Polar Biosci., **15**, 88–96, 2002

Reproductive phenology of subalpine moss, *Polytrichum ohioense* Ren. et Card.

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***Abstract*:** The reproductive phenology of *Polytrichum ohioense* was investigated in a sub-alpine forest at the foot of Mt. Tyausu, in the Yatsugatake Mountains, Central Honshu, Japan. Shoots were collected every 2 weeks from May to October from the study site. Developmental stages of gametangia and sporophytes formed in the current and previous year were registered. The temperature above the turf occasionally dropped below 0℃ before June, while the temperature in the turf did not drop below 0℃. Juvenile antheridia formed about one month earlier than archegonia. Mature antheridia and archegonia are observed from late May to early August and from late June to mid-July, respectively. Fertilization seems to occur from late June to mid-July. Longer persistence of mature antheridia is supposed to contribute to higher efficiency of fertilization to supply its sperm for a relatively long period. And delayed formation of archegonia in the warm season may contribute to the adaptation to the temperature decrease at the beginning of the growing season. Sporophytes were found first at the end of June, then gradually grew and reached the ECI stage by October. The sporophytes seemed to spend the period of snow cover in the ECI stage, and started to grow again in the next growing season in May. Spore dispersal was observed from mid-July to mid-August. Sporophytes took 13 months to mature including a 6 month resting period. The phenological parameters observed in the present study provide a way to adapt to the the short growing season in the sub-alpine zone in Central Honshu, Japan.

key words: moss, phenology, sub-alpine zone, growing period, snow cover

Introduction

Bryophytes show considerable diversity of phenology to adapt to the environments of various habitats. Following the systems established by Greene (1960) for recognizing stages in the reproductive cycles of mosses, many studies have investigated the phenology of many moss species, from tropical to polar regions (*e.g.* Longton, 1972, 1979; Longton and Greene, 1969; Hancock and Brassard, 1974; Odu, 1981; Sagmo Solli *et al.*, 1998; Stark, 1986; Miles *et al.*, 1989 etc.) and especially from low lands and low montane zones in Japan (*e.g.* Tsujimura, 1974; Deguchi and Takeda, 1986; Deguchi and Hidaka, 1987; Imura and Iwatsuki, 1989a, b; Imura, 1994). These phenological studies focused on developmental patterns of gametangia and sporophytes, and discussed adaptations to

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various habitats.

In the subalpine zone, mosses are one of the important components of forest floor vegetation in the coniferous forest (Archibold, 1995). In such a subalpine zone in Central Honshu, Japan, heavy snow covers the ground for about 6 months in general, and the growing season seems to be about 6 months for mosses growing on the ground. This period of snow cover is very close to that in the sub-Antarctic, Arctic and Norwegian boreal zone. In these areas, phenologies of some species were investigated previously (Longton, 1966, 1972, 1979; Sagmo Solli *et al*., 1998). However, no phenological study of mosses in the subalpine zone has been done. This study aims to clarify the reproductive phenology of gametangia and sporophytes of a moss growing in the Yatsugatake Mountains, subalpine zone of Japan.

Materials and methods

Polytrichum ohioense Ren. et Card., distributed in Japan, Siberia, Europe and North America (Noguchi, 1987), was selected for this study. In Japan, *P. ohioense* is common in the subalpine zone, its habitat is on shaded humus in forests, rarely in raised bogs (Osada, 1966). This species is reported to be dioicous or monoicous by Noguchi (1987), and autoicous by Osada (1966). At the study site, shoots with both sexual organs were not observed. Therefore, in this study, this species was recognized as dioicous moss. This is acrocarpous moss and sexual organs are found on apices of shoots.

Temperatures in the *P. ohioense* population and samplings of this species were measured in a forest of *Abies mariesii*, located at elevation of 2140 m on the western foot of Mt. Tyausu (2384 m), in the Yatsugatake Mountains, Nagano prefecture, Central Honshu, Japan (Fig. 1). A vegetationally homogeneous area of *P. ohioense* in a gap (*ca*.

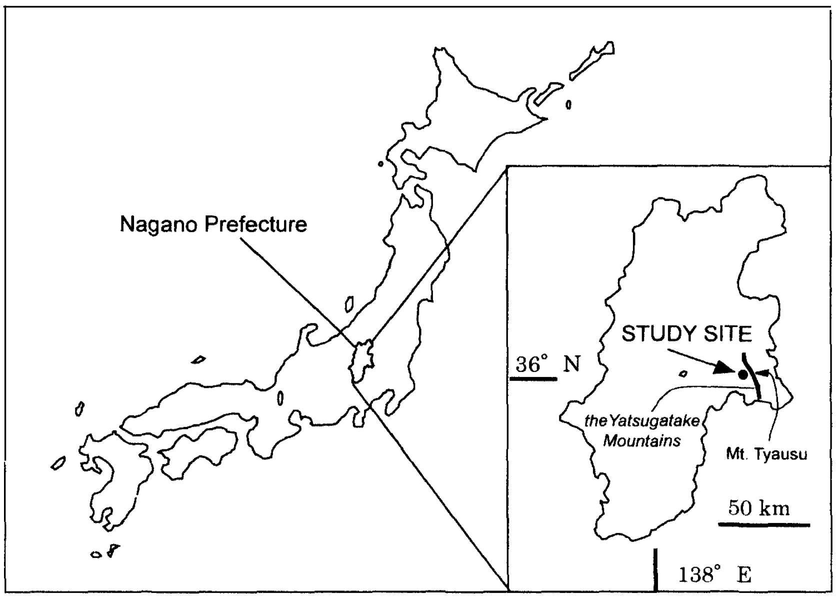

Fig. 1. Map of study site, western foot of Mt. Tyausu (2384 m) in the Yatsugatake Mountains, Nagano Prefecture, Central Honshu, Japan.

6 × 6 m) in the forest was selected for the present study.

In the gap, air temperatures above the turf (15 cm above the surface of turf of *P. ohioense*) and temperature in the turf (*ca.* 1 cm below the surface) were measured and stored in temperature data loggers (Optic Stow Away Temp, Onset Computer Corporation) at intervals of 1 hour from May 14 to October 27, 1998.

Shoots were categorized into four types, as male (with male inflorescences forming splash cups), female with current year sporophytes (with young sporophytes or female inflorescences), female with previous year sporophytes (with developed sporophytes) and vegetative (without any sexual organs). Twenty samples each of male shoots, female shoots with previous year sporophytes were collected every two weeks from May 14 (soon after snow melting) to October 27, 1998. It was difficult to distinguish the female inflorescences from vegetative shoots in the field, thus the thirty to forty shoots without any male inflorescences and sporophytes were collected and the presence of female inflorescences determined after dissection of shoot apices for evaluation of sexuality of the shoots. The female shoots were also used for the investigations of archegonial development as mentioned below.

Developmental stages of antheridia, archegonia and sporophytes were determined according to Greene (1960) (Table 1). The numbers of gametangia per inflorescence in each developmental stages were recorded using a microscope (Nikon ECLIPSE E 400). Developmental stages of sporophytes were determined by the naked eye. To estimate the average development at each sampling time, the maturity index (I) was calculated

Table 1. Developmental stages of gametangia and sporophytes used in this investigation of *Polytrichum ohioense* (following Greene, 1960).

Stage	Index value	Explanation
Gametangia		
Juvenile (J)	1	Small gametangia up to half of full size.
Immature (I)	2	Gametangia from half to full size, green.
Mature (M)	3	Gametangia still green but with open apex. Spermatozoids are visible, in antheridia.
Dehisced (D)	4	Gametangia brown. Antheridia empty and somewhat shrunken.
Sporophytes		
Swollen venter (SV)	1	The lower part of the archaegonium is swollen. This is the first visible sign that the archegonium is fertilized.
Early calyptra in perichaetium (ECP)	2	Calyptra starts to emerge from the bracts.
Late calyptra in perichaetium (LCP)	3	Calyptra from half to almost fully visible above the bracts. Seta no yet visible.
Early calyptra intact (ECI)	4	Calyptra present. Seta ranging from just visible above the bracts to fully elongated. Capsule with the same diameter as the seta.
Late calyptra intact (LCI)	5	Calyptra present. Capsule expanding or has attained full size.
Early operculum intact (EOI)	6	Capsule changes color from green to brown but more than half the capsule is still green.
Late operculum intact (LOI)	7	More than half the capsule is brown but operculum is intact.
Operculum fallen (OF)	8	Operculum fallen and spores are dispersed.
Empty and fresh (EF)	9	Capsules of current cycle with more than 75% of the spores released.

according to the idea of Sagmo Solli *et al*. (1998) as follows:

$$I = \frac{\sum_{i=1}^{s} M_i r_i}{r_{tot}},$$

where M_i is the Index Value of the developmental stage, r_i is the number of gametangia or sporophytes in the developmental stage, r_{tot} is the total number of gametangia or sporophytes and s is the total number of developmental stages (*i.e*. 4 for gametangia and 9 for sporophytes).

Results

Temperature

The pattern of temperature above the turf and the temperature in the turf of *P. ohioense* from May 14 to October 27 are summarized in Fig. 2. The mean temperature above the turf in late May was 9.6°C. Until early June, the temperature above the turf occasionally dropped below 0°C. Toward early August, this temperature increased, the mean temperature in early August reached 14.5°C. After then, it decreased toward the end of the measurements, the mean temperature was 5.4°C in late October. At the beginning of October, it dropped below 0°C and temperature below 0°C was often observed in October. The mean temperature in the turf was usually *ca.* 2°C higher than that above the turf, and the trend was similar to that above the turf. The temperature in the turf did not drop below 0°C throughout the measurement period.

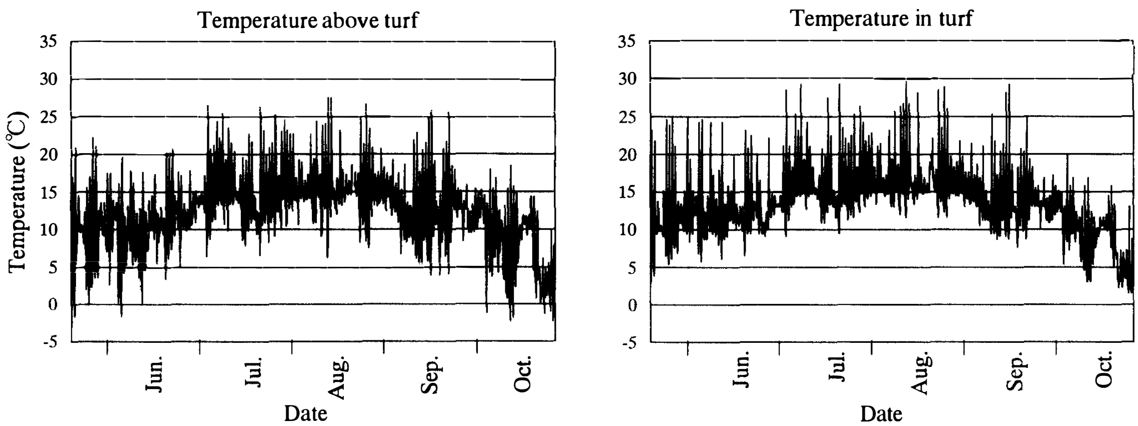

Fig. 2. Temperature above the turf and in the turf of *Polytrichum ohioense* at the study site from May 14 to October 27, 1998. Temperature showed as raw data measured at interval of one hour.

Phenology of gametangia

All antheridia found on May 14 were categorized in the juvenile stage (Fig. 3a). Mature antheridia appeared from May 31 to August 2. Dehisced antheridia appeared from June 12, and 98% of antheridia reached the dehisced stage by August 2. On the other hand, archegonia were not found in May (Fig. 3b). Juvenile and immature archegonia first appeared on June 12. Mature archegonia were found on June 30 and

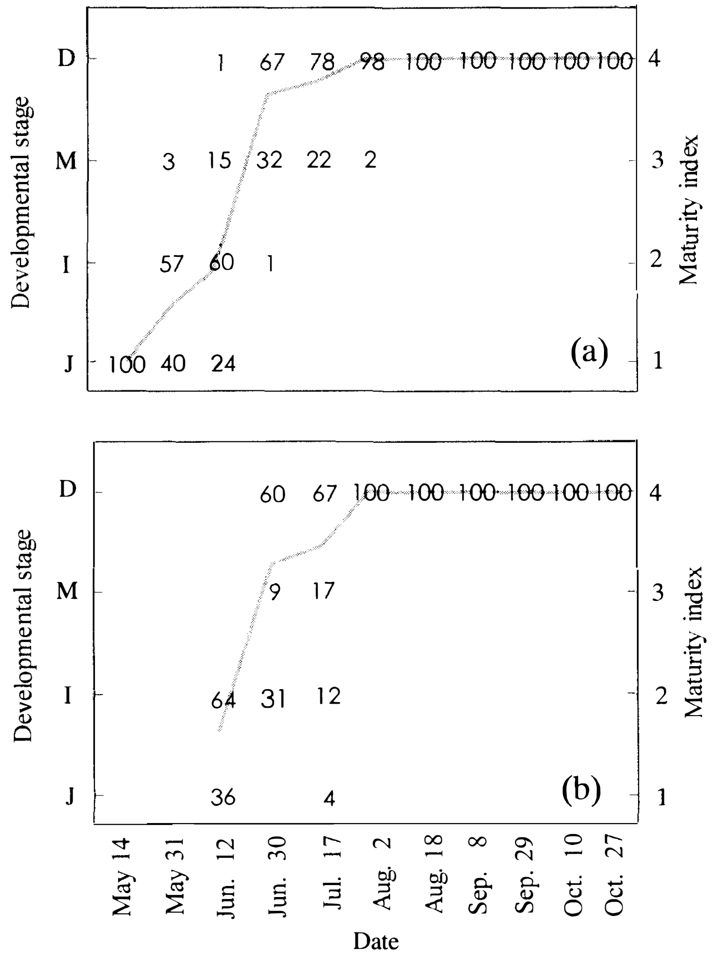

Fig. 3. Development of antheridia (a) and archegonia (b) of *Polytrichum ohioense* from May 14 to Octover 27, 1998. Numbers show percentages of gametangia of different stages on each sampling occasion. The lines show the maturity index for each gametangium. See Table 1 for the definitions of the developmental stages (J, I, M, D).

July 17, however, more than half of the archegonia had already been categorized in the dehisced stage at those times. On June 30 and July 17, fully matured antheridia and archegonia were recognized. Maturity indices (Fig. 3, solid lines) indicated that male and female gametangia took on average at least one and a half months, and at most one month, to reach the mature stage (I=3).

Phenology of sporophytes

All sporophytes collected at the beginning of our sampling were in the early calyptra intact (ECI) stage, which would have been produced the previous year (Fig. 4). During June and July, maturation of the sporophytes progressed, sporophytes produced in the previous year reached the operculum fallen (OF) stage by July 17. Spore dispersal from sporophytes produced in the previous year occurred from July 17 to August 18.

Sporophytes produced in the current year were first recognized on June 30. The

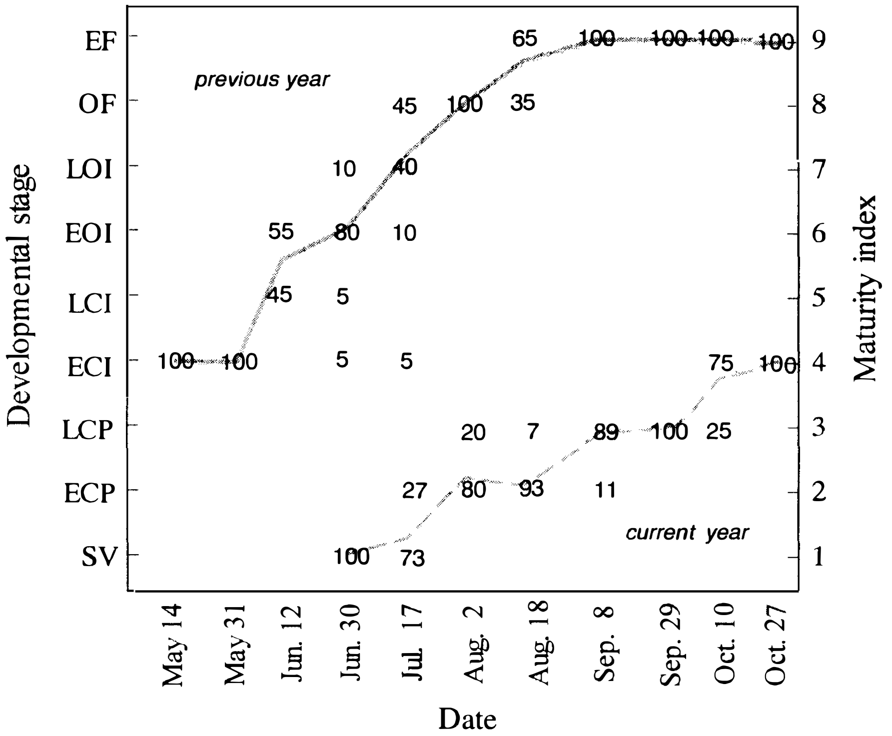

Fig. 4. Development of sporophytes of *Polytrichum ohioense* from May 14 to Oct. 27, 1998. Numbers show the percentages of sporophytes initiated in the current and previous years. The lines show the maturity index for current (dotted line) and previous (solid line) year sporophytes. See Table 1 for the definitions of the developmental stages (SV, ECP, LCP, etc.).

newly produced sporophytes developed gradually during late summer and reached the ECI stage by October 27. The ECI stage was observed both at the beginning and the end of the present study. Sporophytes growing at the study site survived the winter in the ECI stage.

Discussion

The subalpine zone in Central Honshu is one of the heaviest snow areas in Japan. Generally snow covers the ground for almost half of the year (November to April). The growing period of subalpine plants is shortened comparing to the areas with less snow or without snow (Shibata, 1996). Temperature measurement indicated that freezing might have occasionally occurred near the beginning (May) and end (October) of our observation period. Such sub-zero temperature sometimes cause serious growth inhibition as freezing stress (Levitt, 1980), if that happens, then growth of *P. ohioense* at the study site might be limited in May and October.

In spite of occasional sub-zero temperature in May, formation of antheridia started immediately after snow disappearance. Juvenile antheridia were found for a month in further developmental stages, such as the immature and mature stages, after that. It is suggested that antheridia formation on the apex of male shoots continued for a month at the beginning of the growing season, this might be one reason for the relatively prolonged period (late May to early August) of mature antheridia production. On the other hand, archegonia formation seemed to start all at once in the population in June, a month after the antheridia, when the temperature around the population never dropped below 0°C. Mature and dehisced stages of archegonia were seen in late June and mid-July.

Differences of the timing of gametangia formation between antheridia and archegonia are common features of dioicous mosses (*e.g.* Longton and Greene, 1967, 1969; Longton, 1972; Miles *et al.,* 1989; Imura, 1994; Sagmo Solli *et al.,* 1998). Longer existence of mature antheridia is supposed to contribute to high efficiency of fertilization to supply their sperm for a relatively long period to the archegonia, while maturation tends to be delayed by climatic factors. Plants avoid exposure of sensitive tissue to intolerable temperatures by seasonal phenology (Körner, 1999). Delayed formation of archegonia in the warm season may contribute to avoiding the risk of juvenile archegonia being exposed to low temperature. We suggest that such delayed formation of archegonia might increase the fertilization efficiency, by avoiding the risk of sudden temperature decrease in the early growing season in this species. This may an adaptation to the temperature decrease at the beginning of the growing season.

Since the fertilized archegonia (SV) appeared from late June to mid-July, it is inferred that fertilization occurred in this period, corresponding to the rainy season. In this study, fertilization of moss occurred in the rainy season. Since water is indispensable to fertilization, it appears that the gametangial phenology of this moss fits seasonal changes of the study site climate well. However, further studies are required.

Sporophytes of *P. ohioense* in the swollen venter (SV) stage were found first at the end of June, then the sporophytes gradually grew and reached the early calyptra intact (ECI) stage by October, the end of the current growing period. The sporophytes seemed to spend the period of snow cover in the ECI stage, and started to grow again in the next growing season in May. Suspension of development of sporophytes in winter has been noticed in studies of sub-Antarctic and sub-Arctic mosses (Longton, 1966, 1972, 1979; Sagmo Solli *et al*., 1998), and also a few moss species that inhabit the lower montane zone in Japan (Deguchi and Takeda, 1986; Deguchi and Hidaka, 1987) (summarized in Table 2). The resting period of 6 months observed in the present result is one of the longest that have been observed, nearly the same length as that of sub-Antarctic and sub-Arctic mosses. Sub-Antarctic mosses tend to stop sporophyte development at an earlier stage (SV, early calyptra in perichaetium: ECP, late calyptra in perichaetium: LCP) in the current growing season and winter (Longton, 1966, 1972), but mosses growing in Canada and Norway tend to stop development in the LCP and ECI stages (Longton, 1979; Sagmo Solli *et al.,* 1998). Such difference of wintering stage among species in different habitats may partly be attributed to the difference of climatic conditions within a short growing period. In a lower latitudinal habitat with much warmer and better conditions for sporophytes, newly produced sporophytes could reach higher stages. Sub-Antarctic mosses cannot reach a further stage, within the current year possibly due to lower temperature and/or lower solar radiation, especially in autumn.

In summary, *P. ohioense* spent the early growth season in sexual reproduction and post-growth of sporophyte from the ECI to empty and fresh (EF) stages, spore dispersal occur in mid-summer when climatic conditions such as temperature are suitable for moss

Table 2. The periods for maturation (from fertilization to spore release) of sporophytes and resting period of development of sporophytes in winter and spring or summer from previous studies and this study. Numbers show months for maturation and resting.

Species	Period for maturation	Resting period in winter	Resting period in spring or summer	Locallity	Reference
Polytrichum alpinum	12-15	6-8		Sub-Antarctic	Longton (1966, 1972)
Polytrichum alpestre	15	6-8		Sub-Antarctic	Longton (1972)
Polytrichum juniperinum	15	6-8		Sub-Antarctic	Longton (1972)
Polytrichum piliferum	15	6-8		Sub-Antarctic	Longton (1972)
Psilopilum antarcticum	15	6-8		Sub-Antarctic	Longton (1972)
Dicranum majus	14	6		Norway	Sagmo Solli *et al*. (1998)
Polytrichum alpestre	14-15	5-7		Canada	Longton (1979)
Polytrichum ohioense	**13**	**6**		**Japan**	**THIS STUDY**
Ptychomitrium fauriei	9	3		Japan	Deguchi and Takeda (1986)
Entodon challemgeri	9	2		Japan	Deguchi and Hidaka (1987)
Aulacopilum japonicum	11	4	3	Japan	Deguchi and Hidaka (1987)
Trachycystis microphylla	5		4	Japan	Imura and Iwatsuki (1989b)
Astomum crispum	8		3	Japan	Deguchi and Hidaka (1987)
Fabronia matsumurae	11-12			Japan	Deguchi and Hidaka (1987)
Venturiella sinensis	8-9			Japan	Deguchi and Hidaka (1987)
Trematodon longicollis	2			Japan	Deguchi and Hidaka (1987)
Ptychomitrium dentatum	9-10			Japan	Deguchi and Takeda (1986)
Ptychomitrium polyhylloides	9			Japan	Deguchi and Takeda (1986)
Ptychomitrium linearifolium	8			Japan	Deguchi and Takeda (1986)

growth in the subalpine zone in Japan. Sporophytes start growth immediately after fertilization (late June to early July) and reach the ECI stage in the later growth season of the current year. Sporophytes in the ECI stage, which is a wintering form of *P. ohioense* at the study site, rest for 6 months during the period of snow cover. As a result, maturation of sporophytes takes *ca*. 13 months, the longest case among mosses reported in Japan (Table 2). However, taking account of the maturation timing of gametangia and sporophytes, and following spore dispersal in mid-summer, the phenological characteristics of moss observed in the present study form an adaptation to the short growing season in the subalpine zone in Central Honshu, Japan.

Acknowledgments

We thank Dr. T. Fukushima and Dr. Y. Hoshino for their support and encouragement through this study. We are also grateful to Dr. Y. Bekku, Dr. T. Ueno and the members of the laboratory of vegetation management of the Tokyo University of Agriculture and Technology for their support in the field work.

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(Received September 17, 2001: Revised manuscript accepted November 7, 2001)