CHAPTER 4-13

ADAPTIVE STRATEGIES: SPECULATIONS ON SPOROPHYTE STRUCTURE

TABLE OF CONTENTS

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Figure 1. *Bryum argenteum* capsules, representing the sporophyte generation. Photo by Dick Haaksma, with permission.

SPOROPHYTE

The sporophyte is that generation seen as a stalk and capsule (win an unseen foot) perched on top of the gametophyte. During young stages the sporophyte will bear a gametophyte **calyptra** that influences its development.

Vanderpoorten *et al*. (2002) conceded that sporophyte traits in the **Amblystegiaceae** (Figure 2) are more "labile" than previously thought, warning that an understanding of that plasticity is necessary to prevent giving the traits undue emphasis in classification systems.   In fact, many sporophyte characters are strongly correlated with habitat conditions.

Figure 2.   *Hygrohypnum luridum* (**Amblystegiaceae**) capsules in the wet zone. Photo by Michael Lüth, with permission.

Calyptra

The calyptra is not part of the sporophyte. Rather, it is developed from the archegonium after the embryo becomes sufficiently large to force the splitting of the archegonium. The upper portion of the archegonium remains on the developing sporophyte and becomes the calyptra. Its function after it becomes a calyptra influences the sporophyte development, so it is perhaps better discussed here in its influence on the sporophyte, rather than under the topic of gametophyte.

As discussed in the chapter on development, the calyptra creates an environment in which the capsule develops, and it influences the shape of the capsule. If the calyptra is removed too early, the capsule may fail to develop. Split calyptrae (Figure 3) can result in uneven development, leading to curved capsules. It would be easy to design experiments to compare effects of removal or split calyptrae, including effects of timing, on a variety of species representing different groups of bryophytes. The results could be quite interesting.

Figure 3. *Pylaisia polyantha* capsule with split calyptra that can cause the capsule to develop asymmetrically if it splits early enough in development. Photo from Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

But is there any role for the structure of the surface of the calyptra?

Hairs

Lloyd Stark (Bryonet 8 May 2012) observed that the hairs on the calyptra of *Forsstroemia* (Figure 4) result when paraphyses in the female inflorescence resume extension in length upon fertilization. One hypothesis for this trait is that such long hairs help keep the relative humidity high within the perichaetial leaves, thus acting to retard the rate of desiccation for the developing embryo. Then, when the sporophyte is mature, these hairs are retained on the calyptra. I haven't followed the development, but the hairs of at least some taxa seem too large to be just a lingering of the archegonial hairs, suggesting that they enlarge as the calyptra enlarges,

requiring energy and resources. If they continue to extend as the calyptra develops, then there may be some advantage that would favor that prolonged use of energy.

Figure 4. *Forsstroemia trichomitria* with capsules. Photo by Bobby Hattaway (www.discoverlife.org), through online permission.

There appear to be two kinds of hairs, "true" hairs and undeveloped archegonia. In *Fontinalis*, the calyptral "hairs" develop from aborted archegonia (Figure 5) whose eggs were presumably not fertilized (Glime 1983). This results in a small number of hairs near the base of the calyptra.

Figure 5. Aborted archegonium (SEM) on calyptra of *Fontinalis squamosa*. Photo by Janice Glime.

Neil Ellwood (9 May 2012) recounted a story about benthic filamentous Cyanobacteria that may have some relevance for calyptra hairs. These cyanobacterial hairs had no photosynthetic capacity, but they had high phosphatase activity. They were produced at times of phosphorus stress. He suggested that one possibility for the hairs of *Orthotrichum* (Figure 6) might aid in the uptake of nutrients from the moisture trapped among them. As a follow-up to this discussion, Johannes Enroth (Bryonet 9 May 2012) suggested two hypotheses:

1. Hairy calyptrae are more common in nutrient-poor environments.
2. Hairy calyptrae are larger than hairless ones in relative as well as absolute terms.

Figure 6. *Orthotrichum cupulatum* showing hairs on the calyptra. Photo by Jan-Peter Frahm, with permission.

Neil Ellwood (Bryonet 10 May 2012) suggested testing hypothesis number 1 by staining the calyptra with BCIP/NBT (colored stain) or ELF97 (fluorescent) with and without hairs on medium with and without P limitation. The P limitation can be enhanced by augmenting the N concentration. This should be supported by testing tissue levels of N and P. He points out that bacteria, Cyanobacteria, green algae, and diatoms are known to use extensions in response to nutrient limitation. In the biofilm diatom *Didymosphenia*, these enzymes are pushed into the stalks, an extension of the cells. The continuation of this practice in bryophytes might be expected. It is an interesting idea that has never been tested. What other bryophyte structures might serve such a function? Leaf hairs? Stem tomentum?

But Claudio Moya Delgadillo (Bryonet 9 May 2012) raised an interesting point. When the archegonium breaks away from the underlying stem to ride atop the developing sporophyte (forming the calyptra), the capsule has not yet expanded. Hence whatever growth occurs in the calyptra must come from contact with the expanding urn of the capsule – or from its own activity? This raises the question of just when the hairs expand and where they get the energy to do it.

Capsules

Ken Kellman (Bryonet 8 May 2012) recounts his experience searching for bryophytes in pouring rain. He noticed numerous bryophyte species had droplets of water sequestered by their capsules (Figure 7) and was struck by

the need for a hydrophilic surface chemistry to accomplish that phenomenon. Whatever their function, it is likely that different capsule shapes and sizes also affect the ability to hold the water drops. Capsules of *Orthotrichum* (Figure 6) were an exception to holding water and Kellman suggested that perhaps the hairs on the calyptrae helped to disperse the water droplets. On the other hand, perhaps the hairs on the calyptrae help to discourage foraging by slugs that tend to eat capsules.

Figure 7. *Bryum capillare* showing water drop clinging to capsule. Photo © Stuart Dunlop <www.donegal-wildlife.blogspot.com>, with permission.

Capsule Structure

Like Vanderpoorten *et al*. (2002) for the **Amblystegiaceae**, Rose *et al*. (2016) concluded that capsule shape is driven by differences in physiological demands in diverse habitats. Furthermore, they found that sporangium shape is a convergent character associated with habitat type. In fact, "many shifts in speciation rate are associated with shifts in sporangium shape across their 480 million year history."

Stomata

Location, Structure, and Number

Stomata, those openings between a pair of guard cells that are familiar structures of tracheophyte leaves, are also present in the sporophytes of many bryophytes (Paton & Pearce 1957). They are absent among the **Marchantiophyta** (Figure 10) (Crum 2001), but seem to be homologous in the **Bryophyta** (Figure 8) and **Anthocerotophyta** (Figure 9) (Renzaglia *et al*. 2000; Ligrone *et al*. 2012), but apparently with somewhat different selection pressures at play and sometimes a rather different role from that in tracheophytes. Despite their rather widespread presence, they are absent in several highly organized but unrelated genera of bryophytes (Paton & Pearce 1957).
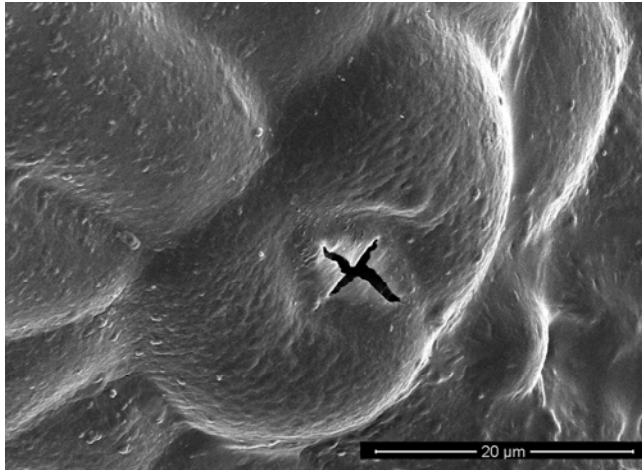
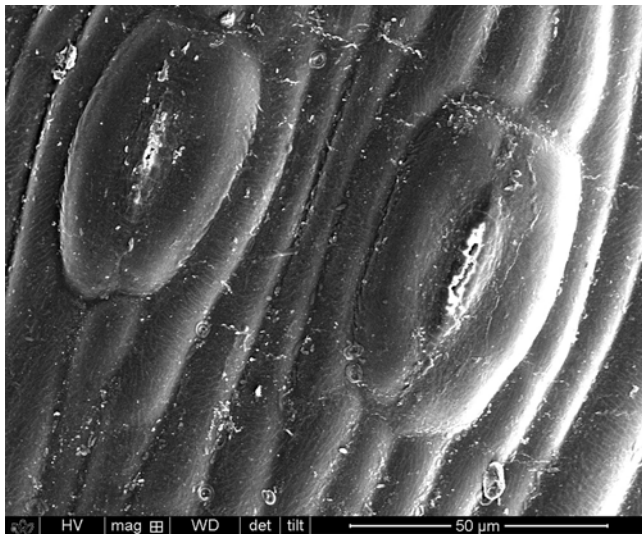Figure 8. *Physcomitrella patens* sporophyte stomata SEM. Photo courtesy of Jeff Duckett and Silvia Pressel.

Figure 9. *Anthoceros punctatus* sporophyte stomata SEM. Photo courtesy of Jeff Duckett and Silvia Pressel.

Figure 10. Liverwort *Fossombronia* cf. *caespitiformis* capsule showing its ability to repel water. Liverwort capsules lack stomata. Photo by Andras Keszei, with permission.

In some mosses, the guard cells are round in cross section, have thick walls, and do not open and close (Ziegler 1987). These occur in species with reduced photosynthetic tissue in the capsule. These have been considered to be evolutionarily reduced, not primitive. It is interesting that, unlike tracheophytes, mosses lack subsidiary cells associated with the guard cells, and the guard cells are larger than the surrounding epidermal cells (Figure 11), two characteristics distinguishing them from the stomatal apparatus of tracheophytes.
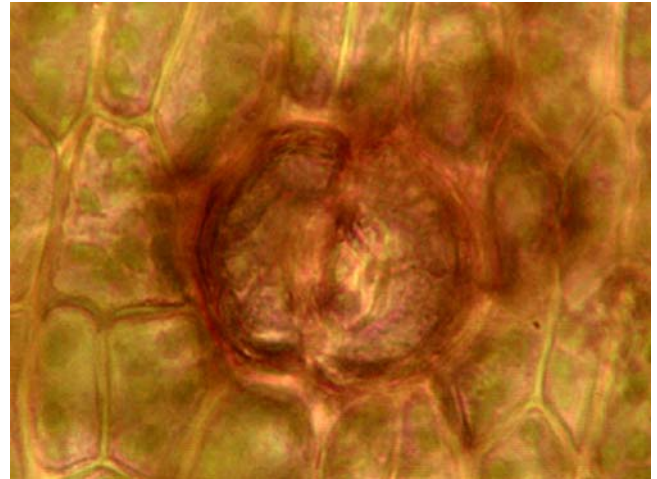

Figure 11. *Orthotrichum affine* stoma showing two guard cells, no subsidiary cells, and larger size of guard cells compared to epidermal cells. Photo by Malcolm Storey, through Creative Commons.

Paton and Pearce (1957) surveyed the stomata of British bryophytes and found that most of the stomata were 20-45 µm wide, but ranged 20 µm to 60 µm or more and were typically 70 µm long or more. The guard cell walls, typically two, may be thick or thin, and the stomata may be round or elongate (Figure 17). Generally the long axis of the stoma is parallel with the long axis of the capsule.

Stomata number varies widely and depends largely on the size of the capsule, with small capsules of *Pleuridium* (Figure 12) and *Acaulon* (Figure 13) having only four and *Polytrichum* (Figure 14) and *Philonotis* (Figure 15) having over 200 (Paton & Pearce 1957; see also Egunyumi 1982 for tropical African mosses). Most, however, at least in Great Britain, have 15 or fewer.

Figure 12. *Pleuridium subulatum* showing small capsules that have only 4 stomata. Photo by Kristian Peters, with permission.

Figure 13. *Acaulon muticum* with small capsules hidden within the perichaetial leaves. Photo by Jan-Peter Frahm, with permission.
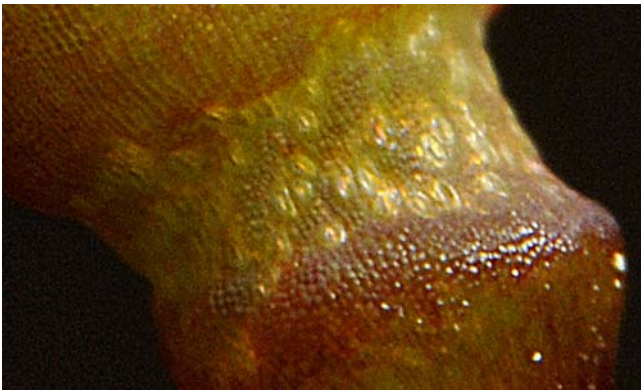

Figure 14. *Polytrichum* stomata at neck of capsule. This genus can have more than 200 stomata. Photo by George Shepherd, through Creative Commons.

Figure 15. *Philonotis revoluta* capsules, a genus with more than 200 stomata on the capsule. Photo by Zen Iwatsuki, with permission.

One might expect the level of the guard cells relative to the capsule surface to be of adaptive significance, and these may be slightly raised (Figure 16), level with the epidermis (Figure 17), or sunken (Figure 18), but most are level with the epidermis (Paton & Pearce 1957). Paton and Pearce (1957) concluded that there was no relationship between sunken stomata and a dry habitat.   Only in *Polytrichum* (Figure 16), where the stomata are in deep, narrow grooves in species from dry habitats and are shallow in those from wet habitats, is there a suggestion of adaptive location (Bünger 1890).
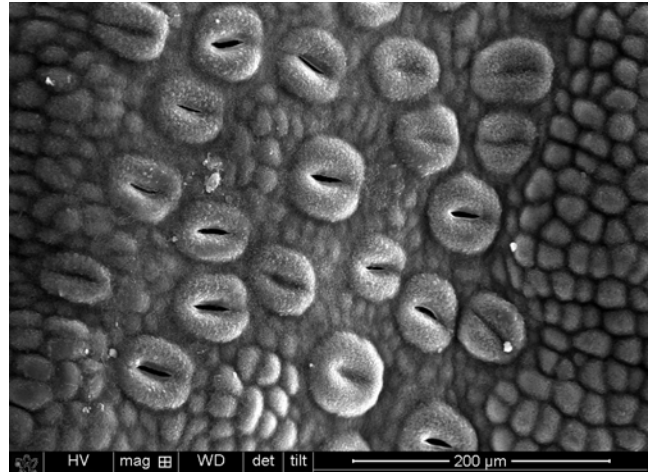

Figure 16. SEM of *Polytrichum juniperinum* stomata at capsule base. Photo courtesy of Jeff Duckett and Silvia Pressel.
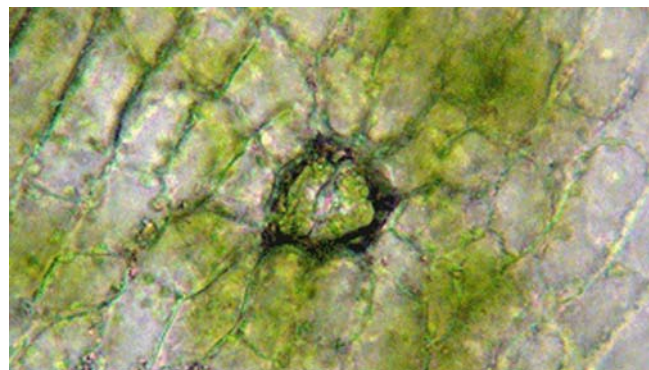

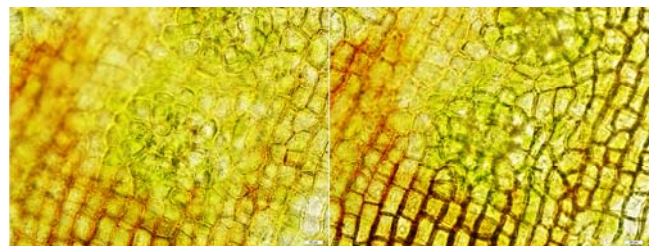Figure 17. *Orthotrichum pusillum* surface stoma. Photo by Bob Klips, with permission.

Figure 18.   *Orthotrichum anomalum* showing sunken stomata. Photo from Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

Stomatal Functioning

The real puzzle came with observations by Haberlandt (1886) on the mechanism of closing the guard cells. Unlike the leaf tissue of tracheophytes, the tissue adjoining the guard cells of moss capsules is very thick and the guard cells cannot bulge into it. Using *Plagiomnium cuspidatum* (Figure 19), Haberlandt showed that only the ventral wall of the guard cell is capable of movement. This causes the width of the guard cell to increase and the depth to decrease as the turgor decreases, closing the pore across the middle. But this meant that the length and width of the stoma remained the same whether it was open or closed. Bünger (1890) made similar observations regarding the behavior in *Polytrichum* (Figure 16), but he found in addition that the upper and lower ridges of the guard cells would come together to close the stoma, reminiscent of the action of the tier of pores in the thallus of *Marchantia* (see Chapter 4-

12). But Paton and Pearce (1957) revealed a caveat for these observations. They were done by immersing the capsules in glycerine, a typical mounting medium at that time. The glycerine kills the cells, so these results might not be indicative of what would happen naturally.

Figure 19. *Plagiomnium cuspidatum* capsules with guard cells that close in response to increased turgor. Photo from Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

Adaptive Significance

Small numbers of small stomata are typical among species of dry habitats. Variations in stomata density in at least some **Polytrichaceae** (Figure 14, Figure 16) can depend on the environment, with those species in moist habitats having more stomata per mm^2 (Szymanska 1931). Paton and Pearce (1957) found the same trend among the wider sampling of British bryophytes. Because the stomata are usually restricted to a very small area of the capsule, usually below the level of the spore mass and often located on the apophysis and neck of the capsule (Figure 14), their restricted locations and small numbers make them difficult to count accurately. It is interesting that Paton and Pearce found a positive correlation between length of seta and number of stomata, a relationship also observed by Egunyumi (1982) for tropical African mosses. But as was seen in subchapter 4-7, the length of the seta is diminished in many taxa of dry habitats. And one might suppose that if capsules are immersed in perichaetial leaves, stomata would be of little value. Indeed, in such taxa as *Pleuridium* (Figure 12) and *Acaulon* (Figure 13), there are only four stomata, but this also correlates with the small capsule size.

It appears that the stomata may serve in water regulation to photosynthetic tissue of the capsule (Paton & Pearce 1957). The stomata seem to be confined to green portions of the capsule, and larger assimilatory portions had more stomata (Haberlandt 1886). Bünger (1890) interpreted the stomata at an older stage to have a waxy plug and thus assumed that the stomata were no longer required because the tissue had ceased being assimilative. Haberlandt made the interesting observation that species with sunken stomata had a poorly developed assimilatory region. He also demonstrated that the guard cells could open and close the stoma, depending on their turgor.

Vaizey (1887) described the movement of water through the sporophyte, with uptake by the foot, transport up the seta, and transpiration through the stomata, suggesting that they could close to minimize the effects of drought. Perhaps it is also important for them to be open to facilitate this upward movement of water and solutes. Blaikley (1932) added credence to this transpiration interpretation by putting vaseline in the stomatal groove of *Polytrichum commune* (Figure 20) and found that the transpiration rate fell to one third of the original rate. However, Paton and Pearce (1957) caution that this experiment also blocked the cuticle, and that the cuticle is known to have considerable transpiration.

Figure 20. *Polytrichum commune* capsules. Photo by Michael Lüth, with permission.

Paton and Pearce (1957) set out to demonstrate the effects of the environment on the closing of the guard cells. They reasoned that they could not examine opening because older guard cells were permanently closed. Their results are interesting:

1. dry vs moist, 24-hr or 16-hr light or continuous dark: stomata tended to be open in moist, closed in dry conditions

2. dry vs moist, 4°C & 35°C: stomata usually remained open

3. dark for 24 & 48 hr, then light for 1/2 & 1 hr: stomata mostly open

4. dark for 48 hr, then CO_2-free atmosphere for 1-24 hr darkness: always some open stomata

5. 1, 3, 6, 12, 24-hr exposure to each combination of light & dark, dry, very dry, and normal air, CO_2-free & 5% CO_2: open stomata in all conditions

When they did a new set of experiments, including some new species, results were similar, with the only closure occurring when the capsules dried out (Paton & Pearce 1957). In one experiment they dried the capsules for 3-4 days, then soaked them overnight, and some of the stomata opened. In their final experiment, they placed *Bryum bicolor* (Figure 21) capsules on a glass slide under the microscope, allowed them to dry, and observed the shrivelled epidermis and closed stomata. When they added water, the epidermal cells again swelled and the stomata

opened. After repeating this response with other species, they concluded that the stomata are capable of opening and closing in response to the water content of the cells.

Figure 21. *Bryum bicolor*, a moss in which stomata close and open in response to drying and rewetting. Photo by Jonathan Sleath, with permission.

Sphagnum - As you have seen in the discussion of the **Sphagnum Explosion** in subchapter 4-9, the stomata can, at least in that genus, play a role in capsule drying, leading to dehiscence. These stomata do not respond to potassium levels, but rather respond to the hormone ABA (Chater *et al*. 2011). Nevertheless, they respond to environmental signals in the same way as guard cells of tracheophytes. This leaves us with the question of whether the stomata have any role in dehiscence in taxa other than *Sphagnum*.

Interpretation of the role of stomata is confused by the rather odd distribution among the taxa. They are present in most of the **Dicranaceae** examined, but absent in *Campylopus* (Figure 22) (Paton & Pearce 1957). They are likewise absent in several very short, ephemeral taxa with cleistocarpous capsules [*Acaulon* (Figure 13), *Micromitrium* (Figure 23], but they are present in the ephemeral, cleistocarpous *Ephemerum* (Figure 24). There seems to be a trend to absence in aquatic taxa: *Octodiceras* (Figure 25), *Cinclidotus* (Figure 26), *Fontinalis* (only 1 species examined; Figure 27). But Paton and Pearce found both stomate and non-stomate capsules among epiphytes and forest floor species, making any habitat conclusions very tenuous.

Figure 23. *Micromitrium tenerum*, a species with cleistocarpous capsules and no stomata. Photo by Amelia Merced, through Creative Commons.

Figure 24. *Ephemerum minutissimum*, a tiny ephemeral species with stomata. Photo by Jan-Peter Frahm, with permission.

Figure 22. *Campylopus nivalis*, a species of **Dicranaceae** with no stomata. Photo by Michael Lüth, with permission.

Figure 25. *Fissidens fontanus*, a species that tends to lack stomata. Photo by Michael Lüth, with permission.

Figure 26. *Cinclidotus fontinaloides* with capsules that lack stomata and have the capsule base buried in perichaetial leaves. Photo by Michael Lüth, with permission.
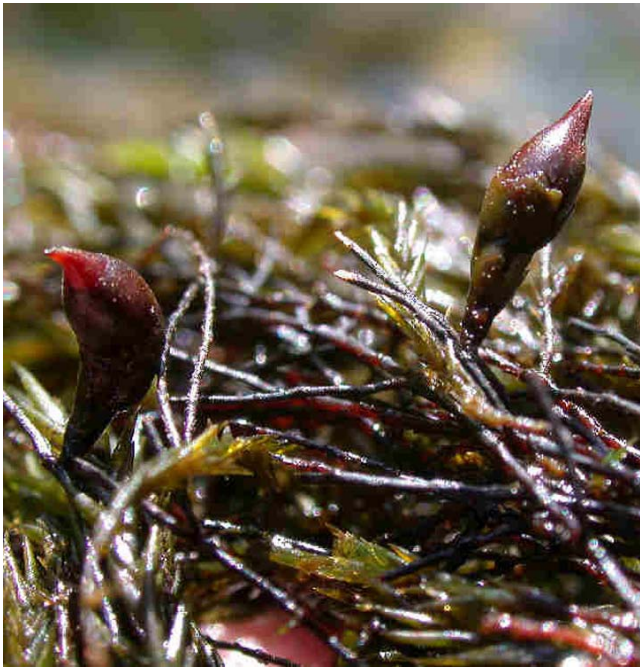

Figure 27. *Fontinalis dalecarlica* with capsules, member of a genus where at least one species lacks stomata. Photo by Janice Glime.

The presence of the stomata should relate to their function if evolutionary processes have had sufficient time to select against those that are less fit. Let's examine this relationship in the **Polytrichaceae**. Haig (2013) reports that the stomata are prevented by the calyptra from functioning in transpiration until that calyptra is pushed upward sufficiently far for the stomata to be exposed (Haig 2013). That raises an interesting question regarding certain members of the **Polytrichaceae**. How can stomata function at all in transpiration in species where the calyptra covers the entire capsule until the capsule reaches maturity? One would expect the transpiration function to be most important during the early stages when photosynthesis is occurring in the capsule.

In the **Polytrichaceae** stomata are absent in *Atrichum* (Figure 28), *Pogonatum aloides* (Figure 29), *P. urnigerum* (Figure 30), and *Polytrichastrum alpinum* (Figure 31), but present in *Oligotrichum* (Figure 32), *Polytrichum strictum* (Figure 33), *Polytrichum commune* (Figure 20), and numerous (nearly 200) in *Polytrichastrum formosum*

(Figure 34) (Paton & Pearce 1957). It appears that the gametophyte and sporophyte may be working at cross purposes here. If indeed the calyptra prevents the stomata from functioning, then why are they present in *Polytrichum strictum* (Figure 33) and *P. commune* (Figure 20) that both have long calyptrae that still cover the whole capsule at maturity, but absent in *Atrichum* (Figure 28) and *Polytrichastrum formosum* (Figure 31) that have abbreviated calyptrae? Is this a gametophyte (calyptra) trait where the coverage of the calyptra is important to the developing capsule in the *Polytrichum* species? This suggests that the stomata of the sporophyte are not sufficiently detrimental, if at all, to cause elimination of that combination. This is perhaps a good illustration of the differing and sometimes conflicting selection pressures on the two generations, with the gametophyte pressure taking precedence here.

Figure 28. *Atrichum undulatum* with capsules and short calyptrae – a genus that lacks stomata. Photo by Martin Hutten, with permission.

Figure 29. *Pogonatum aloides*, a species lacking stomata in the capsule and with the calyptra covering most of the base of the capsule. Photo by Michael Lüth, with permission.

Figure 30. *Pogonatum urnigerum*, a species lacking stomata in its capsules. Photo by Janice Glime.

Figure 31. *Polytrichastrum alpinum*, a species that lacks stomata but has the lower part of the capsule exposed. Photo by Hermann Schachner, through Wikimedia Commons.

Figure 32. *Oligotrichum hercynicum*, a species of **Polytrichaceae** with stomata – and an exposed lower half of the capsule. Photo by Michael Lüth, with permission.

Figure 33. *Polytrichum strictum*, a species of **Polytrichaceae** with stomata, but with the capsule covered at maturity. Photo by Michael Lüth, with permission.

Figure 34. *Polytrichastrum formosum*, a species of **Polytrichaceae** with stomata. Photo by Michael Lüth, with permission.

Ligrone *et al*. (2012) considered the stomata to be a sporophyte innovation with the "possible ancestral functions of producing a transpiration-driven flow of water and solutes from the parental gametophyte." If we consider the importance of stomata in tracheophytes, we know that they provide the end of the transpiration stream that is needed to bring water and minerals to the top of the plant. Since bryophytes take in most of their water through their leaves, this at first might seem like an unnecessary function. But the stomata are not in leaves, they are above the leaves in the sporophyte. And the sporophyte needs to get nutrients, hormones, and possibly even water from the leafy gametophyte. The seta can serve as a capillary organ to help move these materials, but the open stomata could increase this movement in the same way it does in tracheophyte leaves. This would fit with the absence or small numbers of stomata in sessile capsules and likewise in submersed capsules.   But we have no experimental evidence to support this hypothesis.

But Ligrone *et al*. (2012) added a second function - facilitating spore separation before release. This could fit with some of the other theories discussed here, particularly the role of drying in the *Sphagnum* capsule (Figure 35). Drying would help the spores to separate. But would the movement of air, like stirring the pot, provide any facilitation worthy of note?

Figure 35. *Sphagnum plumulosum* showing swollen capsule with operculum, dry compressed capsules, and capsules that have lost their opercula. Photo by David T. Holyoak, with permission.

It is interesting that in mosses the stomata occur only on the sporophyte and in most cases are restricted to that part of the capsule where most of the photosynthesis occurs (Ziegler 1987). This correlation supports the concept of stomata providing a site for CO_2 exchange during the early, photosynthetic stages of capsule development. Figure 22-Figure 33 illustrate the degree of capsule coverage by the calyptra in several species. Those species that do not have stomata in the capsules have a thin capsule epidermis, apparently providing adequate CO_2 exchange.

We are still left with the question of how stomata relates to capsule dehiscence and dispersal. Although the research on *Sphagnum* suggests that the stomata (Figure 36) might play a role in rapid drying of the capsule, leading to dehiscence, there appear to be no data, either observational or experimental, to test this role in other bryophytes. We might even conclude that the wax plugs and other evidence of lost function discussed above (see Stomatal Functioning) would preclude such a function in non-*Sphagnum* capsules. Nevertheless, there could be at least some species in which this function is important. And the absence of stomata in some of the cleistocarpous capsules and some of the aquatic capsules, where they would be of little value might suggest that such a function is being lost where it is not needed. But then that can also be said for its function in capsule transpiration. We need experimentation on a wide range of capsules. And we need to remember that they may serve both functions.
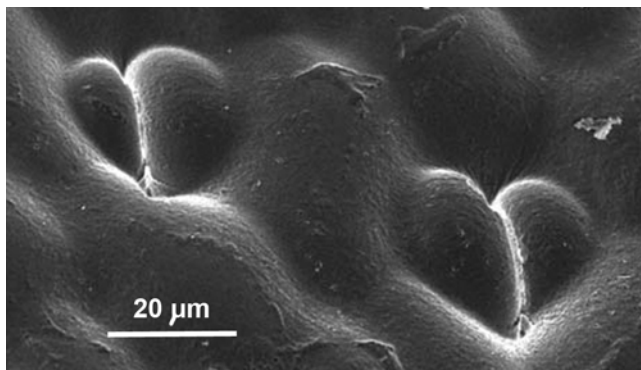

Figure 36. Non-functional stomata of a mature *Sphagnum* capsule. Photo courtesy of Jeff Duckett and Silvia Pressel.

But all of this discussion has been about the Bryophyta. How do the guard cells function in the **Anthocerotophyta** (Figure 37)? What is their role in those horn-shaped sporophytes where dehiscence is continuous and results from splitting that starts at the top and works downward? How can stomata help a sporophyte that is young at the bottom with new spores being produced when the other end of the capsule is wide open? Are they left-overs from functions in their ancestors, or do they have a role we have not even imagined yet?
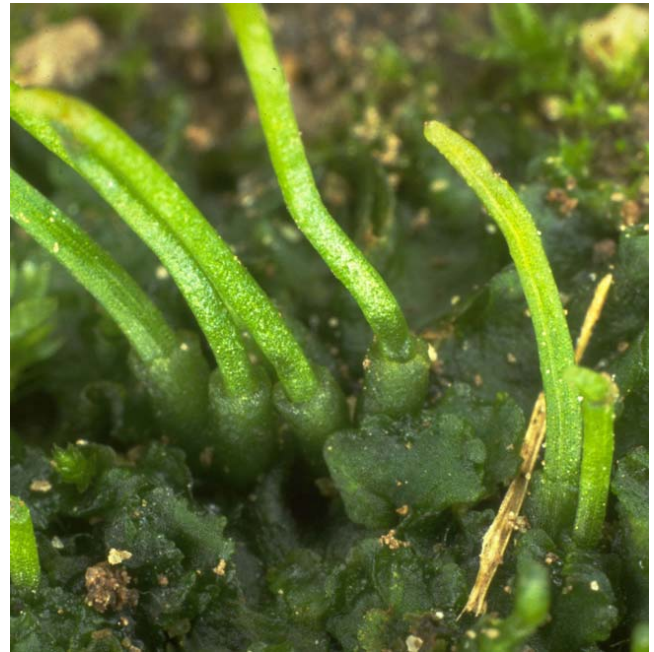

Figure 37. *Anthoceros agrestis* showing involucre at base and elongate capsule. Photo by Jan-Peter Frahm, with permission.

Ziegler considers these hornwort stomata to be true stomata, functioning like tracheophyte guard cells with a substomatal chamber where photosynthetic tissue resides. Raven (2002) suggests one possibility for their importance – that decreasing levels of CO_2 in the environment required special adaptations to maintain sufficiently high levels of CO_2 for photosynthesis. This makes sense for the hornwort stomata that are present at the base of the sporophyte in the young and dividing tissue. The admission of CO_2 through the stomata would permit higher photosynthesis in the part of the sporophyte that needs it.

Duckett *et al*. (2010) consider the hornwort stomata (Figure 38) to function as they do in *Sphagnum* – to facilitate drying of the capsule interior so that the spores can be dispersed, a suggestion made earlier by Lucas and Renzaglia (2002). They support this conclusion by the determination that the stomata open only after they have emerged from the involucre, and that they remain open thereafter. Furthermore, those "air-filled" spaces inside the stomata are initially filled with mucilage and only become air spaces after drying commences.
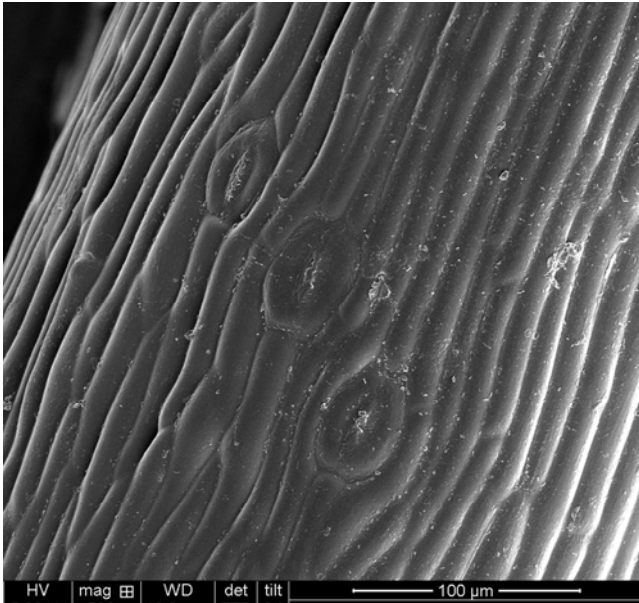
Figure 38.   SEM of *Anthoceros punctatus* sporophyte showing stomata.   Photo courtesy of Jeff Duckett and Silvia Pressel.

Hornwort Capsules

The hornwort capsules are unique among bryophytes in having a basal meristem. This means that the tip of the capsule is mature first, splits open first, and disperses spores first. It also means that while the tip is dispersing spores, the base continues to produce them.   This is certainly an advantage for producing and releasing spores over a longer period of time while at the same time keeping the developing spores near the nutrient sources available from the gametophyte.

But are there other advantages?   Roberto Ligrone (Bryonet) suggested that it might be an adaptation to herbivory on the sporophyte, much like grass grows at the base and survives the herbivory of large grazing mammals.

Are Bryophytes Slow to Evolve?

Their small size and seeming lack of complexity has led us to ask if bryophytes have a slow rate of change and consequent evolution. First, Ann Stoneburner and Robert Wyatt have shown that the rate of bryophyte evolution/genetic change has been as rapid as that in tracheophytes.   But, as we usually conclude, the morphological expression of this evolution appears to be more limited. Hence, we must look for the expression of this evolution elsewhere. Evolutionary treatises designed for the lay public tend to overlook the fact that evolution is not just about morphology. That is only its most obvious expression.   Bryophytes have been "stuck" with one evolutionary problem that has limited their morphological diversity – they lack true lignin.   With this structural compound absent, bryophytes cannot accomplish great height due to lack of support. Proctor (2010) contends that bryophytes are simply too small to have the sorts of complexities developed by tracheophytes.   Bryophytes have a plant body 100X smaller and a millionth the volume of tracheophytes. One could, therefore, argue that they are limited by their small size that prevents them from developing great complexity.

To consider their size "limitation," let us consider the historical fate of the horsetails and lycopods. During the dinosaur days, these groups were represented by tall plants – 30 feet or more, with leaves 3 feet long. Those long leaves were serviced by only one vein down the center. Hence, competition for water with emerging plants that had branched veins (ferns, conifers, ultimately flowering plants) most likely put them at a disadvantage. One could argue that their "answer" to that competition was to become small. To avoid being teleological, we can consider that only the small members (perhaps newcomers) survived the competition and drying of habitats.

But bryophytes, in a world where insects were speciating at a phenomenal rate, faced another serious problem.   Their slow growth rates made them very vulnerable when attacked by hungry herbivores. Hence, those species that were conspicuous survived best if they were endowed with secondary compounds that discouraged herbivory. And many researchers have described hundreds, perhaps thousands, of secondary compounds. Many of these serve both to discourage herbivory and to prevent disease.   These special endowments could permit bryophytes to survive, grow slowly, take advantage of their asexual reproduction to propagate and spread, tolerate cold in winter as C3 plants insulated from extreme cold by snow, and avoid being wiped out by hungry animals, especially right after snowmelt when food is scarce and animals are hungry.

Nevertheless, all these factors favoring smallness and simplicity still seem to evade the question of why they lack structural complexity. Many bryophytes have adapted a strategy of horizontal growth. In that case, support would not seem to be an issue. Why are there no larger structures on these, either above or below ground? What is it that maintains a relatively slow growth rate? To say that their limited photosynthetic tissue prevents them from growing faster would seem to be circular reasoning.   (Sorry, Richard, I actually like your argument that the limited photosynthetic tissue limits them, but why has it stayed limited?)

As pointed out by all our contributors to Bryonet thus far, the bryophytes have "found" a group of niches in which they thrive. They are often in situations where many other plants could not thrive, and in some cases the bryophytes are necessary for other plants to become established. Perhaps the bryophytes, or some of them, have "limiting genes" that restrain their growth rates.   Gerson (1972) showed that a diet of certain bryophytes could prevent the mite *Ledermuelleria frigida* from reproducing. Perhaps at least some bryophytes have highly conserved genes (*e.g.* near the centromere) that do a similar thing by inhibiting their own growth.

Let's consider the alternatives to the current bryophyte strategy.   Diego Knop Henriques (Bryonet 8 February 2011) expressed his opinion that "the very simplicity of bryophyte structures rendered them one of the greatest physiological abilities to survive all those millions of years: the poikilohydrism." Although the flowering plants are the most diverse plant group on the planet, the bryophytes are second.   Furthermore, there are few habitats where no bryophyte can grow. (It is of note that the ocean is one of

them.) Their distributions are generally wider than those of flowering plants, attesting to their good dispersal ability. They have incredible abilities to survive a wide range of conditions, to come back to life from ages past, and to avoid being devoured by advancing herbivores. Diego Knop Henriques sums up his perspective as "simplicity + effectiveness in physiological and reproductive strategies may be the evolutionary way bryophytes followed, while great complexity + intense investments in defenses and specializations in several ranks were one of the paths flowering plants and others followed to struggle for survival. Structural complexity was not a necessary condition for bryophytes to diversify and maintain themselves as one of the living branches of The Tree of Life."

What might be lost if bryophytes were larger or more morphologically diverse? Could they still develop easily from fragments if they had large, showy reproductive organs or complex leaves? Would thick cuticles make regeneration from a leaf impossible, or at least improbable? Would a faster growth rate be at the expense of secondary compounds that prevent herbivory? I agree, they seem to be well adapted for their circumstances, and I think they will outlive most or all other plant groups under radically changing conditions of the planet.

Rod Seppelt (Bryonet 8 February 2011) reminded us of the Baas-Becking hypothesis, "Everything is everywhere, but the environment selects." He reminds us of the morphological diversity in genera such as *Sphagnum* (Figure 40-Figure 42), *Calymperes* (Figure 43-Figure 49), or **Polytrichaceae** (Figure 28-Figure 34), the leaf architecture in **Pottiaceae** (Figure 45-Figure 48), the large size of *Dawsonia superba* (Figure 50) versus the minute size of *Stonea*, *Goniomitrium* (Figure 51), or *Weisiopsis*, the structure of *Ephemeropsis* (Figure 52), the size of some plants of *Fontinalis* (Figure 53), the variation in peristome morphology, variety of vegetative propagules, costal anatomy, cell architecture, a life history that may go from spore to spore in less than 2 weeks. Think of the adaptations of **Splachnaceae** (Figure 54) for attracting insects and spore dispersal, bryological physiological capabilities (desiccation, living in water, salt tolerance, tolerance in some of heavy metals etc.) - and that is without delving into the liverworts (some of which live in highly acidic fumarole streams) and hornworts.

Figure 40. *Sphagnum cuspidatum*, a moss of bog and fen pools and lakes. Photo by Aimon Niklasson, with permission.

Figure 41. *Sphagnum angustifolium* Europe 3 Photo by Michael Lüth, with permission.

Figure 39. *Sphagnum contortum*, a moss of fens and mires. Photo by Jan-Peter Frahm, with permission.

Figure 42. *Sphagnum girgensohnii*, a moss that is common in coniferous forests. Photo by Janice Glime.

Figure 43. *Calymperes tenerum* with gemmae. Photo by Jan-Peter Frahm, with permission.
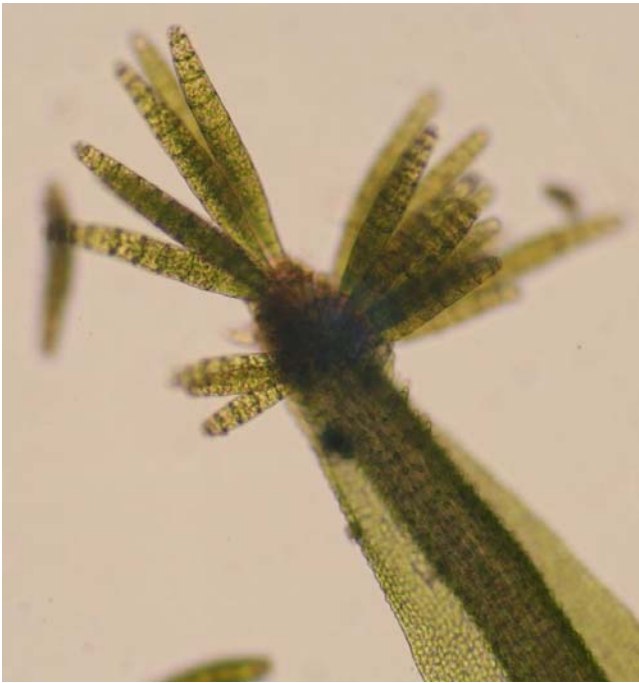

Figure 46. *Anoectangium aestivum* showing diversity in the Pottiaceae. Photo by Michael Lüth, with permission.

Figure 44. *Calymperes erosum* leaf with gemmae. Photo by Li Zhang, with permission.

Figure 47. *Tortella fragilis*, a member of the Pottiaceae showing fragile leaf tips. Photo by Michael Lüth, with permission.

Figure 45. *Aloina ambigua* showing succulent leaves. Photo by John Game, through Flickr Creative Commons.

Figure 48. *Syntrichia intermedia*, a member of the **Pottiaceae** with long leaf awns. Photo by Jan-Peter Frahm, with permission.
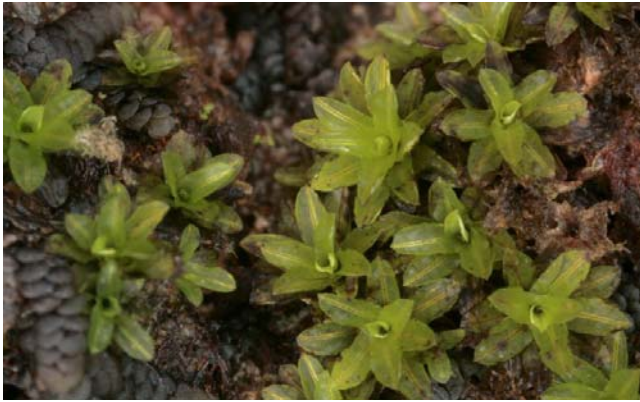
Figure 49. *Calymperes motleyi*. Photo by Jan-Peter Frahm, with permission.

Figure 50. *Dawsonia superba* from New Zealand. Note the ferns between the plants in the foreground, giving reference to the large size of *Dawsonia*. Photo by Jan-Peter Frahm, with permission.

Figure 51. *Goniomitrium enerve*, a very tiny moss. Photo by David Tng, with permission.

Figure 52. *Ephemeropsis trentepohlioides*. Photo by Niels Klazenga, with permission.

Figure 53. *Fontinalis duriaei* showing long, dangling plants; held by Janice Glime. Photo by Zen Iwatsuki, with permission.

Figure 54. *Splachnum rubrum* showing expanded and colorful hypophysis on capsule, used for attracting flies. Photo by Michael Lüth, with permission.

In the case of the bryophytes, as Diego has pointed out, physiological adaptations/modifications may be as important or more important. Bryophytes have such wonderful abilities to dry out and then revive that researchers in agriculture have been attempting to put the bryophyte genes into tobacco, among other things.

To quote Seppelt, "What could be more physiologically challenging than living in acid water (*Solenostoma vulcanicola*), occasional immersion in sea water [*Muelleriella crassifolia* (Figure 55)], or sitting on a rock or desert soil pavement where the diurnal temperature and moisture regime can go from being frozen overnight, being wet from dew in the morning, dehydrate in the sun during the day when soil or rock surface temperatures can reach well in excess of 40°C, becoming moist again in the evening with dew, and refreezing overnight.

Figure 55. *Muelleriella crassifolia*, a moss with a rare tolerance to submersion in sea water. Photo by Juan Larrain, Cape Horn Bryophytes NYBG, with permission.

Perhaps one constraint on morphological diversity is that genes for the gametophyte and for the sporophyte are subjected to different selection pressures (Pokorny *et al*. 2012). If these genes occur on the same chromosomes, selection will work against the greater of two evils, permitting somewhat unfit characters to persist because their chromosomes are needed in the other generation. For example, Pokorny *et al*. (2012) found that in the **Hookeriales** both sporophyte and gametophyte characters are labile, with documented parallel changes and reversals in traits from both generations.

By the time bryophytes evolved, algae already had the five hormones known from plants (Tarakhovskaya *et al*. 2007). But these are hormone groups, and variations within them were on the way. Furthermore, coordination that worked in water might often fail on land due to absence of sufficient water at a critical time. Perfection of timing would necessarily take a long history of trial and error among the species in the many new habitats. And such timing coordination would require enzymes and other forms of controls, responsive to the new cues of the terrestrial environment. Many changes were needed for a diverse and increasing array of niches.

Summary

Sporophytes are perched atop the gametophyte and are dependent on them. This means that they must live with the selection pressures that determine selection on the gametophyte.

Sporophytes begin in the archegonium, which breaks apart to become the calyptra on the upper part of the sporophyte. This gametophyte calyptra structure, surrounding the developing sporophyte, influences its development. The calyptra can completely cover the capsule, be split on one side, or sit only as a short covering at the top. The calyptra may have hairs that may cease importance after the embryo emerges from the base of the archegonium or that may develop further to reduce water loss or defer herbivores.

Capsule stomata occur at the base of the capsule in many genera of mosses and hornworts, but not in liverworts. They may provide openings for CO_2 exchange during early development or permit faster drying to aid spore dispersal. They may open and close in the young capsule; they may remain open in the older capsule; they may become non-functional with the stoma closed with wax at later stages.

Bryophytes have been considered slow evolvers, simple plants. But evidence suggests that they evolve at rates similar to those of other plants. Lack of lignin limits size and small size limits morphological development. But bryophytes invented numerous controls that are timed with environmental changes such as seasons, they developed a range of new hormones, and they developed numerous secondary compounds that protect them from herbivory, bacteria, and fungi. Furthermore, they have interesting mechanisms by which they survive desiccation and winter freezing conditions while their photosynthetic tissue remains above ground. They are not without morphological variability, as demonstrated in Chapter 4-12.

These adaptive values of bryophyte structures are largely speculation, hypotheses waiting to be tested.

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