

## Culture studies on *Caulerpa* (Caulerpales, Chlorophyceae) II. Morphological variation of *C. racemosa* var. *laetevirens* under various culture conditions

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The effects of temperature and light intensity on the morphogenesis of *Caulerpa racemosa* var. *laetevirens* from southern Japan were investigated in unialgal culture under 25 combinations of 5 temperatures (20.0°-30.0°C) and 5 light intensities (0.5-8.0 klux). The morphological variation of the present alga is correlated with environmental factors. The *laetevirens*-type assimilator observed on the mother plants was formed under combinations of low temperatures (20.0°, 22.5°C) and high light intensities (5.0, 8.0 klux). By contrast, under combinations of low and high temperatures (20.0°-30.0°C) and low light intensities (0.5, 1.5 klux) the *peltata*-type assimilator was formed. The intermediate-type between these two types of assimilators was formed under the remaining combinations. The results suggest that *C. racemosa* var. *laetevirens* and *C. racemosa* var. *peltata* are morphological variations (ecophenes or ecads) of a single species.

*Key Index Words:* *Caulerpa*, *Caulerpa racemosa* var. *laetevirens*, *Caulerpales*, *Chlorophyceae*, culture, morphogenesis, morphological variation, plasticity, southern Japan alga.

The genus *Caulerpa* LAMOUROUX is a large group of coenocytic siphonous marine green algae exhibiting a remarkably high degree of morphological variation (plasticity). The thallus is characterized by having a prostrate cylindrical rhizome with branched filamentous attachment rhizoids and assimilators. The assimilator consists of an upright shoot bearing numerous ramuli (branchlets).

The occurrence of intermediate or transitional growth forms between various taxa of *Caulerpa* has often been reported. Moreover, different parts of the same thallus sometimes have features characteristic of two or more taxa. Many investigators

have suggested that this morphological variation may be strongly affected by environmental factors (WEBER-VAN BOSSE 1898, SVEDELIUS 1906, BOERGESEN 1907, GILBERT 1942, EUBANK 1946, TAYLOR 1950, 1960, NIZAMUDDIN 1964, REHM and ALMODOVAR 1971).

TANDY (1934) made field transplant experiments on several taxa of *Caulerpa* and offered evidence that *C. peltata* and *C. fastigiata* were only forms of *C. racemosa*. GILBERT (1942) and EGEROD (1975) emphasized that the use of cultures should prove a most valuable aid to systematic studies in the genus. However, there have been few culture studies on the relationship between morphological variation and environmental factors.

PETERSON (1972) transplanted and cul-

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tured wild plants of several taxa of *C. racemosa* in a light-controlled aquarium. He demonstrated that when a single specimen of *C. racemosa* var. *uvifera* was exposed to different light intensities, it formed new assimilators that were characteristic of other taxa. He stated that the ability of *C. racemosa* to change growth form in altered light environments provides evidence for an environmental control of this kind of morphological variability. Moreover, he suggested that the varieties of *C. racemosa*, such as var. *peltata* and var. *lamourouxii*, should be considered ecological phenotypes (ecophenes or ecads). CALVERT (1976) confirmed these results, showing that when five taxa of *Caulerpa* were transferred into aquaria and cultured with a light intensity lower than that typical of their natural habitat, their newly formed ramuli changed in arrangement from radial to bilateral.

As mentioned above, *Caulerpa racemosa* shows an extreme degree of morphological variability, which has led to taxonomic confusion. In a previous paper on culture studies of reproduction and development in *C. racemosa* var. *laetevirens* (ENOMOTO and OHBA 1987), the authors briefly mentioned the morphological variation of this alga. The present paper provides details of assimilator formation under 25 combinations of temperature and light intensity regimes and offers some taxonomic discussion.

### Materials and Methods

Plants of *Caulerpa racemosa* (FORSK.) J. AG. var. *laetevirens* (MONT.) WEBER-VAN BOSSE (Figs. 1A, 1B) were collected at Muroto-misaki (33°16'N, 134°14'E), Shikoku Island and Ayamaru-misaki (28°28'N, 129°43'E), Amami-ōshima Island in the southern part of Japan. Collections

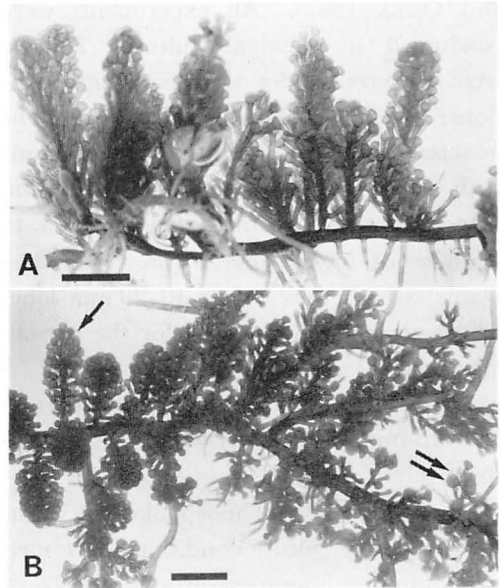


Fig. 1. Wild plants of *Caulerpa racemosa* var. *laetevirens*. A. Typical plant from low tidal mark at Muroto-misaki; B. Morphologically complex plant from 3.0 m depth at Ayamaru-misaki, with *laetevirens*-type assimilators (single arrow) and *peltata*-type assimilators (double arrow). Scale: 10 mm.

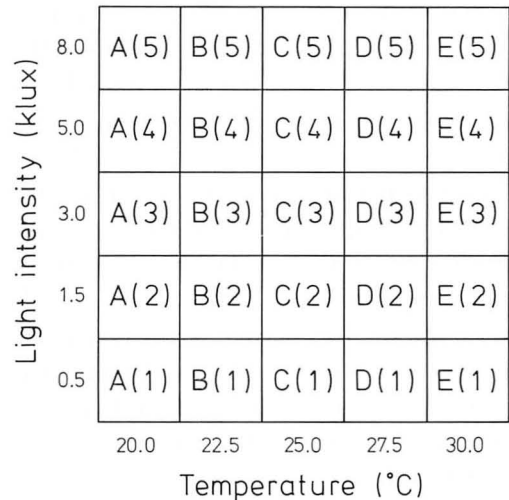


Fig. 2. Schematic representation of the temperature and light intensity gradients used in the present culture experiments. A symbol is given to each combination.

were made during May and July of 1981–1985. The procedures of pre-culture for wild plants and isolation of zygotes are detailed in a previous paper (ENOMOTO

and OHBA 1987). All experiments were conducted in unialgal culture. Zygotes were cultured under the following conditions: 25°C, 1.0–3.0 klux, L:D=14:10 hr in screw-capped glass tubes (18×130 mm) with 15 ml of PROVASOLI'S ES medium. After about two months, zygotes grew to filamentous germlings (protonema-like plants) which were about 10–20 mm long. These germlings were used for the present experiments. Culture strains A and B were isolated from thalli collected at Muroto-misaki and Ayamaru-misaki, respectively.

For the analysis of morphological variation 25 sets of culture conditions were used by combining 5 temperatures (20.0°, 22.5°, 25.0°, 27.5°, and 30.0°C) with 5 light intensities (0.5, 1.5, 3.0, 5.0 and 8.0 klux). These combinations are represented schematically and each is given a symbol in Fig. 2. A daylength of 14 hr (06:00–20:00) was employed. Toshiba daylight fluorescent lamps (6100 K; FL40SD-SDL) were used as the light source. Light intensity was measured at the beginning and the end of an experiment. When germlings reached about 10 mm in length, each was transferred to a separate glass vessel (90×90 mm) containing 350 ml of the same medium (PES). Three vessels were placed under each set of conditions mentioned above. The medium was changed every two weeks. After 4 months, the germlings were analyzed as to assimilator morphology.

The wild plants of *C. racemosa* var. *laetevirens* from Muroto-misaki (Fig. 1A) and Ayamaru-misaki were found to agree well with the description given by OKAMURA (1913, 1936). The rhizome is cylindrical, branched, and intricate, 1.0–3.0 mm in diameter, 10–30 (–50) cm in length. The upright shoots are cylindrical, simple or

rarely branched distally, 20–60 (–100) mm in height, bearing 20–70 (–100 or more) imbricate ramuli. The ramulus is cylindrical with an obtuse or somewhat swollen head, 1.2–6.0 (–8.5) mm in length, 0.5–2.0 mm in diameter at its head, 0.3–0.9 mm in diameter at its base.

On the Pacific coast of Japan, this alga is common between Bôshô Peninsula (35°N, 140°E) and Amami-ôshima Island (28°N, 129°E), but is rare on Okinawa Island (26°N, 128°E) (SEGAWA 1935, OKAMURA 1936, YAMADA and TANAKA 1938). It is usually found on sunny rocks in the lower intertidal zone, but sometimes it grows at a depth of 4–5 m on the waveswept outer margin of the reef where light intensity is relatively high.

Sometimes morphologically complex plants were found (Fig. 1B) in which *laetevirens*-type assimilators were produced by rhizomes exposed to the sun while *peltata*-type assimilators were produced in the shade. Such plants grow at a sun/shade interface, as in hollows and on the undersurface of overhanging rocks in shallow water.

## Results

### *Experiments on strain A*

#### 1. Cross-gradient experiments:

The germlings that had been cultured for two months under 25 different sets of conditions began to form assimilators. After four months, the assimilators were sufficiently mature to allow a morphological analysis to be carried out. The morphology of assimilators varied with the culture conditions (Figs. 3 and 5A).

Three aspects of the assimilators were considered for morphological analyses: shape of the ramuli, arrangement of the ramuli on the upright shoot, and overall appearance. In all instances the rhizomes

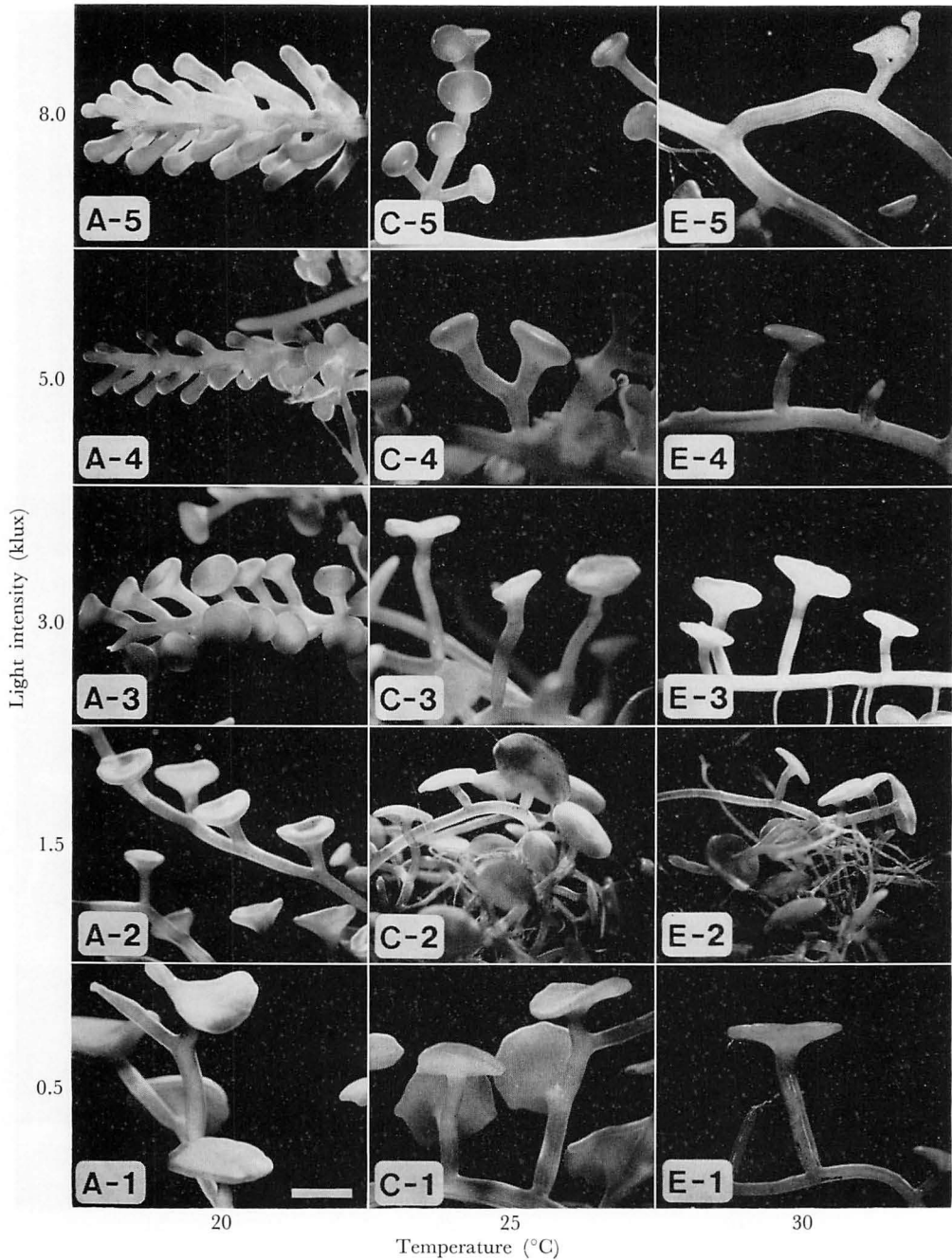


Fig. 3. Morphogenetic response of strain A to the cross-gradients of temperature and light intensity. Scale: 2 mm.

and upright shoots were cylindrical.

Three types of assimilators were recognized:

1) *Laetevirens*-type: The ramuli are cylindrical with obtuse heads and are arranged in a polystichous pattern (tristichous, de-

cussate, or spiroscalate phyllotaxis) on the upright shoot. These two characters appear to be linked. The overall appearance of the assimilators is similar to that of the mother plants. This type developed under 3 sets of conditions, A(4,5) and B(5), i.e.,

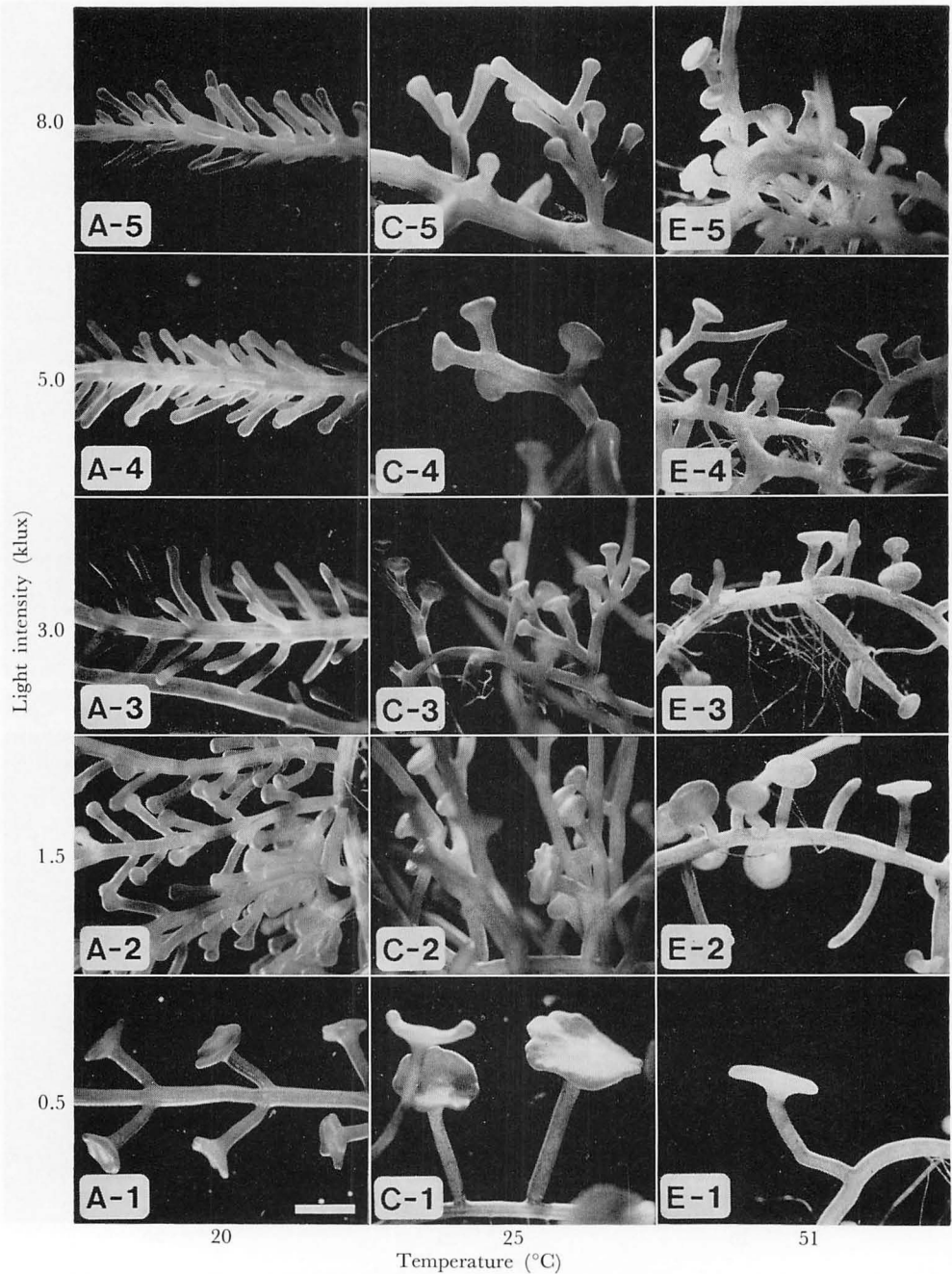


Fig. 4. Morphogenetic response of strain B to the cross-gradients of temperature and light intensity. Scale: 2 mm.

low temperature and high light intensity. This type is shown in Fig. 3 and is represented by the symbol L (C P) in Fig. 5A.

2) *Peltata*-type: The ramuli have the form of a shield, with flat discoidal heads,

and are arranged in a monostichous pattern (solitary or secund) on the upright shoot or the rhizome. The shape of the ramuli was linked to their arrangement on the upright shoot. The overall appearance of the assimilators was similar to that of

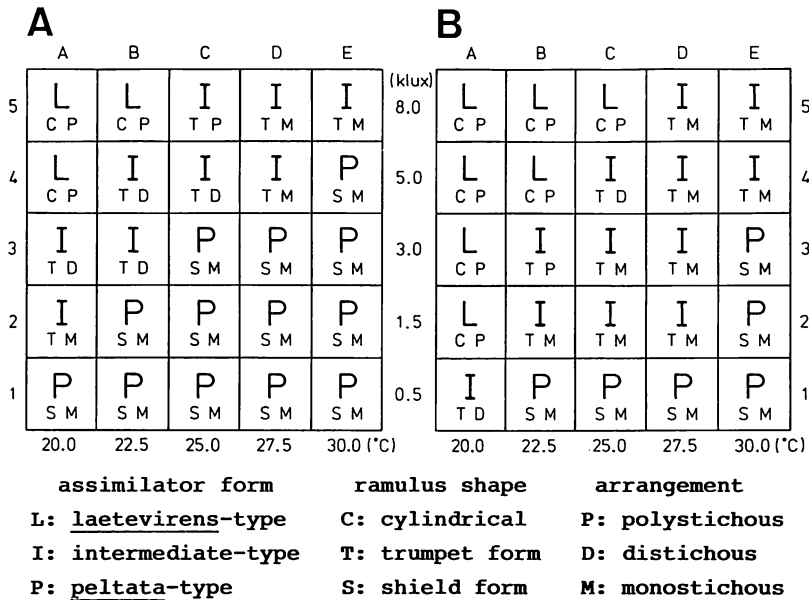


Fig. 5. Diagrammatic representation of the morphogenetic response to the cross-gradients of temperature and light intensity. A. Strain A, B. Strain B.

*C. racemosa* var. *peltata*. This type developed under 13 sets of conditions, A(1), B(1,2), C(1,2,3), D(1,2,3) and E(1,2,3,4), i.e., from low to high temperature and low light intensity. This type is shown in Fig. 3 and is represented by the symbol P (S M) in Fig. 5A.

3) Intermediate-type: The ramuli are trumpet-form with obconical heads. The arrangement of the ramuli on the upright shoots or the rhizomes varied with the culture conditions, allowing the recognition of three kinds of intermediate-type assimilators, as follows:

(a) Intermediate-type assimilator with polystichous arrangement of ramuli. This kind developed under only one set of conditions: C(5), i.e., moderate temperature and high light intensity. It is shown in Fig. 3 and is represented in Fig. 5A by the symbol I (T P).

(b) Intermediate-type assimilator with distichous (opposite or alternate) arrangement of ramuli. This kind developed under 4 sets of conditions: A(3), B(3,4)

and C(4), i.e., low temperature and moderate light intensity. It is shown in Fig. 3 and is represented in Fig. 5A by the symbol I (T D).

(c) Intermediate-type assimilator with monostichous arrangement of ramuli. This kind developed under 4 sets: A(2), D(4,5) and E(5), i.e., low temperature and low light intensity or high temperature and high light intensity. It is shown in Fig. 3 and is represented by the symbol I (T M) in Fig. 5A.

The formation of assimilators was inhibited at 30.0°C, the assimilators being dwarfed.

## 2. Transplant experiment:

When a thallus that had formed *laetevirens*-type (polystichous arrangement) assimilators under 22.5°C, 5.0 klux was transferred and cultured under 20.0°C, 0.5 klux for a month, it formed intermediate-type assimilators on its rhizomes and upright shoots (Fig. 6A).

### Experiments on strain B

#### 1. Cross-gradient experiments:

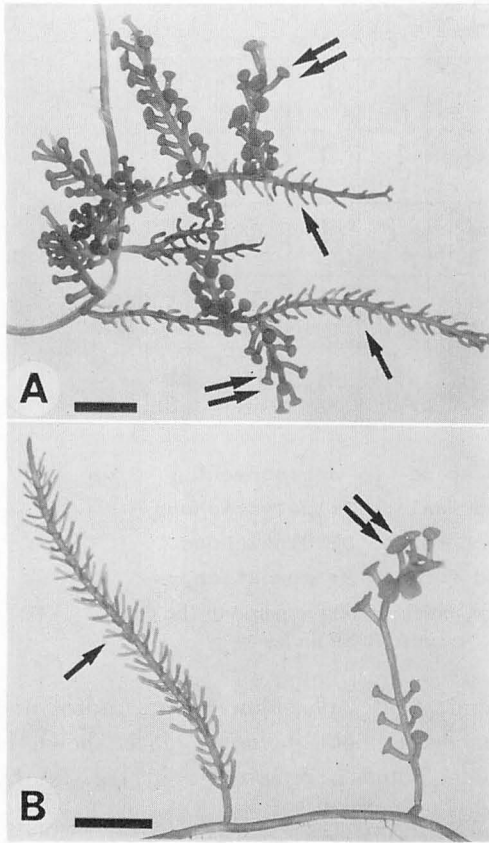


Fig. 6. Transplant experiments. A. Plant (strain A) with *laetevirens*-type assimilators (single arrows) produced in culture under 22.5°C, 5.0 klux after being transplanted to 20.0°C, 0.5 klux. Note newly formed intermediate-type assimilators with trumpet-form ramuli (double arrows). B. Plant (strain B) with a *laetevirens*-type assimilator (single arrow) produced in culture under 20.0°C, 3.0 klux after being transplanted to 22.5°C, 0.5 klux. Note newly formed *peltata*-type assimilators (double arrow). Scale: 10 mm.

The form and arrangement of ramuli and the overall appearance of assimilators produced in strain B under each of the 25 sets of conditions corresponded essentially with those of strain A, but with several minor exceptions. *Laetevirens*-type assimilators occurred under six rather than three sets of conditions and typical *peltata*-type assimilators appeared in only three rather than eight sets of conditions. Results are shown in Figs. 4 and 5B.

## 2. Transplant experiment:

When a thallus that had formed *laetevirens*-type assimilators under 20.0°C, 3.0 klux was transferred and cultured under 22.5°C, 0.5 klux for a month, it formed *peltata*-type assimilators on the original rhizome (Fig. 6B).

### Process of assimilator formation

The development of assimilators of *laetevirens*-type, intermediate-type (with distichous arrangement), and *peltata*-type is shown in Figs. 7 and 8.

1) *Laetevirens*-type: A conical erect shoot was formed on a creeping rhizome (Fig. 8A-a). After 5–7 hr, a pair of tiny whitish outgrowths were formed opposite one another a little below the apex (growing point) of the shoot (Figs. 7A, 8A-b). After about 10 hr, each outgrowth elongated and became an acute, cylindrical, lateral branchlet which curved upward slightly (Figs. 7B, 8A-c,d). Subsequently, it continued to elongate, attaining a size of 0.5 mm diam.  $\times$  2.0–4.0 mm long (Figs. 7C, D, E, 8A-e, f). After about 60 hr, its tip enlarged and developed into a cylindrical ramulus with an obtuse head (Figs. 7F, 8A-g). About 4–5 hr after the appearance of the primary pair of outgrowths, a secondary pair was produced a little below the apex of the upright shoot, i.e., a little above the primary pair. It was formed at an angle of 60°–90° to the primary pair in a horizontal plane (Figs. 7B, 8A-d, e). After about 60–70 hr, the secondary pair of outgrowths developed into ramuli (Figs. 7F, 8A-h). The formation of successive ramuli proceeded in the same sequence (Figs. 7G, 8A-i). About one week after the appearance of the erect shoot, the assimilator had grown to a height of 10–20 mm (Fig. 7H). After 2 weeks, it had grown to 25–30 mm.

2) Intermediate-type with trumpet-form

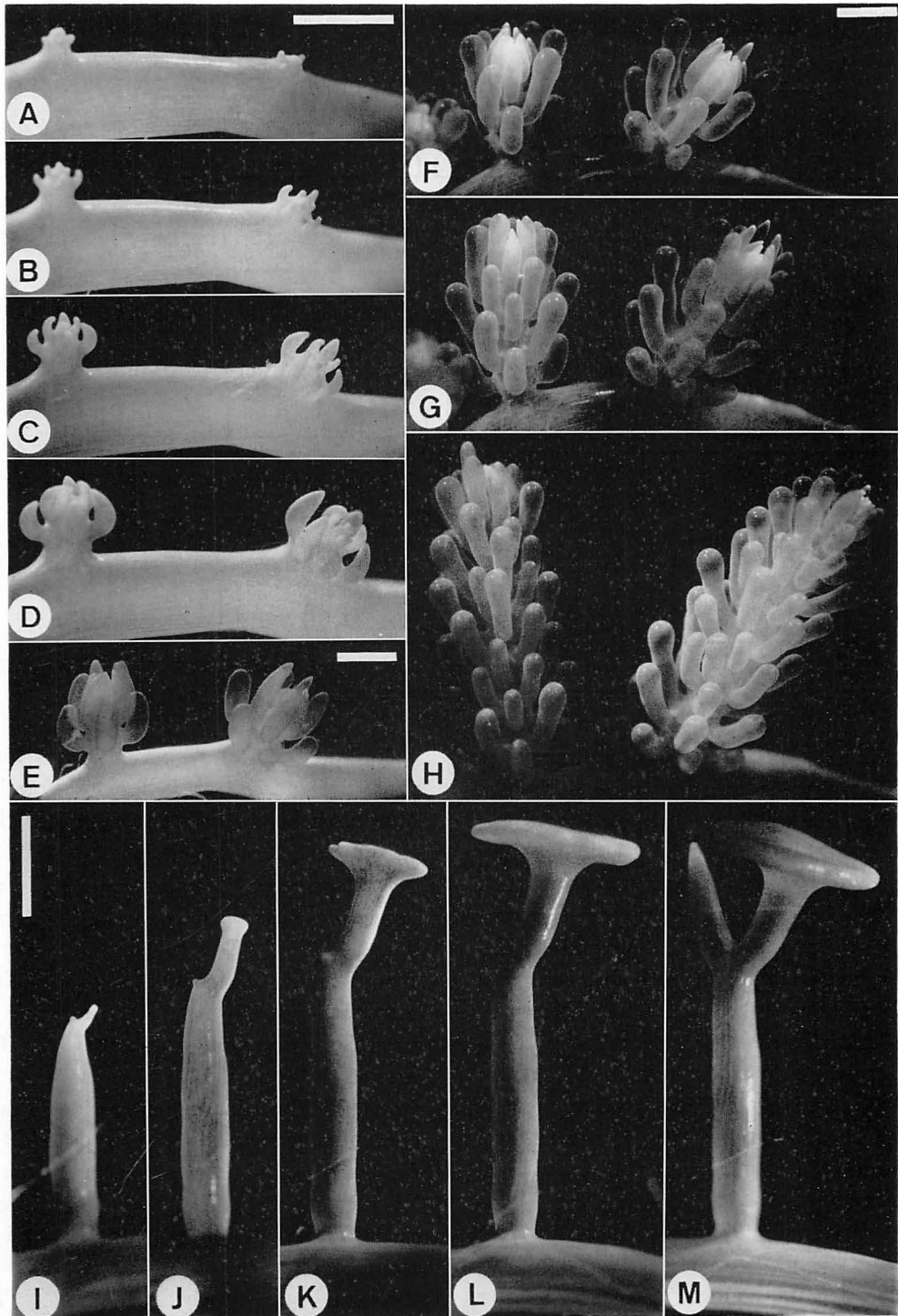


Fig. 7. The process of assimilator formation. A-H. *Laetevirens*-type, I-M. *Peltata*-type. Scale: 2 mm.



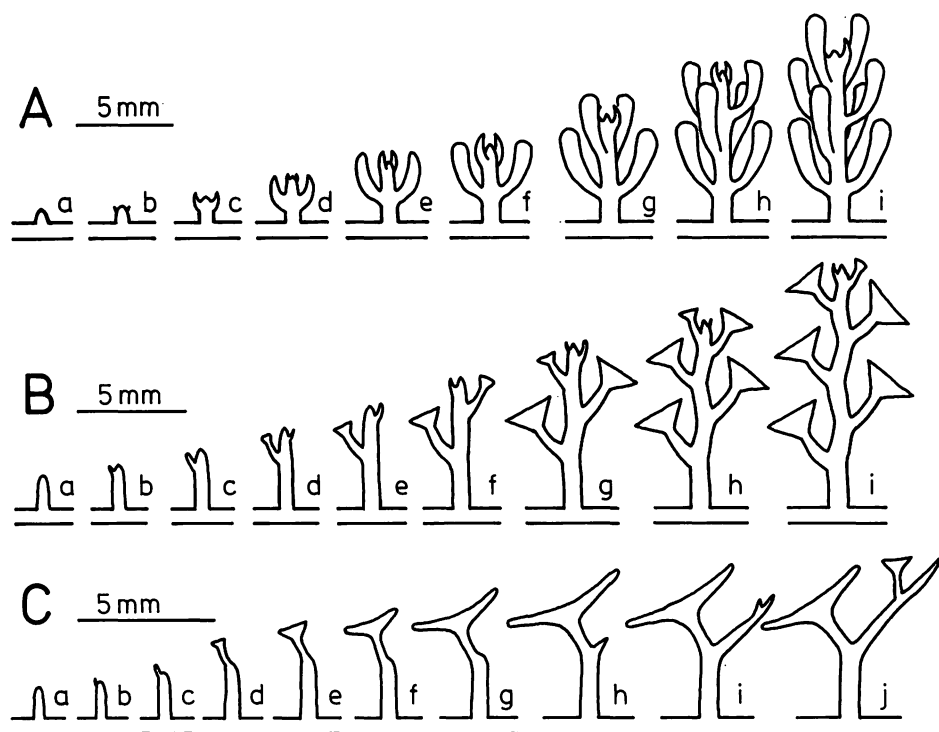


Fig. 8. Schematic representation of the process of assimilator formation. A. *Laetevirens*-type, B. Intermediate-type with trumpet-form ramuli arranged distichously, C. *Peltata*-type.

ramuli: An erect shoot was formed on a creeping rhizome and elongated (Fig. 8B-a). When it grew to a length of 2–3 mm, it produced laterally a tiny whitish outgrowth a little below its apex (Fig. 8B-b). The outgrowth appeared in the morning (around 08:00). After about 10–12 hr, it elongated upward and became a cylindrical protuberance which measured 0.5–1.0 mm in length (Fig. 8B-c). Subsequently, its apex expanded and became an obconical head (Figs. 8B-d, e). After 36–48 hr, it developed into a trumpet-form ramulus which measured 2–3 mm in diameter (Figs. 8B-f, g). During the formation of the primary ramulus, the apex of the erect shoot (growing point) continued to elongate, producing a secondary ramulus in the same sequence (Figs. 8B-e, f). A tertiary ramulus and successive ramuli were formed in the same way (Figs. 8B-

g, h, i). The ramuli are arranged alternately or nearly opposite one another. After about two weeks, the assimilator had grown to 20–25 mm in height.

3) *Peltata*-type: First an erect shoot was formed on the upper surface of a creeping rhizome (Fig. 8C-a). When it grew to about 2 mm in length, a tiny whitish outgrowth was formed a little below its apex (growing point) (Fig. 8C-b). This outgrowth appeared in the morning (around 08:00), after which the erect shoot ceased to elongate. After about 7–8 hr, the outgrowth had attained a length of 0.5–1.0 mm (Figs. 7I, 8C-c). Subsequently, it thickened (Figs. 7J, 8C-d) and its apical portion began to expand into an obconical head (Figs. 7K, 8C-e). The head continued to expand horizontally, becoming a disk measuring 2.0–3.0 mm in diameter (Figs. 8C-f, g). After about

36 hr, the outgrowth had developed into a primary peltate or shield-form ramulus and its head measured 4.0–5.0 mm in diameter (Figs. 7L, 8C-h). Sometimes, when a primary ramulus was completed, the apex of the erect shoot began to elongate again (Figs. 7M, 8C-h, i), producing a secondary and a tertiary ramulus in the same sequence (Figs. 8C-i, j).

## Discussion

The present experiments reveal that the form and arrangement of ramuli in *Caulerpa* may vary considerably with temperature and light intensity. They prove that the morphological plasticity of the present alga is correlated with environmental factors rather than with genetic polymorphism. All cross-gradient experiments were carried out using thalli derived from the same plant at the same time. As shown in Figs. 6A and 6B, when a thallus was transferred into a different set of conditions it developed assimilators of a different shape. The fact that the results of the cross-gradient experiments in strain A are almost identical to those in strain B suggests that morphological plasticity is not the expression of a single genotype and that similar results could be expected if other populations of *C. racemosa* var. *laetevirens* were investigated.

PETERSON (1972) reported that when wild plants of several taxa in *C. racemosa* were transferred into an aquarium, they changed their form in response to different light conditions, and that *C. racemosa* var. *uvifera* f. *intermedia*, after being transferred to high light (21.0 klux), produced *laetevirens*-type assimilators, which were not found on the original plants. CALVERT (1976) also showed that when wild plants of five taxa in *Caulerpa* were kept under low light, the arrangement of their ramuli

varied from radial to bilateral. In the present study, at 20.0° and 22.5°C, the assimilators varied in form with increasing light intensity from *peltata*-type with shield form ramuli through intermediate-type to *laetevirens*-type with cylindrical ramuli. These results suggest that the *laetevirens*-type assimilator is adapted to high light intensity and that the *peltata*-type is adapted to a lower light intensity. SVEDELIUS (1906) thought that radial arrangement was adapted to high light and bilateral arrangement to low light. In the present study, a polystichous arrangement was favored by low temperature and high light intensity.

In the field, *laetevirens*-type assimilators were observed on typical plants of *C. racemosa* var. *laetevirens* growing on sunny rocks in shallow water and on morphologically complex plants growing on sunny rocks in hollows. *Peltata*-type assimilators, on the other hand, were found on typical plants of *C. racemosa* var. *peltata* in deep water and on morphologically complex plants growing on shady rocks in hollows and on the undersurface of overhanging rocks. Light intensity is probably a factor affecting vertical distribution of the three types of assimilators.

In the present experiments, the *laetevirens*-type assimilator appeared at relatively low temperatures (20.0°, 22.5°C), but did not appear at relatively high temperatures, whereas the *peltata*-type appeared at low and high temperatures (20.0°–30.0°C). These results correspond with field observations that plants with typical *laetevirens*-type assimilators are common in relatively cold water at Muroto-misaki and Ayamaru-misaki, but were not observed in warm water at Okinawa Island. Seawater temperature is probably a factor in the geographical distribution of these two types of assimilators. The exist-

ence of morphologically complex plants and the results of the present experiments suggest that *C. racemosa* var. *laetevirens* and *C. racemosa* var. *peltata* are probably ecophenes (ecads) of a single species.

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### 大葉英雄\*・榎本幸人：緑藻スリコギゾタの各種設定条件下での発現形態について

室戸岬および奄美大島産のスリコギゾタの単藻培養体を用い、温度および光照度が形態発現に及ぼす影響を調べた。20°C~30°Cの5温度と0.5~8.0 kluxの5照度を組み合わせ、25の実験区を設定した。低温・高照度(20°C, 8.0 klux)では母藻体と同様のスリコギゾタ型直立部が、高温・低照度(27.5°C, 0.5 klux)では母藻体とは全く異なるタカツキゾタ型直立部が形成される。他の実験区では両者の中間的あるいは移行的な形態を示す直立部が発現した。本藻の著しい形態的変異は遺伝的多型性によるものではなく、生育環境条件によるものと考えられる。またスリコギゾタ、タカツキゾタは生育環境によって生じる同種のエコフェーン(エケード)である可能性が示唆される。(656-24 兵庫県津名郡淡路町 神戸大学理学部臨海実験所。\*現住所: 108 東京都港区港南4-5-7 東京水産大学植物学教室)