BioOne RESEARCH EVOLVED

Examining the Ability of Calyptrae to Produce Protonema in *Funaria hygrometrica*

Author(s): Melissa A. Wynne and Jessica M. Budke
Source: Evansia, 29(2):61-64. 2012.
Published By: The American Bryological and Lichenological Society, Inc.
DOI: http://dx.doi.org/10.1639/079.029.0205
URL: http://www.bioone.org/doi/full/10.1639/079.029.0205

BioOne (www.bioone.org) is a nonprofit, online aggregation of core research in the biological, ecological, and environmental sciences. BioOne provides a sustainable online platform for over 170 journals and books published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Web site, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/page/terms_of_use.

Usage of BioOne content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Examining the ability of calyptrae to produce protonema in *Funaria hygrometrica*

Melissa A. Wynne
Department of Ecology and Evolutionary Biology, 75 North Eagleville Road, University of Connecticut, Storrs 06269-3043, USA
E-mail: **melissa_wynne@hotmail.com**

Jessica M. Budke
Department of Ecology and Evolutionary Biology, 75 North Eagleville Road, University of Connecticut, Storrs 06269-3043, USA
E-mail: **jessica.m.budke@gmail.com**

***Abstract*. Our research assesses the ability of calyptrae to produce protonema in the moss species *Funaria hygrometrica* Hedw. Herein the following question is addressed: how long after detachment from the maternal plant are calyptrae able to produce new individuals by means of protonema? Plants from a local Connecticut population were grown in laboratory conditions until 2, 14, or 28 days post-detachment of the calyptra from the leafy gametophyte. Calyptrae were removed, placed onto sterile media, and observed for protonemal growth for 10 weeks. Calyptrae were found to be alive and produced protonema at all three developmental ages. The youngest calyptrae produced the highest percentage of protonemal growth, whereas the oldest calyptrae produced significantly fewer. Our data provide evidence that calyptrae in a laboratory setting remain alive after detachment from the maternal plant and may have the potential to act as a dispersal unit.**

***Keywords*. calyptra, dispersal unit, *Funaria hygrometrica,* protonema.**

INTRODUCTION

The leafy gametophyte is the vegetative and dominant part of the moss life cycle. The function of the gametophyte is to produce gametes for sexual reproduction. The fusion of egg and sperm results in an embryo that divides to form a mature sporophyte (Fig. 1A). In the vast majority of mosses, the sporophyte apex is covered throughout development by a protective cap of tissue (i.e., the calyptra; Fig. 1B). The calyptra develops from tissues of the maternal gametophyte and completely surrounds the sporophyte early in development. Eventually the calyptra separates from the remainder of the leafy gametophyte and remains atop the sporophyte until capsule maturity. Premature calyptra removal results in abnormal sporophyte development demonstrating that the calyptra is necessary for sporophyte maturation (Zielinski, 1910).

One important characteristic of mosses is that all cells are totipotent: they are able to give rise to new plants in the form of protonema (Goebel, 1905). Asexual reproduction in mosses includes many diverse structures, such as gemmae, protonemal buds, and fragmentation of any vegetative tissue, that serve as dispersal units to regenerate new mosses away from the maternal plant (Newton and Mishler, 1994). Asexual reproduction via gemmae and propagules has been shown to be as effective in producing new individuals as sexually produced spores in *Tetraphis pellucida* (Kimmerer, 1991). Cells of the calyptra have been observed alive at sporophyte maturity (Bopp, 1954; Oehlkers and Bopp, 1957), and thus may have the ability to produce protonema (Meyer, 1942).

This study examines several questions regarding the calyptra lifespan: (1) Can we confirm that calyptrae are able to produce new individuals? (2) How long after detachment from the leafy gametophyte are calyptrae able to produce protonema? (3) Can calyptrae produce protonema after naturally falling off the mature sporophyte late in development? Addressing these questions will enable us to determine if calyptrae have the potential to act as dispersal units.

Materials and Methods

Funaria hygrometrica plants from a local Connecticut population (Budke #145 CONN) were grown in the laboratory under fluorescent lights on a rich/sandy/loam soil mixture with basic pH. Leafy gametophytes were grown for 4 months at room temperature (12/12hrs light/dark), followed by a cold treatment at 10°C (8/16hrs light/dark) for 2 months to stimulate antheridia and archegonia formation (Dietert, 1980). Antheridia were produced after approximately 1 month and archegonia after approximately 2 months. Pots were then flooded with de-ionized water for 24 hours to facilitate fertilization. The cultures were un-flooded and remained in the growth chamber at cold treatment conditions for an additional week. They were then placed on a light cart at room temperature (12/12hrs light/dark) to promote sporophyte growth and development.

Plants were observed daily to check for the time of detachment of the calyptrae from the leafy gametophyte (Fig. 1B). Once the calyptrae were detached, 91 plants (including the leafy gametophyte, sporophyte, and calyptra) with sporophytes 5 to 6 mm tall were randomly assigned to one of three experimental groups (early = 2 days, middle = 14 days, and late = 28 days post-detachment). The plants were maintained in culture conditions and allowed to mature to either 2 (N = 24), 14 (N = 39), or 28 days (N = 28) after detaching from the leafy gametophyte. Once they reached their assigned experimental age the plants were placed in a humidity chamber for several hours to ease calyptra removal. Additionally, a set of calyptrae (N =28) that had naturally fallen from fully developed capsules were collected and processed in the following manner.

After removal, calyptrae were split longitudinally to facilitate sterilization (Fig. 1C) and each was placed in 1 ml of water in a microcentrifuge tube. Calyptrae were then surface sterilized with a 1.0% bleach solution, and rinsed three times with sterile water using a centrifuge. Calyptrae were placed on plates of sterile Knops media, with Gelrite substituted in place of agar as the solidifying agent (Collier and Hughes, 1982). Calyptrae were placed onto media in a laminar flow hood to maintain sterile conditions and then the plates were placed on a light cart at room temperature (12/12hrs light/dark) and observed every two weeks for protonema growth. Individuals were scored for presence or absence of protonema growing from the calyptrae every two weeks until no additional individuals produced protonema.

Results

Protonema production was not observed from any of the calyptrae that had fallen from the fully formed, mature sporophytes. However, protonema growth was observed in the individuals assigned to the three age groups (Fig. 1D). At 4 weeks, 11% of the middle and 7% of the late stage calyptrae showed protonemal growth (Fig. 2). After week 4, the middle stage showed a minor increase (13%) in the number of calyptrae with protonemal growth, whereas, no additional late stage calyptrae produced protonema (Fig. 2). The early stage calyptrae showed 17% protonema production at week 6 and 25% at week 8 (Fig. 2). The early stage (2 days old) at week 8 had the highest percentage of calyptrae with protonemal growth across the experiment (Fig. 2).

Calyptrae at the earliest developmental stage were examined at 10 weeks but no additional growth was observed, consequently data was only collected for 8 weeks for the other two groups. No protonema were produced from any of the calyptrae that were sampled from mature sporophytes that had naturally fallen off late in development.

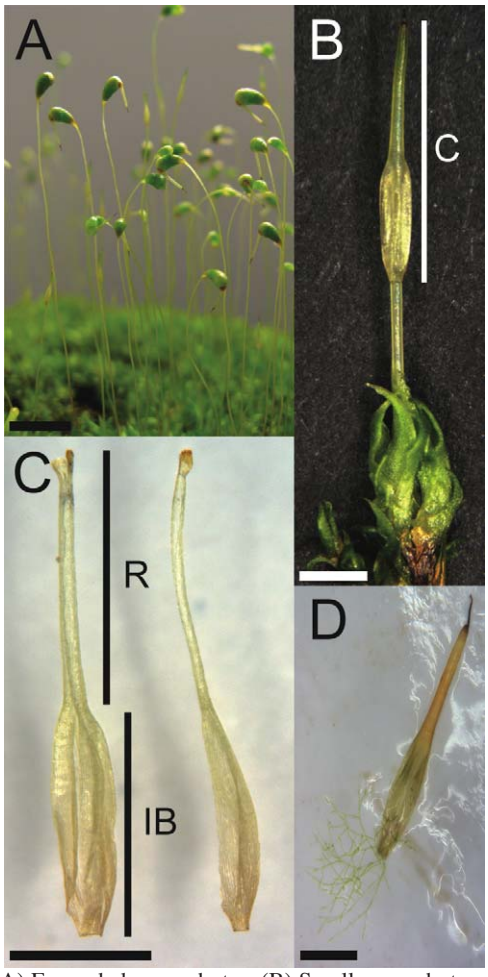

Figure 1. *Funaria hygrometrica* (A) Expanded sporophytes. (B) Small sporophyte with calyptra covering the apex. (C) Calyptra split longitudinally. (D) Calyptra with protonemal growth from the bottom edge of the inflated base. Abbreviations: C, calyptra; IB, inflated base; R, rostrum. Scale bars: A = 1cm, B-D = 1mm.

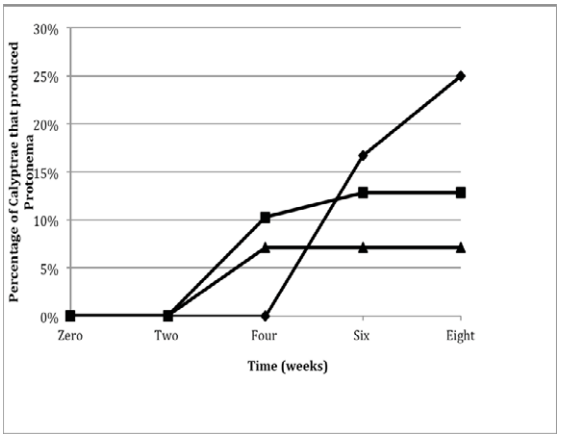

Figure 2. Calyptrae were observed for protonemal growth over an 8 week period every 2 weeks. The percentages of calyptrae producing protonema for each developmental age [early = 2 days (diamond), middle = 14 days (square), and late = 28 days post-detachment (triangle)] are displayed.

Discussion

Calyptrae of *Funaria hygrometrica* are alive and able to produce protonema at 2, 14, and 28 days post-detachment from the leafy gametophyte. The youngest calyptrae have the greatest potential for growth, however, compared to the middle and late stage calyptrae that germinated at 4 weeks after removal, they are delayed and begin to germinate at 6 weeks. These plants were grown in relatively stress-free laboratory conditions, which may not reflect the potential for protonema development from calyptrae in their natural environment. Calyptrae that had naturally fallen off the sporophyte apices late in development did not produce protonema. Thus calyptrae may only have the potential to act as a dispersal unit early in development. However, it is unlikely that calyptrae at this early stage would naturally fall off the sporophyte apex.

All of the protonemal growth originated from the inflated base of the calyptra as opposed to the rostrum (Fig. 1C, D). Moreover, when calyptrae were cut transversely no protonema grew from either the middle of the inflated base or the rostrum (pers. obs.). Based on our study of *F. hygrometrica*, only the cells composing the bottom edge of the calyptra inflated base are able to produce protonema.

The calyptrae of *Funaria hygrometrica* have the potential to produce protonema in a laboratory setting. It is apparent that the younger calyptrae have a greater ability to produce protonema. In order to fully assess the potential of calyptrae to act as dispersal units, wild populations grown under natural conditions must be examined.

Acknowledgements

The authors would like to thank Dr. B. Goffinet and Keith Bowman for comments on earlier versions of this manuscript. Funding was provided by the National Science Foundation (DEB-0919284 to B. Goffinet and C. S. Jones) and the Office of Undergraduate Research at the University of Connecticut. Thanks to Dr. C. Schlichting for use of a growth chamber and light cart, the Ecology and Evolutionary Biology greenhouse staff for soil preparation, Dr. L. Lewis for use of a laminar flow hood, and Dr. C. Jones for use of a camera and dissecting scope.

Literature Cited

Bopp M. 1954. Untersuchungen über Wachstum und Kapselenwicklung normaler und isolierter Laubmoossporogone. Zeitschrift für Botanik 42: 331–352.

Collier P. A. and Hughes K. W. 1982. Life cycle of the moss *Physcomitrella patens*, in culture. Journal of Tissue Culture Methods 7: 19–22.

Dietert M. F. 1980. The effects of temperature and photoperiod on the development of geographically isolated populations of *Funaria hygrometrica* and *Weissia controversa*. American Journal of Botany 6: 369–380.

Goebel K. 1905. Organography of Plants, Part II. Special Organography. (Transl. by I.B. Balfour.) Clarendon Press, Oxford, United Kingdom.

Kimmerer R. W. 1991. Reproductive Ecology of *Tetraphis pellucida* I. Population Density and Reproductive Mode. The Bryologist 94: 255–60.

Meyer S. L. 1942. Physiological studies on mosses. IV. Regeneration in *Physcomitrium turbinatum*. Botanical Gazette 104: 128–132.

Newton A. E. and Mishler B. D. 1994. The evolutionary significance of asexual reproduction in mosses. Journal of Hattori Botanical Laboratory 76: 127–145.

Oehlkers F. and Bopp M. 1957. Entwicklungsphysiologische Untersuchungen an Mossmutanten II. Die Korrelation zwischen Sporogon und Kalyptra bei Mutanten von *Funaria* und *Physcomitrium*. Zeitschrift für induktive Abstammungs- und Vererbungslehre 88: 608–618.

Zielinski F. 1910. Beitrage zur Biologie des Archegoniums und der Haube der Laubmoose. Flora 100: 1–36.