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OBSERVATIONS ON THE LIFE-HISTORY OF BRYOPSIS HYPNOIDES LAMOUR. FROM NEWFOUNDLAND: A NEW VARIATION IN CULTURE[1,2]

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SUMMARY

At its northernmost distributional limit in northeastern America, in Newfoundland, *Bryopsis hypnoides* Lamour. appears to have a heteromorphic, monophasic life-history. Zygotes resulting from fusion of anisogametes develop in culture into prostrate filamentous germlings, these also reproducing sexually by anisogametes to form a new generation of the prostrate type. Erect *Bryopsis* later grew from the prostrate phase: asexual stephanokontic zooids were entirely absent. The life-history in Newfoundland compares in part with that for *B. hypnoides* from northern parts of its European range; the presence of sexual reproduction in the prostrate phase in Newfoundland, however, is unique in *Bryopsis* and is not apparently a temperature dependent phenomenon. In the field Newfoundland *B. hypnoides* reaches sexual maturity in winter at temperatures less than 0 °C. Germling phases studied in culture could not be located in the field. The question is raised as to the possible genetic isolation of Newfoundland *B. hypnoides* from populations in Europe.

1. INTRODUCTION

Bryopsis hypnoides Lamour. (Chlorophyceae, Caulerpales) occurs over a wide range of the northeastern coast of North America, from Bermuda to Newfoundland (TAYLOR 1957; SOUTH & CARDINAL 1970; SOUTH & HOOPER 1972). The occurrence of the species in the cold Newfoundland waters, while not unexpected, was unusual in that sexual plants were found to reach reproductive maturity in winter at water temperatures less than 0 °C (SOUTH & HOOPER 1972). For more southern populations in northeastern North America sexual reproduction is reportedly restricted to the summer months. Extensive studies of life-histories in *Bryopsis* have been made (HUSTEDE 1964; NEUMANN 1969; RIETEMA 1969, 1970, 1971a, 1971b). For *B. hypnoides* the investigations have concerned populations from widely separated parts of the species range in Europe (NEUMANN 1969; RIETEMA 1971b), and comparable information for North American populations appears to be lacking.

According to RIETEMA (1970, 1971b) the life-histories of *B. hypnoides* and *B. plumosa* vary in identical ways at different latitudes along the coasts of Europe. In the Mediterranean germling phases are produced which can both form

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stephanokontic zooids and develop new *Bryopsis* plants directly: the "dormancy" of the germling phase is difficult to break. In more northern populations, the germling phases are only capable of directly developing into new *Bryopsis* plants and in culture the "dormancy" is easy to break after transfer to fresh culture medium: stephanokontic zooids are never produced. RIETEMA (1971b) tended to interpret the diminutive creeping germlings of the northern populations as separate sporophytic phases that had lost the capacity to form stephanokontic zooids. Cytological evidence appears to support this view (NEUMANN 1969; RIETEMA 1971b).

In the present paper a culture study of the life-history of *B. hypnoides* from Newfoundland, the most northerly site presently known for the species from northeastern North America, is described. The results of the study in part support RIETEMA's (1971b) findings for northern European populations, although field and culture evidence suggest that further in situ and culture studies of both phases are still required.

2. MATERIALS AND METHODS

Plants collected by R. G. Hooper from Eastport Causeway, Alexander Bay, Newfoundland [48°39'N; 53°54'W] on January 2, 1972, formed the basis of the present study. The population is the only one to date known for Newfoundland. Plants occur at a depth of 0–3 m subtidally, in a tidal channel subject to extreme currents and estuarine influence. Plants are attached to sediment-covered rocks.

A stock culture was established by R. G. Hooper on January 4, 1972, using a sterile enriched seawater medium with phosphate, nitrate, P_{11} metals, S_3 vitamins and vitamin B_{12} in the concentrations given for ASP_2 medium by PROVASOLI et al. (1957). Sterile branch apices were employed as the inoculum. Further investigations involved sub-culturing from the stock (commencing on February 15, 1972), under a variety of temperature, light and photoregime conditions, as summarized in *table 1*.

Stock cultures were maintained in 500 ml glass dishes at 10°C, 8/16 photoregime conditions (*table 1*). Sub-cultures were maintained in glass jars containing 20 ml medium, or in 5 cm disposable petri dishes containing 5 ml medium, and incubated in Sherer (Sherer-Gillet Co., Michigan) walk-in

Table 1. *B. hypnoides*: summary of culture conditions employed

T°C	light intensity[b]	photoregime (l/d)
2[a], 5, 10	450 1×	8/16
5, 10, 15	450 1×	16/8

[a] Used only for tests with anisogamete suspensions.
[b] Emitted from Sylvania "Cool White" fluorescent tubes.

controlled environment rooms, a reach-in Sherer culture cabinet, or (at 2.0 °C) in a modified refrigerator. Subcultures were initiated from a single stock parent by excising unbranched, 4.0 mm long sterile branch tips and using these as the inoculum. Steam-sterilized medium was employed for subculturing and unialgal cultures were obtained; the culture medium was renewed at approximately weekly intervals. Suspensions of anisogametes, when required, were pipetted into the disposable petri dishes under sterile conditions, for further study.

Cell wall reactions of both mature thalli and germlings to staining with Congo red and zinc-chlor-iodine were investigated.

3. RESULTS

3.1. Culture investigations

The original stock cultures grew rapidly and were once subcultured before the main subculturing series commenced on February 15. Stock plants reached lengths of 5 cm or more in six weeks and morphologically resembled the parent material, although being somewhat more laxly branched. At initiation of the main subculture series stock plants were in part reproductive, the determinate laterals transformed into gametangia. Male gametes were produced in the upper half of each gametangium, female ones in the lower half.

In the complete range of culture conditions employed (*table 1*) all subcultures grew readily. In less than a month, however (March 7), a universal production of gametangia occurred, with a complete cessation of vegetative growth. A massive release of biflagellate anisogametes occurred, and copulation was frequently observed. Development of the zygotes was followed in both the 10 ml and 5 ml dishes, transferring gamete suspensions to the latter, as described. The zygotes resulting from fusion of anisogametes germinated to produce very large numbers of creeping germlings which, after a few weeks, densely covered the bottoms and walls of the culture dishes. The germlings, while remaining as prostrate, branched structures, became highly fertile, releasing biflagellate anisogametes. Subculturing of zygotes obtained from germlings of anisogametes under all culture conditions resulted in production of a further germling stage in culture. At least two germling generations had thus been obtained in the first month.

Approximately six weeks from the original subculturing of February 15, upright *Bryopsis* plants commenced growth, these originating directly from the prostrate germling phases. Production of the uprights eventually occurred under all culture conditions, although more readily at 10 °C and 5 °C than at 2 °C and 15 °C. Growth of the *Bryopsis* continued in culture, the plants eventually again forming gametangia. In some instances it was observed that male gametes were produced proximally rather than distally in the gametangia, as had been observed on the original field material.

Cell walls of the upright gametophytic phase both from the field and culture reacted positively with Congo red and zinc-chlor-iodine. Cell walls of the prostrate phase, however, reacted negatively.

3.2. Field investigations

Intermittent examination of the *B. hypnoides* population in Newfoundland showed that following sexual maturation in January 1972, plants decayed and disappeared from the field. Attempts to locate the prostrate germling phase obtained in culture proved negative. By June 1972, however, a new population had appeared in the same locality, the plants healthy in appearance although sterile. The fate of the plants in the intervening period thus remains uncertain.

4. CONCLUSIONS AND DISCUSSION

B. hypnoides from Newfoundland, reaching sexual maturity in January 1972, at a temperature below 0 °C subsequently disappears from the field until the following June. In culture, successive, sexually reproducing generations of prostrate germlings were obtained, these later giving rise directly to new upright *Bryopsis*.

The results obtained here in part agree with RIETEMA's (1971b) findings for *B. hypnoides* in its northern range in Europe, in that the germling phase does not produce stephanokontic zooids, and that the *Bryopsis* phase develops directly from the germlings. The sexual reproduction of the Newfoundland germlings, however, does not necessarily fit the theory (RIETEMA 1971b) that they represent a sporophyte in which production of stephanokontic zooids has been suppressed. Although suggestive of results comparable to Rietema's, the reliability of the Congo red and zinc-chlor-iodine tests might require further substantiation in the light of our study.

The site of meiosis in Newfoundland *B. hypnoides*, not determined here, could be speculated as being prior to gamete production, in the gametangia of both phases rather than pointing to the possibility of a heteromorphic biphasic life history, as established by RIETEMA (1970, 1971b) for both *B. plumosa* and *B. hypnoides* from Europe, *B. hypnoides* from Newfoundland exhibits a heteromorphic, monophasic life history in culture. The production of stephanokontic zoospores in the germling phase in European *B. plumosa* and *B. hypnoides*, regarded as the sporophyte by RIETEMA (1971b), is not obligate and apparently dependent on temperature. Successful inbreeding of northern and southern *B. plumosa* populations by RIETEMA (1970) indicated that there were no genetic barriers between populations from the two regions. Temperature appeared to have no effect on Newfoundland *B. hypnoides*, the lack of stephanokontic zooids being apparent at all times. It will be important to determine whether or not southern populations of *B. hypnoides* from northeastern North America behave similarly to Newfoundland populations.

To date life-history studies of *Bryopsis* have depended entirely on culture investigations. Attempts to compare culture findings with actual events in the field are lacking. Rietema's work has successfully refuted the original and long-held interpretations of the monophasic, diplontic nature of the *Bryopsis* life-history, but this appears to be only partially true for Newfoundland populations. The behaviour of the species in the field as opposed to culture might be

held as critical by some, while the question remains whether *B. hypnoides* from Newfoundland is distinct genetically from the same species in Europe.

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