Jap. J. Phycol. (Sôrui) 28: 141–150. September 20, 1980

New blade initiation in the perennial red alga *Constantinea rosa-marina* (GMELIN) POSTELS et RUPRECHT (Cryptonemiales, Dumontiaceae)

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LINDSTROM, S. 1980. New blade initiation in the perennial red alga *Constantinea rosa-marina* (GMELIN) POSTELS et RUPRECHT (Cryptonemiales, Dumontiaceae). Jap. J. Phycol. 28: 141–150.

New blade initiation is described for the perennial red alga *Constantinea rosa-marina*. New blades are initiated each autumn from a meristematic region in the center of the previous year's blade. During new blade production cells in the meristematic region begin to elongate in a prescribed sequence and attain lengths to 200 times their original size, thereby forming the medullary filaments of the new blade. A predetermined pattern of cell divisions occurs concomitantly to produce the cortical tissue of the new blade.

New blades are produced under short-day conditions in both the field and the laboratory. A laboratory experiment showed that new blade initiation is halted by long-day conditions.

Key Index Words: Constantinea rosa-marina; *Cryptonemiales*; *development*; *new blade initiation*; *Rhodophyta*.

SETCHELL (1906) distinguished three species of *Constantinea* on the basis of new blade initiation in relation to new stipe production. *Constantinea rosa-marina* (GMELIN) POSTELS et RUPRECHT and *C. simplex* SETCHELL were separated from *C. subulifera* SETCHELL on the basis that the blades of the former two species are peltate (circular, having the stipe attached to the lower surface at the center), "becoming perfoliate only upon the appearance of a new lamina, while in *C. subulifera* the blades are circular and perfoliate from the very beginning." POWELL (1964) and ABBOTT (1968) have described new blade initiation in *C. subulifera* and *C. simplex*, respectively. However, nothing has yet been written on new blade initiation in *C. rosa-marina*, the type species of the genus.

Constantinea rosa-marina is a perennial red alga which arises as a multiaxial blade from a cushion-like disc. Early in its development the blade becomes peltate, and the plant maintains this form throughout its life span of up to 18 years or more (the age of the oldest plant collected during this study).

A new blade is produced each fall in the center of the old blade, and it is this terminal blade which produces the reproductive structures. Following reproduction, the outer reproductive part of each blade is shed, and the inner part of the blade gradually erodes so that after a few years an "annual ring" on the stipe is all that remains of a blade.

In order to better understand the relationship between the species of *Constantinea*,

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I undertook a comparative study of the two species occurring in Japan, *C. rosa-marina* and *C. subulifera*. Since POWELL (1964) has already studied *C. subulifera* rather thoroughly in Puget Sound and it is the less common of the two species in Japan, I concentrated on *C. rosa-marina*. In this paper, I describe the initiation of new blades in *C. rosa-marina*. Reproductive structures and reproductive strategy will be described in a subsequent paper.

Materials and Methods

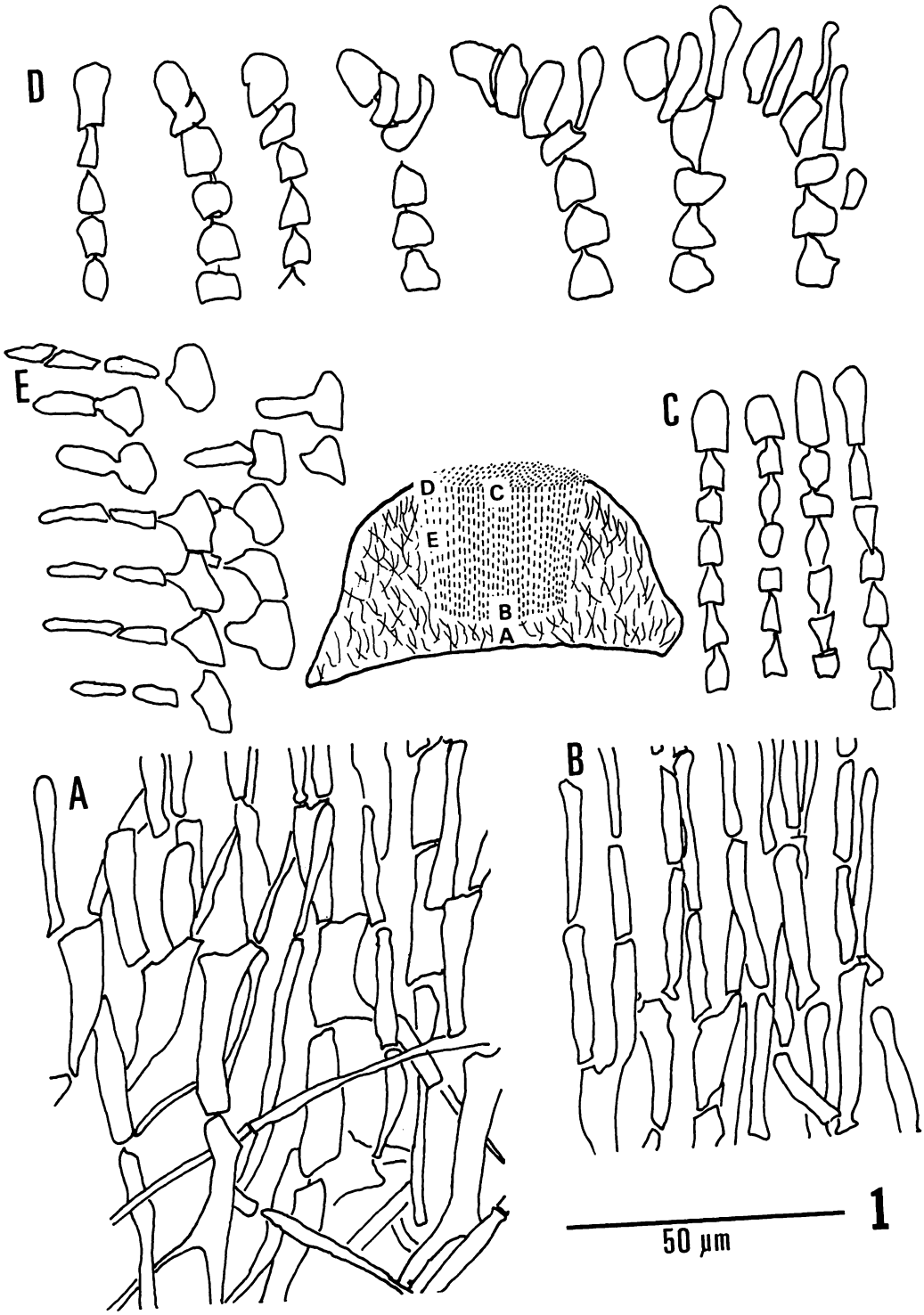
In Japan, *C. rosa-marina* and *C. subulifera* occur along the eastern coast of Hokkaido from Cape Nosappu (43°23′N lat., 145°49′E long.) to Cape Erimo (41°33′N lat., 143°8′E long.). In the present study, *C. rosa-marina* was found subtidally at depths of 4–10 m, and *C. subulifera* from the low intertidal zone to 2 m subtidally, except for one male plant that was cellected at a depth of 5 m at Cape Nosappu. According to Prof. T. MASAKI (pers. comm.), *C. rosa-marina* can be collected in the extreme low intertidal zone of Daikokujima, an offshore island in the cold current region of eastern Hokkaido. Specimens observed during the course of this study came from the following collections:

Date	Location	Depth	Collector
May 26, 1978	Kushiro	4–10 m	N. TAZAWA
June 6, 1978	Cape Nasappu	0–5 m	Author
Aug. 5, 1978	〃 〃	Cast ashore	M. KUROGI
Sept. 5, 1978	〃 〃	〃 〃	Author
Oct. 17, 1978	〃 〃	〃 〃	〃
Nov. 29, 1978	〃 〃	〃 〃	〃
Jan. 26, 1979	〃 〃	〃 〃	〃

Plants were preserved in 10% formalin in seawater. Sections of the meristematic region were made using a freezing microtome, and glycerine-mounted sections were stained with Cotton blue for microscopic examination.

Plants for the photoperiod experiment on new blade initiation in *C. rosa-marina* were collected September 5 at Cape Nosappu. The upper blade and stipe were removed from the rest of the plant. The upper blades were then trimmed so that a rim less than 1 cm wide remained around the stipe. Plants were put into individual 200 m*l* glass storage jars with PROVASOLI's ES medium (PROVASOLI 1968) and maintained in 10°C culture rooms. Eight plants were placed under a $16:\overline{8}$ photoregime, and eight under an $8:\overline{16}$ photoregime. Several plants became moribund during the first month and were terminated. Medium was changed every five days for the first ten weeks and approximately every ten days to three weeks thereafter. Epiphyte growth, especially for plants under long-day conditions, necessitated periodic cleaning of the plants.

Fig. 1. Cross section of mound in center of *Constantinea rosa-marina* blade showing: A. construction of mound below the meristematic zone showing similarity to the blade and stipe below it; B. construction of mound showing transition to meristematic cylinder; C. construction of apical region of meristematic cylinder before actual new blade initiation; D. sequence from left to right of an apical cell switching from transverse to oblique division with the immediately subapical cells subsequently cutting off cells to fill in the space created by deflection of the original apical cell; E. lateral branching of cells of the meristematic cylinder with several branch apices already beginning to divide obliquely.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Results

Morphological observations of C. rosa-marina

New blade initiation in *C. rosa-marina* can be divided into two phases: preparatory and actual. The preparatory phase begins during the summer months when a small mound of tissue appears in the center of terminal blades of *C. rosa-marina* immediately above the stipe. This mound produces a transition from the branched filamentous construction of the blade and stipe below it (Fig. 1A) to the unbranched filamentous construction of a solid cylinder of meristematic tissue at the top of the mound (Fig. 1 C). As the meristematic cylinder increases in depth, the apical cells cut off shorter and shorter intercalary cells so that the earlier formed cells at the base of the meristematic cylinder average about 18 μm in length (Fig. 1 B) compared to 6.5 μm for cells just below the apical cells (Fig. 1 C) just prior to actual new blade initiation.

Actual blade initiation involves three processes: (1) branching of the apical cells of the meristematic cylinder to form the cortex of the upper surface of the blade, (2) elongation of the intercalary cells of the meristematic cylinder to form the medulla of the blade and stipe, and (3) branching of the intercalary cells of the meristematic cylinder to form (a) the cortex of the lower surface of the blade and stipe, (b) the secondary cortical filaments of the blade which cross from cortex to cortex, and (c) the secondary medullary filaments of the stipe. These processes are initiated in a prescribed sequence but, once initiated, occur simultaneously.

The first sign of actual blade initiation occurs when the meristematic cylinder is about 75–90 cells deep. At this time, the apical cells of the meristematic cylinder begin to branch (Fig. 1 D). This occurs in each apical cell by the formation of an oblique rather than a transverse cell wall. The apical cell is cut off toward the outside of the cylinder, and the immediately subapical cell begins to elongate at its distal end to fill the space left by the oblique division of the apical cell. Eventually, the distal protrusion of this subapical cell is cut off to form another apical cell, which begins to divide obliquely as the initial apical cell also continues to do. Other subapical cells (to 3–4 cells below the cell in which an oblique division first occurred) may also cut off new apical cells in a similar manner, the net effect being the creation of a layer of cortical tissue which is being pushed out from the center of the new blade by production of new apical (cortical) cells.

As the blade begins to grow in diameter, the intercalary cells of the meristematic cylinder elongate in a prescribed pattern to keep pace with the proliferation of apical cells, and stipe elongation occurs concomitantly (Fig. 2).

As the processes described above continue, the cortex of the lower surface of the new blade is initiated by cells near the outer edge of the meristematic cylinder producing lateral branches at right angles (Fig. 1 E). These lateral branches continue to elongate by transverse division, but once they reach the outer surface of the new blade their apical cells begin to divide obliquely in the same manner as the apical cells of the upper surface of the blade (i. e., with the apical cell always cut off toward the margin of the new blade). The formation of these lateral branches keeps pace with production of new cortical cells on the upper surface of the new blade. In addition to forming the cortex of the lower surface of the new blade, lateral branches developing from the outer edge of the meristematic cylinder near its base form the cortex of the stipe, and lateral branches arising from cells within the meristematic cylinder become the filaments which cross from cortex to cortex. Cells of these filaments may form secondary pit connections with other cortical cells. In the center of the meristematic cylinder, these lateral branches tend to grow downward and form the secondary medullary filaments

of the stipe.

By the end of May, when blade and stipe growth appeared to have ceased, the medullary cells of the stipe averaged about 150–500 μm in length (depending on their position and the length of the stipe), whereas most of the medullary cells in a typical blade (radius = 70 mm) ranged from 1100–1500 μm in length, with some cells over 2 mm in length. From October to May cells near the base of the meristematic cylinder therefore elongated only about 10 times, from about 18 μm to 160–230 μm, but those originally near the top of the meristematic cylinder elongated about 200 times, from about 6.5 μm to 1100–1500 μm. Cell width increased by only 4–8 times to about 20–30 μm during the same period.

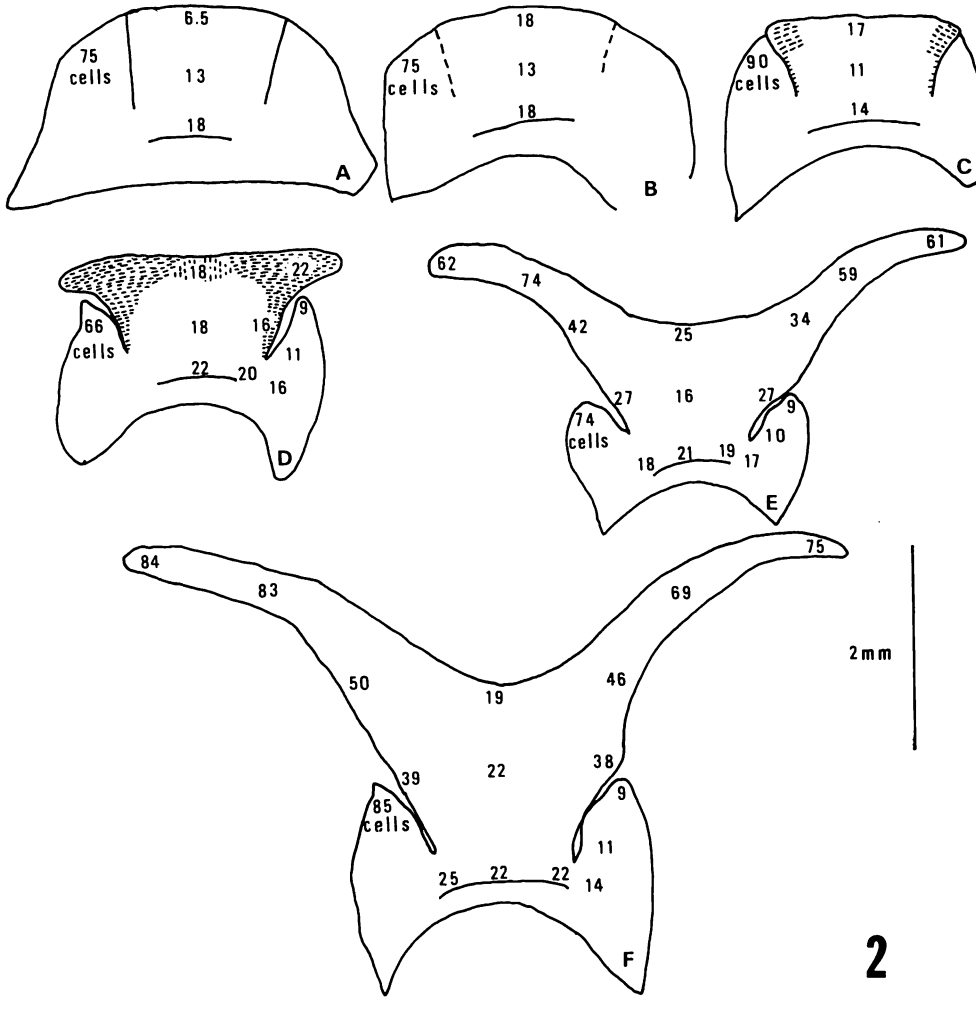

Fig. 2. A series of cross sections of the mound in the center of the *C. rosa-marina* blade showing new blade production and the concomitant elongation of cells in various parts of the meristematic cylinder. Broken lines in B–D indicate areas where lateral branches are beginning to form. Numbers indicate average lengths in μm of 10 cells in that part of the meristematic cylinder. The depth of the meristematic cylinder at the time apical cell branching was initiated is indicated on the left of each mound. Numbers in the lower right corner of D–F show cell lengths of peripheral cells of the meristematic cylinder which did not take part in new blade production.

The oblique cell divisions responsible for forming the upper surface of a *Constantinea* blade occur about 14–16 times as indicated by the number of cell rows exhibiting that kind of branching in the center of the new blade just above the stipe. Since the cortex of *Constantinea* is only 6–8 cells thick, the first 8–10 rows of cortical cells cut off by oblique divisions must have elongated together with the intercalary cells of the meristematic cylinder to help form the medullary filaments of the blade.

Morphological observations of C. subulifera

Compared to *C. rosa-marina* the new blades of *C. subulifera* fall apart much more readily when preserved and sectioned. It was therefore not possible to obtain a similar sequence of early blade development stages in *C. subulifera* as in *C. rosa-marina*. However, some comparisons can be made:

The new blade is initiated at the top of the stipe which protrudes from the previous year's blade. A meristematic zone develops at the top of the stipe similar to that in *C. rosa-marina* except that it occurs in a slight depression (probably formed by the cells comprising the meristematic zone elongating less than the surrounding stipe cells). Also, the meristematic zone was only about 50 cells deep in the specimens examined. Development of the new blade appears to proceed as in *C. rosa-marina* except that fewer cells at the base of the meristematic zone contribute to growth in stipe length, most contributing directly to new blade production. Because of the delicate nature of mature *C. subulifera* blades, it was not possible to determine the final lengths of the medullary filaments derived from the cells of the meristematic zone as was done in *C. rosa-marina*.

Field observations

In mid October, I observed a wide variety of stages in new blade initiation in *C. rosa-marina*. Some specimens appeared to show no further development than plants collected in early September. However, some specimens showed evidence of very young blades with diameters up to 3.2 mm, hardly as wide as the stipe itself! By late November, all specimens had new blades. In contrast, new blades of *C. subulifera* first appeared in late September or early October. The following table shows average new blade diameter, old stipe length, and new stipe length for terminal blades of specimens of *C. rosa-marina* and *C. subulifera* collected in the fall and early winter.

	Sept. 5	Oct. 17	Nov. 29	Jan. 26
C. rosa-marina			n=72	n=56
new blade diameter[1]	0	(3.2 max)	9.9±4.5	31.5±11.9
old stipe length[2]	~1.5	~1.5	~1.5	~1.5
new stipe length	0	0	0.5	3.8±1.1
C. subulifera	n=20	n=13	n=10	n=35
new blade diameter	0	10.6±5.0	30.8±9.2	56.3±16.6
old stipe length	6.2±1.5	7.8±1.7	NM[3]	6.8±2.1
new stipe length	0	0	NM	2.5±1.4

1) All measurements in mm.
2) Although the mound of tissue cannot technically be called a stipe, it contributes to overall stipe length since the new blade arises out of the upper surface of the mound.
3) NM=not measured.

Photoperiod experiment

Under laboratory conditions, one short-day plant terminated October 12 showed early signs of new blade initiation (i. e., branching of the apical cells and production of laterals along the upper outer edge of the meristematic cylinder), but the first macroscopic evidence of blade initiation in short-day plants did not appear until late October or early November.

When it was evident that all short-day plants were producing new blades but no long-day plants were, the cultures were rearranged so that all long-day plants were moved to short-day conditions, and some of the short-day plants were moved to long-day conditions. Long-day plants moved to short-day conditions showed macroscopic

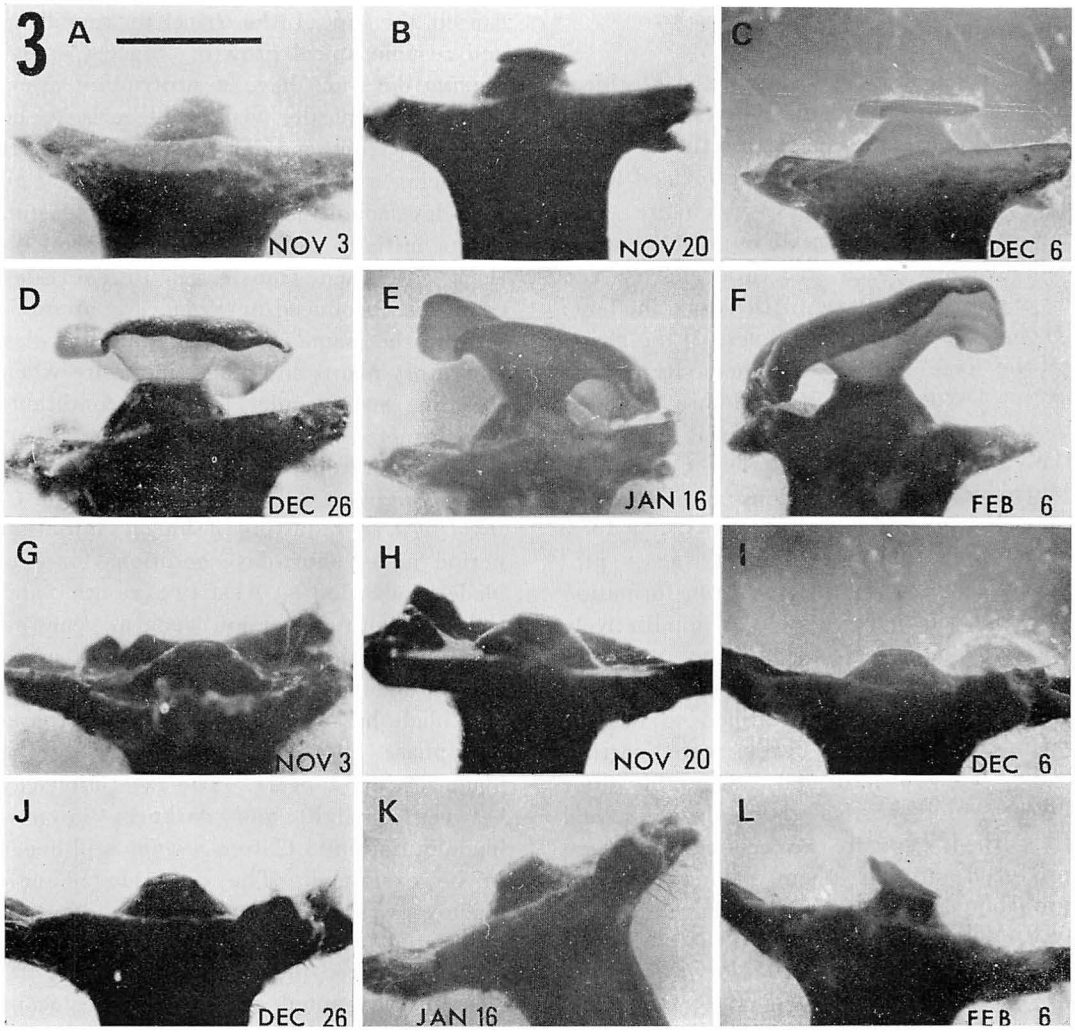

Fig. 3. A–F. Blade center cultured under short-day ($8:\overline{16}$) conditions from Sept. 6-Dec. 6 and under long-day ($16:\overline{8}$) conditions from Dec. 6-Feb. 6. G–L. Blade center cultured under long-day conditions from Sept. 6-Dec. 6 and under short-day conditions from Dec. 6-Feb. 6. Photographs taken after 8 weeks (Nov. 3), 11 weeks (Nov. 20), 13 weeks (Dec. 6), 16 weeks (Dec. 26), 19 weeks (Jan. 16), and 22 weeks (Feb. 6). Scale = 5 mm.

evidence of new blade initiation after another two months. This period of time corresponded to how long it had taken plants in nature and the plants originally cultured under short-day conditions to initiate new blades after the collection date (Fig. 3). Growth of blades initiated under short-day conditions slowed and eventually appeared to halt when moved to long-day conditions (Fig. 3).

Discussion

As DIXON (1971) has stated, the Florideophycideae are particularly well suited to morphological analysis of growth and development because all thalli are formed by an aggregation of filaments, which are, with few exceptions, produced by strictly apical cell division. Such structural analysis can be expressed in terms of three parameters: (1) the disposition of the axes, (2) the shape of the axes, and (3) the longevity of the axes. DIXON (1971) himself has confined most of his analyses to uniaxial species in the Nemalionales and the Ceramiales. Morphological observations of multiaxial taxa (NORRIS and KIM 1972; CODOMIER 1972) have shown that medullary tissue is produced sequentially by the transformation of older cortical tissue. A qualitatively different situation appears to exist in *Constantinea rosa-marina* and *C. subulifera*. Here, cells which are destined to become medullary filaments never were cortical cells. They are formed before the outer investment of cortical tissue develops, and they then elongate as cortical cells are produced around them. This situation probably arose because of the peltate and perennial nature of the genus.

BOWEN (1971) states that *Maripelta rotata* (DAWSON) DAWSON, a peltate perennial red alga from southern California and Mexico, undergoes repeated cycles of blade abscission and new blade formation, which she states is unique in the Rhodophyta but nevertheless shows some similarity to vesicle formation in the closely-related genus *Botryocladia*. NEUSHUL *et al.* (1967) have cultured *Sciadophycus stellatus* DAWSON, another peltate red alga from southern California and Mexico, but no work has been done on new blade initiation in this species.

POWELL (1964) found a critical daylength of about 11–14 hours for between 21 and 28 days was required to completely trigger new blade initiation in *C. subulifera*. Longer daylengths, interruption of the dark period, or a shorter initiation period all caused the tip of the stipe to round up and resume apical growth. Since *C. rosa-marina* does not have a protruding stipe, no macroscopically visible effect is to be expected from moving a plant not yet producing a new blade from short-day to long-day conditions. In my experiment, plants initially put under long-day conditions and then transferred to short-day conditions produced new blades at approximately the same time as did short-day laboratory plants and plants in nature when the time spent under long-day conditions is subtracted from the total time spent under laboratory conditions. Therefore, *C. rosa-marina* appears to differ from *C. subulifera* in requiring a longer initiation period under short-day conditions for new blade production (at least two months) and lacks a definite reversion (such as renewed stipe elongation in *C. subulifera*) when placed under long-day conditions after a new blade has been initiated (the "preparatory phase" in this paper) but before the blade actually appears. The exact interplay between daylight and darkness in new blade initiation in *C. rosa-marina* still needs to be examined. The cessation of new blade growth in *C. subulifera* in the spring was noted by NEUSHUL and POWELL (1964) in field-cultured plants.

As already noted, some differences occur in new blade initiation in *C. rosa-marina* and *C. subulifera*. POWELL (1964) briefly dealt with new blade initiation in *C. subulifera* at the microscopic level. He refers to a flattening of the center of the stipe with concomitant thickening of the "cortical zone" at the tip of the stipe to 50–60

cells. This description and his accompanying figures agree with observations I made on new blade initiation in Japanese *C. subulifera*.

New blade initiation in *C. simplex* also shows some differences from *C. rosa-marina*. According to ABBOTT (1968), *C. simplex* shows a slight depression in the mature blade which marks the meristematic region rather than its occurrence in a mound rising out of the mature blade as in *C. rosa-marina*. In *C. simplex*, ABBOTT distinguished two types of cells in the meristematic zone, central and peripheral, which are responsible for stipe elongation and new blade production, respectively. However, such a clear-cut distinction of cell types is not evident in *C. rosa-marina* or *C. subulifera* where central and peripheral cells both contribute to new stipe production (when it occurs in conjunction with new blade initiation in *C. subulifera*), and also both contribute to the medulla of the new blade.

Some of the morphological differences cited by SETCHELL (1906) for distinguishing the three species of *Constantinea* have been found to not hold up when specimens from various seasons and habitats are examined. Whereas SETCHELL stated that the blades of *C. subulifera* are perfoliate "from the very beginning," POWELL (1964) and the present author (unpublished observations) have found that the stipe does not begin to protrude through a new blade until March to May, leaving a period of five to seven months when the terminal blade is peltate, as in *C. rosa-marina* and *C. simplex*. The development of a small mound in the center of terminal *C. rosa-marina* blades during the summer, while perhaps not making the blade perfoliate, contrasts SETCHELL's statement that a new blade originates in a kind of depression in the center of the old blade. SETCHELL further stated that the internode between the old and the new blade in *C. rosa-marina* "elongates soon after the new blade is fairly well formed," but in the present study stipe elongation was found to occur simultaneously with new blade production. Furthermore, in many low intertidal specimens of *C. rosa-marina* from Southeast Alaska, I have found that the internodes fail to elongate, an important characteristic cited by SETCHELL for distinguishing *C. simplex* from *C. rosa-marina*. However, my observations should be considered as a clarification of SETCHELL's because the same three species he dealt with then remain valid today, and the best vegetative characteristics for distinguishing them, while not as clear-cut as SETCHELL originally indicated, remain those he stated in 1906, namely, the protruding stipe of *C. subulifera* and the long versus the short internodes of *C. rosa-marina* and *C. simplex*, respectively.

Acknowledgements

The author wishes to thank Prof. Munenao KUROGI of Hokkaido University, Dr. Nobuo TAZAWA of Hokkaido Kushiro Fisheries Experimental Station and Mr. Tokio SUZUKI and the staff of the Seaweed Culture Station of Nemuro Fisheries Cooperative Association for their help in obtaining the specimens examined in this study. I also want to express my appreciation to Prof. KUROGI, Ms. Natasha CALVIN, Dr. Joseph POWELL and Dr. Michael J. WYNNE for their critical reviews of the manuscript. This study was carried out under the foreign student scholarship program of the Japanese Government.

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S・リンドストローム: 多年生紅藻オキツバラ *Constantinea rosa-marina* の新葉形成

多年生紅藻オキツバラ (*Constantinea rosa-marina*) の新葉の形成を記述した。新葉は前年の葉の中央部にある形成部位から秋に開始される。新葉の形成中，形成部位の細胞は予定の様式で伸長してもとの大きさの200倍の長さに達し，新葉の髄部となる。同時に既定の様式で細胞分裂が起って新葉の皮層部が形成される。

新葉は天然でも実験室でも短日条件下で形成される。実験室の長日条件下で新葉の形成は停止する。(060 札幌市北区北10条西8丁目 北海道大学理学部植物学教室，現住所: Division of Biological Sciences, The University of Michigan, Ann Arbor, Michigan 48109, U. S. A.)