

A REVIEW OF LIFE HISTORY PATHWAYS IN *BRYOPSIS*

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(Communication presented by Prof. Giacomo Tripodi)

ABSTRACT. The genus *Bryopsis* comprises siphonous green algae widely distributed from tropical to polar seas. Despite the early reports on the simplicity of its life history, subsequent culture observations showed variety of life history patterns, even within a single species. Karyological data and reports on DNA quantification led to somewhat contradictory conclusions about the ploidy level of the two life history phases and about the moment of meiosis. Long term observations on Mediterranean species highlighted new alternatives in recycling of the two morphological phases. Looking at all published experimental data, we summarize all life history pathways of *Bryopsis* species.

1. Introduction

The genus *Bryopsis* J.V. Lamouroux [1] comprises green algae consisting of tubular multinucleate (siphonous) axes, lacking cross walls, variously branched with a feather-like appearance. Species are widely distributed from tropical to polar seas. Despite the early reports on the simplicity of its life history [2, 3], subsequent culture observations showed a more complex cycle [4-9].

A discovery of variety of life history patterns, even within a single species, and of new reproductive characters [7, 8, 10] led to the establishment of new genera: *Pseudobryopsis* Berthold in Oltmanns [11], a *Bryopsis*-looking alga differing because of peculiar pyriform gametangia, and *Bryopsisidella* J. Feldmann [12], an alga with two alternating morphological phases, previously reported as different taxa, namely *Bryopsis halymeniae* Berthold [13], the gametophyte, and *Derbesia neglecta* Berthold [14], the sporophyte. These studies resulted in a consequent taxonomic reorganization of the family Bryopsidaceae [see also 15]. Among *Bryopsis* species, karyological data and reports on DNA quantification led to somewhat contradictory conclusions about the ploidy level of the two life history phases and about the moment of meiosis [3, 16-19].

Long term observations carried by the authors on Mediterranean species highlighted new alternatives in recycling of the two morphological phases [20, 21]. Looking at all

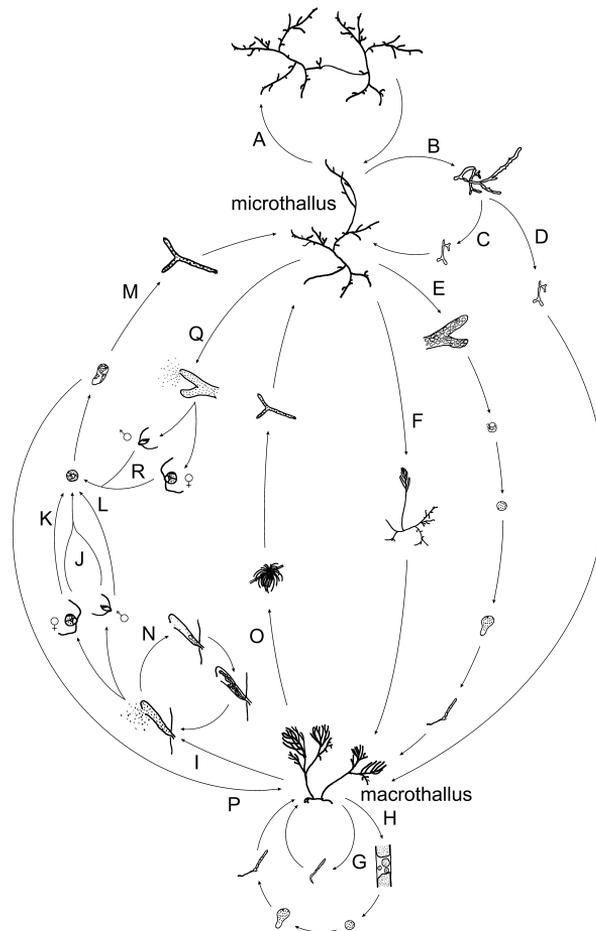


FIGURE 1. Life history pathways of *Bryopsis* species: (A) Fragmentation of microthallus; (B) Propagulum differentiation; (C) Propagulum growing in microthallus; (D) Propagulum growing in macrothallus; (E) Holocarpic differentiation of stephanokont zoospores; (F) Direct development of macrothallus from microthallus; (G) Detachment of vegetative ramuli; (H) Protoplast formation; (I) Differentiation of gametangia in macrothallus; (J) Fusion of gametes; (K-L) Parthenogenesis; (M) Germination of zygote in microthallus; (N) Reorganization of protoplasts in gametangia; (O) Production of microthalli directly from macrothalli siphons (MPMP); (P) Germination of zygote in macrothallus; (Q) Differentiation of gametangia in microthallus; (R) Fusion of gametes.

published experimental data we summarize all life history pathways of *Bryopsis* species (Fig. 1).

To our knowledge, such diversity is unseen in any other genus of green seaweeds, or even algae in general.

2. Life history of *Bryopsis*

Early reports on the biology of a population of *B. plumosa* (Hudson) C. Agardh from Naples (Italy, Mediterranean Sea) [2, 3] described a life history with a single diploid generation (monobiontic cycle) producing biflagellate anisogametes in vegetative-like ramuli (Fig. 1, I-J-P) [22]. In the first half of the 20th century this pattern was regarded as typical for this genus [23, 24].

Both types of gametes may be produced either on separated thalli (dioecious species) or on the same thallus (monoecious species), in different gametangia or in distinct sectors of the same gametangium. Only macrogametes have an eyespot. Gamete release occurs through one or more subapical papillae [22].

Later, observations on different European populations of *B. plumosa* (Hudson) C. Agardh and *B. hypnoides* J.V. Lamouroux showed that life history of these algae is dibiontic, that is with two strongly heteromorphic alternating generations. Zygotes germinated into uninucleate microthalli, microscopic creeping irregularly branched siphons with a single giant nucleus, alternating with macroscopic plumose thalli (Fig. 1, I-J-M). Mainly two different patterns may occur to recycle macrothalli. In the first pathway, macrothalli develop directly from microthalli, arising as upright siphons in continuity from creeping ones (direct development, Fig. 1, F). This was first observed on a population of *B. hypnoides* from Helgoland (Germany, North Sea) [19], with uninucleate microthalli (“protonema” in the original paper) becoming multinucleate before the production of erect macrothallus axes. A similar life history pattern was detected in *B. plumosa* populations from the North Sea and the Mediterranean Sea [4, 9, 25], and in *B. hypnoides* from the Baltic Sea, the North Sea and the Mediterranean Sea [6, 8].

In the second pathway, microthalli produce holocarpically stephanokont zoospores, which germinate into macrothalli, as shown by the observations on a population of *B. plumosa* from Roscoff (France, Atlantic Ocean) (Fig. 1, E) [9]. The same pattern occurs in *B. plumosa* populations from the Atlantic Ocean and the Mediterranean Sea [4, 16-18, 26], in *B. hypnoides* populations from the Atlantic Ocean and the Mediterranean Sea [5, 6, 18, 20, 21, 27], in *B. corymbosa* J. Agardh populations from the Mediterranean Sea and the Atlantic Ocean [20, 28, 29], and in *B. muscosa* J.V. Lamouroux from the Atlantic Ocean [28].

Both direct development and zoosporogenesis may occur in one population, as observed in *B. plumosa* from Naples (Mediterranean Sea) [4]. Populations with different development pattern, belonging to the same species, can hybridize and give fertile hybrids, and microthallus development seems genetically stated [4, 9]. According to Brück and Schnetter [30], only a few of microthalli undergo sporogenesis in the field.

2.1. Life history pattern and latitudinal distribution. Rietema [6] pointed out a correlation between life history pattern of the studied European populations and their latitudinal distribution. In Mediterranean isolates, from Naples and Banyuls [4, 6], both

direct development and zoosporogenesis may occur and microthalli undergo a long and hard-breaking dormancy period as uninucleate stage before completing their development. Microthalli isolates from high latitudes, namely from Atlantic coast of France, can only produce zoospores and their dormancy is shorter and easier to break [4, 6, 9]. Finally, northern populations from several localities in the North Sea and the Baltic Sea show a direct development and a shorter dormancy [8, 9].

Observations on populations of *B. hypnoides* from Helgoland [19] and from Banyuls [31], and of *B. plumosa* from Brittany [26], agree with this pattern, but dormancy is longer in the latter isolate. Differently, a population of *B. hypnoides* from Wales [27] does not match completely Rietemas model. It is a northern population with a “Mediterranean-like” life history pattern, because it can have both direct development and zoosporogenesis, even if its dormancy is easier to break than that of Mediterranean isolates. Finally, the life cycle of a population of *B. plumosa* from Iceland [25] completely disagrees with that model. Microthalli undergo a very long dormancy, eighteen months or more, before developing into macrothalli directly. In culture, the author was not able to correlate microthallus development to any physical factors, such as photoperiod, temperature or irradiance.

2.2. Parthenogenesis. Parthenogenesis (i.e. the direct development of gametes into zygotes) was observed on macrogametes of *B. plumosa* from Lake Ganzirri (Italy, Mediterranean Sea) (personal observations), of *B. muscosa* and *B. corymbosa* from Japan (Strait of Korea) [28], of *B. muscosa* from Brittany (Atlantic Ocean), on macrogametes and exceptionally on microgametes of *B. plumosa* from Brittany [26, 32], and on both gamete types of *B. plumosa* from North Carolina (Atlantic Ocean) (Fig. 1, K-L) [33].

Occurrence of parthenogenesis was differently interpreted by some researchers: it might be due to anomalous diploid gametes [18, 25] or, on the basis of the observation of rare macro- or microgametes in gametangia of the opposite sex, it would be only apparent and an in situ fertilization would occur [28, 33].

2.3. Vegetative reproduction. Few field observations are known about seasonality and induction of reproduction in *Bryopsis* species. It is not plain how frequent sexuality is in the field, and multiplication is commonly vegetative [21, 27].

As for macrothalli, several alternative ways commonly occur, as fragmentation of thallus, cutting off ramuli, in correspondence of a newly formed cross wall (Fig. 1, G), or cytoplasts formation (Fig. 1, H), sometimes referred to as aplanospores. Injured siphons extruded protoplasm and generated spontaneously numerous protoplasts which reorganized their wall and eventually developed into mature plants.

Vegetative multiplication is frequent in culture conditions, as well as in the field [20, 21, 23, 27, 33-35]. Ramuli detachment is a very efficient way to spread towards new habitats on a wider range than gametes and spores. It usually precedes seasonal or occasional disappearance of a population in a site, making reasonable that it is a response to unfavourable growth conditions [21, 33].

Production of new erect axes from creeping old ones is very common. Particularly, on a population of *B. corymbosa* from Reggio Calabria (Italy, Mediterranean Sea), reorganization of protoplasm was observed in a few empty gametangia, as well as in non releasing ones, in culture conditions (personal observations, Fig. 1, N). After gamete release, residue cytoplasm rarely reorganizes in small protoplasts, which fuse together and regenerate new

thin axes with irregular branching patterns growing inside old gametangium wall. Such regenerated siphons grow out into macrothallus siphons.

Brück and Schnetter [30] described for the first time fragmentation of microthalli on a population of *B. plumosa* from Canary Islands. We also found such a vegetative multiplication on *B. corymbosa* and *B. hypnoides* from the Strait of Messina [20, 21]. Particularly, Brück and Schnetter reported for the first species that microthalli have a region specialized for vegetative multiplication, a thin branch that produce an adventitious thallus similar to parental one (Fig. 1, A). Later, by necrosis of the connecting axis, this “duplex structure” separates into independent plants. In species we investigated, multiplication is due to adventitious branched vegetative propagula that are cut off in a defined abscission zone and grow to dimensions of mature thalli (Fig. 1, B-C). As for their development and morphology, these branched propagula remind of “stellate propagula” of *Bryopsidella ostreobiformis* Calderón-Sáenz and Schnetter [15], observed only on this species and considered typical of this genus by the authors.

2.4. Alternative pathways. Alternatives to the generally observed life history models are known.

A study on a population of *B. hypnoides* from Newfoundland [36] showed the occurrence of sexual reproduction in both morphological phases (Fig. 1, I-Q). Zygotes, arising from fusing anisogametes from either macrothalli or microthalli, germinated into the creeping phase (Fig. 1, J-R-M), while plumose plants are produced by direct development (Fig. 1, F); zoosporogenesis never occurs.

Furthermore, observations on a population of *B. plumosa* from Canary Islands brought out the possibility of production of microthalli directly from macrothalli siphons (“MPMP” in the original paper), without the occurrence of gamete differentiation, both in culture conditions and in the field (Fig. 1, O) [17].

Finally, we occasionally observed the differentiation of adventitious branched propagula from microthalli growing in macrothalli on an isolate of *B. corymbosa* from Reggio Calabria (Italy, Mediterranean Sea) (personal observations, Fig. 1, B-D).

2.5. The moment of meiosis. As for the moment of meiosis and the ploidy level of the two morphological phases, direct (karyological and DNA quantification data) and indirect (life history observations) evidences for both sporogenic and gametogenic meiosis were published [3, 4, 6, 8, 9, 16-19, 36].

Karyological evidences of gametogenic meiosis were published by Zinnecker [3] for *B. plumosa* from Naples, by Neumann [19] for *B. hypnoides* from Helgoland, who also observed the first mitotic division of the macronucleus of the microthallus, and by Kermarrec [18] for *B. plumosa* from Brittany.

The thesis of the occurrence of meiosis in gametangia is also supported by indirect evidences from observations on *B. hypnoides* from Newfoundland [36], in which both macro- and microthalli produce gametes. Even if the moment of meiosis was not experimentally determined, the authors supposed that it occurred at gamete differentiation in both morphological phases. The occurrence of sexual reproduction in both morphological phases does not seem explainable in a life history in which the two phases have different ploidy levels.

Besides, the simultaneous production of both macrothallus-producing and vegetative propagula even from one microthallus in a population of *B. corymbosa* from Italy seems hardly explainable in a haplo-diplontic life cycle pattern.

Conversely, studies on the evaluation of nuclear DNA content by microspectrophotometry brought evidences for sporogenic meiosis. Observation on isolates of *B. plumosa*, *B. pennata* J.V. Lamouroux and *B. hypnoides* from North Carolina [16] showed that the ploidy level of microthallus nuclei was the same of gametes nuclei, proving that macrothalli were haploid.

Further evidences came from a study on a population of *B. plumosa* from Canary Islands [17]. The authors proved that macrothallus nuclei had the same ploidy level of gametes and that microthalli were diploid. Only at a late stage before zoospore formation, haploid nuclei were present in microthalli. Meiosis was supposed to occur late in microthallus development, presumably the last division before zoospore differentiation. Furthermore, the authors excluded the hypothesis that the microthallus primary nucleus was polyploidy because they observed an irregular diminution of the DNA content during the division process of the secondary nuclei and the simultaneous existence of nuclei with large differences in DNA content. As an alternative, they suggested that rDNA amplification may occur, which may be necessary because of the large quantity of cytoplasm associated with this solitary nucleus. The fragmentation of the nucleolus observed in some population [8, 18, 19] may be an indication of the multiplication of the nucleolus organizer.

Microthalli-producing macrothallus parts (MPMPs) were the only portions of macrothalli having diploid nuclei. The authors supposed somatic autogamy to occur, but fusing nuclei were not observed.

Brück and Schnetter also speculated on the cytological cycle of monobiontic populations of *Bryopsis* species and inferred that ploidy levels of morphological phases were to be similar in both models of life cycle [6, see also 9]. Somatic meiosis might occur in the late plurinucleate stage, before erect axes are brought out. This hypothesis is supported because mono and dibiontic isolates can hybridize and the resulting microthalli have sometimes a mixed development, in which a part of one plant produces zoospores while the residual part of the same plant gives directly erect axes [6, 8, 9]. This behaviour might be due to the presence of different haploid nuclei in one thallus, because of meiotic segregation.

Indirect evidences supporting sporogenic meiosis may come from observations on culture studies. According to Rietema's findings [4, 9], in dioecious species, zoospores produced from one thallus give about 50% of male macrothalli, 50% of female macrothalli and a few monoecious. Moreover resulting macrothalli may have different vegetative morphology that is kept in vegetative subcultures. From these observations, Rietema inferred that meiosis occurred anywhere in microthallus phase, probably during zoospore differentiation.

3. Conclusions

Further observations are necessary to clarify the cytological cycle and the reproductive biology of different *Bryopsis* species. Particularly, microthallus development should be investigated, both in the field and in controlled conditions, in order to test which physical

or physiological factors control the induction of sexual versus asexual reproduction and the seasonality of the two phases.

In our opinion, uninucleate microthalli of *Bryopsis* should to be considered perennial and autonomous thalli, with their own development cycle, independent from sporogenesis and capable of vegetative multiplication, as it can be also inferred on the basis of previous observations [17, 20], rather than a specialized zygote, as pointed by van den Hoek et al. [37]. In accordance with such interpretation, we find meaningless to consider as “dormancy” the period of time between the moment in which microthalli reach of a definite size and sporogenesis, since it is neither a period of ceased growth and reduced physiological activity (thalli grow and fragmentize), nor merely a gap in which microthalli survive under unfavourable conditions waiting for optimal growth conditions to reproduce and close their life history.

In such perspective, macrothalli would be just a heteromorphic expression of the perennial phase, needed for sexual reproduction and enhancing the dispersion ability of the population in the field.

Acknowledgements

The authors are deeply indebted to prof. Flora De Masi who dedicated much of her time to discuss such topic and to encourage the first author in her career. Great acknowledgments are also due to prof. Giacomo Tripodi for the review of the present paper and especially for the support given at any time.

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Presented 25 November 2009; published online 20 September 2010

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Atti Accad. Pelorit. Pericol. Cl. Sci. Fis. Mat. Nat., Vol. LXXXVIII, No. 2, C1A1002005 (2010) [9 pages]