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Spore Germination and Early Stages of Development in *Hypnea musciformis* (Rhodophyta, Gigartinales)

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Abstract

The early stages of development of the carpospores and tetraspores of *Hypnea musciformis* (Wulfen) Lamouroux have been investigated. Both types of spores germinated immediately after liberation. The spores segmented repeatedly into 2, 3, 4 cells, etc. until a multicellular ball of cells was produced. The germination pattern was thus of the discal type, i.e., *Typus discalis mediatus* (Inoh, 1947) or the "*Dumontia*-type" in the terminology of Chemin (1937). Subsequently, a relatively expansive attachment holdfast was produced from each sporeling. This was followed by the development of up to 4 or more shoot axes arising from the basal disc. These findings are discussed with reference to an earlier study by the senior author on the developmental biology of other species of *Hypnea* Lamouroux.

Introduction

Hypnea musciformis (Wulfen) Lamouroux is the best-known species in the genus *Hypnea* Lamouroux (Rhodophyta, Gigartinales). The alga has been reported from many tropical and subtropical shores in the Atlantic Ocean (Taylor, 1928), the Indian Ocean (Weber-van Bosse, 1928; Rao, 1970) and the Pacific Ocean (Lucas and Perrin, 1947; Chapman, 1971). The major diagnostic feature of the seaweed is the presence of hook-like processes on the ends of the main axes. The incurved structures function as tendrils in "fixing" the seaweed onto other seaweeds for support (Fritsch, 1945).

Most reports on *Hypnea musciformis* are of floristic nature only. However, with the recent realization that the alga is a producer of commercial seaweed colloids (Humm and Williams, 1948), more attention has been drawn towards its ecology and economic aspects (Lawson, 1957; Rao, 1970). Together with these aspects, information on the developmental biology of the seaweed is desirable. This is especially important since seaweed cultivation from spores is considered to be a potential method of raising commercial crops of economic seaweeds (Krishnamurthy, 1965; Imada *et al.*, 1972; Mshigeni, 1974).

Although *Hypnea musciformis* was known even before the turn of the 19th century (as *Fucus musciformis* Wulfen: see Kylin, 1956), nothing has so far been known about any aspect of its developmental biology. In the present study, therefore, the authors attempted to advance knowledge in this neglected area. The major focus of the study was to reveal the unknown aspects of spore germination pattern and early stages of holdfast and shoot differentiation in *H. musciformis*.

Materials and Methods

Cystocarpic and tetrasporic fronds of *Hypnea musciformis* (Wulfen) Lamouroux were collected from Oyster Bay, Dar es Salaam, Tanzania during spring low tides. The plants were found growing mainly as epiphytes attached to other plants at about low-water mark or in intertidal pools. Cystocarpic plants were recognized by their protruding cystocarpic swellings about 1.0 mm in diameter. Tetrasporic plants were recognized by their swollen stichidial branchlets. In addition to being swollen, the bases of the stichidial branchlets were more darkly pigmented than vegetative branchlets.

After collection, the fertile fronds were immediately transferred to the laboratory where they were thoroughly cleaned of adhering surface contaminants. This was done with an artist's brush

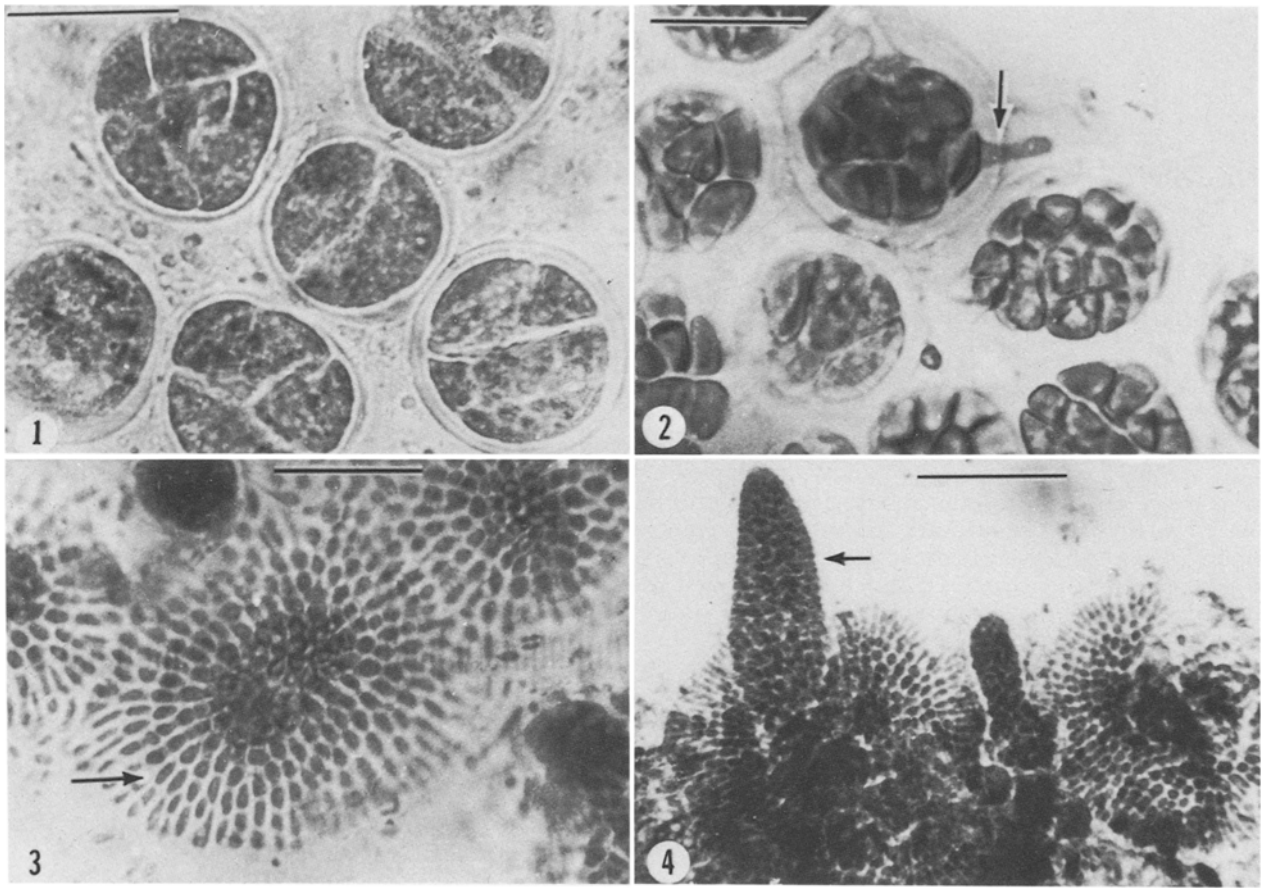

Fig. 1. *Hypnea musciformis*. *1*: Carpospore germling (1 day) at 1- to 3-celled stage (scale = 25.0 µm); *2*: tetraspore germling (8 days old) showing morula-like multicellular balls of cells with some of surface cells metamorphosed to rhizoids (arrowed) (scale = 26.0 µm); *3*: carpospore germlings (21 days old) with expansive disc (arrowed) but no elongated shoot (scale = 70 µm); *4*: tetraspore germlings (21 days old) with expansive attachment disc and differentiated shoots (arrowed) (scale = 85 µm)

followed by rinsing with Millipore-filtered seawater. The plants were then placed in Pyrex culture dishes containing 200 ml of a modified Instant Ocean culture medium, which consisted of 50% seawater (collected from natural *Hypnea musciformis* habitat) by volume, 50% Instant Ocean (Stein, 1973) and 50 mg/l sodium nitrate. Many microscope cover glasses were placed at the bottom of the dishes to hold the spores after liberation. Tetrasporic and cystocarpic fronds were placed in separate culture containers. The fertile plants were then incubated in a chamber maintained at 26°C and provided with a light intensity of 200 ft-c from cool-white fluorescent light tubes.

On the following day, the cover glasses were examined under the microscope to detect whether spores had been shed. If so, the parent fronds were removed and the cover glasses with their attached spores were transferred to other culture dishes and incubated under conditions identical to those described above. The culture medium was changed every 3 days throughout the study period.

During the first week, carpospore and tetraspore germlings of *Hypnea musciformis* were sampled daily and examined under a Zeiss compound microscope to study the germination pattern. Measurements of carpospore and tetraspore germling size were made using a micrometer scale inserted in the eye piece of the camera. Mean spore diameter and its confidence limits (P = 0.05) were subsequently calculated statistically (Snedecor and Cochran, 1967). A total of 100 spores of each type was measured.

Between the first and second week of culture, the sporelings were examined at 3-day intervals. Thereafter they were examined once every week. The observed developmental patterns were photographed using a Minolta SRT 101 camera and microscope adaptor.

Results

The appearance of the freshly liberated carpospores and tetraspores of *Hypnea musciformis* was similar. The mean diameters of the two types of spores were 26.9 ± 0.6 and 24.2 ± 0.4 µm, respectively. Thus, the carpospores were slightly larger than the tetraspores.

Both types of spores displayed a similar germination pattern. Soon after liberation the spores were segmented into 2 cells by formation of a median wall, then a second wall was formed, usually perpendicular to the first one (Fig. 1: *1*), and further walls were added, until a morula-like mass of cells was produced (Fig. 1: *2*). Some of the surface cells of this multicellular mass metamorphosed into colourless rhizoidal extensions (Fig. 1: *2*); this process was observed towards the end of the first week of spore germination.

By the end of the second week, holdfast and shoot initials had been differentiated in some of the sporelings. At 3 weeks, old shoots of these sporelings were appreciably elongated. In the majority of cases, however, shoot differentiation was delayed to the end of the third or fourth week. In these cases, shoot development was preceded by the formation of a very expansive disc (Fig. 1: *3*, *4*). The expansive multicellular holdfast discs were 250 to 450 µm in diameter.

The number of shoots differentiating from the basal attachment discs is very variable. In some cases only a single shoot developed, in some 2 shoot axes were differentiated (Fig. 2: *7*), in some 3 (Fig. 2: *5*, *6*), while in others (Fig. 2: *8*) 4 or more shoot axes were differentiated. As shown in Fig. 2: *8*, the shoot axes are not necessary differentiated at the same time: others can be initiated much later. Thus, the attachment disc can produce many shoots than illustrated in the present paper. The shoots were primarily purplish green in colour and resembled the parent plants in this respect. Even though aeration was not provided in the culture dishes, a very large number of sporelings remained alive and healthy in the laboratory for as many as 3 months.

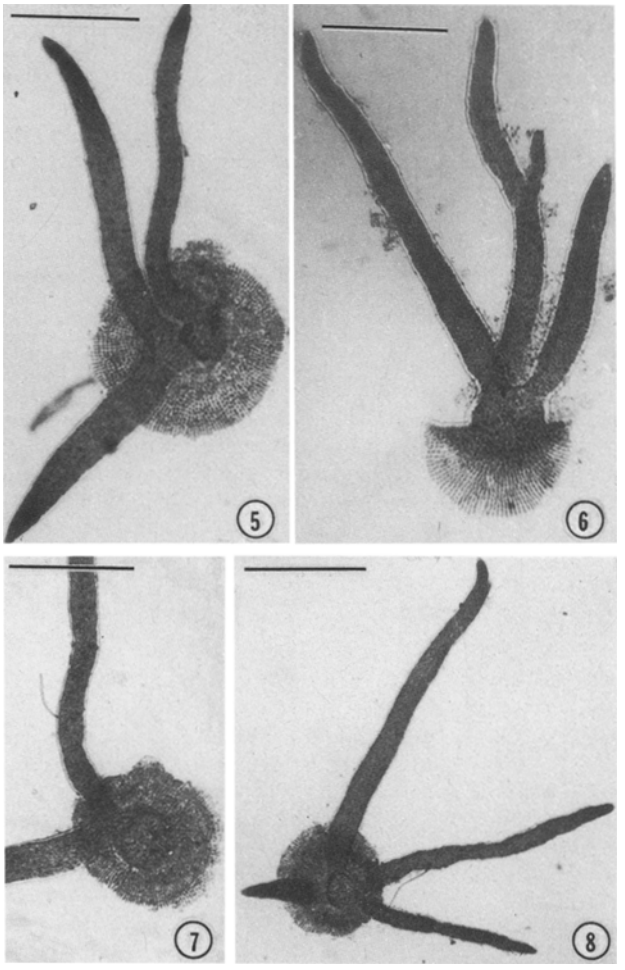

Fig. 2. *Hypnea musciformis*. *5*: carpospore germling (8 weeks old) with expansive attachment disc (500 µm diameter) and 3 shoot axes arising from it; *6*: carpospore germling (8 weeks old) with less expansive attachment disc (320 µm diameter) and 3 shoot axes (one branched); *7*: tetraspore germling (8 weeks old) with expansive disc (450 µm diameter) and 2 shoot axes arising from it; *8*: carpospore germling (8 weeks old) with expansive attachment disc (460 µm diameter) and 4 shoot axes of different ages developing from it

Discussion

The findings reported above on the germination of *Hypnea musciformis* carpospores and tetraspores are in agreement with those recently reported for *H. cervicornis* J. Agardh and *H. chordacea* Kuetzing from Hawaii (Mshigeni, 1974). Thus, the spore germination pattern of this seaweed falls within the framework of Chemin's (1937) "*Dumontia*-type" or Inoh's (1947) "*Typus discalis mediatus*".

The observation that the majority of *Hypnea musciformis* sporelings produced expansive attachment discs (Figs. 1 and 2) before shoot differentiation is similar to the recently reported observation (Mshigeni, 1974) for *H. chordacea* from Hawaii. Also worth noting is the fact that both species, although belonging to the Rhodophyta, are dominantly green in colour. These facts suggest that the two species show close phylogenetic relationships, and hence support the systematic

grouping of Agardh (1876) who placed them together (*H. chordacea* Kuetzing as a synonym of *H. spicifera* Suhr) in the section Virgatae.

The fact that very many sporelings of *Hypnea musciformis* remained viable in laboratory culture dishes for as many as 3 months without aeration suggests that spores of this seaweed are easy to grow and could be suitable seeding material in the marine agronomy of the alga for its carrageenan.

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