REGENERATIVE DEVELOPMENT IN CULTURE OF ANTARCTIC PLANTS OF *Ceratodon purpureus* (Hedw.) Brid.*

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Abstract: Regenerative development of *Ceratodon purpureus* (Hedw.) Brid. collected in Langhovde, East Antarctica, was studied with two month culture under controlled conditions, and the branching system was analyzed and discussed based on the native plants collected in the Antarctic, Subantarctic and two temperate sites of the Northern Hemisphere.

Developmental features of secondary protonemata, rhizoids and new shoots produced from cultivated plants varied with conditions of culture medium, temperature and light. The development was generally much better in the liquid culture than in the other cultures, and the optimal temperature for the plant growth was 10°C in the sandy soil culture and 15°C in the liquid and agar cultures under the light condition of 800 lx. The initiation and development of new shoots were possible even under extreme conditions such as 0°C and continuous darkness.

The present study has further offered other conclusions that Antarctic plants of *Ceratodon purpureus* have stronger potential for regeneration in stem cells than its temperate plants and that the character of less divergent, round leaves specific to Antarctic plants of this species is only phenotypic and has not been fixed genetically.

1. Introduction

Horikawa and Ando (1961) first treated the moss flora of the Ongul Islands and vicinity, East Antarctica. After that, Matsuda (1963, 1964a, b, 1968), Horikawa and Ando (1967), Nakanishi (1977) and a few others have contributed to further advancement of both taxonomical and ecological studies of the mosses in this region. Matsuda (1964a, 1968) observed the temperature at the level of moss communities in East Ongul Island, and he proved that the temperature in mosses is higher than the air temperature, hence the environmental conditions are much better for the moss growth there, and that the diurnal change of temperature at plant level is gradual enough to reserve the life of microorganisms in the moss community. Matsuda (1964b, 1968) also studied the developmental process

of a colony of *Bryum inconnexum* and found that a striped pattern by rhizoidal belts seen in the longitudinal section of the colony indicated a periodicity of moss growth. In a colony of *Ceratodon purpureus*, however, he was unable to detect such a pattern of rhizoid formation.

Results of these studies suggest the peculiar mechanism of Antarctic moss life adapted to severe environmental conditions, and they call a necessity of experimental studies for further ecological information. Autecological studies on Antarctic mosses with experiments under controlled conditions have recently been advanced especially by British botanists and certain workers of the United States. In Japan, however, experimental studies so far made on Antarctic mosses include only two brief notes: one is on *Bryum inconnexum* by TAKAKI (1962) and the other on *Ceratodon purpureus* and *Bryum* spp. by HORIKAWA and ANDO (1967).

In the present paper, I report several developmental features of gametophyte in Antarctic plants of *Ceratodon purpureus* cultivated under controlled conditions. Furthermore, discussions are made on the results of the experiment in relation to the morphological data obtained from exsiccated specimens of wild plants.

2. Material and Methods

The material of *Ceratodon purpureus* (HEDW.) BRID. used for this study, which is a well-known cosmopolitan moss and occurs most commonly in the Ongul region, was collected in Langhovde by a member of the 15th Japanese Antarctic Research Expedition (1973–1974). It had, before the experiment, been stored frozen at −20°C for about 2.5 years.

The temperature condition was controlled at 15°C, 10°C, 0°C and at the room temperature (25–35°C). The light condition was: 1) under 800 lx of continuous white light from a fluorescent lamp (6 W), 2) under natural light by the window (about 300 lx on an average in the daytime), and 3) under continuous darkness, but interrupted by a short exposure to room light for observation every 10 days. Combinations of these conditions were: 15°C-800 lx, 10°C-800 lx, 0°C-800 lx, 15°C-darkness, and the room temperature (25–35°C) under natural light.

The culture media employed were: 1) liquid of, 2) agar of, and 3) sandy soil moistened with one-half-strength Knop's solution ajusted to pH 5.6. The sandy soil used, which had been brought back from the Antarctic, was autoclaved before the experiment. Each medium was held under aseptic condition in closed petri dishes of 9 cm in diameter.

Individual plants disentangled from a colony of *Ceratodon purpureus* about 1 cm in thickness were washed with distilled water and then were placed about 50 per dish horizontally on the medium. The culture was initiated on the 10th of July, 1977 and continued for two months (60 days).

3. Results and Discussion

3.1. General aspect of regenerative development

In a few days after the beginning of the culture (15°C-800 lx), shoots placed on every medium first produced secondary protonemata around them which showed distinct phototropism.   At the same time or a little later, small buds of new shoots less than 1 mm in length were observed on stem surface under microscope.

After a week or ten days, the protonemata became longer, and the buds on stems were visible even with the naked eye.   In a month, the new shoots (branches), which were about 5 per old stem in number, reached 1–1.5 cm in length.

After 50 days, the primary new shoots formed secondary branch primordia on their surface above a leaf base.   In this experiment, the development of new shoots on secondary protonemata was rather infrequent.

According to ANDO and ICHIDA (unpublished), in an agar culture of Japanese plants of *Ceratodon purpureus*, buds of new shoots were abundantly formed on secondary protonemata, while they were only rarely produced from stems.   It seems that Antarctic plants of this species have stronger potential for regeneration in cells of their stem itself.

LONGTON and MacIVER (1977) found that in Antarctic cultures of *Bryum argenteum*, production of shoots was more prolific, and furthermore, their development occurred more rapidly than those of the plants from North American populations.   From those results, they suggested that Antarctic plants of *B. argenteum* adapted themselves by facilitating their establishment during the short growing season of the Antarctic.   The same adaptation seems to be also applicable to Antarctic *Ceratodon purpureus*.

3.2. Comparison of developmental features in various combinations of controlled conditions

Developmental features of secondary protonemata, rhizoids and new shoots produced in the culture were compared among different combinations of controlled conditions.   They are shown in Fig. 1 by star diagrams.   The extent of each feature is indicated in five grades based on the value at the last stage of the experiment.

In the liquid culture, the development was in general much better than in the others, especially under the conditions of 15°C-800 lx and 10°C-800 lx.   In the agar culture, the development was reduced and the poor production of secondary protonemata was remarkable.

On the sandy soil, the development showed very irregular patterns varying with temperature and light conditions.   For example, under the condition of 15°C-800 lx, no new rhizoids were formed, but at 10°C under 800 lx, rhizoid formation was normal.   This trend, however, may possibly be nonessential.   It is noteworthy but not understandable that the number of new shoots in the sandy soil culture was
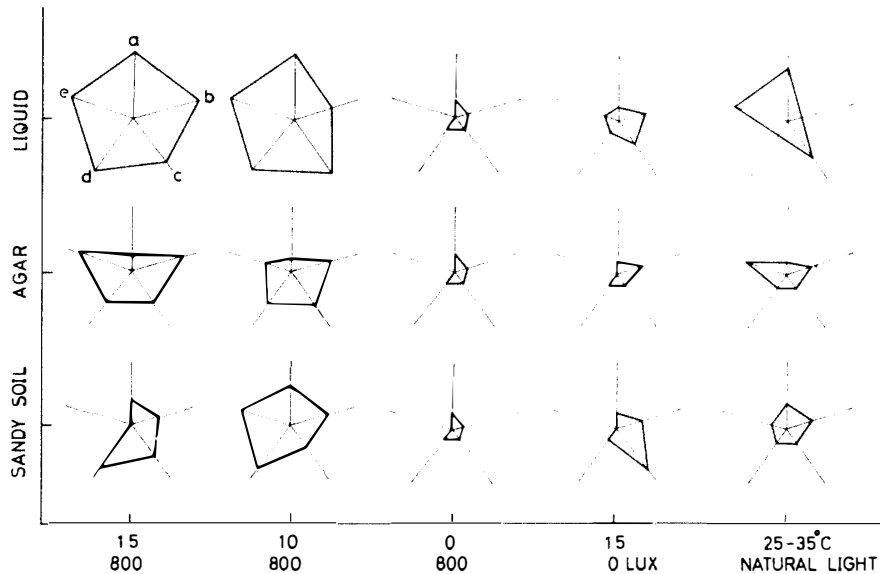
Fig. 1. Star diagrams showing developmental features in the various combinations of controlled conditions. The extent of development is indicated in five grades based on the value at the last stage of the experiment. The five axes are a. development of secondary protonemata (estimated by the general expansion), b. length of new shoots, c. number of new shoots, d. length of young leaves, and e. development of young rhizoids (estimated by the general expansion).

largest under the condition of darkness.

In the cultures under 800 lx at both 15°C and 10°C, the development is higher and patterns of the diagrams are considerably similar to each other except the degree of rhizoid formation, while in the cultures under natural light at room temperature, the development generally decreased with irregular patterns. Such a feature in the room culture may have been caused by the too high temperature which often became as high as 35°C and by the much weaker average light intensity.

The most remarkable feature of regenerative development was shown in the growth of new shoots. Creation of buds and growth of new shoots in the various conditions is shown in Fig. 2. The growth of new shoots was estimated by their length on an average measured every ten days. In the liquid and agar cultures, the growth showed the similar pattern except the case under the room condition, 25°C–35°C and natural light. In the liquid culture under the room condition, formation of buds was weak and their development was very slow. It is noteworthy that the growth was best at 10°C in the sandy soil culture in contrast with most vigorous growth at 15°C on the other media.

Figure 2 indicates another fact that the time of bud formation is different with conditions of every medium. On the liquid medium, buds appeared after 10 days in the dark condition and after 20 days at 0°C. On the agar, they were produced
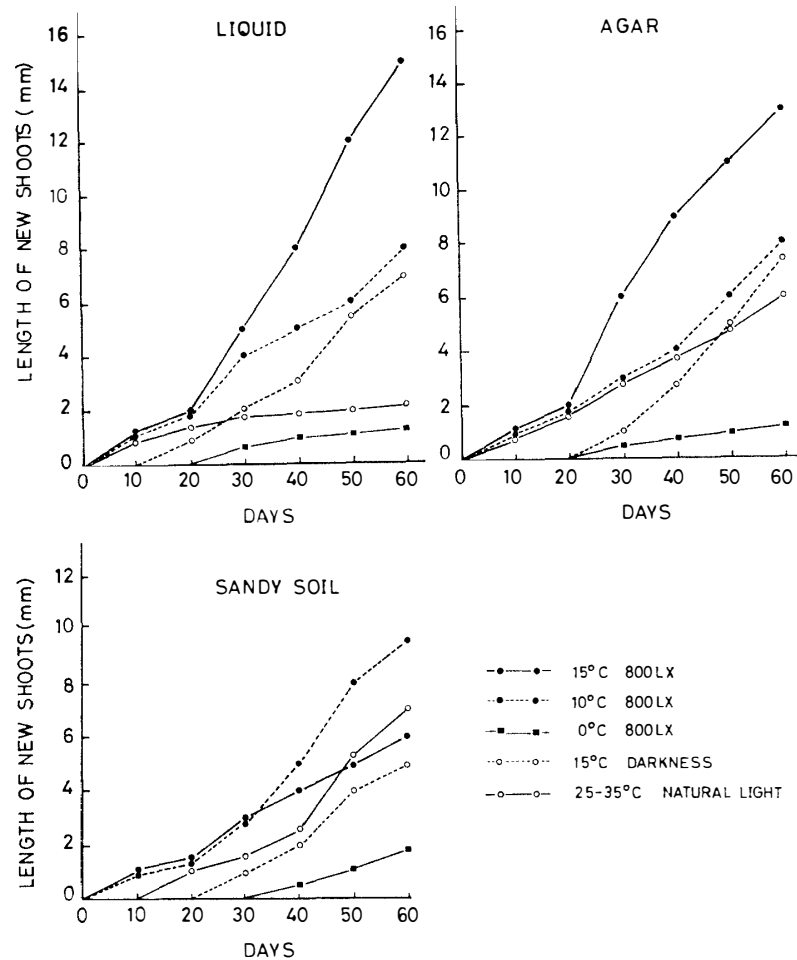
Fig. 2. Growth of new shoots under five different conditions on the three kinds of media.

after 20 days both under darkness and at 0°C.   On the sandy soil, bud formation occurred much later, except in the culture under 15°C-800 lx and 10°C-800 lx.   In this culture, it required more than 10 days for bud formation even under room temperature-natural light.   It is very interesting that bud formation began almost simultaneously on the three media when plants were cultivated under 15°C-800 lx or 10°C-800 lx, but its time was different when they were cultivated in the other conditions.

According to an experimental study on East Antarctic mosses by RASTORFER (1970), optimal temperatures for net photosynthesis were 25°-30°C and 15°-20°C for *Bryum argenteum* and *B. antarcticum*, respectively.   On the other hand, LONGTON and MACIVER (1977) proved that the highest value of colony expansion and shoot production of *Bryum argenteum* from the continental Antarctic was

obtained under the temperature regime of 15°C (night)/22°C (day), occasionally 10°C/17°C. The same fact was observed by them also in the population from North America. In the case of Antarctic plants of *Ceratodon purpureus*, the optimal temperature for its growth seems to be 10°–15°C as far as the present study is concerned.

It is of interest that the temperature condition of 0°C did not inhibit the production of new shoots. In an experimental study made by CLARKE and GREENE (1971) on the reproductive performance of *Pholia nutans* collected in Great Britain and in South Georgia, there was proved an evidence of a lower temperature optimum for maturation in South Georgia (Subantarctic) than in British plants and of a lower frost tolerance in the temperate plants. COLLINS (1977) observed relatively high rates of net photosynthesis even at −5°C in *Polytrichum alpestre* from Signy Island. As RASTORFER (1970) pointed out, Antarctic mosses do not require low temperature to survive the harsh environment, but have a capacity to endure severe protoplasmic desiccation and freezing.

From the result of the present experiment, it may be concluded that Antarctic plants of *Ceratodon purpureus* show a specialized adaptation to low temperature with a positive growth even at the freezing point. It must be further noticed that the regeneration of the *Ceratodon* was very active despite that its specimens had, before the experiment, been deposited frozen at −20°C for 2.5 years. Such strong tolerance for long freezing of Antarctic mosses was previously reported by HORIKAWA and ANDO (1967), LONGTON and HOLDGATE (1967), and GREENE and LONGTON (1970).

It is of another interest that formation of new shoots occurred even in the cultures under continuous darkness. Induction of buds in dark-grown culture of *Ceratodon purpureus* was already reported by SZWEYKOWSKA (1963). He induced some buds on protonemata arizen from spores in a culture using organic medium containing sugar and kinetin. The same culture for dark-induction of buds was also tried by CHOPRA and GUPTA (1967) with *Funaria hygrometrica*. The present experiment, however, is remarkable in that the buds were produced on the media without organic substances, although it remains possible that short exposures to room light for observation might have affected the bud-induction.

An interesting fact was furthermore observed that morphological features of stems and leaves in new shoots differed with the nature of media and light condition (Fig. 3). In the liquid culture under 15°C-800 lx, leaves of new shoots were larger and somewhat reflexed with acute apices. These features of leaves are similar to those seen in temperate populations of this species. However, in the agar culture under the same conditions, leaves developed more poorly and were usually straight with rather round apices. Such a round form of leaves is characteristic of Antarctic plants of this species as reported by HORIKAWA and ANDO (1961, 1967). In the liquid culture at the same temperature as the above but under
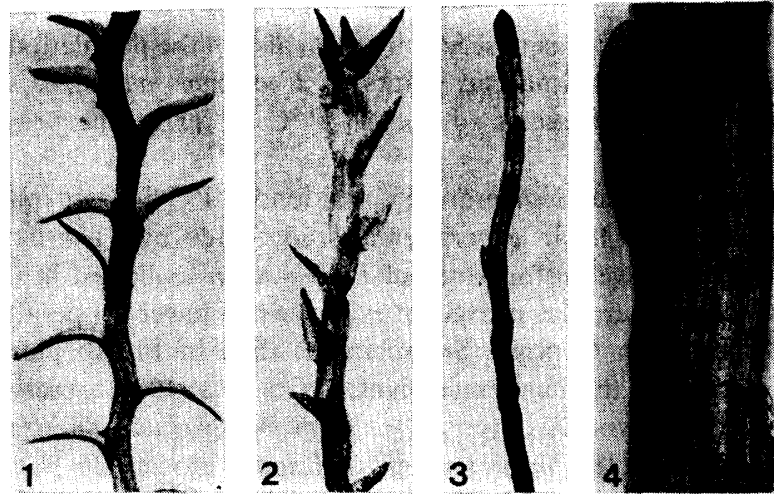
Fig. 3. Three different forms of young shoots observed in the culture under different conditions:
1. 15°C-800 lx on liquid medium, 2. 15°C-800 lx on agar medium, and 3, 4. 15°C-
darkness on liquid medium. 1–3, ×7; 4, ×47.

darkness, new shoots were etiolated, showing thinner stem and with smaller, whitish leaves that are loosely arranged. Such thinner shoot form of poor foliation simulates the slender basal part of branches seen in natural Antarctic plants which correspond to the initial shoot developed under darker condition of early summer. It seems clear that the less divergent round leaf character specific to Antarctic plants of Ceratodon purpureus in only phenotypic and has not been fixed genetically.

3.3. Branch and rhizoid formation from stems

There are, in mosses, known two ways of bud formation from parent plants, namely, buds of new shoots are formed directly from the stem or produced on secondary protonemata developed from the old plants. In the present culture of Ceratodon purpureus, buds of new shoots from cultured stems became visible seven to ten days after the start of the culture. After about 50 days, buds of secondary new branches were initiated on the primary new shoots (Fig. 4: 1–4).

In wild plants of this species, branching is usually limited to the upper part of shoots, but in the culture of shoots horizontally placed on the medium, buds of new shoots were produced even from the lower part. This fact suggests that the potentiality of branch formation is distributed widely over the stem. It is well known that the growth form of mosses has a close connection with environmental conditions. The growth form is determined primarily by the mode of branching, and the mode of branching is correlated with the pattern of bud formation. Thus, to elucidate the ecological meaning of the growth form of mosses, there is a need of further experimental studies in which we analyze the potential ability of branch
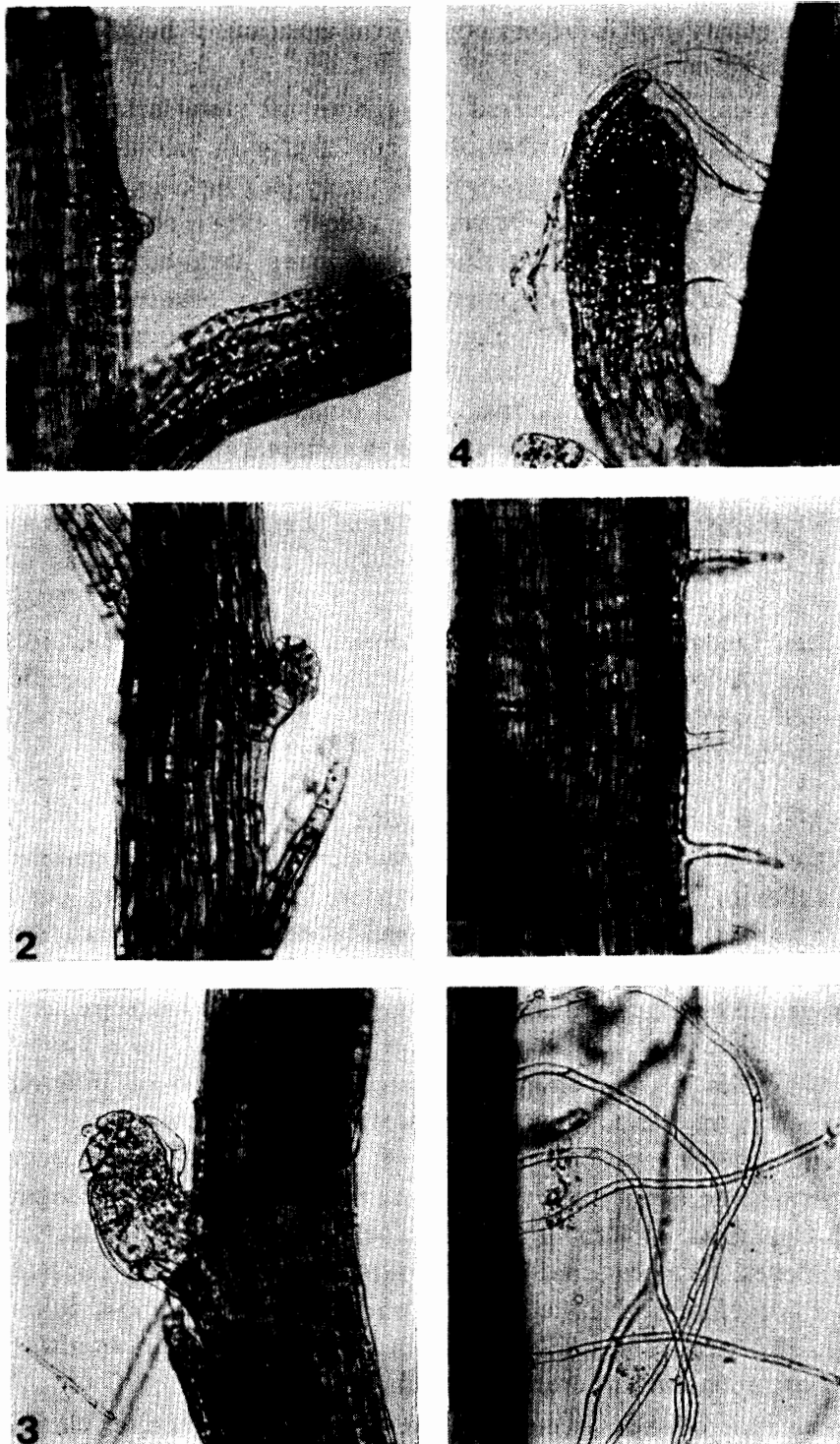
*Fig. 4. Buds of secondary new branches and rhizoids produced from the primary new shoots.
1–2. Branch primordia initiated on the stem surface above a leaf base; 3–4. Small
branches with slightly differentiated young leaves; 5–6. Rhizoids derived from epidermal
cells of stem.   1–4, ×50; 5–6, ×125.*

formation and clarify which factors control the position of budding or arouse the dormant branch primordia.

In this species, rhizoids occurred throughout the stem-surface of new shoots (Fig. 4: 5–6). Young rhizoids were often confused with secondary protonemata, but the former was distinguished from the latter by lacking chloroplasts and having oblique septa in cell-filaments. MATSUDA (1964b, 1968) observed in Antarctic plants of *Ceratodon purpureus* that rhizoids occurred irregularly on the stem and did not show the periodical development such as seen in *Bryum inconnexum*. This indicates that the rhizoid formation may be controlled differently among species.

As already shown in Fig. 1, rhizoid formation in *Ceratodon purpureus* is most active in the liquid culture and poorest on the sandy soil. Accordingly, the rhizoid formation is not considered to be related to the adaptation of facilitating the water absorption. The contrastive difference in rhizoid development between the sandy soil cultures of 15°C-800 lx and of 10°C-800 lx (Fig. 1) suggests that the rhizoid formation is affected by delicate factors, even if such a result was only by an accidental error.

From these facts, it is known that the correlation between the rhizoid formation and environmental conditions is highly complicated, and further detailed studies, combining field observations with experiments under controlled conditions, are required to bring light on this problem.

3.4. Branching system and periodicity of growth

The branching system of *Ceratodon purpureus* includes two fundamental forms, monopodial (having one main axis of growth) and sympodial (with axis formed of successive branches). These forms are usually controlled by the formation of gametangia. Antarctic plants of this species are rarer in forming gametangia than those in moderate climate, and therefore, the branching is more frequently monopodial.

Some examples of branching in *Ceratodon purpureus* of different origins, namely, from two temperate sites of the Northern Hemisphere, and Subantarctic and Antarctic regions, are shown in Fig. 5. In both the Subantarctic and the Antarctic plants, the shoots show a more remarkably and shortly segmented appearance. The basal portion of every branch is thinner with poorly developed leaves. Branches are occasionally long-stoloniferous; these are considered to be etiolated ones developed adventiously from the lower part of moss colony. Thinner parts are also seen intercalarily on a stem, although they are not always distinct. These segments apparently represent annual increments.

The same type of shoot segments were detected in Antarctic plants of *Polytrichum alpestre* by LONGTON and GREENE (1967), LONGTON (1972) and COLLINS (1976). They analyzed the growth pattern of the *Polytrichum* by tracing the arrangement of those segments. MATSUDA (1964b, 1968) also recognized the annual seg-
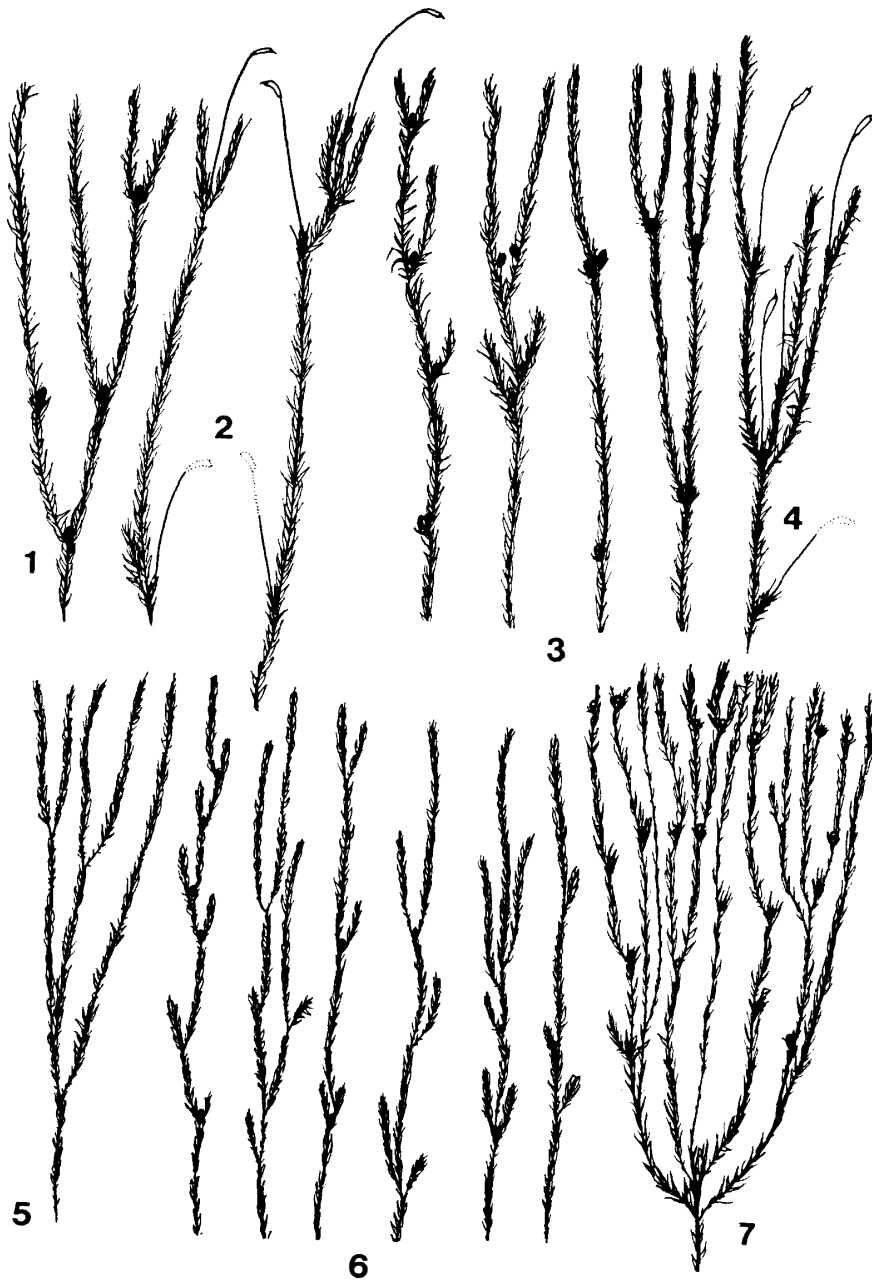
Fig. 5. Branching patterns and formation of gamentangia in plants of different origins: *1-2. Hokkaido, Japan*; *3-4. Scotland*; *5-6. Langhovde, East Antarctica*; *7. South Georgia, Subantarctic.* *All* ×3.

ments in *Bryum inconnexum* which were differentiated by the feature of leaves and by periodical formation of rhizoids.

HAGERUP (1935) studied the periodicity and shoot branching in European plants of *Ceratodon purpureus*, and pointed out that this species shows a bicyclic

periodicity, namely, it has two growth periods a year: December to March and July to September. Antarctic plants of *Ceratodon purpureus*, however, are apparently not bicyclic but unicyclic in growth periodicity. The periodicity of growth is marked by several morphological features in many mosses (MEUSEL, 1935; LACKNER, 1939) and this is most pronounced in Antarctic mosses whose growth is limited to the summer season.

Acknowledgments

References

ANDO, H. and ICHIDA, C. (unpublished): An experimental study on ecological differences by sex in *Ceratodon purpureus* (HEDW.) BRID.

CHOPRA, R. N. and GUPTA, U. (1967): Dark-induction of buds in *Funaria hygrometrica* HEDW. Bryologist, **70**, 102–104.

CLARKE, G. C. S. and GREENE, S. W. (1971): Reproductive performance of two species of *Pohlia* from temperate and sub-Antarctic stations under controlled environmental conditions. Trans. Brit. Bryol. Soc., **6**, 278–295.

COLLINS, N. J. (1976): Growth and population dynamics of the moss *Polytrichum alpestre* in the maritime Antarctic. Oikos, **27**, 389–401.

COLLINS, N. J. (1977): The growth of mosses in two contrasting communities in the maritime Antarctic: Measurement and prediction of net annual production. Adaptations within Antarctic Ecosystem, ed. by G. A. LLANO. Washington, Smithsonian Institution, 921–933.

GREENE, S. W. and LONGTON, R. E. (1970): The effects of climate on Antarctic plants. Antarctic Ecology, Vol. 2, ed. by M. W. HOLDGATE. London, Academic Press, 786–800.

HAGERUP, O. (1935): Zur Periodizität im Laubwechsel der Moose. Kgl. Dansk. Vidensk., Biol. Meddr., **11**, 1–88.

HORIKAWA, Y. and ANDO, H. (1961): Mosses of the Ongul Islands collected during the 1957–1960 Japanese Antarctic Research Expedition. Hikobia, **2**, 160–178.

HORIKAWA, Y. and ANDO, H. (1967): The mosses of the Ongul Islands and adjoining coastal areas of the Antarctic Continent. JARE Sci. Rep., Spec. Issue, **1**, 245–252.

LACKNER, L. (1939): Über die Jahresperiodizität in der Laubmoose. Planta, **29**, 534–616.

LONGTON, R. E. (1972): Growth and reproduction in northern and southern hemisphere population of the peat-forming moss *Polytrichum alpestre* with reference to the estimation of productivity. Proc. 4th Int. Peat Congr. I–IV, Helsinki, 259–275.

LONGTON, R. E. and GREENE, S. W. (1967): The growth and reproduction of *Polytrichum alpestre* HOPPE on South Georgia. Philos. Trans. R. Soc. London, Ser. B, **252**, 295–322.

LONGTON, R. E. and HOLDGATE, M. W. (1967): Temperature relationships of Antarctic vegetation. Philos. Trans. R. Soc. London, Ser. B, **252**, 237–250.

Longton, R. E. and MacIver, M. A. (1977): Climatic relationships in Antarctic and Northern Hemisphere populations of a cosmopolitan moss, *Bryum argenteum* Hedw. Adaptations within Antarctic Ecosystem, ed. by G. A. Llano. Washington, Smithsonian Institution, 899–917.

Matsuda, T. (1963): Nankyoku Higashi Onguru-tô no sen-rui bunpu ni tsuite (The distribution of mosses on East Ongul Island, Antarctica). Hikobia, 3, 254–265.

Matsuda, T. (1964a): Nankyoku Higashi Onguru-tô ni okeru sen-rui gunraku no bikishô ni tsuite (Microclimate in the community of mosses near Syowa Base at East Ongul Island, Antarctica). Nankyoku Shiryo (Antarct. Rec.), 21, 12–24.

Matsuda, T. (1964b): Nankyoku Ranguhobude chiku ni okeru sen-rui gunraku no seitaigaku-teki kenkyû (Ecological studies on the community of mosses at Langhovde region, Antarctica). Nankyoku Shiryo (Antarct. Rec.), 21, 25–38.

Matsuda, T. (1968): Ecological study of the moss community and microorganisms in the vicinity of Syowa Station, Antarctica. JARE Sci. Rep., Ser. E (Biol.), 29, 58 p.

Meusel, H. (1935): Wuchsformen und Wuchstypen der europäischen Laubmoose. Nova Acta Leopold., Neue Folge, 3, 123–277.

Nakanishi, S. (1977): Ecological studies of the moss and lichen communities in the ice-free areas near Syowa Station, Antarctica. Nankyoku Shiryo (Antarct. Rec.), 59, 68–96.

Rastorfer, J. R. (1970): Effects of light intensity and temperature on photosynthesis and respiration of two East Antarctic mosses, *Bryum argenteum* and *Bryum antarcticum*. Bryologist, 73, 544–556.

Szweykowska, A. (1963): Kinetin-induced formation of gametophores in dark cultures of *Ceratodon purpureus*. J. Exp. Bot., 14, 137–141.

Takaki, N. (1962): Nankyoku-san sen-rui no baiyô (Culture of an Antarctic moss). Sentai Chii Zappo (Misc. Bryol. Lichenol.), 2, 154.