



Illuminating the role of the calyptra in sporophyte development

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Abstract

The study of moss calyptra form and function began almost 250 years ago, but calyptra research has remained a niche endeavor focusing on only a small number of species. Recent advances have focused on calyptra cuticular waxes, which function in dehydration protection of the immature sporophyte apex. The physical presence of the calyptra also plays a role in sporophyte development, potentially via its influence on auxin transport. Progress developing genomic resources for mosses beyond the model *Physcomitrium patens*, specifically for species with larger calyptrae and taller sporophytes, in combination with advances in CRISPR-Cas9 genome editing will enable the influence of the calyptra on gene expression and the production of RNAs and proteins that coordinate sporophyte development to be explored.

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Introduction

Successful development and reproduction are central processes for biological organisms. In the vast majority of plants, the results of fertilization are highly branching, free-living diploid sporophytes, whereas in bryophytes, the diploid sporophytes are unbranched, physically attached to and dependent on the parental gametophyte throughout their life [1]. Bryophyte sporophytes are

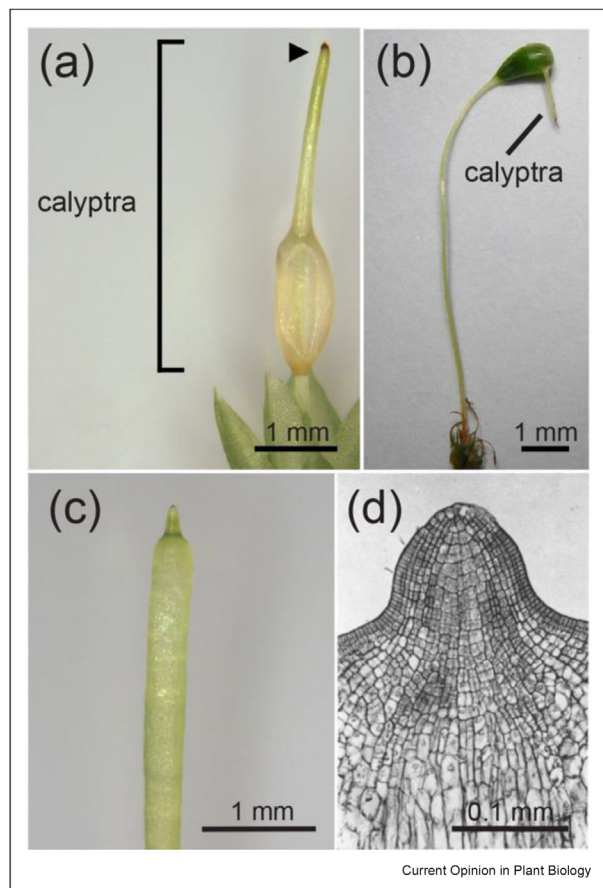
covered by a waxy cuticle which is impervious to water uptake and thus they acquire water and mineral nutrients from their parental gametophyte [2]. While bryophyte sporophytes can photosynthesize, most taxa also acquire the majority of their photosynthates from their parental gametophyte [3]. All of these resources are transferred through the foot, which is located at the base of the sporophyte [4]. Thus the survival and development of bryophyte sporophytes are intimately connected with the parental gametophyte.

In mosses, the apical region of the sporophyte also interacts with the gametophyte. During the early stages of development, the sporophyte apex is covered by a cap of gametophyte tissue, which is called the calyptra (plural = calyptrae; [Figure 1a](#); [5]). The calyptra forms early during development from the archegonium and, in some taxa, also from the subtending gametophyte stem ([Figure 2](#); [6]). In some species, the calyptra separates from the leafy gametophyte below via a ring of dehiscent cells (e.g., *Funaria* in Ref. [7]). Once disconnected from the rest of the gametophyte the calyptra persists atop the sporophyte apex during capsule expansion ([Figure 1b](#)) and can remain alive for a time, but does not continue to grow and ultimately dies [8,9].

Sporophyte development and the calyptra

The calyptra plays a critical role in moss sporophyte development ([Figure 3](#)). When calyptrae are removed during early development under high humidity conditions the apical region remains undifferentiated and the sporophyte does not transition to capsule expansion and differentiation ([Figure 1c,d](#); [10,11]). Typically during this transition, the seta meristem ceases cell divisions. However, calyptra removal results in this meristem continuing to divide [12]. Without the calyptra it no longer builds a narrow cylindrical seta and instead produces an expanded, obconic-shaped stalk ([Figure 1c](#)). To test whether this was a physiological and/or physical interaction, calyptrae were experimentally removed, boiled using multiple solvents to extract any physiologically active compounds, and then replaced on the sporophyte apex [13]. Even after this experimental manipulation, the sporophytes continued through their regular developmental transitions, producing both a seta and capsule with normal morphology. These

Figure 1



Funaria hygrometrica sporophytes with and without calyptrae. (a,b) Reproduced with permission from Ref. [21]. (a) Parental gametophyte calyptra covering an immature, unexpanded sporophyte. Arrow indicates sporophyte apex. (b) Moss calyptra on the top of a mature sporophyte capsule. (c,d) Calyptra removed from sporophyte apex in a high humidity chamber. Sporophyte continues to grow via the activity of the seta meristem and the capsule does not expand or differentiate. (c) Instead of producing a narrow cylindrical seta the meristem produces an expanded obconic-shaped stalk. Reproduced with permission from Ref. [5]. (d) Median longitudinal section of the sporophyte apex. Reproduced with permission from Ref. [12].

observations point toward the physical presence of the calyptra coordinating both seta development and capsule differentiation, but further exploration of potential physiological influences has not been undertaken.

The plant hormone auxin also plays a role in moss sporophyte development (Figure 3). During early development, auxin is transported basipetally through the sporophyte and later, during capsule differentiation, it is transported both acropetally and basipetally [14,15]. Experimental disruption of the basipetal flow of

auxin results in sporophytes with multiple sporangia, demonstrating its role in branching suppression in the moss sporophyte [15,16]. The acropetal flow of auxin has been proposed to play a role in capsule differentiation [14], and thus calyptra removal during early development, which results in a sporophyte that does not transition to capsule differentiation (Figure 1c,d), may be due to disruption of this auxin flow. Further studies of auxin transport in moss sporophytes are needed to determine which tissues auxin is moving through (i.e., epidermis, cortex, and/or central strand), the role transmembrane proteins play (e.g., auxin efflux PIN-FORMED [PIN] proteins; [17]), and how the physical presence of the calyptra may influence auxin transport.

The moss calyptra also protects the undifferentiated sporophyte apex from dehydration (Figure 3). Researchers long observed that when calyptrae are removed during early development under low humidity conditions, the apex withers and the moss sporophyte dies [18,19]. The ability of the calyptra to protect the sporophyte from dehydration was attributed to a waxy cuticle [7,20]. Despite the early articulation of this hypothesis, it took nearly 100 years to confirm that a waxy cuticle is present on the calyptra [21] and that it develops precociously relative to the cuticle of the sporophyte [22]. Under low humidity conditions, experimental removal of calyptra cuticle waxes negatively impacts survival, development, and fitness of the sporophyte, thus demonstrating the importance of the calyptra cuticle for dehydration protection [23].

Calyptra morphological diversity

Comparative chemistry may enable us to better understand the structure and function of the calyptra cuticle. In *Funaria hygrometrica* the calyptra was found to have thicker cuticle wax coverage in comparison to the leafy gametophyte ($2.0 \mu\text{g cm}^{-2}$ versus $0.94 \mu\text{g cm}^{-2}$, respectively; [24]). While the calyptra of this species has a smooth morphology (Figure 1a,b), other taxa, such as members of the Polytrichaceae and Orthotrichaceae, have calyptrae that are covered in epidermal hairs (Figure 4a–c). Ongoing research in our laboratory is testing the hypothesis that species with smooth calyptrae have thicker layers of cuticle waxes in comparison to species with hairy calyptrae, due to the added dehydration protection provided by the hairs. In a separate study, a comparison of three Polytrichaceae species indicated potential differences in wax chemical composition between two species with hairy calyptrae (*Pogonatum pensilvanicum* and *Polytrichum juniperinum*) and one species with smooth calyptrae (*Polytrichadelphus pseudopolytricum*; [25]). Considering the results from these studies together

Figure 2

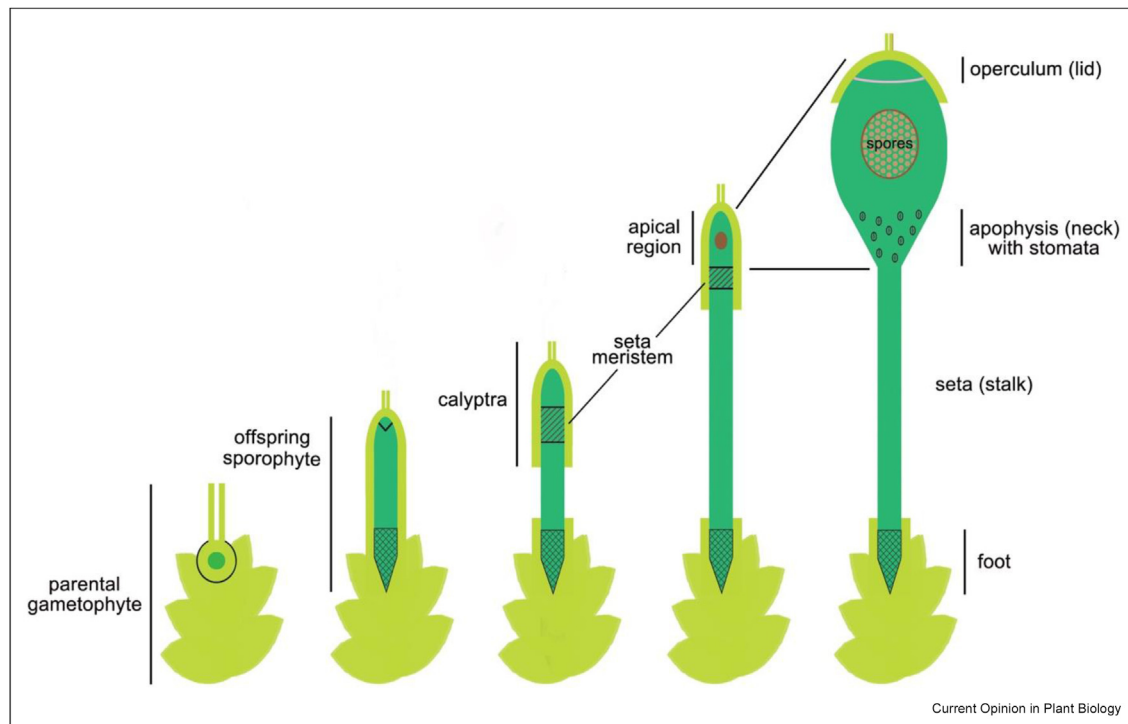


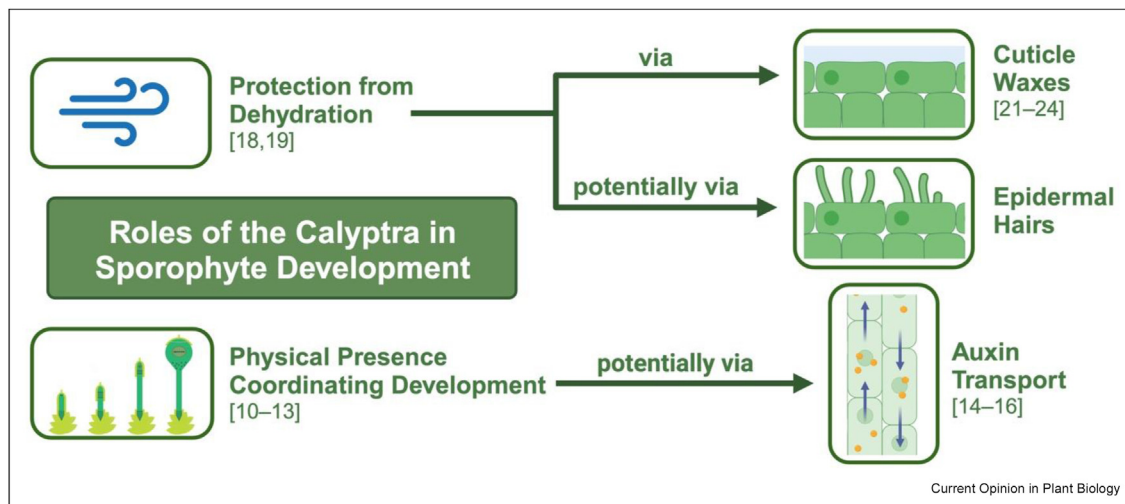
Diagram of moss sporophyte development. Sporophytes begin development completely surrounded and protected by tissues of the parental gametophyte. Initially the sporophyte grows by divisions of a single apical cell. Later a second meristematic region, the seta meristem, produces the seta/stalk, which elevates the undifferentiated apical region. This region later differentiates into the sporangium/capsule. The sporangium includes cells that will undergo meiosis to produce haploid spores, a operculum/lid that enables spore release, and an apophysis/neck that, if present, is where stomata are located in some species. Throughout sporophyte development the apical region and seta meristem are covered by the gametophyte calyptra. Reproduced with permission from Ref. [5].

[24,25] a potential pattern emerges. The two species with smooth calyptrae (*F. hygrometrica* in Figure 1a,b and *P. pseudopolytricum*) have wax mixtures that include alcohols and low levels of alkanes, but lack aldehydes. In contrast, the two species with hairy calyptrae (*P. pensilvanicum* and *P. juniperinum*) lack alcohols, have high levels of alkanes, and aldehydes are present. These differences in wax composition suggest that cuticles on smooth and hairy calyptrae may function differently, though our current level of understanding does not enable us to determine the precise functional differences for these compounds. Further exploration of calyptrae with different hairiness levels will enable us to determine if these patterns are consistent across a wider array of species.

In addition to having different levels of calyptra hairiness, calyptrae also vary in size and shape across the approximately 13,000 species of mosses [26]. Calyptra size ranges from very small (0.2 mm) in *Physcomitrium* (*Physcomitrella*) *patens* (Hedw.) Mitt. [27] to relatively large (up to 20 mm) in *Dawsonia polytrichoides* R. Br. [28].

Evolutionary reductions in calyptra size appear to be correlated with smaller sporophytes and faster life cycles, but this hypothesis remains to be tested in a phylogenetic context. Calyptra shape ranges from species that have a narrow rostrum apically that abruptly transitions to a wider inflated base (Figure 4d,e) to those that become gradually wider from the top to bottom (Figure 4f–h) to species that have a narrow tube-shape throughout (Figure 4i–k). These morphologies have historically been divided into two broad categories. Cucullate calyptrae have a slit up one side (Figure 4i–k) and mitrate calyptrae lack a prominent single slit, but can have one to multiple small slits at the bottom edge (Figure 4d–h). These morphological disruptions occur after calyptra development is complete. They are caused by the expansion of the underlying sporophyte and are influenced by capsule morphology, which can range from upright, resulting in mitrate calyptrae, to inclined, resulting in cucullate calyptrae. Connecting this structural diversity in size, shape, and hairiness of the calyptrae to their functional abilities, in terms of protection and developmental coordination as well as

Figure 3



Roles of the calyptra in sporophyte development. These roles include protection from dehydration [18,19] and the physical presence of the calyptra coordinating development [10–13]. Cuticular waxes on the surface of the parental calyptrae [21,22,24] have been demonstrated to provide dehydration protection for the sporophyte [23]. Epidermal hairs on the calyptrae may also play a role in dehydration protection, but this has yet to be studied experimentally. Auxin transport in moss sporophytes occurs basipetally during the early development and later, during capsule differentiation, auxin is transported both acropetally and basipetally [14,15]. Disruption of the basipetal flow of auxin results in sporophytes with multiple sporangia, demonstrating its role in branching suppression [15,16]. The physical presence of the calyptra may play a role in coordinating sporophyte development by influencing auxin transport, but this hypothesis has yet to be test. This illustration was created with [BioRender.com](https://www.biorender.com).

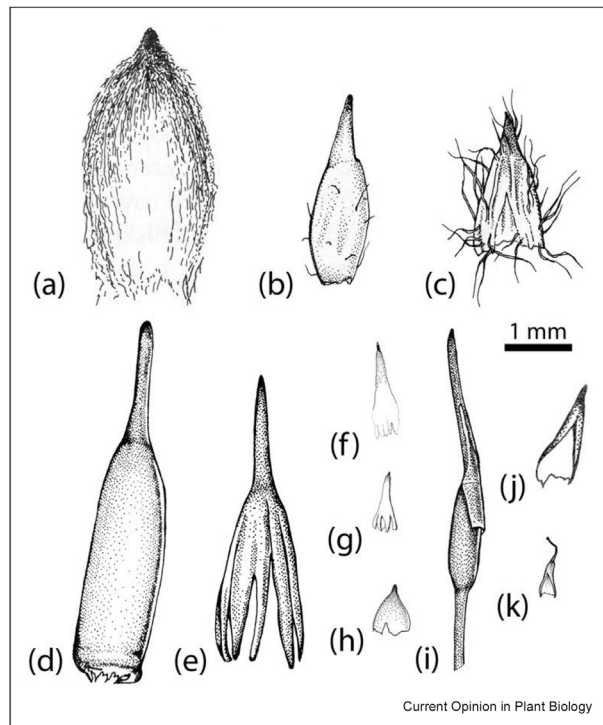
the diverse habitats these species occupy remains an underexplored area of study.

Model systems for studying calyptra function

The majority of research studies that integrate functional genomics and development in mosses focus on the model species *Physcomitrium patens* (Funariaceae; [29–33]). This species was developed as a model system due to its rapid life cycle [34], sequenced genome [35], and its ability to be genetically transformed using homologous recombination [36]. Research employing *P. patens* has expanded our understanding of plant growth and development, but unfortunately this species is not an optimal system for studying the functional relationships between the parental calyptra and sporophyte. In this species, both the calyptra and sporophyte are morphologically reduced (0.2 and 0.4 mm in height, respectively) and the capsule lacks structures that aid in spore dispersal, including peristome teeth and an operculum [27]. The rapid life cycle also results in a short time span for studying sporophyte development in relation to the calyptra. Due to these challenges, there do not appear to be any published studies focusing on calyptra cuticle waxes or the calyptra–sporophyte relationship in *P. patens*.

Species with larger calyptrae and taller sporophytes devote more time to sporophyte development, enabling this relationship to be studied at multiple developmental stages [22] and their larger size facilitates easier manipulation experiments [11,23]. These larger species are also often morphologically complex, enabling explorations of the developmental influence of the calyptrae on structures such as the peristome, which has been identified as a key innovation in the evolution and diversification of mosses [37]. Genomic resources have been developed for several species that have both relatively large calyptrae and sporophytes, as well as complex sporophyte morphologies, including *Ceratodon purpureus* (Hedw.) Brid. [38], *Syntrichia caninervis* Mitt. [39], *Takakia lepidozoides* S.Hatt. & Inoue [40], *Entodon seductrix* (Hedw.) Müll. Hal. [41], *Hypnum curvifolium* Hedw. [41], *F. hygrometrica* Hedw. [42], and *Physcomitrium pyriforme* (Hedw.) Brid. [42]. The latter two species, along with *P. patens*, are in the Funariaceae and thus are well positioned for comparative developmental studies [43]. Combining these genetic resources with advances in CRISPR-Cas9 genome editing [44] will enable us to develop these species as model systems to study the influence of the calyptra on gene expression and the production of RNAs and proteins that coordinate sporophyte development.

Figure 4



Calyptra morphological diversity. (a–c) Calyptrae with hairs on the epidermis. (a) *Polytrichum hyperboreum* R.Br. (b) *Orthotrichum laevigatum* J.E.Zetterst. (c) *Ulotia crassa* (Hedw.) Bridel. (d–h) Mitrate calyptrae lack a prominent single slit, but can have one to multiple small slits at the bottom edge. (d) *Encalypta texana* Magill. (e) *Ptychomitrium gardneri* Lesq. (f) *Callicostella pallida* (Hornsch.) Ångstr. (g) *Campylostelium saxicola* (F.Weber & D.Mohr) Bruch & Schimp. (h) *Pterygoneurum kozlovii* Laz. (i–k) Cucullate calyptrae have a single slit up one side. (i) Calyptra on top of sporophyte capsule of *Didymodon bistratosus* Hébr. & R.B.Pierrot. (j) *Bartramioopsis lescurei* (James) Kindb. (k) *Weissia muhlenbergiana* (Sw.) W.D.Reese & B.A.E.Lemmon. (a,b,d,e,g,k) Reproduced with permission from Ref. [49]. (c,f) Reproduced with permission from Ref. [50].

Conclusions and future directions

Despite the fact that studies of calyptra form and function began almost 250 years ago [45,46], calyptra research has remained a niche endeavor that has focused on only a few taxa [5]. Enhancing our understanding of the calyptra will necessitate an examination of a wider array of species, including taxa with diverse sporophyte morphologies (Figure 4), different sexual systems that result in different levels of relatedness between the gametophytes and sporophytes and thus different levels of parent-offspring conflict [47], and diverse growth forms that range from parental gametophytes that support the development of a single sporophyte at a time (e.g., acrocarpous) to gametophytes that support multiple sporophytes concurrently (e.g., pleurocarpous; [48]). Examining the relationship between these biotic features and abiotic aspects of the habitats where these species live, such as substrate and moisture, in

combination with phylogenetic comparative methods will enable us to expand both our ecological and evolutionary understanding of moss calyptrae.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jessica M. Budke reports financial support was provided by US National Science Foundation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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- This study confirms that moss sporophytes are homiohydric, in agreement with the results in [2]. In contrast to vascular plants, the stomata for the 11 moss species examined in this study did not respond to treatments with and changes in the levels of abscisic acid (ABA), plasmolysis, CO₂ concentration, or light. They demonstrated that moss sporophytes do undergo photosynthesis and the rate of CO₂ assimilation was linearly related to the relative water content (RWC). They also found a relationship between CO₂ assimilation and stomatal density across 10 moss species. By experimentally sealing the stomata and disrupting the cuticle of the capsule they showed that the majority of the water lost from the capsule is via the stomata and that the moss sporophyte cuticle is relatively impermeable.

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