D. viridis (Fig. 55 A) ; The gametophytes grew well at concentrations ranging from 0.5 to 50 mg/*l*. At 100 mg/*l* boron cell-division did not occur. Slight growth occurred in

Table 30 Percentage of mature branches formed on *D. viridis* gametophytes after 14 days at different boron concentrations.

Boric acid Wt. /*l* (as B)	% of mature branches	% of antheridia branches	% of oogonial branches	% of oogonial branches with a sporophyte
0	99.5	22.8	76.7	2.8
100 μg	98.7	36.7	62.0	9.1
500 μg	92.9	30.0	62.9	24.0
2 mg	83.6	38.1	45.5	42.4
10 mg	72.4	37.6	34.8	27.3
50 mg	77.2	38.1	39.1	30.5
100 mg	56.1	29.5	26.6	2.1

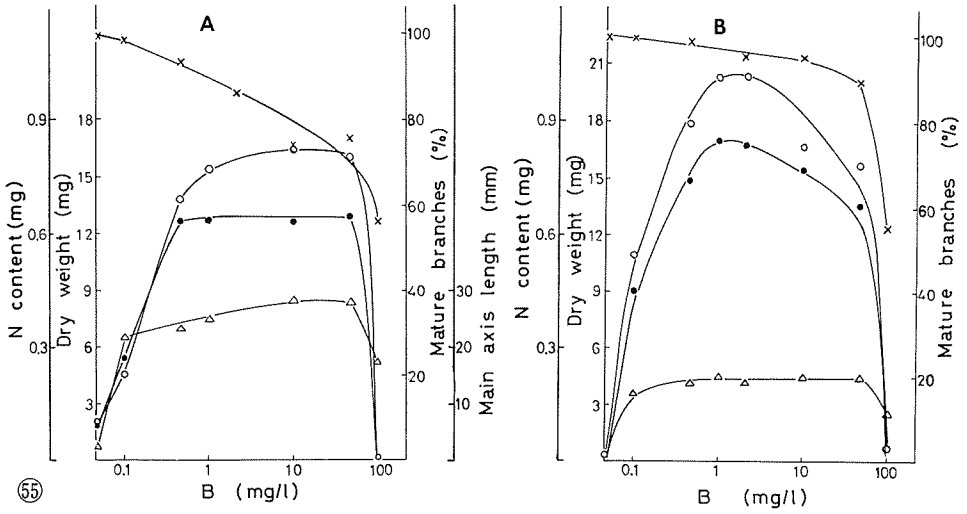

Fig. 55 Effects of boron concentrations on growth and maturation in *Desmarestia*. A. *D. viridis*. B. *D. ligulata*.

boron-free media. Complete maturation of the gametophytes occurred at concentrations lower than 2 mg/*l*. At the higher concentrations inhibition of maturation occurred. In boron-free media, all branches formed gametangia and the oogonial branches were more abundant than in the cultures with boron (Table 30). However, most eggs degenerated after extrusion and sporophytes were not produced. The sporophyte grew well at 0.1 to 50 mg/*l*. The growth did not occur in boron-free media and was inhibited at 100 mg/*l*.

D. ligulata (Fig. 55 B) ; The gametophytes grew well at 0.5 to 10 mg/*l* and slightly better growth was obtained at 1 to 2 mg/*l*. No growth occurred in boron-free media and concentrations higher than 10 mg/*l* inhibited growth. Complete maturation of the gametophytes occurred at concentrations lower than 0.5 mg/*l*. At concentrations less than 0.5 mg/*l*, all branches formed gametangia but sporophytes were rarely formed and most eggs degenerated. At a concentration of 100 mg/*l*, clusters of antheridia were produced but the formation of the oogonial branches was inhibited (Table 31). The sporophytes grew well at 0.1 to 50 mg/*l*. The growth did not occur in boron-free media and was inhibited at100mg/*l*.

2) Iodine

Iodine was added as a freshly prepared solution of potassium iodite at levels of 0.001 to 10 mg/*l* iodine.

D. viridis (Fig. 56 A) ; Growth of the gametophytes was not affected by the addition of iodine at concentrations ranging from 1 μg/*l* to 10 mg/*l*. The final yeild of the nitrogen content was constant up to 10 mg/*l*, and the dry weight increased slightly at concentrations

higher than 0.1 mg/*l*. Maturation of the gametophyte and sporophyte growth was not affected by iodine concentration.

D. ligulata (Fig. 56 B); Gametophyte growth was slightly promoted at concentrations higher than 10 μg/*l* and the greatest growth was obtained at concentrations ranging from 1 to 10 mg/*l*. However, the growth at 10 μg/*l* iodine was the same as in the control. Maturation of the gametophyte and sporophyte growth was not affected by iodine concentration.

3) Molybdenum

Molybdenum was added as a freshly prepared sodium molybdate at levels of 0.01 to 100

Table 31 Percentage of mature branches formed on *D. ligulata* gametophytes after 14 days at different boron concentrations.

Boric acid Wt. /*l* (as B)	% of mature branches	% of antheridia branches	% of oogonial branches	% of oogonial branches with a sporophyte
0	100	56.4	43.6	0.4
100 μg	100	55.4	44.6	8.7
500 μg	99.1	62.2	36.9	5.0
2 mg	95.1	54.3	40.8	38.6
10 mg	96.2	52.8	43.4	32.5
50 mg	89.5	49.1	40.4	32.0
100 mg	57.6	54.0	3.6	2.0

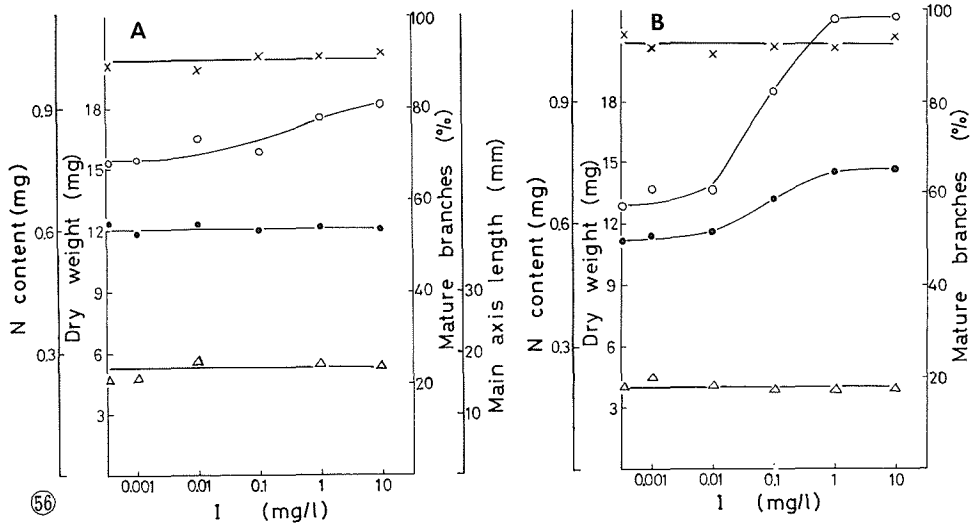

Fig. 56 Effects of iodine concentrations on growth and maturation in *Desmarestia*. A. *D. viridis*. B. *D. ligulata*.

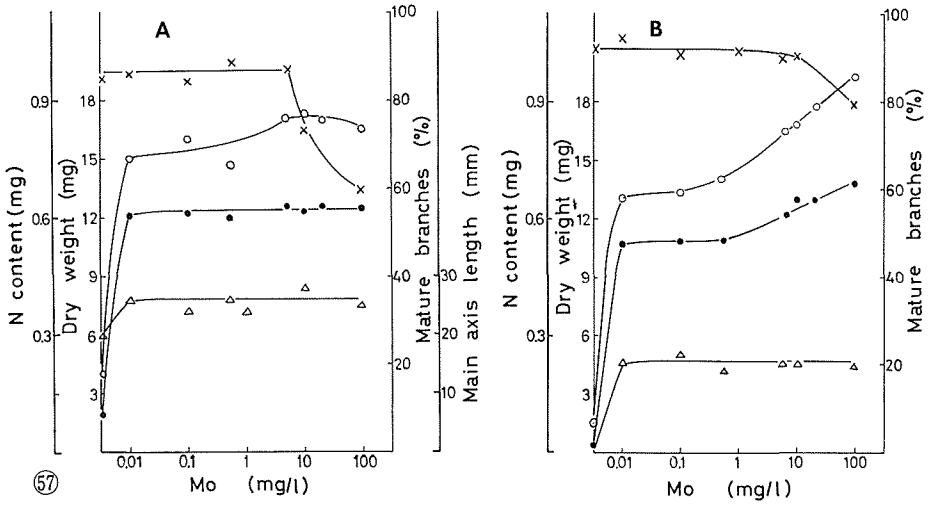

Fig. 57 Effects of molybdenum concentrations on growth and maturation in *Desmarestia*. A. *D. viridis*. B. *D. ligulata*.

mg/*l* molybdenum.

D. viridis (Fig. 57 A) ; The gametophytes grew well at 0.01 to 100 mg/*l* and the dry weight increased slightly at concentrations higher than 0.5 mg/*l*. Molybdenum-free cultures grew for a short time and bleached within two months. Complete maturation of the gametophytes occurred at concentrations lower than 5 mg/*l*. Concentrations of 10 mg/*l* and more slightly inhibited maturation. The sporophytes grew well at 0.01 to 100 mg/*l*. Growth in molybdenum-free media occurred during the first month, but the plants then bleached.

D. ligulata (Fig. 57 B) ; The gametophytes grew well at 0.01 to 100 mg/*l* and better growth was obtained at concentrations higher than 0.5 mg/*l*. Plants in molybdenum-free media grew for a short time and then bleached within one month. Complete maturation of the gametophytes occurred at concentrations lower than 10 mg/*l*. Concentrations of 100 mg/*l* slightly inhibited maturation. The sporophytes grew well at 0.01 to 100 mg/*l*. The molybdenum-free cultures grew for a short time and the plant bleached within one month.

4) Other trace metals.

The addition of iron (0.01–5 mg/*l*) as ferric chloride, manganese (0.01–10 mg/*l*) as manganese chloride, zinc (5–100 μg/*l*) as zinc chloride, cobalt (1–50 μg/*l*) as cobalt chloride, bromine (0.1–100 mg/*l*) as sodium bromide, strontium (0.05–50 mg/*l*) as strontium chloride, rubidium (10–500 μg/*l*) as rubidium chloride and lithium (10–500 μg/*l*) as lithium chloride had no effect upon growth and maturation of the gametophytes and growth of the sporophytes in either species.

Chelators

Preliminary experiments on *Desmarestia* showed that ASP_{12} medium was good for maturation of the gametophytes and growth of the sporophytes. Further, ASP_{12} NTA medium was good for growth of the gametophytes. These media were used with either EDTA or EDTA+NTA. In the present study three chelators, EDTA, NTA and hydroxy-

Table 32 Effects of chelators on maturation and growth of gametophytes and growth of sporophytes in *D. viridis*.

Chelators	Wt. /*l*	% of mature branches of gametophytes after 14 days	Dry weight, mg, of gametophytes after 150 days	Length, mm, of sporophytes after 50 days
None		57	14.1	20.5
NTA	100 mg	86	14.4	28.7
NTA	200 mg	76	15.6	28.7
Na_2 EDTA	5 mg	84	14.4	28.3
Na_2 EDTA	10 mg(ASP_{12})	91	14.2	29.0
Na_2 EDTA	20 mg	88	14.0	27.5
Na_2 EDTA	50 mg	85	14.1	18.5
Hydroxy-EDTA	10 mg	76	13.2	32.0
Na_2 EDTA + NTA	5 mg 100 mg	88	14.6	28.5
Na_2 EDTA + NTA	10 mg 100 mg(ASP_{12}NTA)	76	15.2	30.0

Table 33 Effects of chelators on maturation and growth of gametophytes and growth of sporophytes in *D. ligulata*.

Chelators	Wt. /*l*	% of mature branches of gametophytes after 14 days	Dry weight, mg, of gametophytes after 150 days	Dry weight, mg, of sporophytes after 90 days
None		57	15.1	0.9
NTA	100 mg	65	11.6	3.2
NTA	200 mg	68	12.2	3.4
Na_2 EDTA	5 mg	85	13.8	4.2
Na_2 EDTA	10 mg(ASP_{12})	91	12.6	3.9
Na_2 EDTA	20 mg	84	13.2	2.6
Na_2 EDTA	50 mg	76	14.7	3.0
Hydroxy-EDTA	10 mg	62	14.1	2.1
Na_2 EDTA + NTA	5 mg 100 mg	67	13.1	3.4
Na_2 EDTA + NTA	10 mg 100 mg(ASP_{12}NTA)	58	13.4	3.2

-EDTA, were tested at different concentrations. Growth of the gametophytes was estimated from the cultures grown under Set 7 conditions after 150 days.

D. viridis (Table 32); The gametophytes grew well at all tested levels of the chelators. The control lacking chelators grew well but the color of the thalli was a pale brown. Complete maturation of the gametophytes occurred in the medium containing 10 mg/*l* Na_2 EDTA (similar to ASP_{12}). The sporophytes grew well in all chelators, except in 50 mg/*l* of Na_2 EDTA. The control plants were about two-thirds as long as the sporophytes grown in ASP_{12}.

D. ligulata (Table 33); The gametophytes grew well in the media containing Na_2 EDTA, hydroxy-EDTA and Na_2 EDTA+NTA. The addition of 100 mg/*l* and 200 mg/*l* NTA slightly inhibited gametophyte growth. The control plants lacking chelators grew well but its color was pale brown. Complete maturation of the gametophytes occurred in the medium containing 10 mg/*l* Na_2 EDTA (similar to ASP_{12}). The sporophytes grew well at 5–10 mg/*l* Na_2 EDTA. The control plants were about one-fourth of the dry weight of the sporophytes grown in ASP_{12}.

Nitrogen

Ammonium, nitrite, nitrate, urea and amino acid were investigated as nitrogen sources. Nitrite inhibited the gametophyte growth and maturation and sporophyte growth at concentrations greater than 1 mg/*l* nitrogen (N).

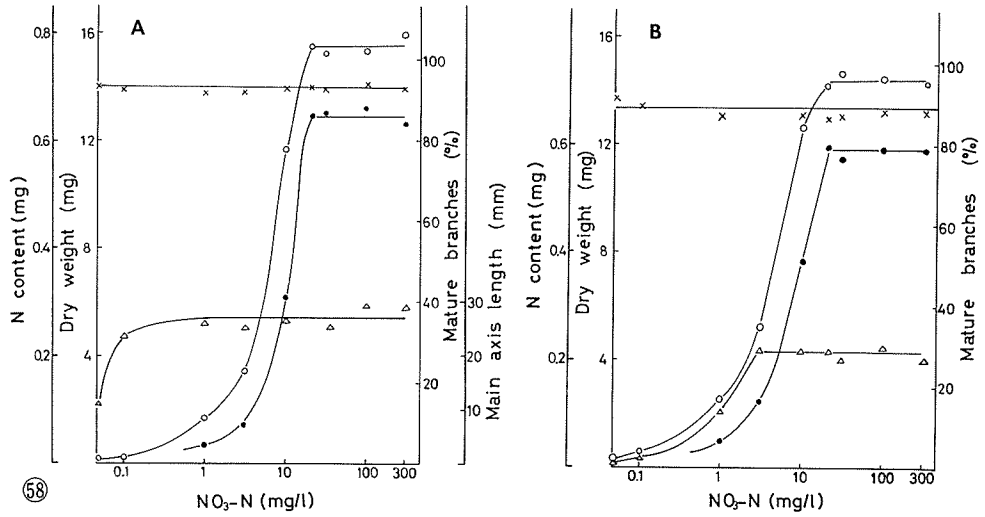

Fig. 58 Effects of nitrate concentrations on growth and maturation in *Desmarestia*. A. *D. viridis*. B. *D. ligulata*.

1) Nitrate

Nitrate was added as a freshly prepared solution of sodium nitrate at 0.1–300 mg/*l* N.

D. viridis (Fig. 58 A); The gametophytes grew well at nitrogen concentrations ranging from 20 to 300 mg/*l* N. The control plants in nitrogen-free media grew for a short time and then bleached within one month. Complete maturation of the gametophytes occurred at concentrations of 0.1 to 300 mg/*l* N and also in control lacking nitrogen. The sporophytes grew well at 1 to 300 mg/*l* N. At concentrations of 0.1 mg/*l* N and lower, they grew for a short time and bleached within one month.

D. ligulata (Fig. 58 D); The gametophytes grew well at 20 to 300 mg/*l* N. The control plants lacking nitrogen grew for a short time and then bleached within one month. Complete maturation of the gametophytes occurred from 0.1 to 300 mg/*l* N and also in control lacking nitrogen. The sporophytes grew well from 3 to 300 mg/*l* N. The control plants lacking nitrogen grew for a short time and bleached within one month.

2) Ammonium

Ammonium was added as a freshly prepared solution of ammonium chloride at levels of 0.1 to 100 mg/*l* N.

D. viridis (Fig. 59 A); Most gametophyte cells died within 10 days after inoculation at concentrations of ammonium higher than 1 mg/*l* N. However, a few survived and grew into filamentous thalli at concentrations between 1–30 mg/*l* N. The greatest growth was obtained at 10 mg/*l* N. Concentrations higher than 10 mg/*l* N inhibited growth and no growth occurred in cultures at 50 mg/*l* N. Maturation of the gametophytes was inhibited at

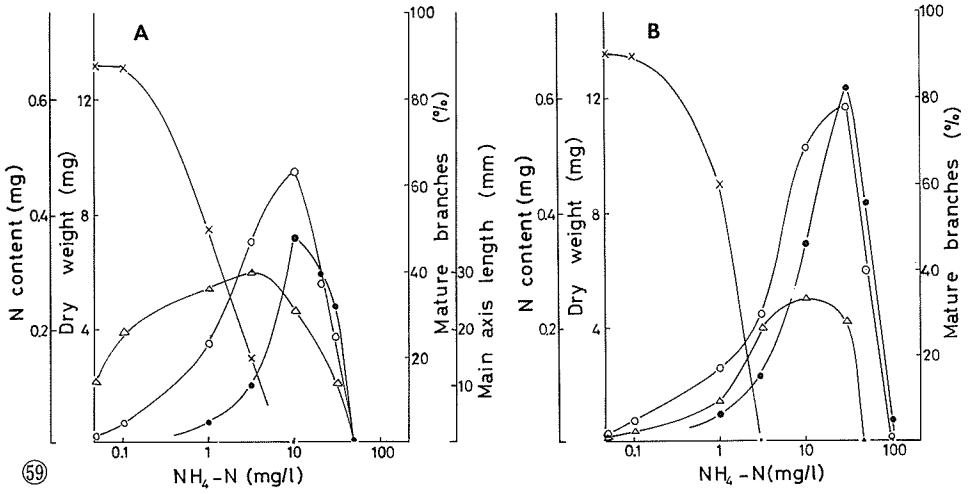

Fig. 59 Effects of ammonium concentrations on growth and maturation in *Desmarestia*. A. *D. viridis*. B. *D. ligulata*.

concentrations higher than 0.1 mg/*l* N. The sporophytes grew at 1 to 10 mg/*l* N and the greatest growth was obtained at 3 mg/*l* N. At concentrations of 0.1 mg/*l* N and lower, they grew for a short time and then bleached within one month. Concentrations higher than 10 mg/*l* N inhibited growth and no growth occurred at 50 mg/*l* N.

D. ligulata (Fig. 59 B) ; Most gametophyte cells died within 10 days after inoculation at concentrations of ammonium higher than 1 mg/*l* N. However, a few survived and grew at concentrations less than 50 mg/*l* N. The greatest growth was obtained at 30 mg/*l* N. Concentrations higher than 30 mg/*l* N inhibited growth and no growth occurred at100mg/*l* N. Maturation of the gametophytes was inhibited at concentrations higher than 0.1 mg/*l* N. The sporophytes grew well at 3 to 30 mg/*l* N and the greatest growth was obtained at 10 mg/*l* N. At concentrations less than 0.1 mg/*l* N, the sporophytes grew for a short time and bleached within one month. No growth occurred at 50 mg/*l* N.

3) Urea

D. viridis (Fig. 60 A) ; Most gametophyte cells died within 10 days after inoculation at concentrations of urea higher than 1 mg/*l* N. After 20–30 days, a few surviving cells grew into filamentous thalli. The greatest growth was obtained at 30 to 50 mg/*l* N. No growth occurred at 100 mg/*l* N. Maturation of the gametophytes was inhibited at concentrations higher than 1 mg/*l* N. The sporophytes grew well at 1 to 10 mg/*l* N. At concentrations of 0.1 mg/*l* N and lower, they grew for a short time and bleached within one month. Concentrations higher than 10 mg/*l* N inhibited growth. No growth occurred at 50 mg/*l* N.

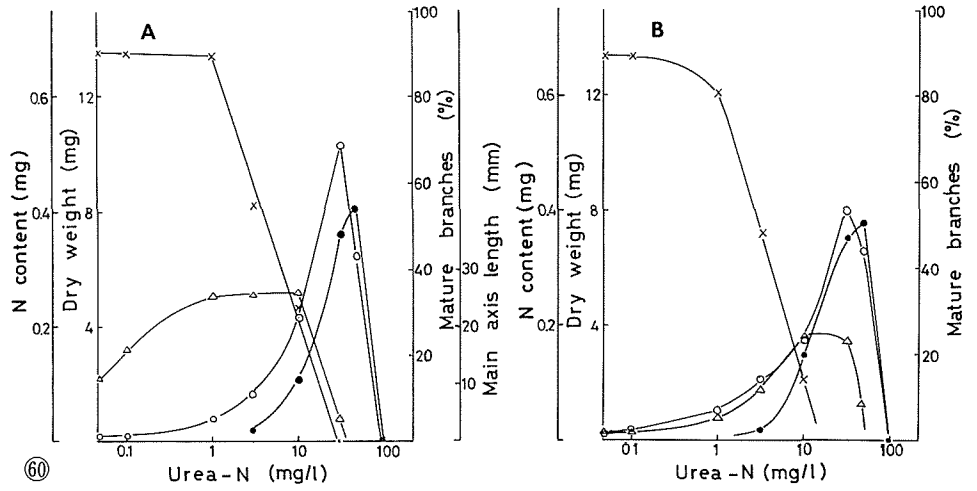

Fig. 60 Effects of urea concentrations on growth and maturation in *Desmarestia*. A. *D. viridis*. B. *D. ligulata*.

D. ligulata (Fig. 60 B) ; Most gametophyte cells died within 10 days after inoculation at concentrations of urea higher than 1 mg/*l* N. After 20–30 days, a fer surviving cells grew into filamentous thalli. The greatest growth was obtained at 30 to 50 mg/*l* N. No growth occurred at 100 mg/*l* N. Maturation of the gametophytes was inhibited at concentrations higher than 1 mg/*l* N. The sporophytes grew well at 10 to 30 mg/*l* N. At concentrations of 0.1 mg/*l* N and lower, they grew for a short time and bleached within one month. Concentrations higher than 50 mg/*l* inhibited growth and no growth occurred at 100 mg/*l* N.

4) Amino acids

Twenty-one amino acids were tested at a concentration of 10 mg/*l* N.

D. viridis (Table 34) ; A slight growth of the gametophytes was observed in cultures with cysteine, cystine, tyrosine and tryptophan. Maturation of the gametophytes was slightly inhibited by these four amino acids. The sporophytes utilized these four amino acids.

D. ligulata (Table 35) ; A slight growth of the gametophytes was also obtained in

Table 34 Effects of amino acids on maturation and growth of gametophytes and growth of sporophytes in *D. viridis*.

Nitrogen sources	Wt. /*l* (as N)	% of mature branches of gametophytes after 14 days	Growth of gametophytes after 150 days		Growth of sporophytes after 50 days Length, mm.
			Dry weight, mg.	Nitrogen amount, mg.	
None		91	0.2		8.8 (bleached)
$NaNO_3$	10 mg	92	11.6	0.308	26.3
Cysteine	10 mg	60	3.6	0.035	7.0
Cystine	10 mg	73	8.0	0.117	18.5
Tyrosine	10 mg	72	1.5	0.023	13.5
Tryptophan	10 mg	84	1.8	0.085	14.9

Table 35 Effects of amino acids on maturation and growth of gametophytes and growth of sporophytes in *D. ligulata*.

Nitrogen Sources	Wt. /*l* (as N)	% of mature branches of gametophytes after 14 days	Growth of gametophytes after 150 days		Growth of sporophytes after 90 days Dry weight, mg.
			Dry weight, mg.	Nitrogen amount, mg.	
None		97	0.3		0.2 (bleached)
$NaNO_3$	10 mg	90	12.6	0.380	4.3
Cysteine	10 mg	89	5.2	0.061	2.0
Cystine	10 mg	98	6.2	0.122	2.0
Tyrosine	10 mg	99	1.6	0.055	0*
Tryptophan	10 mg	92	3.5	0.150	0.2

* slightly grew with deep color.

the cultures with these four amino acids. Maturation of the gametophytes with the addition of amino acids was the same as in the control to which no amino acid was added. Whereas, the sporophytes could utilize cysteine and cystine. In cultures containing tryptophan, growth of the sporophytes was not promoted compared with the control but they had deep color in 90 days. The addition of tyrosine inhibited sporophyte growth but they developed a deep color in 90 days.

Phosphorus

Tripotassium phosphate and sodium glycerophosphate were used as phosphorus sources.

1) **Tripotassium phosphate**

Concentrations ranging from 0.1 to 50 mg/*l* phosphorus (P) were examined.

D. viridis (Fig. 61 A); The gametophytes grew well at 1 to 50 mg/*l* P. The control plants lacking phosphorus grew for a while and then bleached within three months. Complete maturation of the gametophytes occurred at these concentrations of phosphorus and also in control lacking phosphorus. The sporophytes grew well at 0.5 to 50 mg/*l* P. At concentrations of 0.1 mg/*l* P and below, the sporophytes grew for a short time and bleached within one month.

D. ligulata (Fig. 61 B); The gametophytes grew well at 3 to 50 mg/*l* P. The control plants lacking phosphorus grew for a while and bleached within three months. Complete maturation of the gametophytes occurred at these concentrations of phosphorus

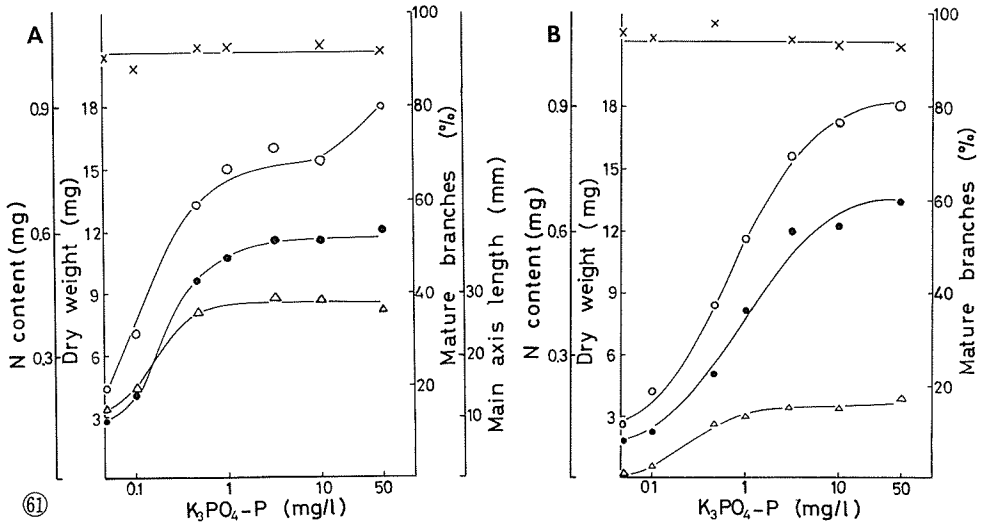

Fig. 61 Effects of tripotassium phosphate concentrations on growth and maturation in *Desmarestia*. A. *D. viridis*. B. *D. ligulata*.

and also in control lacking phosphorus. The sporophytes grew well at 1 to 50 mg/*l* P. At concentrations of 0.1 mg/*l* P and below, the sporophytes grew for a short time and bleached within one month.

2) **Sodium glycerophosphate**

Concentrations ranging from 0.1 to 50 mg/*l* phosphorus were examined.

D. viridis (Fig. 62 A); The gametophytes grew well at 0.5 to 50 mg/*l* P. Complete maturation of the gametophytes occurred at these additions of phosphorus and also in control lacking phosphorus. The sporophytes grew well at 0.5 to 50 mg/*l* P.

D. ligulata (Fig. 62 B); The gametophytes grew well at 10 to 50 mg/*l* P. Complete maturation of the gametophytes occurred at these additions of phosphorus. The sporophytes grew well at 10 to 50 mg/*l* P.

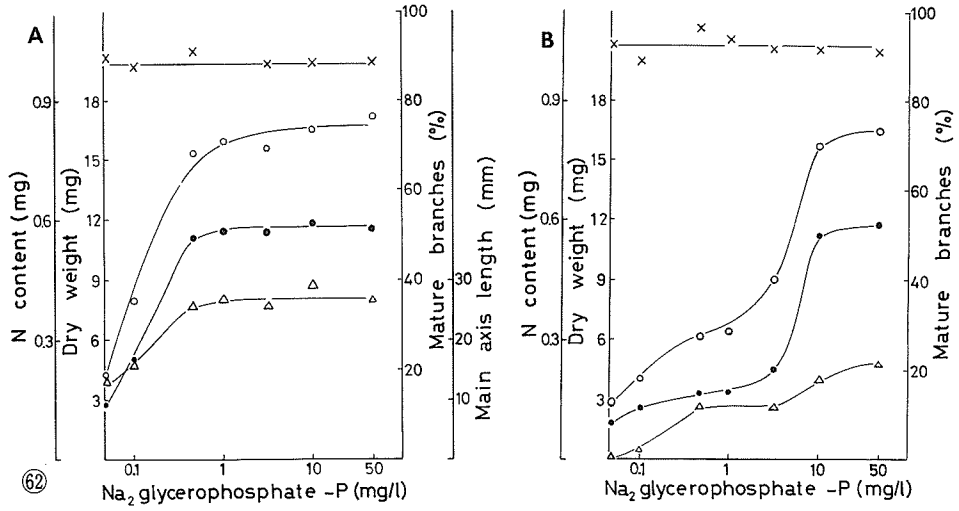

Fig. 62 Effects of sodium glycerophosphate concentrations on growth and maturation in *Desmarestia*. A. *D. viridis*. B. *D. ligulata*.

Organic substances

1) **Carbon sources**

The following organic carbon sources were added at levels of 0.2 g/*l* and 1 g/*l*; glucose, saccharose, sodium acetate, glycolic acid, pyruvic acid, citric acid and lactic acid.

D. viridis (Table 36); Growth of the gametophytes was slightly inhibited by the addition of 1 g/*l* sodium acetate. The addition of other carbon sources did not affect the growth. No growth was obtained in the dark condition in the presence of these carbon sources. Maturation of the gametophytes was inhibited at concentrations of 1 g/*l* glucose, 0.2 and 1 g/*l* citric acid and 1 g/*l* lactic acid. Growth of the sporophytes was inhibited at

Table 36 Effects of various carbon sources on maturation and growth of gametophytes and growth of sporophytes in *D. viridis*.

Carbon sources	Wt. /*l*	% of mature branches of gametophytes after 14 days	Dry weight, mg, of gametophytes after 150 days	Length, mm, of sporophytes after 50 days
None		82	16.1	26.0
Glucose	0.2 g	90	15.4	25.0
	1 g	75	16.7	26.0
Saccharose	0.2 g	89	15.6	26.5
	1 g	88	17.0	24.0
Na acetate	0.2 g	65	15.6	0
	1 g	66	12.8	0
Glycolic acid	0.2 g	86	17.1	25.0
	1 g	53	15.9	21.0
Pyruvic acid	0.2 g	86	13.2	17.0
	1 g	90	18.8	0
Citric acid	0.2 g	62	17.7	17.7
	1 g	70	16.6	0
Lactic acid	0.2 g	84	15.5	20.1
	1 g	53	15.6	16.5

Table 37 Effects of various carbon sources on maturation and growth of gametophytes and growth of sporophytes in *D. ligulata*.

Carbon sources	Wt. /*l*	% of mature branches of gametophytes after 14 days	Dry weight, mg, of gametophytes after 150 days	Dry weight, mg, of sporophytes after 90 days
None		98	13.1	3.8
Glucose	0.2 g	99	13.5	3.4*
	1 g	93	9.7	4.8*
Saccharose	0.2 g	97	11.5	3.8
	1 g	99	12.5	2.9
Na acetate	0.2 g	99	14.9	0
	1 g	99	13.4	0
Glycolic acid	0.2 g	99	10.2	3.3
	1 g	70	10.0	3.3
Pyruvic acid	0.2 g	99	12.1	3.8
	1 g	66	15.2	0
Citric acid	0.2 g	58	12.0	3.2
	1 g	54	12.1	0
Lactic acid	0.2 g	99	10.9	3.3
	1 g	99	11.0	3.0

* Nearly normal thallus was formed

concentrations of 0.2 and 1 g/*l* sodium acetate, pyruvic acid and citric acids. No growth was obtained in the dark condition with any additions of these carbon sources.

D. ligulata (Table 37); Growth of the gametophytes was slightly inhibited by additions of 1 g/*l* glucose, 0.2 and 1 g/*l* glycolic acid and 0.2 and 1 g/*l* lactic acid. No growth was obtained in the dark condition with any additions of these carbon sources. Maturation of the gametophytes was inhibited at concentrations of 1 g/*l* glycolic acid, 1g/*l* pyruvic acid, and 0.2 and 1 g/*l* citric acid. Growth of the sporophytes was inhibited by additions of 0.2 and 1 g/*l* sodium acetate, 1 g/*l* pyruvic acid and 1 g/*l* of citric acid. In cultures containing glucose, the cortical filaments of the sporophytes grew in a cross apposition to the main axis and formed many layered cortex, and developed into nearly normal thalli.

2) Vitamins

The following vitamins were tested; 0.1 mg/*l* thiamine, 1 mg/*l* nicotinic acid, 1 mg/*l* Ca-pantothenate, 0.4 mg/*l* pyridoxine, 5 μg/*l* biotin, 5 mg/*l* coline, 0.1 mg/*l* *p*-aminobenzoic acid, 10 mg/*l* inositol, 50 μg/*l* riboflavin, 0.2 mg/*l* pyridoxamine, 25 μg/*l* folic acid and 0.5 μg/*l* vitamin B_{12}. The gametophytes were depleted by transplanting to new media without vitamins. After three months they were inoculated. The sporophytes germinated in the medium without vitamins were employed as inoculum. Growth and maturation of the gametophytes and growth of the sporophytes with addition of these vitamins were the same as those in their absence.

Discussion

Many seaweeds have been successfully cultivated in undefined and defined media, but their nutrient requirements are not well understood. The seawaters enriched with nutrients are good for all seaweeds so far tested in impure culture under suitable light and temperature conditions. However, many seaweeds grow pooly in the defined media, some do not develop in axenic cultures. In *Ectocarpus confervoides*, BOALCH (1961a, 1961b) obtained only two-thirds of the yield in a defined medium in axenic culture compared with that in enriched seawater. The microthalli of some heteromorphic species developed normally in axenic culture but the macrothalli of them did not develop in the same medium (DRUEHL and HSIAO, 1969; NAKAHARA and TATEWAKI, 1971; FRIES, 1980). This could be explained by a lack of organic growth regulators or a lack of some trace elements in the defined medium (FRIES, 1982). *Ulva*, *Enteromorpha* and *Monostroma* grow normally only in the presence of their accompanying microflora or other red and brown seaweeds (PROVASOLI, 1964; PROVASOLI and PINTNER, 1964; TATEWAKI and KANEKO, 1977). *Ectocarpus fasciculatus* and *Pylaiella littoralis* need kinetin and cytokinin for growth and normal development in bacteria-free cultures (PEDERSÉN, 1968, 1973). Phenylacetic acid and *p*-hydroxyphenylacetic acid stimulate growth of axenic *Fucus spiralis* (FRIES, 1977).

The concentrations of major elements and ratio among them are important factors to

grow successfully in defined media cultures. These elements are different among media which were devised by several investigators. It is necessary to use the suitable basal medium for objective species to clarify the nutrient requirements and their suitable concentrations. For axenic red algae, FRIES used modified ASP_6 (ASP 6F) in a series of her nutritional studies (1963, 1964, 1966, 1968, 1970, 1974). TATEWAKI and PROVASOLI (1964) employed ASP_6 for the basal medium for the experiments with vitamins and IWASAKI (1965, 1967) and FRIES and IWASAKI (1976) used ASP_{12} NTA. Regarding the brown algae, PEDERSÉN (1969a, 1969b, 1973) used ASP 6F in her axenic cultures of *Ectocarpus* and *Pilaiella* and FRIES (1977, 1982) used ASP 6F2 for *Fucus*. MOTOMURA and SAKAI (1981) used ASP_{12} NTA for basal medium for *Laminaria* gametophytes. In the present experiment, the gametophytes of *Desmarestia viridis*, *D. ligulata*, *Laminaria japonica* and *Alaria crassifolia* grew well axenically in ASP_{12} NTA as well as the enriched seawater medium (PESI medium). The sporophytes of *D. viridis* grew well normally in ASP_{12} and their growth was better than that in PESI medium. The sporophytes of *D. ligulata* grew normally at early stage in ASP_{12} but later developed into thalli with slightly abnormal filamentous structure, which was the same as those grown at higher temperature in impure culture. Nearly normal morphology could be obtained in ASP_{12} medium to which glucose had been added. The sporophytes of *L. japonica* and *A. crassifolia* did not grow axenically more than 50 cells in either enriched seawater nor artificial media.

Complete maturation of the gametophytes of *D. viridis* and *D. ligulata* occurred in ASP_{12} at 10°C in a 10-hr photoperiod. The gametophytes of *L. japonica* and *A. crassifolia*

Table 38 Optimal concentrations of major ions for growth of gametophytes and sporophytes and complete maturation of gametophytes in *D. viridis* and *D. ligulata*.

		NaCl (g/*l*)	Mg (g/*l*)	Ca (g/*l*)	K (g/*l*)
D. viridis	Growth of gametophytes	28-32	1.17-5.0	0.2-0.4	0.37-2.0
	Complete maturation of gametophytes	12-28	0.25-1.17	0.1-0.6	0.2 -1.0
	Growth of sporophytes	28	1.17-2.5	0.2-0.4	0.37-2.0
D. ligulata	Growth of gametophytes	28-36	1.17-5.0	0.2-0.6	0.2 -1.0
	Complete maturation of gametophytes	5-32	0.05-1.17	0.2-0.6	0.2 -0.37
	Growth of sporophytes	24-28	1.17-2.5	0.2-0.4	0.37-2.0
Seawater[1]		27	1.27	0.4	0.38

[1] Data from LYMAN and FLEMING, 1940

matured completely in ASP_{12} at 10°C conditions. The favorable conditions for maturation in axenic culture were the same with those in impure culture.

The tolerance or need of seaweeds for major elements of seawater are one of important factors in their vertical and horizontal distribution. PROVASOLI (1958) noted that most littoral organisms need far less Ca and Mg than the environment offers them, one-half the seawater concentration of Mg and one-quarter the concentration of Ca elicit good growth, and Na-tolerance may be the decisive parameter which distinguishes among neritic, supralittoral and estuarine species. The present experiments of *D. viridis* and *D. ligulata* show that the favorable concentrations of major elements were different among the stages of life cycles (Table 38). The maximum growth of the gametophytes occurred at higher concentrations of NaCl than that of the sporophytes. The gametophytes and sporophytes of both species of *Desmarestia* preferred slightly higher concentrations of Mg for growth than the seawater concentrations. However, maturation of gametophytes occurred more abundantly at Mg concentrations lower than the seawater. The favorable concentrations of Ca for growth of the gametophytes and sporophytes were lower than the seawater concentration. The minimum concentration of Ca for optimal growth of the gametophytes and sporophytes was one-half the seawater concentration. Complete maturation of the gametophytes of *D. viridis* occurred at one-quarter the seawater concentration of Ca. The range of favorable concentrations of NaCl was the narrowest among the major elements. Among the stages of life cycles, the range of NaCl, Mg and Ca for complete maturation of the gametophytes was the widest and that for growth of the sporophytes was the narrowest. On the other hand, that of K for complete maturation of the gametophyte was the narrowest and that for growth of the sporophytes was the widest.

It is pointed out that although sulfate is present in relatively high concentrations (2.76 g/*l*) in seawater, most marine algae apparently require sulfate at a level 1/100-1/200 of natural seawater concentration (LEWIN and DUSBY, 1967). The thallus of *Desmarestia* has been shown to accumulate sulfate in high concentrations (BLINKS, 1951), relatively high sulfate requirements were expected. However, the sulfate concentrations for the maximum growth of the gametophytes and sporophytes of *D. viridis* and *D. ligulata* were the same range as those employed in media for the culture of fresh water algae.

Several trace metals are essential for growth and maturation of seaweeds. It is well known that brown algae are rich in iodine (SHAW, 1962). The requirement of iodine in axenic culture has been studied by several investigators (FRIES, 1966, in some red algae; IWASAKI, 1967, in *Porphyra tenera*; PEDERSÉN, 1969, in three species of brown algae; WOOLERY and LEWIN, 1973, in *Ectocarpus siliculosus*). According to them, *Polysiphonia urceolata*, *Porphyra tenera*, *Ectocarpus fasciculatus* and *E. siliculosus* appeared to have an absolute demand for iodine. In *E. siliculosus*, iodine concentrations which required for any appreciable vegetative growth and normal formation and maturation of plurilocular sporangia were different. However, some species proved to be indifferent to iodine additions.

MOTOMURA and SAKAI (1981) found that the gametophyte of *Laminaria angustata* formed oogonia in high concentrations of iron, but was growing vegetatively in lower ones. Zinc is an essential element for *Porphyra tenera* (IWASAKI, 1967). The gametophytes and sporophytes of *D. viridis* and *D. ligulata* grew well in the cultures without addition of iron, manganese, zinc, bromine, strontium, rubidium, lithium and iodine. These results do not prove that these metals are not needed, but they demonstrate that the requirement is low and is satisfied by the impurities brought in by the chemically pure salts of the medium. The addition of boron and molybdenum stimulated the growth of the gametophytes and sporophytes of both species up to 50 mg/*l* boron and up to the highest concentration used, 100 mg/*l* molybdenum. Maturation of the gametophytes of either species was inhibited at high concentrations of boron and molybdenum. In boron-free culture, all terminal cells of branches of gametophytes formed gametangia.

The artificial seawater media, ASP_1, ASP_2, ASP_2NTA, ASP_6, ASP_7, ASP_{12} and ASP_{12} NTA support the growth of various seaweeds (PROVASOLI, 1964). They contain trace metals and 10–30 mg/*l* Na_2 EDTA, 10 mg/*l* hydroxy-EDTA or 10–30 mg/*l* Na_2 EDTA+100 mg/*l* NTA. The addition of a trace metal chelator mixture minimizes impurities (toxic and favorable) and furnishes trace metals (PROVASOLI, 1964). FRIES (1963) reported that in axenic culture, *Nemalion multifidum*, with ASP_6, grew better, if hydroxy-EDTA was omitted and 100 mg/*l* NTA was the only chelator. For *Conchocelis* stage of *Porphyra tenera*, Na_2 EDTA at 10 mg/*l*, was effective for growth (IWASAKI, 1967). In the present experiment, the addition of chelators did not promote the growth of the gametophytes of the either species of *Desmarestia*. However, complete maturation of the gametophytes occurred when 10 mg/*l* Na_2EDTA was added. The growth of the sporophytes of *D. viridis* was promoted by the addition of 100–200 mg/*l* NTA, 5–20 mg/*l* Na_2 EDTA, 10 mg/*l* hydroxy-EDTA and 100 mg/*l* NTA+5−10 mg/*l* Na_2 EDTA. That of *D. ligulata* was promoted by the addition of 5−10 mg/*l* Na_2 EDTA.

Seaweeds have been found to differ in their capabilities of utilizing various nitrogen sources (DEBOER, 1982). Nitrate and ammonium can be utilized as nitrogen sources for growth. *Fucus spiralis* grew similarly on either ammonium or nitrate (BIRD, 1976). *Gracilaria foliifera* and *Neoagardhiella baileyi* exhibited higher growth rates when supplied with ammonium as compared with nitrate (DEBOER *et al.*, 1978). The gametophytes and sporophytes of both species of *Desmarestia* could utilize not only inorganic nitrogen, nitrate and ammonium, but also some organic nitrogen, such as urea and several amino acids. Nitrate nitrogen levels below 20 mg/*l* limited the growth of the gametophytes of both species. This concentration was nearly the same as reported for other seaweeds, *Goniotrichum* and *Nemalion* (FRIES, 1963) and *Conchocelis* stage of *Porphyra* (IWASAKI, 1967). On the other hand, the growth of the sporophytes was stimulated at low nitrate nitrogen concentrations and limited at nitrate nitrogen levels below 1 mg/*l* in *D. viridis* and 3 mg/*l* in *D. ligulata*. Complete maturation of the gametophytes of both species occurred at very low concen-

trations of nitrate and higher concentrations of nitrate did not suppress the maturation.

Ammonium rapidly becomes toxic in alkaline media (PROVASOLI, 1958). It was reported that ammonium can be toxic at 0.84 mg/*l* N for *Ulva* (WAITE and MITCHELL, 1972) and at 0.42 mg/*l* N for juvenile *Macrocystis* sporophytes (HAINES and WHEILER, 1978). The cell-divisions of the gametophytes of both species were inhibited by the addition of ammonium for a while at concentrations above 1 mg/*l* N and then the gametophytes grew gradually below 30 mg/*l* N in *D. viridis* and 50 mg/*l* N in *D. ligulata*. The maximum growth of the gametophytes with ammonium was obtained at 10 mg/*l* N in *D. viridis* and 30 mg/*l* N in *D. ligulata* and the nitrogen content of the gametophytes was the same as observed at 10 mg/*l* N of nitrate and 30 mg/*l* N of nitrate. The cell-divisions and growth of the sporophytes were not inhibited by the addition of ammonium up to 10 mg/*l* N in *D. viridis* and 30 mg/*l* N in *D. ligulata*. The maximum growth of the sporophytes of either species with ammonium was slightly better that than with nitrate. The optimal concentrations of ammonium for growth of the gametophytes and sporophytes of both species were similar to those of *Conchocelis* stage of *Porphyra* (IWASAKI, 1967). Maturation of the gametophytes of both species was inhibited at concentrations above 1 mg/*l* N.

Phosphorus is available in the sea almost entirely in the form of orthophosphate ions. However, seaweeds can utilize organic forms of phosphorus (FRIES, 1963). The minimum requirement of phosphorus of tripotassium phosphate for good growth of the gametophytes and sporophytes of *D. viridis* was the same as for glycerophosphate. This requirement was lower for *D. ligulata*, *Ectocarpus* (BOALCH, 1961b), *Goniotrichum*, *Trailliella* and *Nemalion* (FRIES, 1963) and *Conchocelis* stage of *Porphyra* (IWASAKI, 1967). The minimum requirement for phosphorus of glycerophosphate for growth of the gametophytes and sporophytes of *D. ligulata* was higher than that of tripotassium phosphate and was nearly the same as that of glycerophosphate for growth of *Nemalion* and that of dipotassium phosphate of *Conchoceris* stage of *Porphyra*. Complete maturation of the gametophytes of both species occurred at very low concentrations of phosphorus.

III General Discussion

1. Alternation of generations and alternation of nuclear phases

The life cycle of many brown algae consists of an alternation of an asexual with a sexual generation each having a different morphological form. In most species, the alternation of generations corresponds with the alternation of nuclear phases, but some species alternate between different morphological forms without the alternation of nuclear phases. There are two kinds of thalli on the diploid asexual generation, macrothallus and microthallus which resemble to their sexual generation (plethysmothalli), in *Haplogloia andersonii* (WYNNE, 1969), *Cladosiphon okamuranus* (SHINMURA, 1977) and *Tinocladia crassa* (YOTSUI, 1978). *Pogotrichum yezoense* alternates between macrothallus and microthallus but alternation of

nuclear phases never occurs (SAKAI and SAGA, 1981). The morphological change without alternation of nuclear phases was also observed in *Petalonia fascia* (WYNNE, 1969; HSIAO, 1969; NAKAMURA and TATEWAKI, 1975), *Isthmoplea sphaerophora* (EDERSTEIN *et al.*, 1971; PEDERSÉN, 1975), *Delamarea allenuata* (PEDERSÉN, 1974b) and *Hapterophycus canaliculatus* from Muroran (NAKAMURA and NAKAHARA, 1977).

It was found in some species that swarmers from gametophytes never acts as gametes and do not copulate under certain environmental conditions. Under warm conditions swarmers from gametophytes of *Sphaerotrichia devaricata* (AJISAKA and UMEZAKI, 1978) and *Spermatochnus paradoxus* (MÜLLER, 1981) never copulated. These swarmers developed into microthalli (gametophytes). Swarmers from gametophytes (erect thalli) of *Scytosiphon* did not fuse at the beginning of its growing season (CLAYTON, 1980, 1981).

WYNNE (1969) found in *Melanosiphon intestinales* that the frond with unilocular sporangia and that with plurilocular sporangia were the same morphologically and it repeated or alternated each other without sexual fusion. In some minute filamentous species only one morphological form was observed in life cycle. The thallus formed only plurilocular sporangia from generation to generation and swarmers from these sporangia developed into plants ultimately identical with parent (*Pylayella gardneri*, WYNNE, 1969; *Sorocarpus micromorus*, PEDERSÉN, 1974a; *Pogotrichum filiforme*, PEDERSÉN, 1978). The thalli formed only unilocular sporangia and swarmers from them developed into the same plants with the parents (*Pilayella littoralis* f. *rupincola*, WEST, 1967; *Petroderma maculiforme*, WYNNE, 1969; *Isthmoplea sphaerophora*, RUENESS, 1974). WYNNE (1969) listed various species in which this type of development had been recorded. This may be explained by the supposition that there is here no reduction divisions in the unilocular sporangia (FRITSCH, 1945).

The fusion of swarmers from unilocular sporangia has been observed by several investigators (FRITSCH, 1945; WYNNE, 1969). This phenomenon was regarded as a short-circuiting of the usual pattern of life cycle. Sometimes, however, incomplete separation of swarmers from unilocular sporangia was confused with fusion of swarmers. According to his many years culture experience of *Ectocarpus siliculosus*, MÜLLER (1975) hesitated to accept reports of sexual reproduction in isogamous brown algae unless the actual fusion of fully independent gametes was observed and complemented with developmental studies and chromosome counts in plants derived from such zygotes and unfused zoids. LOISEAUX (1964, 1966, 1967) found in the Myrionemataceae fusion occurred between swarmers of unilocular sporangia of diploid plants. According to her, *Haecatonema foecundum* had one asexual generation which was composed of a diploid discoidal frond and two sexual generations composed of filamentous thalli, one being diploid and the other haploid. The diploid asexual plant formed plurilocular sporangia and the diploid sexual plant formed both plurilocular and unilocular sporangia. Sexual fusion took place between haploid swarmers liberated from unilocular sporangia. The haploid sexual plant formed plurilocular sporangia only.

Unfertilized eggs of *Laminaria japonica* (FANG *et al.*, 1978) and *Arthorocladia villosa*

(MÜLLER and MEEL, 1982) developed into haploid normal sporophytes and formed unilocular sporangia which produced swarmers apomeiotically. In the present experiment, unfertilized eggs of *Desmarestia viridis*, *Agarum cribrosum* and *Alaria crassifolia* developed into haploid sporophytes and formed swarmers apomeiotically. Vegetative cells of the male gametophytes of *Laminaria japonica*, *Ag. cribrosum* and *A. crassifolia* developed into haploid sporophytes and vegetative cells of diploid sporophytes of *L. japonica*, *Ag. cribrosum* and *A. crassifolia* developed into diploid gametophytes. These experimental evidences indicate that the difference in ploidy level cannot account fully for the morphological difference between sexual and asexual generations and the plant is sexual or asexual.

Unfused gametes of several species can germinate and develop into haploid plants. It is interesting which generations are formed from unfused gametes. As mentioned above, haploid sporophytes were formed when unfertilized eggs germinated, but sometimes diploid ones were formed. In many isogamous and anisogamous species, either haploid sporophytes or gametophytes, or both were formed from unfused gametes. MÜLLER (1966) observed in *Ectocarpus siliculosus* that unfused gametes developed into haploid sporophytes. He also found that some haploid plants alternate between gametophytes and sporophytes without the alternation of nuclear phases and some haploid plants repeated sporophytes by swarmers from unilocular or plurilocular sporangia. KORNMANN (1956) observed that unfused gametes of *Ectocarpus confervoides* developed into haploid sporophytes. In some haploid sporophytes, however, vegetative cells developed into gametophytes aposporously.

It was reported in some species, unfused gametes developed into both gametophytes and sporophytes. According to VAN DEN HOEK and FLINTERMAN (1968), unfused female gametes of *Sphacelaria furcigera* developed into female gametophytes and haploid sporophytes with unilocular sporangia. Unfused gametes of *Spermatochnus paradoxus* developed into both gametophytes and sporophytes (MÜLLER, 1981). In the Scytosiphonales, NAKAMURA and TATEWAKI (1975) observed that unfused gametes of both sexes developed into crusts (sporophytes) and sometimes into erect fronds (gametophytes) depending mainly upon temperatures. In *Analipus japonicus*, most of unfused female gametes developed into female gametophytes and a few developed into haploid plants with both unilocular and plurilocular sporangia. Most of the male gametes developed into haploid plants with unilocular and plurilocular sporangia, and a few developed into male gametophytes. Assuming that the plants with unilocular sporangia are sporophytes and the plants with plurilocular sporangia only are gametophytes, the following successions of the parthenogenetic line are distinguishable in *A. japonicus*. The plants repeatedly produce gemetophytes or haploid sporophytes and the plants alternate between gametophytes and sporophytes without alternation of nuclear phases.

In some small algae, unfused gametes developed into only gametophytes. This type of development of unfused gametes was reported in *Ectocarpus siliculosus* from Sylt (KORNMANN, 1956), *Haplogloia andersonii* (WYNNE, 1969), *Stictyosiphon subsimplex* (FIORE,

1977), *Sphaerotrichia divaricata* (AJISAKA and UMEZAKI, 1978) and *Tinocladia crassa* (YOTSUI, 1978), all of which were epiphytes. Gametophytes repeatedly produced by unfused gametes were important for maintenance of population living on other algae.

As above-cited, the life cycle of lower brown algae with unilocular sporangia and/or plurilocular sporangia can not be interpreted by the concept that the plant with sporophytic form is diploid and the plant with gametophytic form is always haploid and sexual. It is now necessary to establish the criteria upon which the various types of life cycles of brown algae can be interpreted. It is clear that the plants are distinguishable by morphological forms, nuclear phases and modes of reproduction. However, a generation in a life cycle is defined as a part of this cycle characterized by an individual, independent or not, which begins with germination of a cell, spore or zygote, and ends with the production of another cell type, either zygote or spore, different from the initial one (RESENDE, 1967). For that reason, it is more important to define the generations of brown algae that the plant is diploid or haploid and sexual or asexual.

From this point of view, four kinds of generations can be distinguished; haploid sexual and asexual generations, diploid asexual and sexual generations. There are two morphological forms, one gametophytic and the other sporophytic. The gametophytic form is morphologically similar to a haploid sexual plant or juvenile filamentous form with reproductive organs in the same species. The sporophytic form is morphologically similar to diploid asexual plants with unilocular sporangia, in which meiosis occurs. In some species both forms are similar (isomorphic species) and in others dissimilar (heteromorphic species).

Haploid sexual generation

The haploid sexual plant takes one morphological form, gametophytic form, and bears plurilocular sporangia. Swarmers from the plurilocular sporangia are gametes. Zygotes develop commonly into diploid asexual plants and sometimes develop into diploid sexual plants (*Hecatonema foecundum* and *Myrionema strangulans*, LOISEAUX, 1968). Some unfused gametes develop into haploid sexual plants, some develop into haploid asexual plants and some develop into both, and some develop into diploid asexual plants.

Haploid asexual generation

The haploid asexual plant forms either plurilocular sporangia or unilocular sporangia, or both but meiosis does not occur in the unilocular sporangia. The plants with plurilocular sporangia usually take gametophytic forms (*Sphaerotrichia*, AJISAKA and UMEZAKI, 1978; *Acrothrix*, AJISAKA, 1979; *Scytosiphon*, CLAYTON, 1980, 1981; *Spermatochnus*, MÜLLER, 1981). The plants with unilocular sporangia takes sporophytic forms (*Acrothrix*, AJISAKA, 1979; *Spermatochnus*, MÜLLER, 1981). Swarmers from the plurilocular sporangia develop into haploid asexual plants or haploid sexual plants according to environmental conditions. Swarmers from the unilocular sporangia also develop into haploid sexual plants or asexual plants with gametophytic forms.

As mentioned above, some species repeat the plants with unilocular sporangia and do not have sexual fusion. These may possess only the haploid asexual generation in their life cycles. Some species have a life cycle in which they alternate between gametophytic forms with plurilocular sporangia and sporophytic forms with unilocular sporangia, but their swarmers do not fuse. In these species, the plants with sporophytic forms and the plants with gametophytic forms are determined by environmental conditions. The life cycle of these plants may consist of the haploid asexual generation only.

In some cases, a haploid sexual plant may change into a haploid asexual plant and sometimes a part of a individual may change (*Ectocarpus confervoides*, KORNMANN, 1956; *E. siliculosus* from Naples, MÜLLER, 1966). Diploid asexual generation with a sporophytic form is formed directly on haploid asexual generation with a gametophytic form by spontaneous diploidization (*Elachista stellaris*, WANDERS *et al.*, 1972).

Diploid asexual generation

The diploid asexual plant forms either plurilocular sporangia or unilocular sporangia, or both. The plurilocular sporangia are sometimes formed on the gametophytic form (plethysmothallus), sometimes on the sporophytic form (known as accessory sporangia of Ectocarpales and Chordariales). The unilocular sporangia are usually formed on the sporophytic form and meiosis occurs in them. Diploid swarmers from plurilocular sporangia commonly develop into diploid asexual plants again and sometimes into diploid sexual plants. The haploid swarmers from unilocular sporangia are zoospores which develop into haploid sexual plants and rarely into haploid asexual plants. Some of the species which repeat the plants with plurilocular sporangia and never occur sexual fusion may consist of diploid asexual generation only in their life cycle.

Diploid sexual generation

The diploid sexual plant forms unilocular sporangia or unilocular and plurilocular sporangia. The unilocular sporangia, as well as the plurilocular sporangia, are formed on the gametophytic form (*Hecatonema foecundum*, LOISEAUX, 1968) or on the sporophytic form (*Myrionema strangulans*, LOISEAUX, 1968) and meiosis occurs in the unilocular sporangia. Sexual fusion occurs among swarmers from unilocular sporangia and zygotes develop into diploid sexual plants or diploid asexual plants. Unfused swarmers develop into haploid sexual plants. The diploid swarmers from plurilocular sporangia develop into diploid sexual plants or diploid asexual plants.

In oogamous brown algae, the haploid sexual plant has a gametophytic form bearing oogonia and antheridia. The haploid asexual plant has a sporophytic form bearing unilocular sporangia, in which meiosis does not occur. The diploid asexual plant has a sporophytic form bearing unilocular sporangia, in which meiosis occurs. The diploid sexual plant has a gemetophytic form bearing oogonia and antheridia. The morphological forms correspond to the fact that the plant is sexual or asexual. The gametophytic form appears in sexual generations and the sporophytic form in asexual generations.

2. Differences in growth and nutrition between generations or morphological forms in culture

Most brown algae which alternate between heteromorphic generations possess two morphologically different forms in a life cycle, microthallus (a gametophytic form) and macrothallus (a sporophytic form) which may appear in different seasons. Futhermore, sometimes both forms are changeable each other according to growth conditions. This possibility indicates that each form might respond in different way to physical or chemical factors. Physiological differences between the morphological forms are shown in differences of growth rate, resistance to various environmental factors and favorable condition for growth.

It is well known that cell-divisions of macrothalli occur faster than those of microthalli under the conditions where the microthalli become fertile. Many investigators demonstrated in their figures and plates that the cell-divisions of the macrothalli took place faster than those of the microthalli (e. g. *Chordaria flagelliformis*, CARAM, 1955; *Petalonia fascia*, WYNNE, 1969). In the present experiment, the growth rates of gametophytes and sporophytes of *Desmarestia viridis* and *D. ligulata* during early stages of development were examined in axenic culture. In *D. viridis* the growth constant k was 0.057 in gametophytes at 14°C in a 14-hr photoperiod and 0.10 in sporophytes at 10°C in a 14-hr photoperiod. These are equivalent to 0.19 cell-divisions/day and 0.33 cell-divisions/day, respectively. In *D. ligulata* k values were 0.061 in gametophytes at 14°C in a 14-hr photoperiod and 0.11 in sporophytes at 10°C in a 14-hr photoperiod. These are equivalent to 0.22 cell-divisions/day and 0.37 cell-divisions/day, respectively. In three species of Laminariales, the growth rates were calculated in 1 to 10-day-old germlings from zoospores and fertilized eggs in bacterized

Table 39 Growth rate (cell-divisions/day) of three species of Laminariales in cultures in a 14-hr photoperiod.

		10℃	18℃
L. japonica	♀	0.14	0.25
	♂	0.33	0.36
	sporo.	1.06	
Ag. cribrosum	♀	0.19	0.19
	♂	0.19	0.19
	sporo.	0.87	
A. crassifolia	♀	0.11	0.14
	♂	0.45	0.46
	sporo.	1.05	

culture. The growth rates of sporophytes were more than twice as fast as gametophytes (Table 39). KAIN (1965) reported that the growth rate of young sporophytes of *Laminaria hyperborea* in the laboratory was 0.5 cell-divisions/day at 10°C and also she (1969) observed 0.6 cell-divisions/day in sporophytes of *Laminaria digitata* at 10°C and 17°C.

Macrothalli do not grow normally and die in certain conditions where microthalli grow well. SEGI and KIDA (1957, 1958) observed that in *Undaria undarioides* only 5% of sporophytes survived at 4500 lux under 15–21°C conditions and 70% of sporophytes survived at 2500 lux after 35 days. At 15–24°C, 60% of gametophytes survived at 4500 lux and 80–90% survived at 2200–3200 lux after 30 days. In *Alaria esculenta* gametophytes grew and became sterile at 4°C in 300 lux in Erd-SCHREIBER medium which was renewed every three months but sporophytes died in this treatment (SUNDENE, 1962). KAIN (1969) reported that at 20°C gametophytes of *Laminaria digitata* and *Saccorhiza polyschides* survived but sporophytes were not persistent. When the temperature rose to 35°C for a two-hour period, blades of *Petalonia fascia* were killed. However, crusts and holdfasts of blades survived (WYNNE, 1969). In the present experiment, gametophytes of *D. viridis* and *D. ligulata* grew well at 22°C but sporophytes died within 15 days.

Sporophytes did not grow under low salinity conditions, where gametophytes grew and became fertile. (*Corda filum* and *Saccorhiza polyschides*, NORTON and SOUTH, 1969; *Macrocystis angustifolia*, SANBONSUGA and NEUSHUL, 1978). At concentrations of sodium chloride lower than 12 g/*l*, sporophytes of *D. viridis* and *D. ligulata* never developed and died within a few days but gametophytes grew and became fertile.

The favorable conditions for growth of microthalli are different from those of macrothalli. The macrothalli (blades) of *Scytosiphon lomentaria* grew faster at 10°C conditions than at 14–18°C conditions. However, the microthalli (crusts) grew faster under warm conditions (NAKAMURA and TATEWAKI, 1975) In *Sphaerotrichia devaricata* the microthalli grew rapidly at higher temperature conditions but the macrothalli grew greater at cooler conditions. (AJISAKA and UMEZAKI, 1978). In *D. viridis* gametophytes grew at 5–22°C and well at higher than 14°C in long-day conditions. Sporophytes grew at 5–18°C and were not affected by photoperiod. During the younger stage, they grew well at 10–14°C but later the growth was suppressed at 14°C and the best growth was obtained at 10°C. In *D. ligulata* gametophytes grew well at 14–22°C and in long-day conditions. In short-days and lower temperatures they grew and soon became fertile. Sporophytes grew at 5–18°C and were not affected by photoperiod. The younger stages grew well at 5–14°C but later stages were suppressed at 14°C and the best growth was obtained at 5–10°C. In *D. tabacoides* gametophytes grew well at 14–22°C, in long days and sporophytes grew well at 14–18°C but were not affected by photoperiod. In *Laminaria japonica*, *Agarum cribrosum* and *Alaria crassifolia*, gametophytes grew well at 14–18°C and sporophytes at 5–10°C.

HSIAO (1969), studying life history and iodine nutrition of *Petalonia fascia*, found that development of zooids from blades into protonema, plethysmothalli or *Ralfsia*-like crusts

depended upon the iodide concentration. According to him, the development of *Ralfsia*-like crusts and blades required was $50.76\times10^{2}\,\mu g/l$ and $50.76\times10^{1}\,\mu g/l$ as the minimal iodide concentrations respectively. A slight difference of nutritional requirements for growth of gametophytes and sporophytes was recognized in axcenic cultures. Gametophytes of *L. japonica* and *A. crassifolia* grew well and became fertile in ASP_{12} and PESI medium in axenic culture but sporophytes did not grow. The ranges of optimal concentrations of major ions, except potassium, for growth of gametophytes of *D. viridis* and *D. ligulata* were wider than those for growth of sporophytes of the both species. Optimal concentrations of micro -nutrients for growth of gametophytes and sporophytes were slight. Sporophytes of two *Desmarestia* species grew well under lower concentrations of nitrate and ammonium than gametophytes (Table 40). These sporophytes died within 5 days in ASP_{12} medium with 0.2 -1 g/*l* of sodium acetate, 1 g/*l* pyruvic acid and 1 g/*l* of citric acid. Gametophytes of both species were not affected by the addition of these carbon sources. *D. ligulata* did not produce a normal sporophytic thallus in ASP_{12} in axenic culture. When 0.2-1 g/*l* of glucose were added, the sporophytes developed into nearly normal thalli. The addition of 0.2 g/*l* glucose had no effect on the gametophytes but 1 g/*l* glucose slightly inhibited their growth.

Complete maturation of gametophytes of *D. viridis* and *D. ligulata* occurred at lower concentrations of various nutrients and the ranges of the nutrients, except potassium, for maturation were usually wider than that for growth of gametophytes and sporophytes. The ranges of potassium concentrations for complete maturation of gametophytes were narrowest.

Recently, several species were cultured from generation to generation and favorable conditions for maturation of the both generations were cleared. In some species, microthalli became fertile under a certain conditions which were different from favorable conditions for

Table 40 Optimal concentrations of various nutrients for growth of gametophytes and sporophytes and complete maturation of gametophytes in *D. viridis* and *D. ligulata*.

		SO_4 (g/*l*)	B (mg/*l*)	Nitrate (mg/*l* N)	Ammonium (mg/*l* N)	P (mg/*l*)
D. viridis	Growth of gametophytes	0.02-4.72	10 -50	20 -300	10	0.5-50
	Complete maturation of gametophytes	0.05-4.72	lower than 2.0	0.1-300	lower then 0.1	0.1-50
	Growth of sporophytes	0.05-4.72	0.5-10	1.0-300	1.0-10	0.5-50
D. ligulata	Growth of gametophytes	0.05-4.72	0.1-50	20 -300	30	10 -50
	Complete maturation of gametophytes	0.05-4.72	lower than 50	0.1-300	lower than 0.1	0.1-50
	Growth of sporophytes	0.05-4.72	1.0-2.0	3.0-300	3.0-30	10 -50

vegetative growth but macrothalli matured under the same conditions where they grew well. NAKAMURA and TATEWAKI (1975) observed in *Scytosiphon lomentaria* that erect thalli became fertile under cool conditions with long or short day-length in which they grew well. However, crusts grew well under warm and long-day conditions in which unilocular sporangia never formed. These crust became fertile when transferred to short-day conditions. In the present investigation, both generations of *D. viridis*, *D. ligulata*, *D. tabacoides*, *Ag. cribrosum* and *A. crassifolia* became fertile and it was found that favorable conditions for maturation were different between generations. Complete maturation of gametophytes of *D. viridis* and *D. ligulata* occurred at 5–10°C in 10-hr photoperiods. Under these conditions sporophytes grew well. In *D. tabacoides* complete maturation of gametophytes occurred at 5–10°C and 10-hr photoperiod but sporophytes grew best at 14–18°C. The formation of unilocular sporangia of *D. viridis* and *D. ligulata* occurred in 2–6-month cultures at 10–18°C in 10- and 14-hr photoperiods. Sporophytes of *D. tabacoides* became fertile in 4–6-month cultures at 14–22°C in 10- and 14-hr photoperiods. These temperature ranges were wider than those for good growth of sporophytes.

In *L. japonica*, *Ag. cribrosum* and *A. crassifolia*, complete maturation of gametophytes and good growth of sporophytes occurred at 10°C in a 14-hr photoperiod. Under higher temperature conditions, maturation of gametophytes and growth of sporophytes were suppressed. The formation of unilocular sporangia of *Ag. cribrosum* was observed in 8-month cultures at 10°C in a 14-hr photoperiod, where gametophytes became fertile and young sporophytes grew well. The formation of unilocular sporangia of *A. crassifolia* was observed in 11-month cultures at 10°C in a 10-hr photoperiod where complete maturation of gametophytes occurred. SANBONSUGA and HASEGAWA (1967) reported the formation of unilocular sporangia of sporophytes of Laminariales in laboratory cultures. According to them, 8-month-old sporophytes of *Undaria pinnatifida* and 10-month-old sporophytes of *Costaria costata* formed unilocular sporangia in March (room temperature rose above 22°C) in a 18-hr photoperiod at 2400 lux. This temperature is higher than the favorable temperature for growth of sporophytes and maturation of gametophytes, which was 15°C in both species. In a more detailed study, they found that in *Costaria costata* the unilocular sporangia were formed either at low temperature (10–12°C) or high (18–22°C), either at low light intensity (2500 lux) or high (5000 lux), and furthermore, either at long light-period (18-hr a day) or short (6-hr a day) (SANBONSUGA and HASEGAWA, 1969).

In several small short-lived species, however, both generations became fertile under wide environmental conditions, lower and higher temperatures and short and long days. FIORE (1977), studying life history of *Stictyosiphon subsimplex*, found that both generations formed reproductive organs under 3–30°C conditions. AJISAKA (1979) observed that microthalli and macrothalli of *Acrothrix pacifica* became fertile at 5–20°C.

In the species which alternate between isomorphic generations, growth of diploid generations is slightly faster than that of haploid generations at younger stage. However,

favorable conditions for growth and development are not markedly different between the generations. MÜLLER (1966) found that gametophytes and sporophytes of *Ectocarpus siliculosus* grew well and became fertile under 13–20°C conditions. In *Analipus japonicus*, the development of germlings from zygotes took place faster than that from zoospores or unfused gametes. The erect fronds were produced from prostrate thalli of both haploid and diploid plants under 5–14°C and short-day conditions. Under 14–22°C and long-day conditions, the prostrate thalli grew well but never produced the erect fronds. Good growth of the erect fronds of both haploid and diploid plants occurred at 5–10°C. At 18–22°C, growth of the erect fronds was suppressed. The physiological differences as shown between generations of heteromorphic species were recognized between the erect frond and the prostrate thallus. The erect frond grew faster than the prostrate thallus but the ranges of favorable temperatures for growth of the erect frond were narrower. Conditions where erect fronds can survive were narrower than those of prostrate thalli. Most plants having one thallus type in a life cycle, their holdfasts were more resistant than blades to extreme conditions as observed in *Phaeostrophion irregulare* (MATHIESON, 1982).

Summary

Seven species of brown algae were investigated by culturing unialgally from generation to generation in order to clarify life cycles, alternation of nuclear phases, effects of temperatures and photoperiods on growth and maturation of sporophytes and gametophytes. Nutritional differences between both generations were studied axenically for four species.

1. *Analipus japonicus* exhibits a life cycle with an alternation of isomorphic generations. The erect fronds are formed under cool and short-day conditions, and do not grow under warm conditions. The prostrate thalli develop under warm and long-day conditions. No differences in development of haploid and diploid plants are observed.

Most of unfused female gametes develop into haploid female plants and a few develop into haploid plants with both unilocular and plurilocular sporangia. Most of unfused male gametes develop into haploid plants with both unilocular and plurilocular sporangia and a few develop into haploid male plants. The erect fronds with both types of sporangia are formed unilocular sporangia most abundantly at low temperatures. Meiosis does not occur in the unilocular sporangia of the haploid plants. In the second and third parthenogenetic generations, there are three kinds of the parthenogenetic lines; 1) repeat plants with plurilocular sporangia only, 2) alternate between plants with plurilocular sporangia only and plants with both types of sporangia, and 3) repeat plants with both types of sporangia.

2. Three species of Desmarestiales were cultured from generation to generation; *D. viridis, D. ligulata* and *D. tabacoides*. The gametophytes are monoecious and they become fertile under cool and short-day conditions. Fertilization occurs when eggs remain in the oogonia and sporophytes germinate *in situ*. The sporophytes grow well under cool

conditions and form unilocular sporangia under warm conditions. The sporophytes die under high temperature conditions, whereas the gametophytes grow well. The cell-divisions of the sporophytes occur more rapidly than those of the gametophytes. In the gametophytes, single cells isolated from any parts of the frond regenerate but in the sporophytes only the apical cells of rhizoids regenerate in *D. viridis* and *D. ligulata*. Unfertilized eggs of *D. viridis* develop into sporophytes, most of which are haploid and a few diploid. The haploid sporophytes produce swarmers without meiosis in the unilocular sporangia.

3. Three species of Laminariales were studied ; *Laminaria japonica, Agarum cribrosum* and *Alaria crassifolia*. The gametophytes become fertile under cool conditions. The gametophytes grow well vegetatively under warm conditions and the sporophytes grow well under cool and long-day conditions. The cell-divisions of the sporophytes occur more rapidly than those of the gametophytes. The sporophytes of *Ag. cribrosum* form unilocular sporangia at 10℃ in a 14-hr photoperiod and those of *A. crassifolia* at 10℃ in a 10-hr photoperiod. The fruiting sporophytes of *A. crassifolia* in culture form sporangial sori in the lower and marginal part of the blade, not giving rise to sporophylls.

The haploid sporophytes are obtained from unfertilized eggs (parthenogenesis) and also from vegetative cells of male gametophytes (apogamy). However, some unfertilized eggs develop into diploid sporophytes. In *Ag. cribrosum* and *A. crassifolia*, some haploid sporophytes derived from unfertilized eggs become fertile and form unilocular sporangia, in which meiosis does not occur. The swarmers liberated from these haploid plants develop into female gametophytes. The diploid gametophytes are formed from the vegetative cells of the sporophytes derived from zygotes (apospory). All of the diploid gametophytes of *L. japonica* and *Ag. cribrosum* and most of *A. crassifolia* are monoecious but they form antheridia more abundantly than oogonia. Diploid gametophyts which formed oogonia only are obtained in *A. crassifolia*. The triploid and tetraploid sporophytes are formed after fertilization of eggs and sperms of haploid and diploid gametophytes. The teraploid gametophyte of *A. crassifolia* are monoecious but the sperms non-functional.

4. In axenic culture, the gametophytes of *D. viridis, D. ligulata, L. japonica* and *A. crassifolia* and the sporophytes of *D. viridis* grow well normally in ASP_{12} and $ASP_{12}NTA$ media but the sporophyts of *D. ligulata* grow abnormally and those of *L. japonica* and *A. crassifolia* do not grow at all. Nearly normal thalli of *D. ligulata* are formed when 0.2 or 1 g/*l* of glucose are added. In *D. viridis* and *D. ligulata*, requirements and optimal concentrations of various nutrients are demonstrated. The optimal concentrations of various nutrients for growth of gametophytes and sporophytes and for maturation of gametophytes are different. Boron is essential for growth of gametophytes and sporophytes. However, maturation of the gametophytes is inhibited by the addition of boron higher than 2 mg/*l* which promotes gametophyte and sporophyte growth. Growth of sporophytes is stimulated at lower concentrations of nitrate than that of gametophytes. Complete maturation of

gametophytes occurs at very low nitrate concentrations. Ammonium supports the sporophyte growth at concentrations 10 mg/*l* N in *D. viridis* and 30 mg/*l* N in *D. ligulata*. However, maturation of the gametophytes of the either species is inhibited at concentrations greater than 0.1 mg/*l* N of ammonium.

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PLATE I

Analipus japonicus (HARVEY) WYNNE

A. Erect frond initials from zoospores from 40-day-old culture grown at 10℃ in a 14-hr photoperiod.

B. Prostrate thallus initials from zoospores from 40-day-old culture grown at 14℃ in a 14-hr photoperiod.

C. Young erect fronds from zoospores from 60-day-old culture grown at 10℃ in a 14-hr photoperiod.

D. Fertile erect frond with plurilocular sporangia from a zoospore from 120-day-old culture grown at 10℃ in a 14-hr photoperiod.

E. Prostrate thallus from a zoospore from 6-month-old culture grown at 14℃ in a 14-hr photoperiod.

F. Cross section of fertile erect frond from a zygote, bearing unilocular sporangia.

G. Cross section of fertile erect frond from a female gamete, bearing unilocular and plurilocular sporangia.

H. Hand crushing of fertile erect frond from a male gamete, bearing unilocular and plurilocular sporangia.

I. Cross section of fertile erect frond from a female gamete, bearing plurilocular sporangia only.

J. Cross section of fertile erect frond from a male gamete, bearing plurilocular sporangia only.

K. Chromosomes of erect frond from a zygote; forty chromosomes are evident. Inset: drawing showing arrangement of 40 chromosomes.

L. Chromosomes of erect frond from a zoospore; twenty chromosomes are evident. Inset: drawing showing arrangement of 20 chromosomes.

Scale in A applies also to B–C; scale in D applies also to E; scale in J applies also to F–I; scale in L applies also to K.

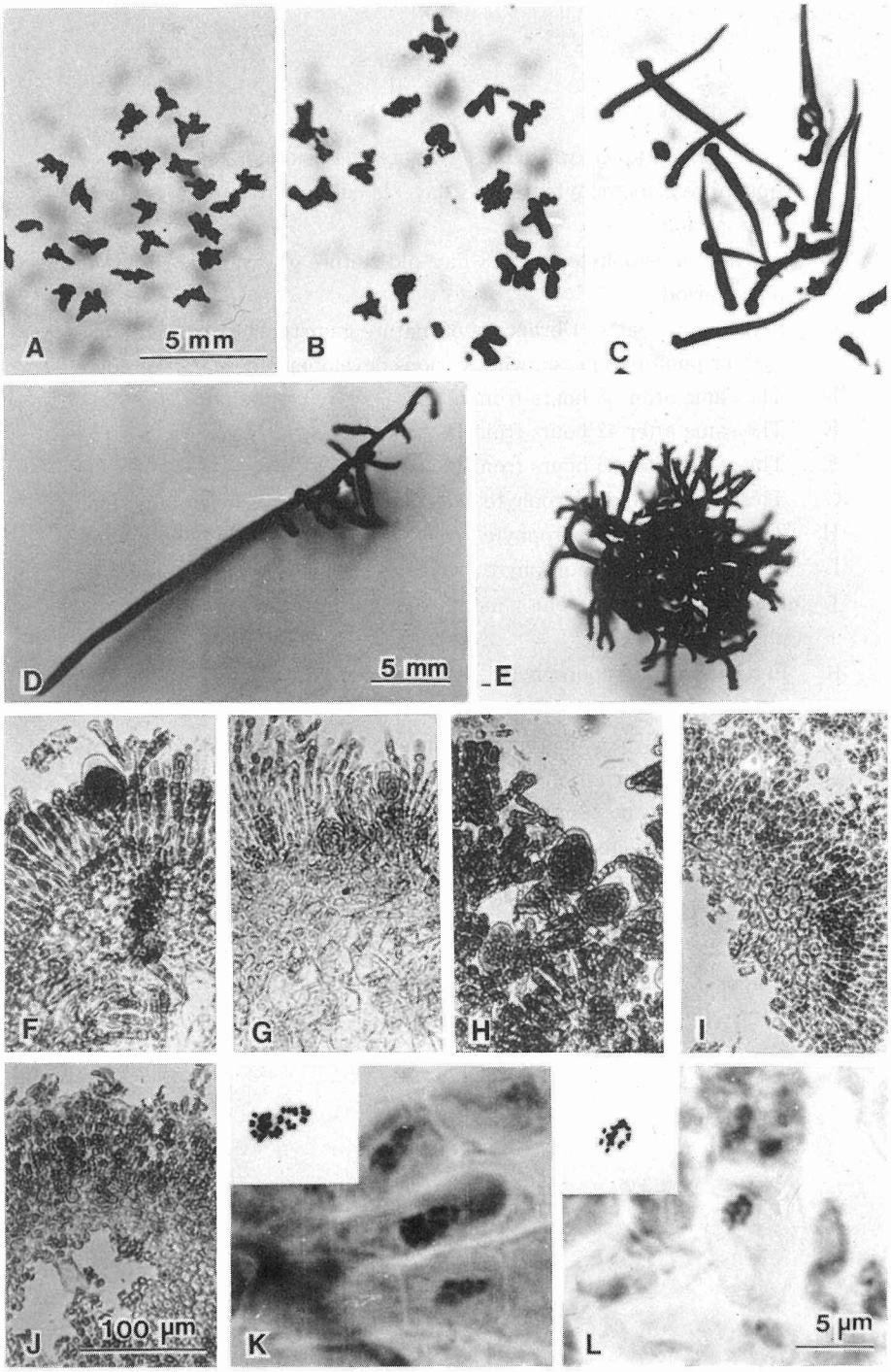

A
5 mm
B
C
D
5 mm
E
F
G
H
I
J
100 µm
K
L
5 µm

PLATE II

Desmarestia viridis (MÜLLER) LAMOUROUX

A. Immature gametophyte from 18-day-old culture grown at 14℃ in a 14-hr photoperiod.

B. Mature gametophyte from 18-day-old culture grown at 12℃ in a 10-hr photoperiod.

C. Some apical parts of branches of mature gametophyte grown at 10℃ in a 12-hr photoperiod, showing various developmental stages of oogonia.

D. The same after 48 hours from C.

E. The same after 42 hours from D.

F. The same after 36 hours from E.

G. Thirteen-day-old sporophyte grown at 10℃ in a 14-hr photoperiod.

H. Three-month-old sporophyte grown at 10℃ in a 14-hr photoperiod.

I. Three-month-old sporophyte grown at 18℃ in a 14-hr photoperiod.

J. Fertile sporophyte from 4-month-old culture grown at 10℃in a 14-hr photoperiod.

K. Fertile haploid sporophyte derived from an unfertilized egg, from 4-month-old culture grown at 12℃ in a 10-hr photoperiod.

L. Chromosomes of gametophyte. Inset: drawing showing arrangement of about 22 chromosomes.

M. Chromosomes of sporophyte derived from a zygote. Inset: drawing showing arrangement of about 44 chromosomes.

N. Chromosomes of sporophyte derived from an unfertilized egg. Inset: drawing showing arrangement of about 22 chromosomes.

Scale in C applies also to D-F; scale in H applies also to I; scale in K applies also to J; Scale in N applies also to L-M.

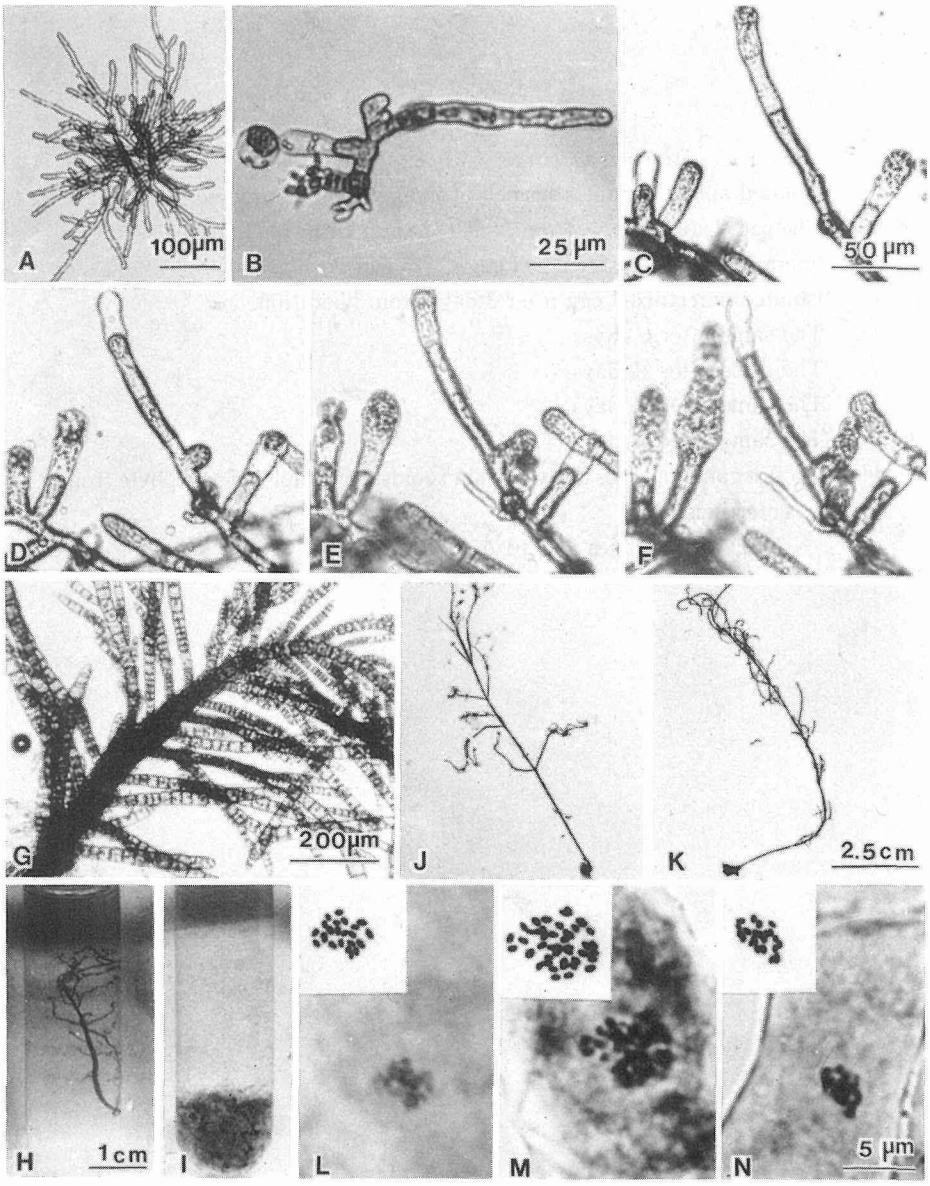

A
100μm
B
25 μm
C
50 μm
D
E
F
G
200μm
J
K
2.5cm
H
1cm
I
L
M
N
5 μm

PLATE III

Desmarestia viridis (MÜLLER) LAMOUROUX

A. Isolated apical part of a branch of immature gametophyte.

B. The same after 5 days from A at 12℃ in a 10-hr photoperiod, showing liberation of free unfertilized egg.

C. Isolated unfertilized egg after 3 days from liberation.

D. The same after 6 days.

E. The same after 15 days.

F. The same after 26 days.

G. The same after 46 days.

H-I. Mature gametophytes derived from zooids from haploid sporophyte from an unfertilized egg.

Scale in B applies also to A, C-E & H-I.

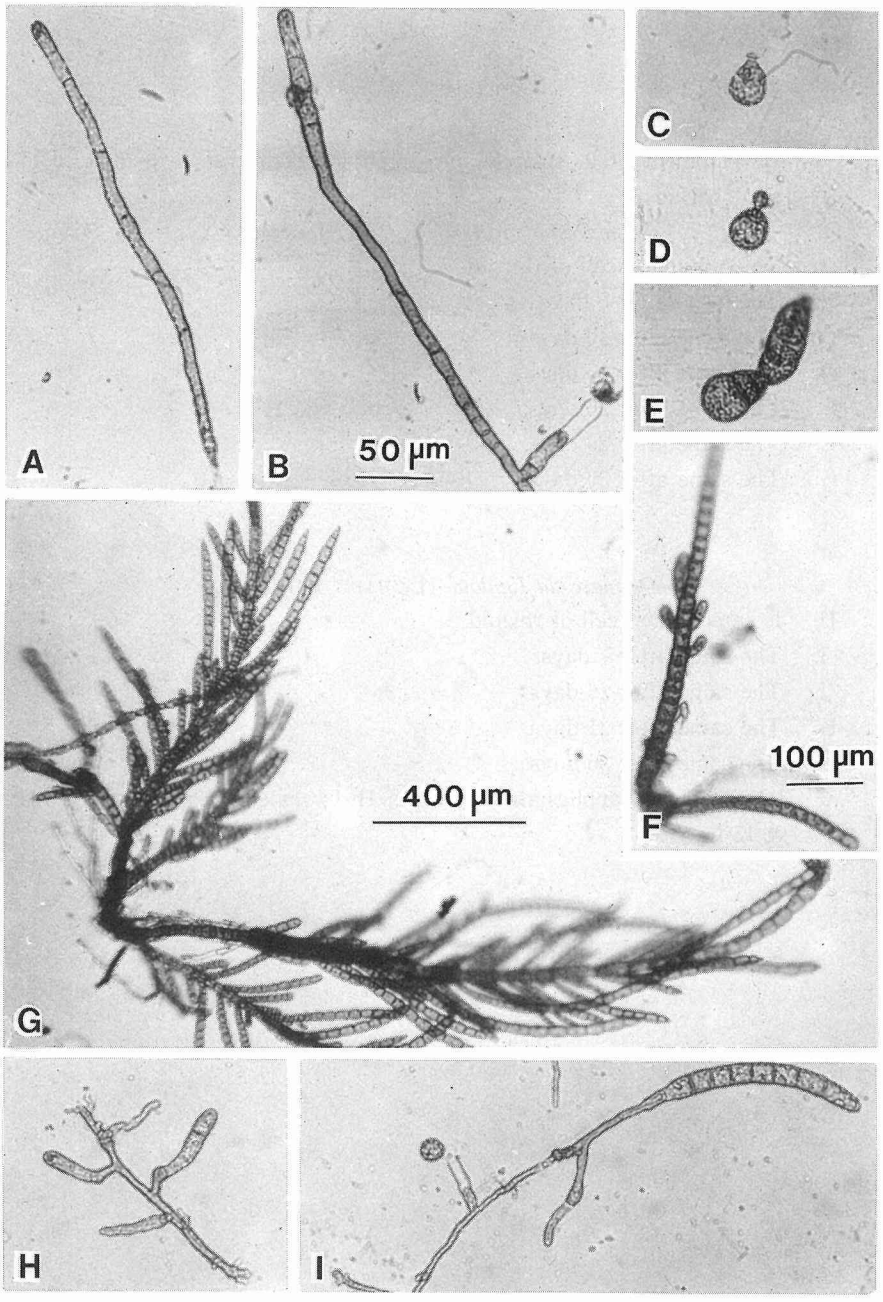

A
B
50 µm
C
D
E
F
100 µm
400 µm
G
H
I

PLATE IV

Development of a isolated apical cell of rhizoid at 12℃ in a 10-hr photoperiod.

Desmarestia viridis (MÜLLER) LAMOUROUX

A. Isolated apical cell of rhizoid.
B. The same after 5 days.
C. The same after 10 days.
D. The same after 15 days.
E. The same after 20 days.
F. The same after 25 days.
G. The same after 30 days.

Desmarestia ligulata (LIGHTF.) LAMOUROUX

H. Isolated apical cell of rhizoid.
I. The same after 7 days.
J. The same after 26 days.
K. The same after 31 days.
L. The same after 36 days.

Scale in C applies also to A-B & H-J ; scale in E applies also to D & K-L.

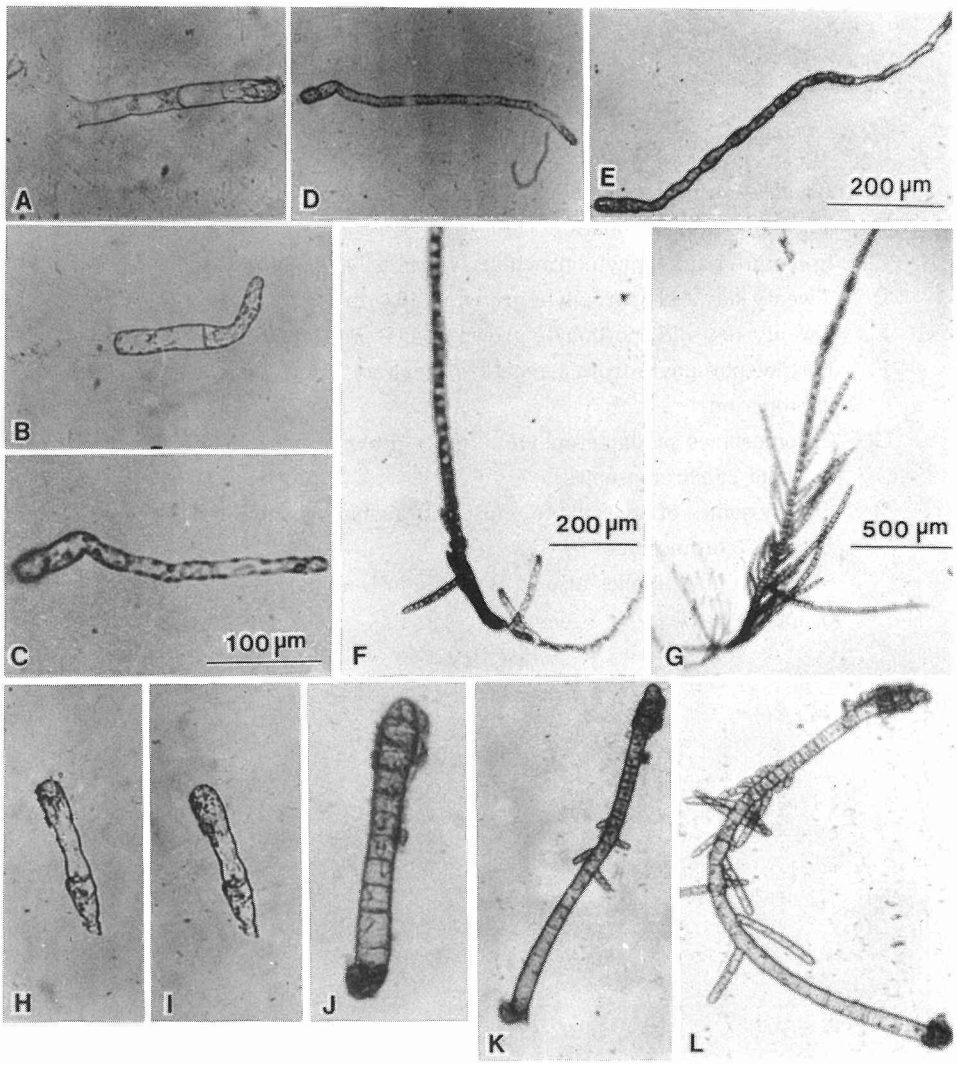

A
D
E
200 µm
B
C
100 µm
F
200 µm
G
500 µm
H
I
J
K
L

PLATE V

Desmarestia ligulata (LIGHTF.) LAMOUROUX

A. Branches of mature gametophyte grown at 12℃ in a 10-hr photoperiod.

B. The same after 34 hours.

C. Immature gametophyte grown at 14℃ in a 14-hr photoperiod.

D. Twenty-day-old sporophyte grown at 10℃ in a 14-hr photoperiod.

E. Twenty-day-old sporophyte grown at 14℃ in a 14-hr photoperiod.

F. Fertile sporophyte from 4-month-old culture grown at 10℃ in a 14-hr photoperiod.

G. Chromosomes of gametophyte. Inset: drawing showing arrangement of about 26 chromosomes.

H. Chromosomes of sporophyte. Inset: drawing showing arrangement of about 52 chromosomes.

Scale in A applies also to B; scale in D applies also to E; scale in G applies to H.

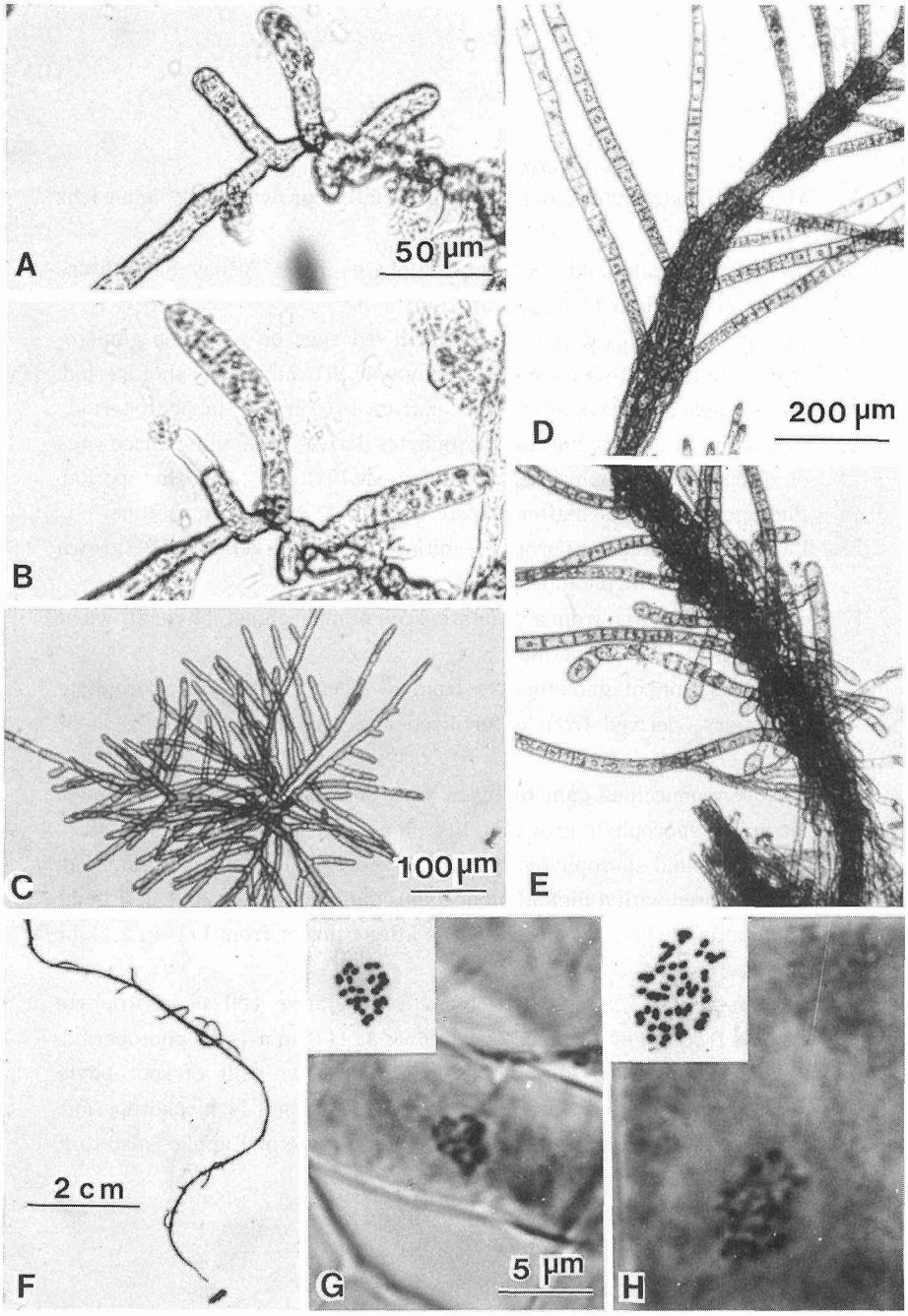

A
50 µm
B
C
100µm
D
200 µm
E
2 cm
F
G
5 µm
H

PLATE VI

Laminaria japonica ARESHOUG

A. Mature gametophytes from 11-day-old culture grown at 10℃ in a 14-hr photoperiod.

B. Immature female and male gametophytes form 26-day-old culture grown at 18℃ in a 14-hr photoperiod.

C. Young sporophytes derived from fertilized eggs on a female gametophyte cultured with a male gametophyte at 10℃ in a 14-hr photoperiod. Photographed 20 days after transfer from 14℃ in a 14-hr photoperiod.

D. Young normal and abnormal sporophytes derived from unfertilized eggs on a female gametophyte cultured alone at 10℃ in a 14-hr photoperiod. Photographed 20 days after transfer from 14℃ in a 14-hr photoperiod.

E. Large elliptical cell (sporophyte initial) on a male gemetophyte grown at 10℃ in a 14-hr photoperiod.

F. Sporophyte derived from a vegetative cell of male gametophyte grown at 10℃ in a 14-hr photoperiod.

G-H. Germination of gametophyte from a vegetative cell of sporophyte (apospory) derived from a fertilized egg grown at 14℃ in a 14-hr photoperiod.

I-J. Diploid monoecious gametophytes with oogonia and antheridia and a tetraploid sporophyte grown at 10℃ in a 14-hr photoperiod.

K. Young triploid sporophytes from fertilized eggs on a female gametophyte cultured with a diploid monoecious gametophyte at 10℃ in a 14-hr photoperiod. Photographed 20 days after transfer from 14℃ in a 14-hr photoperiod.

L. Germination of gametophyte from a vegetative cell of sporophyte derived from an unfertilized egg, grown at 14℃ in a 14-hr photoperiod.

M. Germination of new sporophytes from vegetative cells of sporophyte derived from an unfertilized egg, grown at 10℃ in a 14-hr photoperiod.

Scale in D applies also to B-C & K-L ; scale in J applies also to E & I.

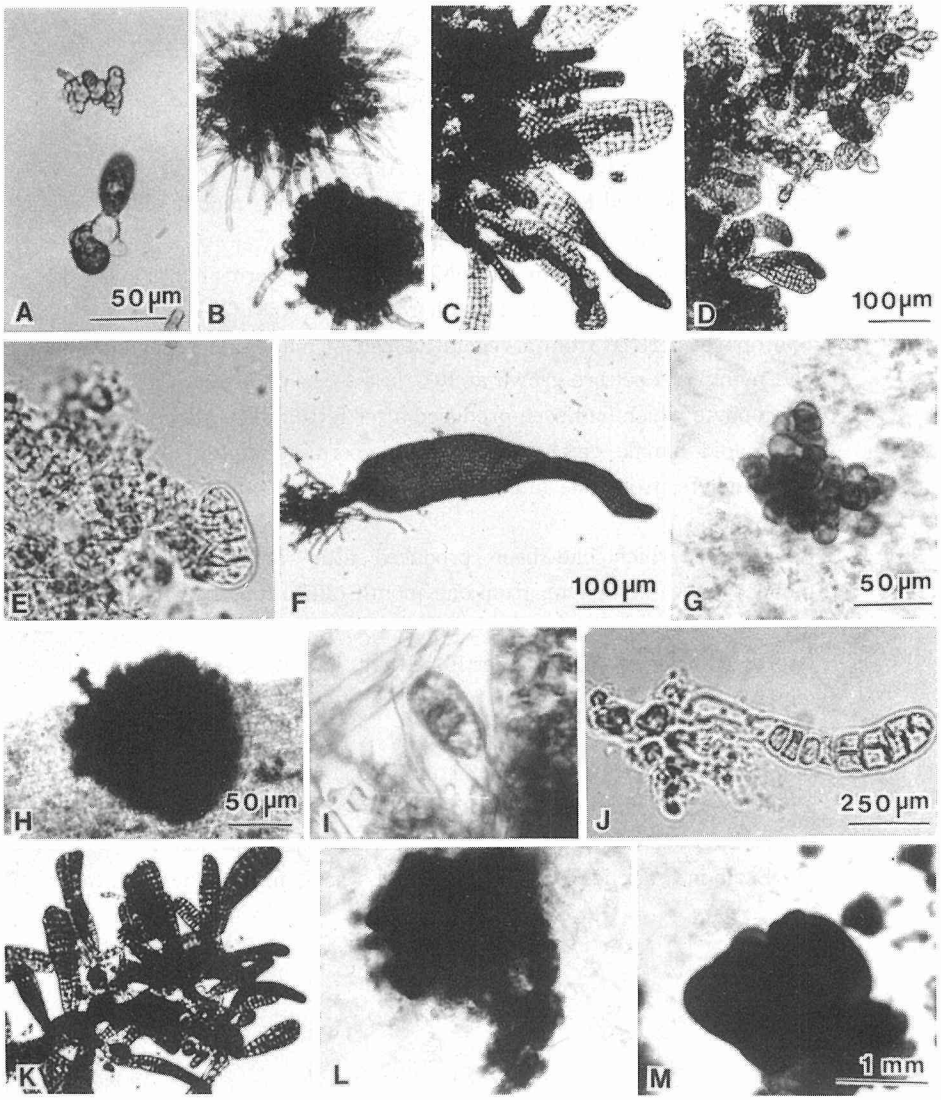

A
50 µm
B
C
D
100 µm
E
F
100 µm
G
50 µm
H
50 µm
I
J
250 µm
K
L
M
1 mm

PLATE VII

Laminaria japonica ARESHOUG

A. Sporophyte derived from a fertilized egg, from one-month-old culture grown at 10℃ in a 14-hr photoperiod.

B. Sporophyte derived from an unfertilized egg, form one-month-old culture grown at 10℃ in a 14-hr photoperiod.

C. Sporophyte derived from a vegetative cell of male gametophyte, from one-month-old culture grown at 10℃ in a 14-hr photoperiod.

D. Sporophyte which had been produced after fertilization between an egg of haploid female gametophyte and a sperm of diploid monoecious gametophyte, from one-month-old culture grown at 10℃ in a 14-hr photoperiod.

E. Sporophyte which had been produced after autogamy of diploid monoecious gametophyte, from one-month-old culture grown at 10℃ in a 14-hr photoperiod.

F. Sporophyte derived from a fertilized egg, having several aposporous gametophytes (arrow) on the bleached area, grown at 14℃ in a 14-hr photoperiod.

G. Sporophyte derived from an unfertilized egg, having many aposporous gametophytes (small arrow) and some new sporophytes (large arrow) on the bleached area, grown at 14℃ in a 14-hr photoperiod.

Scale in C applies also to A-B & D-E; scale in F applies also to G.

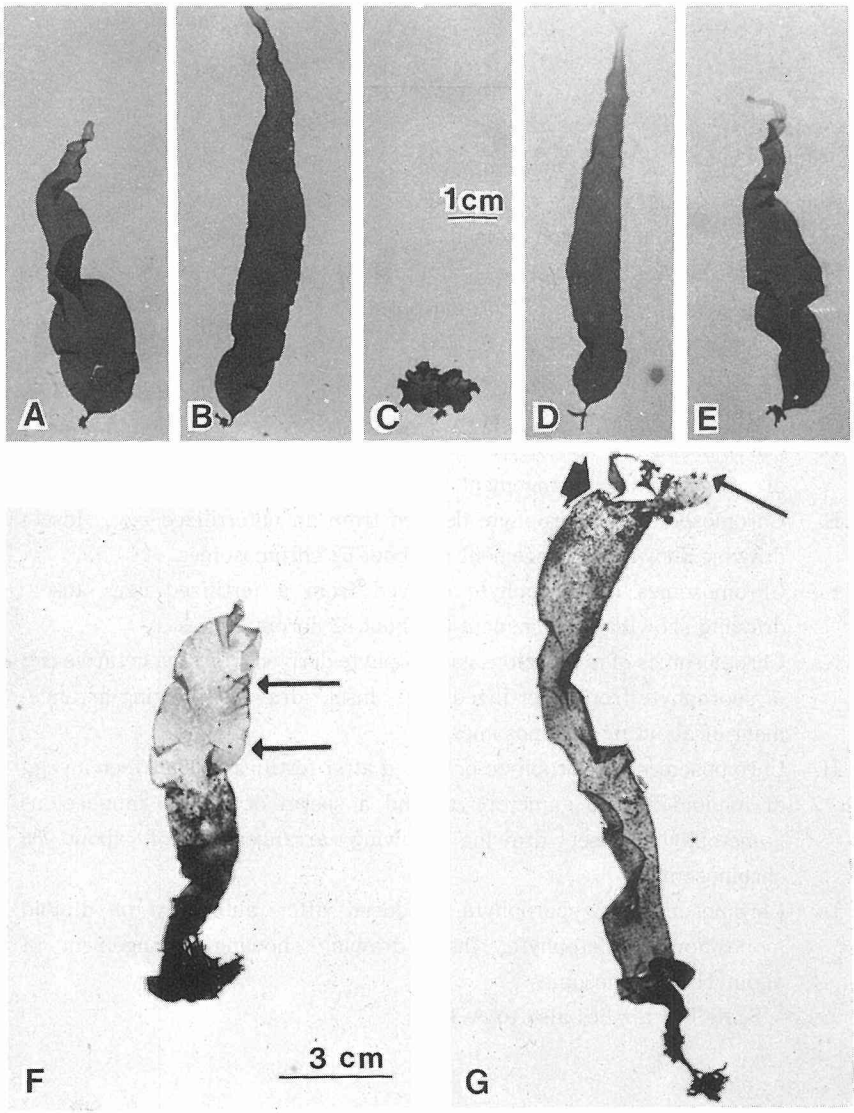

A
B
C
1cm
D
E
F
3 cm
G

PLATE VIII

Laminaria japonica ARESHOUG

A. Chromosomes of male gametophyte. Inset: drawing showing arrangement of about 31 chromosomes.

B. Chromosomes of female gametophyte. Inset: drawing showing arrangement of about 31 chromosomes.

C. Chromosomes of sporophyte derived from a vegetative cell of male gametophyte. Inset: drawing showing arrangement of about 31 chromosomes.

D. Chromosomes of sporophyte derived from an unfertilized egg. Inset: drawing showing arrangement of about 31 chromosomes.

E. Chromosomes of sporophyte derived from an unfertilized egg. Inset: drawing showing arrangement of about 62 chromosomes.

F. Chromosomes of sporophyte derived from a fertilized egg. Inset: drawing showing arrangement of about 62 chromosomes.

G. Chromosomes of monoecious gametophyte derived from a vegetative cell of sporophyte from a fertilized egg. Inset: drawing showing arrangement of about 62 chromosomes.

H. Chromosomes of sporophyte produced after fertilization between an egg of haploid female gametophyte and a sperm of diploid monoecious gametophyte. Inset: drawing showing arrangement of about 86 chromosomes.

I. Chromosomes of sporophyte produced after autogamy of diploid monoecious gametophyte. Inset: drawing showing arrangement of about 114 chromosomes.

Scale in I applies also to A–H.

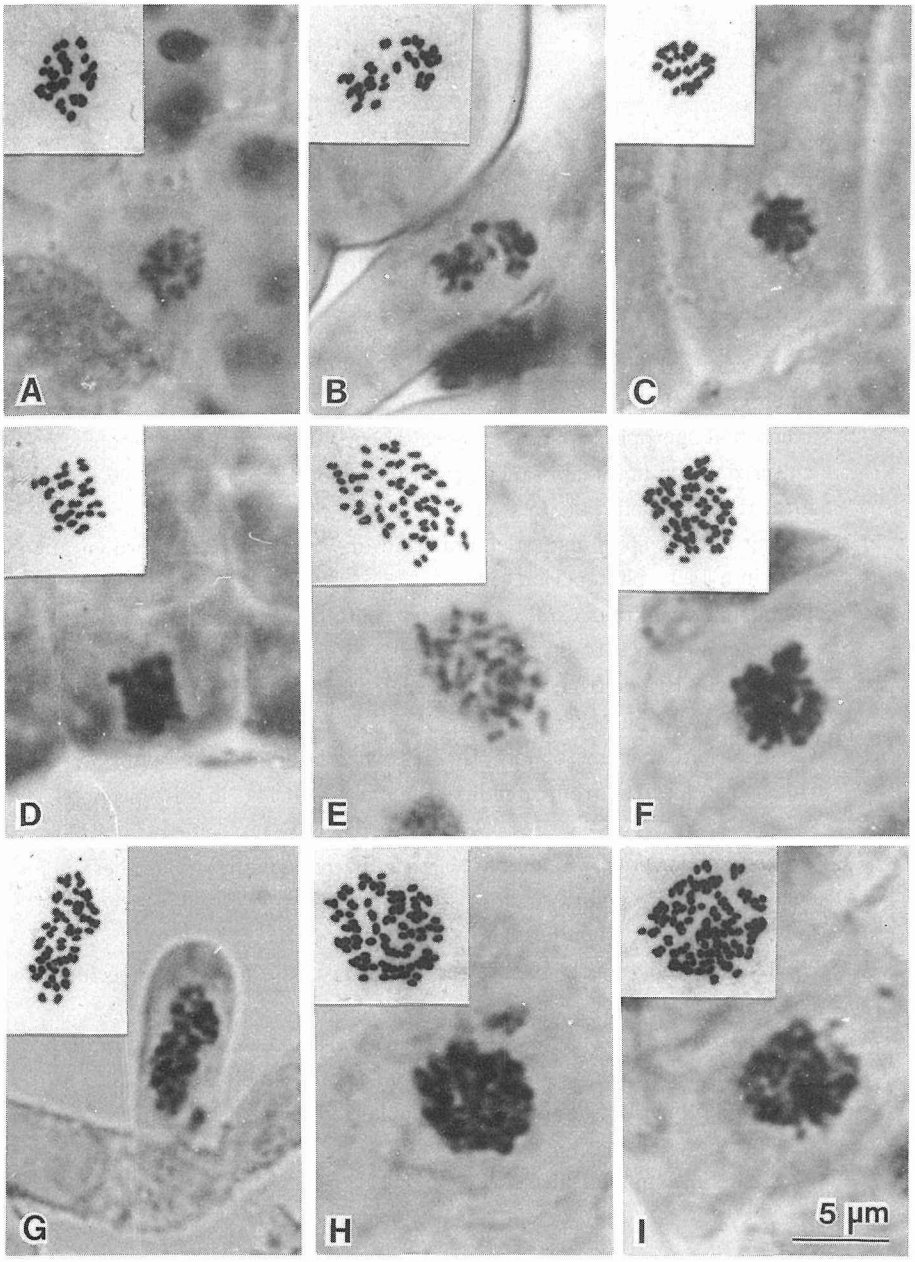

A
B
C
D
E
F
G
H
I
5 µm

PLATE IX

Agarum cribrosum BORY

A. Immature female and male gametophytes from 20-day-old culture grown at 14℃ in a 14-hr photoperiod.

B. Mature gametophytes from 30-day-old culture grown at 10℃ in a 14-hr photoperiod.

C. Young sporophytes derived from fertilized eggs on a female gametophyte cultured with a male gametophyte at 10℃ in a 14-hr photoperiod. Photographed 20 days after transfer from 14℃ in a 14-hr photoperiod.

D. Abnormal sporophytes and degenerated eggs on a female gametophyte cultured alone at 10℃ in a 14-hr photoperiod. Photographed 20 days after transfer from 14℃ in a 14-hr photoperiod.

E. Young normal sporophyte derived from an unfertilized egg, grown at 10℃ in a 14-hr photoperiod.

F. Divided large elliptical cell (sporophyte initial) on a male gametophyte grown at 10℃ in a 14-hr photoperiod.

G. Sporophyte derived from a vegetative cell of male gametophyte grown at 10℃ in a 14-hr photoperiod.

H. Germination of gametophyte from a vegetative cell of sporophyte (apospory) derived from a fertilized egg grown at 14℃ in a 14-hr photoperiod.

I. Diploid monoecious gametophyte with an oogonium and antheridia derived from a vegetative cell of sporophyte from fertilized egg, grown at 10℃ in a 14-hr photoperiod.

J. Young sporophyte produced after autogamy of diploid monoecious gametophyte.

K. Young triploid sporophytes derived from fertilized eggs on a female gametophyte cultured with a diploid monoecious gametophyte at 10℃ in a 14-hr photoperiod. Photographed 20 days after transfer from 14℃ in a 14-hr photoperiod.

Scale in A applies also to B, C, D, H & K.

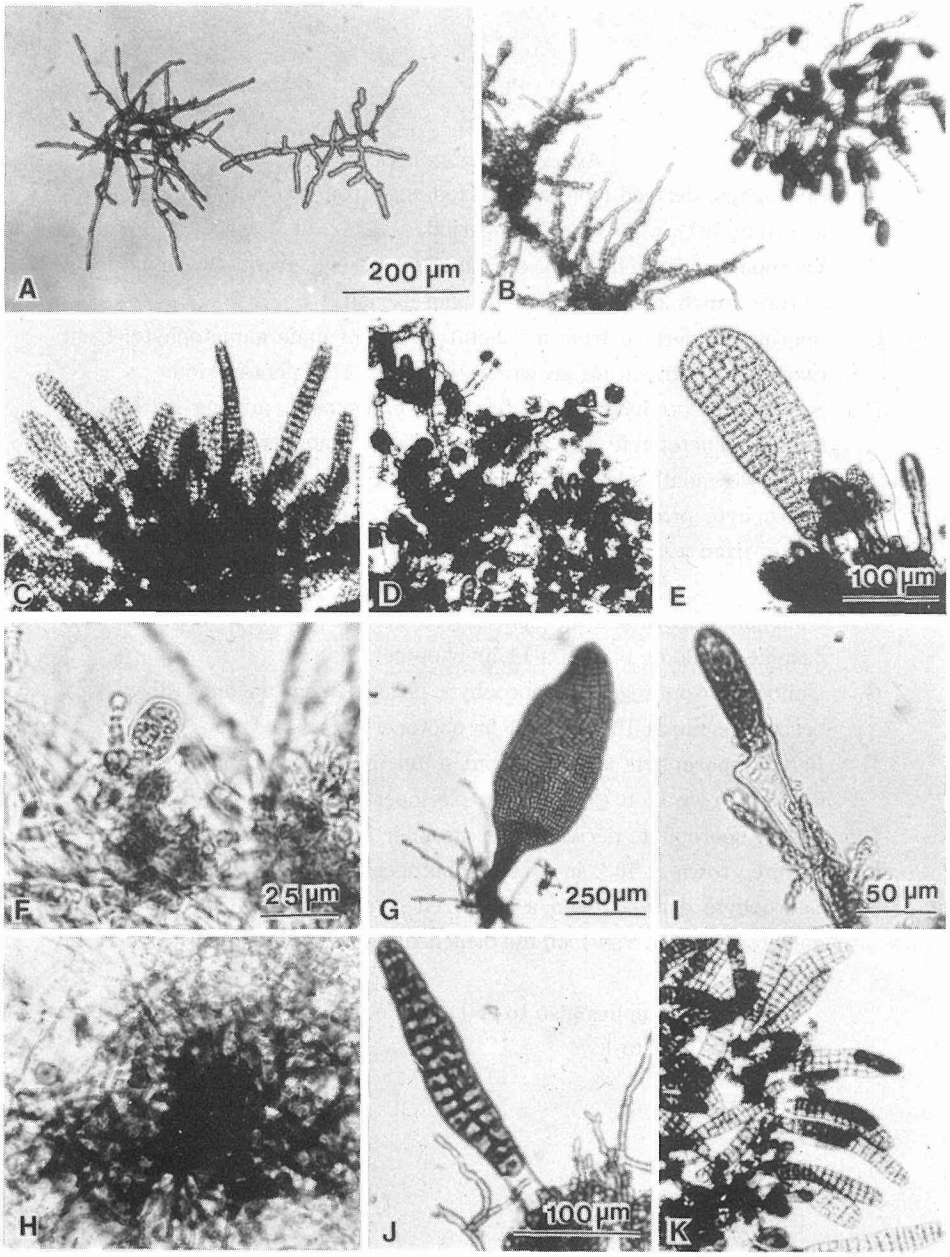

A
200 µm
B
C
D
E
100 µm
F
25 µm
G
250µm
I
50 µm
H
J
100 µm
K

PLATE X

Agarum cribrosum BORY

A. Sporophyte derived from a fertilized egg, from two-month-old culture grown at 10℃ in a 14-hr photoperiod.

B. Sporophyte derived from an unfertilized egg, from two-month-old culture grown at 10℃ in a 14-hr photoperiod.

C. Sporophyte derived from a vegetative cell of male gametophyte, from two-month-old culture grown at 10℃ in a 14-hr photoperiod.

D. Sporophyte produced after fertilization between an egg of haploid female gametophyte and a sperm of diploid monoecious gametophyte, from two-month-old culture grown at 10℃ in a 14-hr photoperiod.

E. Sporophyte produced after autogamy of diploid monoecious gametophyte, from two-month-old culture grown at 10℃ in a 14-hr photoperiod.

F. Unilocular sporangia of sporophyte derived from a fertilized egg, become fertile at 10℃ in a 14-hr photoperiod.

G. Unilocular sporangia of sporophyte derived from an unfertilized egg, become fertile at 10℃ in a 14-hr photoperiod.

H. Fertile sporophyte derived from a fertilized egg, from 8-month-old culture grown at 10℃ in a 14-hr photoperiod.

I. Fertile sporophyte derived from an unfertilized egg, from 8-month-old culture grown at 10℃ in a 14-hr photoperiod.

J. Sporophyte derived from a fertilized egg, having several aposporous gametophytes (arrow) on the bleached area, growing at 14℃ in a 14-hr photoperiod.

Scale in E applies also to A–D; scale in F applies also to G; scale in J applies also to I.

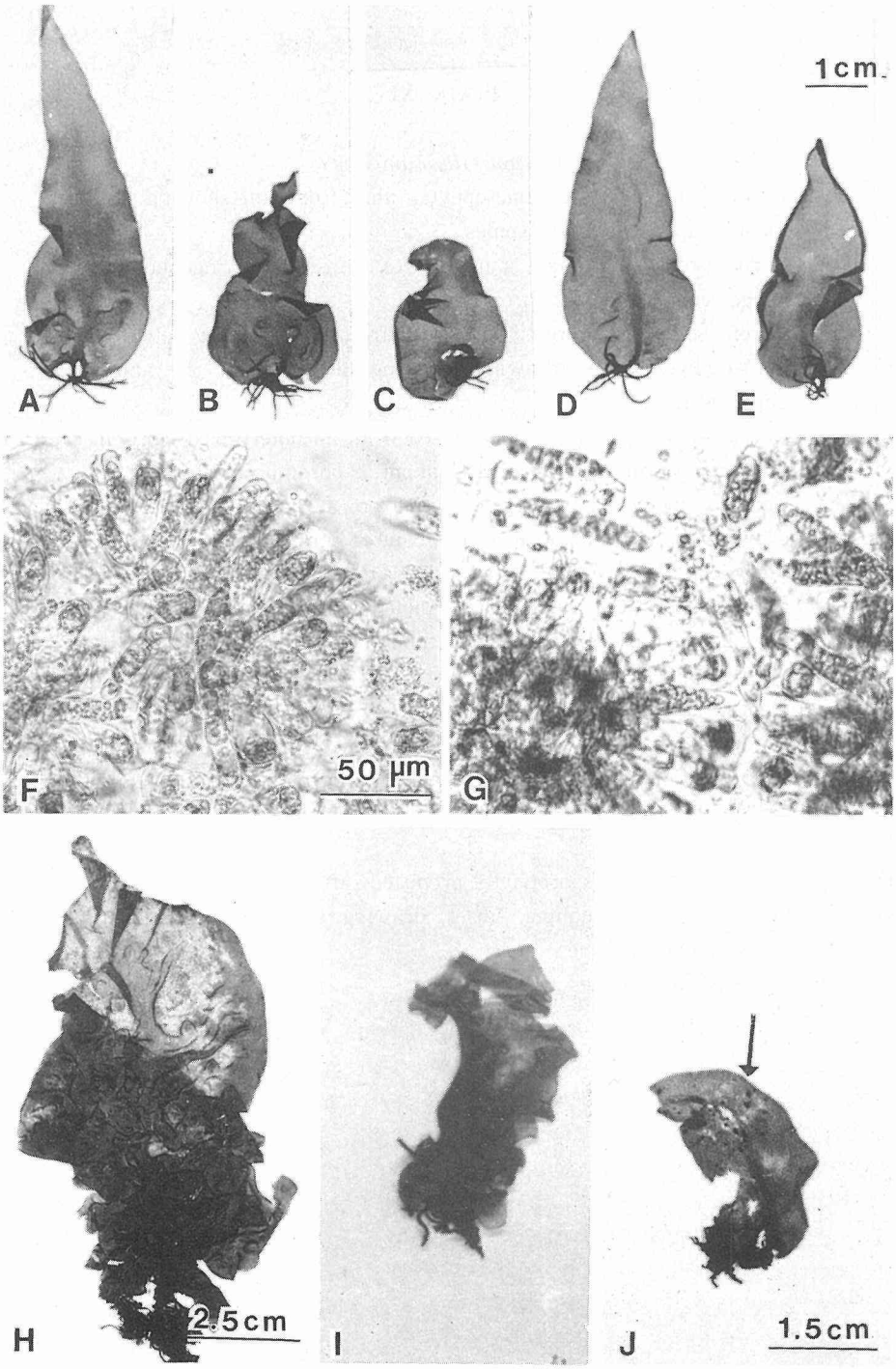

1cm
A
B
C
D
E
F
50 μm
G
H
2.5cm
I
J
1.5cm

PLATE XI

Agarum cribrosum BORY

A. Chromosomes of male gametophyte. Inset : drawing showing arrangement of about 22 chromosomes.

B. Chromosomes of female gametophyte. Inset : drawing showing arrangement of about 22 chromosomes.

C. Chromosomes of sporophyte derived from a vegetative cell of male gametophyte. Inset : drawing showing arrangement of about 22 chromosomes.

D. Chromosomes of sporophyte derived from an unfertilized egg. Inset : drawing showing arrangement of about 22 chromosomes.

E. Chromosomes of sporophyte derived from an unfertilized egg. Inset : drawing showing arrangement of about 44 chromosomes.

F. Chromosomes of sporophyte derived from a fertilized egg. Inset : drawing showing arrangement of about 44 chromosomes.

G. Chromosomes of monoecious gametophyte derived from a vegetative cell of sporophyte from fertilized egg. Inset : drawing showing arrangement of about 44 chromosomes.

H. Chromosomes of sporophyte produced after fertilization between an egg of haploid female gametophyte and a sperm of diploid monoecious gametophyte. Inset : drawing showing arrangement of about 68 chromosomes.

I. Chromosomes of sporophyte produced after autogamy of diploid monoecious gametophyte. Inset : drawing showing arrangement of about 88 chromosomes.

Scale in I applies also to A–H.

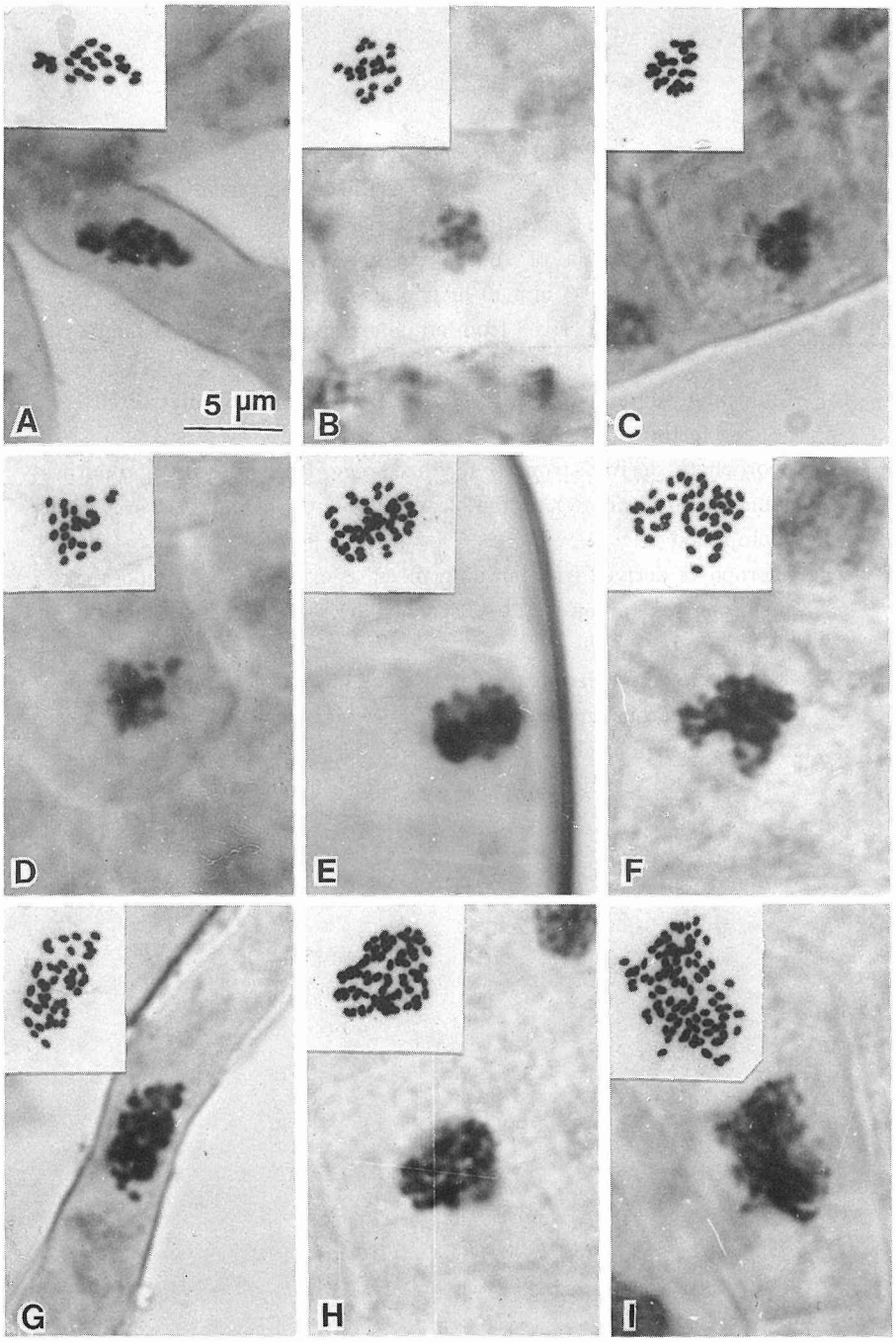

A
5 μm
B
C
D
E
F
G
H
I

PLATE XII

Alaria crassifolia KJELLMAN

A. Fertile sporophyte derived from a fertilized egg, from 10-month-old culture grown at 10℃ in a 10-hr photoperiod.

B. Unilocular sporangia of sporophyte derived from a fertilized egg, become fertile at 10℃ in a 10-hr photoperiod.

C. Fertile sporophyte derived from an unfertilized egg, from 9-month-old culture grown at 10℃ in a 10-hr photoperiod.

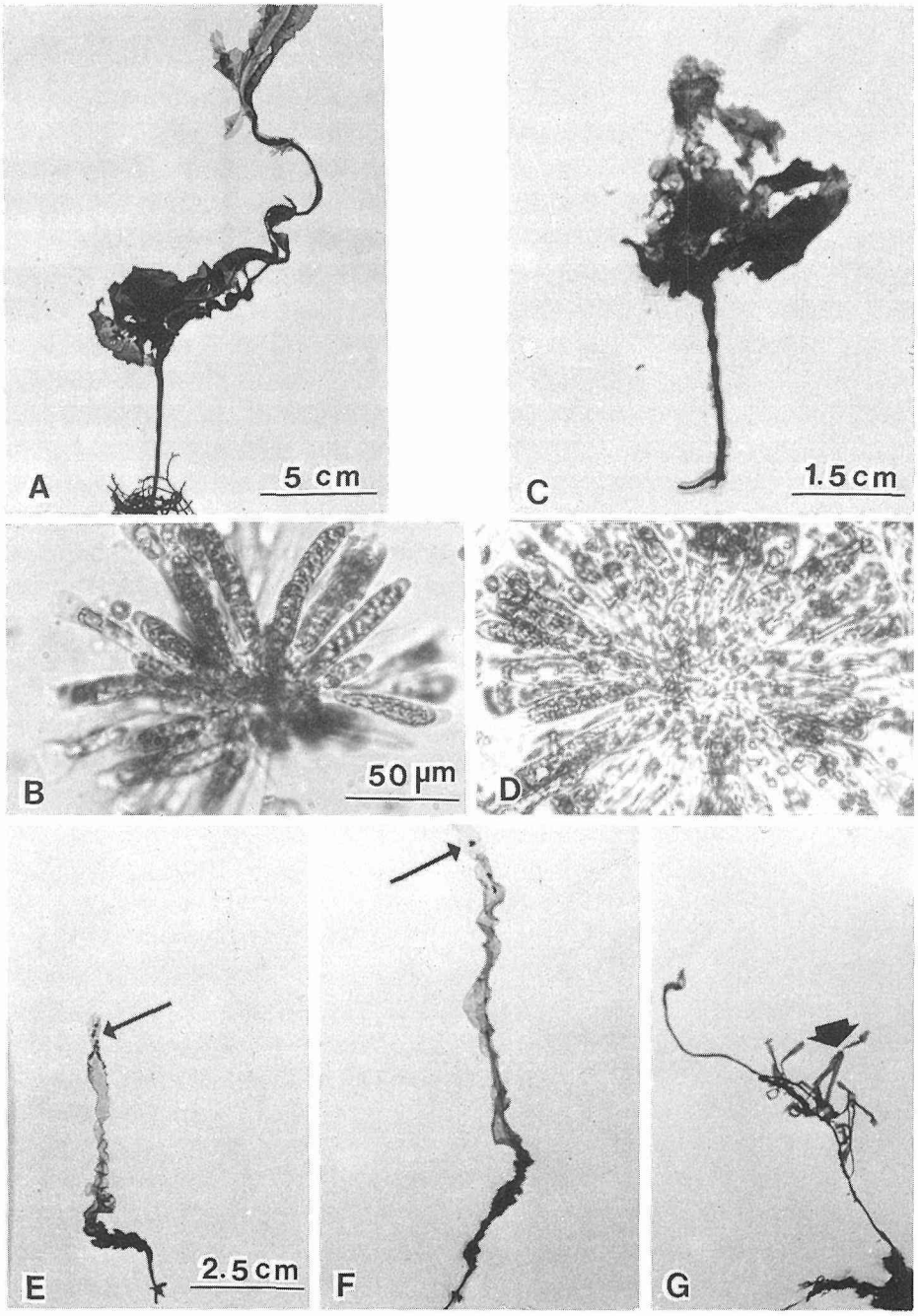
D. Unilocular sporangia of sporophyte derived from an unfertilized egg, become fertile at 10℃ in a 10-hr photoperiod.

E. Sporophyte derived from a fertilized egg, having some aposporous gametophytes (arrow) on the bleached area, grown at 14℃ in a 14-hr photoperiod.

F. Sporophyte derived from an unfertilized egg, having some aposporous gametophytes (arrow) on the bleached area, grown at 14℃ in a 14-hr photoperiod.

G. Sporophyte derived from a vegetative cell of male gametophyte, having some aposporous gametophytes and several new sporophytes (large arrow) on the bleached area, grown at 10℃ in a 14-hr photoperiod.

Scale in B applies also to D; scale in E appiles also to F-G.


A
5 cm
C
1.5 cm
B
50 µm
D
E
2.5 cm
F
G