

CHAPTER 5-9

ECOPHYSIOLOGY OF DEVELOPMENT: SPOROPHYTE



Figure 1. Sporophytes with capsules of the moss *Aloina rigida*. Photo by Michael Lüth.

Sporophyte

The sporophyte of a bryophyte is composed of a foot imbedded in gametophyte tissue, a stalk (seta), and a capsule. Watson (1974) reminds us that it is the sporophyte generation of bryophytes that must be compared to tracheophytes. In this regard, we find that the moss seta has **hydroids** and sometimes **leptoids**, forming a conducting strand. The outer part of the seta has thick walls that provide support. Even an endodermis-like structure is present in *Dawsonia polytrichoides*, a member of the Polytrichales. Although there seems to be no lignin like that of higher plants, the capsule does have a cuticular covering.

The sporophyte is usually at least partly dependent on the gametophyte (Figure 1). *Mnium hornum* relies on the gametophyte for 80% of its assimilate; *Pleuridium* requires up to 90% (Schofield 1985). *Funaria hygrometrica* has capsules that are somewhat dependent while they are young, become almost as productive as the gametophyte at maturity, then drop their production rapidly when the capsule dehisces (Schofield 1985); they may rely on stored

food in the capsule at maturity when they are no longer green, since the **transfer cells** linking them to the gametophyte disintegrate at that time, closing the route from the gametophyte.

Krisiko and Paolillo (1972) suggested that weight gain in the capsule was directly and linearly related to weight loss of the seta. However, capsule weight gain is also a linear function of the length of the gametophyte explant, and in the presence of dextrose, the seta loss is suppressed, suggesting that the gametophyte is the most important source of carbon/weight gain for the capsule.

Sporophyte development, like gametangial development, is a seasonal phenomenon in most mosses. Sporophyte development can be relatively short, with its timing controlled largely by the fertilization process, or it can require 15-18 months and have timing signals separate from those for fertilization. The factors that promote or retard gametophyte buds from the protonema also affect sporophyte development. For example, relatively dry culture conditions promote the formation of setae and the

transformation of the callus into sporangia in *Physcomitrium pyriforme* (Bauer 1963). However, sporophyte development can require environmental characteristics that contrast sharply with those used for gametophyte growth. This permits energy to be diverted into the sporophyte.

A case in point is that of the moss *Physcomitrella patens*. At 15°C and 8-hour photoperiod (20 $\mu\text{mol m}^{-2} \text{s}^{-1}$) – conditions simulating spring – it produced the highest number of sporophytes in the lab, but at 25°C and a 16-hour photoperiod – conditions simulating summer – that number was greatly reduced (Hohe *et al.* 2002). Predictably, the vegetative growth was reduced under conditions favoring sporophyte production; one can assume that this was due to nutrient transfer to the developing sporophyte. It appears that the MADS-box gene PpMADS-S is involved in this sporophyte production, as the RNA production associated with it was 2-3 times higher during the conditions that stimulated sporophyte development.

Once fertilization occurs, this one-time egg, now zygote, continues development to look not like its parent tissue, but like a sporophyte. What is it that makes tissue become sporophyte instead of gametophyte? True, there are two sets of chromosomes, but there is no new or unique information in those two sets as opposed to one, only different combinations and ways of expressing genes for the same type of trait. A most striking bit of evidence regarding control of sporophyte development is the ability of kinetin to stimulate the production of sporophyte buds on the protonema, at least in *Physcomitrium* (Menon & Lal 1974). But something has to determine that such kinetin is available to be the stimulus. Perhaps we can gain some insight from examining experiments with callus tissue that induce it to become gametophyte or sporophyte in character. Bopp (1968) has elaborated on the physiological conditions that determine the life cycle stage developing from callus tissue. At concentrations above 1 g/l of glucose only sporophytes form from sporophyte callus. With no sugar, this same sporophyte callus produces gametophytes, as does gametophyte callus. The most intriguing and informative event is that with the addition of sugar or coconut milk, a gametophyte callus will produce sporophytes. Clearly, it is not the kind of information gained by the second set of chromosomes that makes the difference.

One can easily imagine how these responses could relate to effects of surrounding tissues. Isolated cells must be self-sufficient in their production of glucose, whereas a cell (zygote) retained within an archegonium can use the resources of the rest of the plant. If sugar has already been mobilized for gametogenesis and fertilization, the zygote can easily become a target for this resource. In fact, could it be that the dividing embryo behaves physiologically like a dividing meristem? In tracheophytes, actively dividing cells of meristematic regions typically result in the metabolism of starch to glucose and the mobilization of glucose to the dividing cells. If dividing embryo cells send the same message as dividing meristems, one would expect the same arrival of sugars to these cells. Had the zygote been shed from the parent plant before the cells began to divide, as is the case in the algae, these food reserves would not have been available.

Control of Generation

It is normally the case that the embryo, safely inside the archegonial tissues and in constant contact with its parent, will develop into a foot, stalk, and capsule atop the gametophyte. However, in early and cleverly designed experiments, Pringsheim was able to regenerate gametophytic structures from sporophytic tissue (Bryan 2001), evidence that the environment, not the duplication of genetic information, is the dominant force in determining what the generation will look like. Thus we can be certain that the parent tissues are supplying this special environment and most likely influencing the development of the embryo by controlling moisture, light, nutrients, energy availability, and hormones, at the very least.

Arnaudow (1925) performed tedious experiments in which gametophyte tissue was placed into the archegonium of a moss. By doing this, he showed that a gametophyte so placed could develop the morphological characteristics of a sporophyte. Meiosis, of course, would fail due to the lack of pairs unless the moss happened to be polyploid. He then reversed the procedure and removed zygotes from the archegonium to develop without the influence of gametophyte tissue. These developed into gametophytes. This evidence supports the homology theory that both generations are essentially the same. It is the developmental environment that differs.

Seta Elongation

In liverworts, the capsule forms and then the seta elongates (Figure 2, Figure 3). In mosses, it is the reverse; setae elongate and then the capsule forms (Figure 4, Figure 5). In *Sphagnum*, as well as in some of the Bryopsida, the seta fails to elongate. However, unlike the Bryopsida, in *Sphagnum* the gametophyte forms a **pseudopodium** that elongates after the capsule matures (Figure 6).



Figure 2. Maturing sporophyte of the leafy liverwort *Lophocolea heterophylla* before seta elongation. Photo by Paul Davison, University of North Alabama.



Figure 3. Elongated setae on *Lophocolea heterophylla*. Photo by Jan-Peter Frahm.



Figure 4. Young sporophytes of the moss *Funaria hygrometrica* with setae and calyptrae, but no capsules yet. Photo by Michael Lüth.



Figure 5. Mature capsules of *Funaria hygrometrica*. Photo by Michael Lüth.

Thomas *et al.* (1970) found that liverwort setae respond in a manner similar to that of stems in higher plants; elongation of setae in *Lophocolea* was promoted by low concentrations of IAA and inhibited at higher ones. Soon after that, Kaufman *et al.* (1982) determined that cells in the stalk of *Conocephalum conicum* and seta of *Pellia epiphylla* exhibited acid growth, much like that of *Avena* (oats), implicating involvement of IAA.

The **pseudopodium** (actually gametophyte tissue, not equivalent to a seta) of *Sphagnum palustre* (Figure 6) also shows rapid growth in low IAA concentrations (0.01 ppm) but no growth at higher ones (0.5, 1.0 ppm) (Patterson 1957). The pseudopodia grows even longer in low concentrations than in the controls. But Patterson found a puzzling lack of response at any concentration of IAA by setae of *Frullania inflata* and *F. tamarisci* ssp. *asagrayana*.



Figure 6. **Upper:** Capsules of *Sphagnum* before pseudopodium elongation. Photo by Zen Iwatsuki. **Lower:** Elongated pseudopodia of *Sphagnum palustre*. Photo by Janice Glime.

While comparing the responses of two liverworts, *Pellia epiphylla* (Figure 8) and *Conocephalum conicum* (Figure 7), to that of oats, Kaufman and coworkers (1982) discovered that a tenfold increase in the growth rate of oats (*Avena*) internodes appeared about three hours after application of 10^{-5} M GA_3 , but that 10^{-5} M IAA had no effect. On the other hand, in the liverworts, the setae responded to 10^{-5} M IAA with a two-fold increase in growth rate within 10-15 minutes. Thomas *et al.* (1982) demonstrated the production of auxin (IAA) and ethylene by cells of elongating setae of *Pellia epiphylla*, adding more support to the suggestion that at least IAA may exercise control over seta elongation, and that most probably IAA and ethylene operate in tandem to control seta growth (Thomas *et al.* 1983).

IAA may play another important role in the seta. Thomas *et al.* (2002), using radioactively labelled IAA and infrared video recording of *Pellia epiphylla* setae, have shown that IAA in donor blocks moved preferentially to the lower sides of horizontally placed setae. Upward gravitropic curvature occurred within 50-60 minutes, while growth rates on the top side of the setae dropped (see Figure 8).



Figure 7. Archegoniophores of *Conocephalum conicum*. Photo by Janice Glime.



Figure 8. Elongated setae of *Pellia neesiana*. Note the generally upright positions of the setae despite the nearly 45° slope of the substrate. Photo by Janice Glime.

Consistent with cell elongation in many other plant organs, the seta cells of the leafy liverwort *Lophocolea heterophylla* (Figure 9) increased their osmotic potential to -6 bars, concomitantly experiencing a 16-fold increase in water content, and consequently in length (Thomas 1977). This increase in osmotic potential followed a period in which osmotic potentials were as low as -29 to -37 bars in unelongated seta cells. In this species, at least, the seta elongates as a simple expansion of individual cells (Thomas & Doyle 1976). These cells experienced a 25-fold increase in length while increasing cell wall carbohydrate by only 2-fold. Nevertheless, starch diminishes during elongation, and polyfructosans and sucrose are replaced by fructose and glucose, suggesting that in addition to transport of wall precursors from the gametophyte, carbohydrate reserves in seta cells supply some of the structural materials needed for elongation.

The elongation of the seta of *Lophocolea heterophylla* occurs through rapid cell elongation with no net lipid loss (Thomas 1975). Rather, lipids are converted from glycerolipids and sterol esters in the unelongated seta to phospho- and glycolipids during elongation. At this time, unusual polyunsaturated fatty acids such as arachidonic and eicosapentaenoic acids appear.

Temperature is also important in seta elongation. In *Atrichum undulatum*, seta cells in 12-22°C were three times longer than those in 3-12°C (Stevenson *et al.* 1972). Setae of *Pellia epiphylla*, though, grew longer in cooler temperatures (5°C) (Slade 1965). Those at higher temperatures did have a faster growth rate but the overall length was less.



Figure 9. Young sporophytes on *Lophocolea heterophylla*. Photo by Michael Lüth.

At least some mosses exhibit tropisms in their setae, but little is known of the mechanisms in this organ. In *Oligotrichum hercynicum*, setae bend upward, most likely with a gravitropic response, but possibly also with a light response (Figure 10)



Figure 10. Upward bending of the setae of *Oligotrichum hercynicum*, most likely as a gravitropic response. Photo by Michael Lüth.

Energy Source

The young sporophyte is mostly dependent on the gametophyte for energy and nutrients. **Transfer cells** occur at the juncture of the gametophyte and sporophyte and are typically endowed with extensive **wall labyrinths** (Figure 12) with trapped pockets of cytoplasm in the epidermal cells of the sporophyte foot (Figure 11; Lal & Chauhan 1981). Electron microscopy has revealed these labyrinths in such widely divergent taxa as *Funaria hygrometrica* (Monroe 1965b; Wiencke & Schulz 1975; Browning & Gunning 1977, 1979), *Physcomitrium cyathicarpum* (Lal & Chauhan 1981), *Mnium* (Eymé & Suire 1967), *Polytrichum* (Maier 1967), *Dawsonia* and *Dendroligotrichum* (Héban 1975), and *Sphaerocarpos* (Kelley 1969). Although the labyrinth begins development during seta elongation, maximum development occurs during meiosis (Lal & Chauhan 1981). These transfer cells are a site of intense enzyme activities (Lal & Chauhan 1981), especially phosphatases that activate ATP (Maier & Maier 1972), and facilitate transfer of substances between the two generations, or at least from gametophyte to sporophyte. Wiencke and Schulz (1975) demonstrated that there is some division of labor, with the basal part of the

foot mainly participating in water uptake from the gametophyte and the middle part mainly absorbing nutrients. Radiolabelled sucrose is known to travel both directions in these leptoids in *Polytrichum commune* (Eschrich 1975).

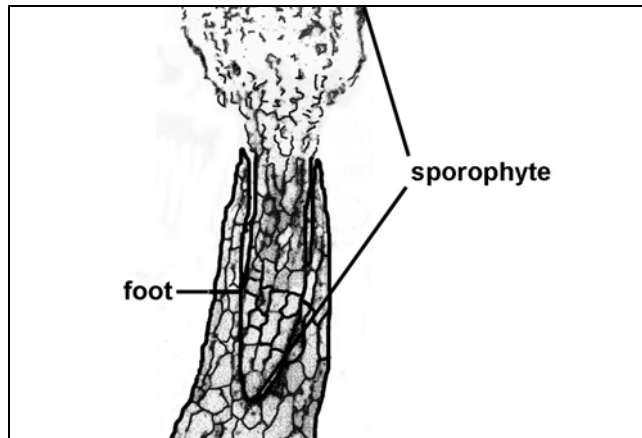


Figure 11. Junction of gametophyte and sporophyte showing haustorial foot of sporophyte. Drawn from Lal & Chauhan (1981).

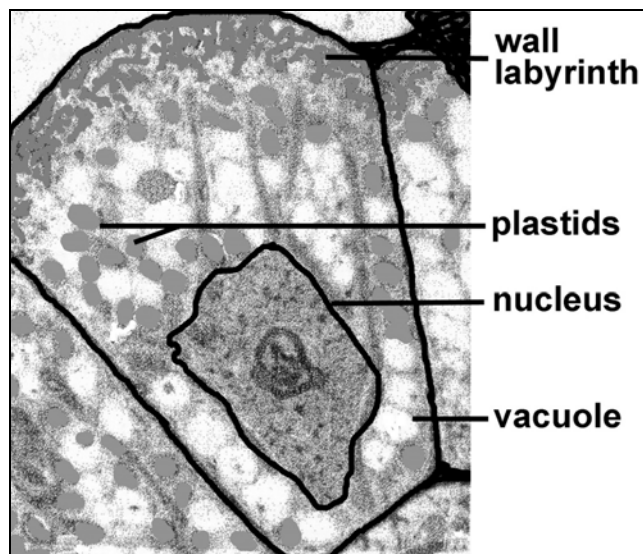


Figure 12. Foot epidermal cell showing labyrinth in cell wall of *Physcomitrium cyathicarpum*. Drawing based on electron photomicrograph in Lal & Chauhan (1981).

Whereas the seta is little more than naked stem tissue requiring minimal resources, the formation of the capsule can be expected to have a high energy cost. Taylor and coworkers (1972) have shown that in several liverworts the sporophyte has a higher concentration of chlorophyll than does the gametophyte. Yet the excised sporophyte requires an exogenous carbon source, suggesting that it is nevertheless dependent on the gametophyte for at least part of its resources. Courtice and coworkers (1978) have shown that sugars move from the gametophyte to the sporophyte in *Physcomitrella*. Apparently the demands of the sporophyte are greater than its own production capacity. If we put these demands into an ecological and temporal context, the need for a gametophytic supplement becomes obvious. For example, sporophytes of *Polytrichum* can require up to 13 months to develop in some localities

(Arnell 1905), spanning a multitude of environmental conditions. When embryo development begins, environmental conditions can easily be less than favorable for photosynthetic activity. Patterson and Baber (1961) found that many temperate mosses were dormant in late summer and autumn. Such a dormant period, if it affects the sporophyte as well, greatly reduces its opportunity to provide its own food. The sporophyte furthermore has little exposed surface area for photosynthesis, and what surface there is, at least throughout most of the development, has its long axis oriented in the same direction as the light, thus minimizing its utility as a light-absorbing organ. It is reasonable, then, that the gametophyte, which is sensitive to moisture that must be available for growth and that has a large photosynthetic surface, can provide the food and the signals for the sporophyte. Hughes (1954) has demonstrated that it is the gametophyte and not the sporophyte that responds to photoperiod to control sporophyte development in *Pogonatum aloides* and *Polytrichum piliferum*.

In *Fontinalis* most species in the northeastern United States have mature gametangia in the autumn. This means that sporophyte development begins as the temperatures drop for winter (Figure 13). During my field observations in New Hampshire, capsule maturity in *Fontinalis novae-angliae* occurred between February and April. By the end of April the capsules were gone. Under these cold conditions, productivity is reduced, although the greater light availability may offset this low temperature effect somewhat. By drawing on the reserves of the gametophyte, sufficient food could be provided for the wintertime capsule development.

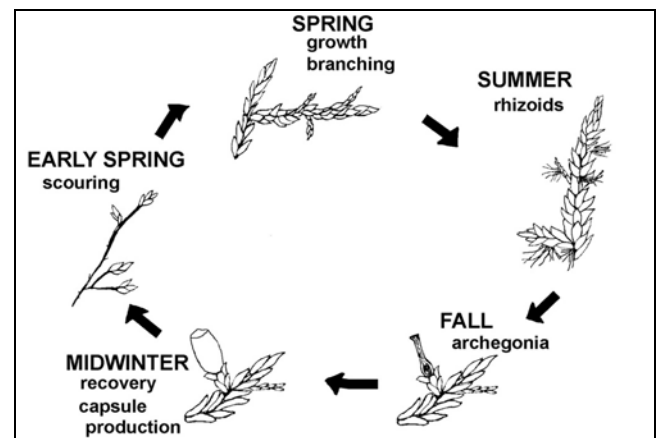


Figure 13. Seasonal cycle of *Fontinalis dalecarlica* and *F. novae-angliae*. Drawing by Janice Glime.

In *Polytrichum juniperinum* and *P. ohioense*, the capsule takes weight from the seta in culture if no dextrose is supplied to the capsule, but little seta loss occurs in the presence of dextrose (Krisko & Paolillo 1972). When photosynthetic sporophyte and gametophyte cultures of *Physcomitrium pyriforme* and *Funaria hygrometrica* are maintained, only the gametophyte is autotrophic. Glucose or some other sugar must be supplied to the sporophyte or all new growth lacks chlorophyll, produces a yellow wall pigment, and dies (Bauer 1963; Krupa 1969). These examples all seem to demonstrate the high energy requirement of the capsule and its dependence on the gametophyte.

The Anthocerotophyta may have rather different energy availability from that of the mosses and liverworts. Thomas *et al.* (1978) found that the sporophytes had a photosynthetic rate almost double that of the gametophytes on a fresh weight basis. They estimated that this was sufficient for maintenance but that supplementation from the gametophyte was needed for growth. They found that this transfer from the gametophyte was facilitated by enzymatic activity in the transfer cells. The carbon transferred ultimately accumulated in the spores.

If photosynthate from the gametophyte is required for sporophyte development, why is there such a high chlorophyll content in the developing sporophyte? We could blame the imperfections of evolution for this phenomenon. If the sporophyte is genetically the same as the gametophyte, it has the potential to form chlorophyll. It has the light necessary. Perhaps no mechanism has evolved to suppress it. Or could it be a mask against ultraviolet light and high light intensity that could otherwise damage dividing cells during sporogenesis?

Proctor (1977) found that the capsule does indeed contribute considerably to the photosynthesis and energy needs of the sporophyte, providing 10-50% of the energy needed for capsule development while it is still green. Perhaps it is just that an extraordinarily high energy requirement for producing spores requires not only the energy of sporophyte photosynthesis, but also that transferred from the gametophyte. The resulting spores must carry sufficient energy to remain viable, even to travel, for long periods before producing the protonemal thread that permits them to once more be photosynthetic.

Light

Early in its life the capsule is green and photosynthetic, typically gaining phenolic compounds that color it with age. Eventually it loses its photosynthetic capability and depends on stored reserves and sometimes the gametophyte. This ability to photosynthesize obviously requires light.

It is interesting that the translocation of carbohydrates (as glucose) to the sporophyte of *Funaria hygrometrica* occurs in response to light (French & Paolillo 1976). French and Paolillo found that capsule morphology of was abnormal in the dark because the spore sac failed to expand. Relatively low light intensity corrected these problems, and the authors felt that photoreceptors might be localized in the capsule. They agreed with Haberlandt (1886) that light affects more than just photosynthesis in the expansion of *Funaria* capsules, and that translocation is especially important in low light. This light relationship might explain why Rydgren and Økland (2002) found more capsules on segments in larger size classes and more identifiable females without them in smaller size classes (Figure 14), but this relationship also could imply that more energy is required than that available in the smaller segments (also possibly related to light availability), or that smaller segments had not yet reached the required degree of maturity. We have already discussed the need for a minimum size, or threshold, for the development of gametangia. It then follows that this same minimum size is necessary for the production of sporophytes, since sporophytes are not possible without an archegonium to house the egg, zygote, and embryo. This size requirement

is supported by the study of Rydgren and Økland (2002) on *Hylocomium splendens* (Figure 14). Size thresholds for the archegonia are discussed earlier in the chapter on gametogenesis.

Photosynthesis is probably not the only light need of the capsule. Krisko and Paolillo (1972) demonstrated that capsule expansion also requires light, with red light being more effective than white, blue, or green. But, then, red light is the most effective wave length for photosynthesis in plants.

In the liverworts *Fossombronia foveolata*, *Lophocolea heterophylla*, *Pellia epiphylla*, *Ptilidium pulcherrimum*, and *Riella affinis*, light was essential for sporophyte development, but surgically removed sporophytes develop slowly, with little increase in dry weight (Thomas *et al.* 1979). Nevertheless, sporophytes of all five of these species fix CO₂ in the light. In excised sporophytes of *Lophocolea heterophylla*, 40% of the fixed CO₂ can be attributed to photosynthetic activity of haploid spores, but when the sporophyte is intact, the calyptra and pseudoperianth inhibit this photosynthesis by as much as 50%. Organic nutrients such as glucose, therefore, are supplied predominantly by the gametophyte.

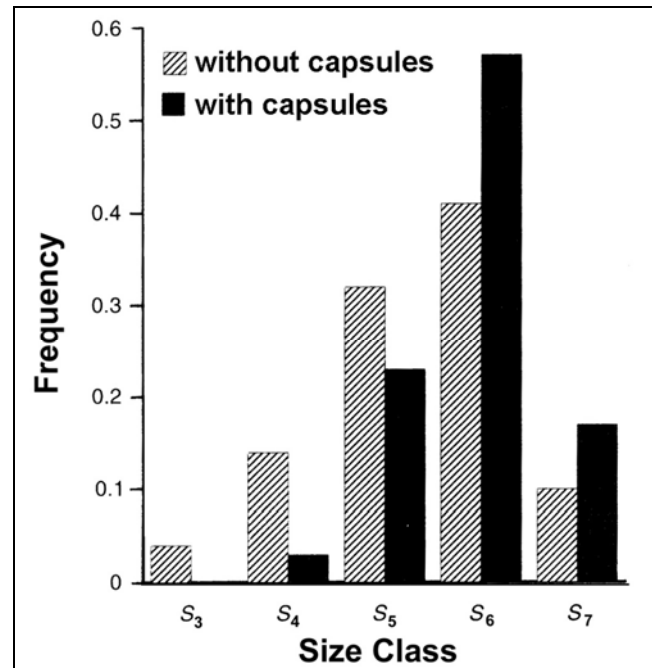


Figure 14. Relationship of frequency of occurrence of number of female segments without capsules compared to those with capsules in five adult size classes of *Hylocomium splendens* over a five-year period. Redrawn from Rydgren & Økland (2002).

Light quality and photoperiod both play roles in sporophyte development in callus cultures (Bauer 1963). Constant light causes metabolic products to accumulate and damage the cultures. Short days down to 4 hours favor seta formation, whereas long days (16 hours) favor retention of the callus form; with fewer than 4 hours of light, the tendency to form protonemata increases. In total darkness, the entire callus forms a protonema. Light quality affects the sporophyte callus growth by retaining the callus form in blue light and forming a linear chain of cells in red light.

Light quality in the field varies with habitat, microhabitat, and season. In *Ceratodon purpureus*, setae develop in far-red light but not in red light (Hoddinott & Bain 1979). Since the far-red:red ratio increases with shading, the greatest seta expansion should occur under a green canopy. *Ceratodon*, however, more typically grows in the open, and setae are abundant there. Perhaps the far-red light stimulus is through the snow (setae are produced soon after the snow disappears), which increases the ratio of far-red:red light (Winchester pers. comm.). This could result in the abundant elongated setae we see early in spring as soon as the snow is gone, but at least some of this elongation occurs in the preceding autumn. If there is growth that responds to the far-red light under snow, we should expect a longer seta in the north than in the tropics, at least for open habitat things. Hmm... That should be relatively easy to check with a herbarium study. In fact, this ubiquitous north temperate moss seems rather rare in most of the tropics, where it is replaced by *C. stenocarpus* (Crum & Anderson 1981). And, this one study gives us no concept of the variability of this trait.

Hughes (1969) found that yellow light enhanced sporophyte development. In *Phascum cuspidatum* (Figure 15), yellow-filtered fluorescent light greatly increased the frequency of sporophyte development. In this case, daylight (white light) favored archegonia, and an early return to fluorescent light (which tends to increase the green to red balance relative to sunlight) restored vegetative growth at the apex, causing the archegonia to become lateral. Daylight resulted in the development of sporophytes in fertilized haploid plants, but it favored vegetative growth of diploid plants. On the other hand, a yellow filter caused diploid plants to produce sporophytes. But what does this yellow-light effect mean in nature?



Figure 15. Capsules forming in the white light of daylight in the natural habitat of *Phascum cuspidatum*. Photo by Michael Lüth.

Almost nothing is known about the effects of yellow light on plants. It is difficult to suggest how a white light:yellow light shift might occur in nature in any predictable way, but a color change caused by archegonial tissue, acting as a filter, could shift light to yellow before it reaches the embryo. Markham *et al.* (1978) have shown that gametogenesis in *Marchantia polymorpha* is coupled with high production of flavonoids, and many species have a golden color in mature archegonia. Capsules of many taxa, including *Marchantia polymorpha* and *Phascum cuspidatum*, are yellow, so perhaps the wave length stimulus is an endogenous one.

Nutrients

Another controlling factor in sporophyte development could be the conversion of nutrients from the inorganic form to the organic form by the gametophyte before the nutrients reach the sporophyte. The sporophyte is not adapted for extensive surface absorption, and so it is dependent upon the highly adapted gametophyte for this function.

Nutrient needs between the gametophyte and sporophyte differ, particularly as the sporophyte is developing. For example, in *Funaria hygrometrica* the developing sporophyte has a greater need for K than for Ca, with spores having a higher K and lower Ca concentration, whereas the degenerating gametophyte loses K and gains Ca (Brown & Buck 1978).

Bauer (1963) found that callus sporophyte cultures of *Physcomitrium pyriforme* X *Funaria hygrometrica* can be maintained on 9.1 M sugar plus yeast extract. The yeast supplies nitrogen in an organic form, which is superior to nitrate or ammonia. But individual amino acids can have harmful effects on the sporophyte. The gametophyte, on the other hand, grows better with inorganic nitrate. If these cultures are given suboptimal nitrogen, sugar promotes differentiation, mostly into young setae, but some protonemata also develop (Bauer 1963). In *Polytrichum formosum*, the sporophyte increases in arginine (an amino acid) concentration as the gametophyte concentration decreases, suggesting a translocation from the gametophyte (Whel 1975). As an annual shuttle species (During 1979), moving from one short-lived habitat to another in the space of 1-2 years, *Physcomitrium pyriforme* might benefit from a signal such as low organic nitrogen, coupled with a sugar supply from the gametophyte, so that spore production could take the species to new sites or remain dormant until suitable conditions return.

Setae of *Lophocolea heterophylla* (Figure 16) increase in protein during elongation, causing a decrease in soluble amino acids (Thomas 1976). When setae were severed from the gametophyte, they decreased in protein and seta elongation was attenuated, suggesting that the synthesis of protein in the seta is necessary for its elongation. Since the gametophyte prefers inorganic nitrogen, and the sporophyte must ultimately obtain its organic nitrogen from the gametophyte, it is reasonable to guess that depletion of inorganic nitrogen in the habitat results in decreased organic nitrogen available for the sporophyte. (We know that in higher plants nitrogen is transported in an organic form.) However, initially the ratio of organic to inorganic nitrogen would increase, and this ratio change could provide the signal for sporophyte production. One difference Bauer (1963) noted between gametophytes and sporophytes is that sporophytes have a much higher content of the amino acid adenine. The relationship between adenine and the inorganic nitrogen content could provide the nitrogen signal. During (1979) placed *Splachnum ampullaceum* in the annual shuttle group, based on its need to find a new substrate once it matures. Since its dung substrate is initially high in organic nitrogen, it is possible that the breakdown of the substrate and the use of nitrogen by the moss is again an adaptive signal for sporophyte production. More speculation! What role does the environment have in providing these signals?



Figure 16. Expanding setae with capsules on *Lophocolea heterophylla*. Photo by Michael Lüth.

Since the sporophyte is dependent upon fertilization, the signal for fertilization, to be adaptive in mosses with short life cycles, must be coupled with the signal for sporophyte formation. Interesting information might result from testing responsiveness of mature gametophytes to sugar and N concentrations as signals for gametogenesis. Since early sporophyte development usually follows a consistent time sequence after gametogenesis, it is reasonable to hypothesize that signals for seta formation and gametogenesis are the same in many species, especially annual ones.

Water Needs

The seta functions to transfer water from the gametophyte to the developing sporophyte. In some mosses (*Funaria* and *Polytrichum*), the center of the seta is a hydroid cylinder with a leptoid sheath surrounding it (Héban 1977). However, it appears that the majority of moss setae have only the hydroid cylinder (Vitt 1981). Héban (1977) suggested that the foot acts as a pump to drive water and other substances upward toward the developing capsule.

The maturation of the sporophyte, although appearing to be a relatively dry structure at maturity, is dependent on available water. Sporophyte abortion often results from insufficient water at a crucial developmental time. In *Sphagnum*, Sundberg (2002) found that sporophyte production was positively correlated with precipitation amount during the previous summer, suggesting that it was sensitive to drought during gametangium formation and fertilization. He found that larger patches had higher probability of producing sporophytes, suggesting that the likelihood of having both sexes was greater, but could it also be possible that retention of moisture was facilitated by larger patches? Sporophyte maturation was likewise negatively affected during their summer of maturation when droughts caused them to dry prematurely. He suggested that some species could benefit from early maturation that permitted them to mature before effects of drought could abort development.

In the Mojave Desert, the opposite effect appears to be true. Following an unusually heavy summer rainstorm, approximately 50% of the sporophytes of *Grimmia orbicularis* aborted at a time when they were still in the

seta elongation phase. Stark (2001) suggested that the abortions may have been due to the dehydration-rehydration cycle during the hot summer when setae were at an abnormally advanced stage of development. Repair from prior desiccation under hot conditions could be too great a cost in energy or nutrients, preventing sporophyte maturation.

Hormones

In addition to requirements for carbohydrates and nutrients from the gametophyte, capsule development seems to be controlled by growth regulators. Or are these growth regulators controlled by the carbohydrates and nutrients? There is evidence that sugar stimulates hormone production. Protonemata can be maintained from sporangia tissue culture by re-culturing every few days (Bauer 1963). Buds from these protonemata yield gametophores. Glucose can be used to stabilize the sporangium factor in the protonema, and when the protonema is allowed to bud, the sporangium factor becomes active. Bauer concluded that the control factor is not a hormone-like substance passed from the sporangium to the protonema, because after numerous culturings of the protonema the supply would be exhausted. Therefore, the substance must propagate itself in the presence of the sugar supply. Likewise, gametophyte callus tissue under culture with high sugar will produce sporophytes (Bopp 1968). Could it be cytokinins that delay capsule expansion upon a seta on a growing gametophytic moss?

Once the capsule develops, it provides a feedback mechanism, some sort of regulator, that inhibits seta development (Redfearn & Meyer 1949). Removal of *Funaria hygrometrica* capsules results in cessation of seta elongation (French & Paolillo 1975 a, b). However this elongation can be restored by application of benzyl adenine (BA) alone or with indole acetic acid (IAA). When capsules were retained, BA prolonged seta meristematic activity and suppressed capsule expansion. And, as suggested above, high cytokinin levels antagonize capsule expansion (French & Paolillo 1975a).

IAA and photoperiod also influence seta elongation. Setae of *Pogonatum aloides* grew longer in long days (18 hours) than in short days (6 hours) (Hughes 1962). This growth was due to an increased cell length. *Pellia epiphylla*, though, had maximum seta elongation in short days when sprayed with aqueous IAA and GA₃ (Kaufman *et al.* 1982). These applied hormones may have overcome the auxin oxidases present, which would be inhibited by long days.

Crombie and Paton (1958) suggested that age affects sporophyte elongation in *Pellia epiphylla*. Hormones may accumulate until their concentrations are high enough to stimulate growth. Certain inhibitors may also need time to break down and be removed.

We have shown that IAA is important in seta development in another way as well. Gravitropism of the seta in *Pellia epiphylla* exhibits lateral redistribution of IAA, with movement to the lower side of a horizontal seta (Thomas *et al.* 2002). This is an important aspect of orienting sporophytes that are originally positioned horizontally, such as those growing on vertical or slanting substrata. However, not all bryophytes have vertically oriented setae on vertical substrata (Figure 17).



Figure 17. Setae and capsules of *Uloa coarctata* on a vertical substrate, demonstrating apparent lack of gravitropism in these setae. Photo by Michael Lüth.

Capsule Shape

Capsule shape is under genetic control of the sporophyte, as demonstrated by the transplant experiments of Arnaudow (1925), but the shape can also be highly influenced by the calyptra. In *Polytrichum juniperinum*, the splitting of the inner sheathing layer of the calyptra causes the capsule to develop bilateral symmetry, whereas in *Funaria hygrometrica* splitting of the calyptra has no effect on capsule shape (Paolillo 1968).

This behavior suggests to me that ethylene could be a controlling factor. The capsule expands the most on the side of the slit. Ethylene inhibits cell division and, at some stages, also cell expansion. Since ethylene is a gas, it can escape more easily on the side with the slit than on the closed side, thus permitting more growth on the split side. The fact that *Funaria* does not respond to a split calyptra could result from its smaller, thinner calyptra and the fact that the calyptra covers very little of the expanding capsule, whereas the calyptra of *Polytrichum* covers the entire capsule.

Neoteny

Neoteny (retention of juvenile characters in adult) occurs in such mosses as *Buxbaumia* (Figure 18) and several species of *Pogonatum* (Figure 19) where the gametophore is reduced and persistent protonemata support the sporophyte. The genetic control of such a phenomenon could be an evolutionary and physiological revelation. Is neoteny the result of the loss of a gene necessary to begin the gametophore process, or is there a gene that results in something that blocks the development? Theoretically, if this link were altered to "normal" condition, the moss would develop into the leafy gametophore typical of its ancestors. Being able to override this neoteny mechanism would be particularly instructive in the case of *Buxbaumia* (Figure 18), which has a unique capsule structure and seems to have no close relatives.

The development of a sporophyte is dependent upon the surrounding tissue of the calyptra, and premature removal of a calyptra can result in capsule abortion or abnormalities. But what is the effect of the surrounding gametophore tissues on the development of the young sporophyte? Surely perichaetial leaves surrounding a developing embryo within an archegonium must exert some influence as that embryo emerges from the

archegonium. But how has this absence of gametophyte leaves influenced the appearance of a *Buxbaumia* sporophyte? And what property causes the *Buxbaumia* sporophyte to exhibit its strong bilateral symmetry? Since the capsules seem to orient themselves with their flat surfaces facing the light, perhaps we should expect it to be controlled by a hormone that responds to light. Are there cryptochromes or phytochromes in the capsule that cause the directional response?



Figure 18. Sporophyte of *Buxbaumia aphylla* growing directly from archegonia on the protonema. Photo by Michael Lüth.



Figure 19. Persistent protonemata with plants of *Pogonatum aloides*. Photo by Michael Lüth.

In some species where the seta fails to elongate, the calyptra is retained throughout capsule development and expands as the capsule does, covering it completely at maturity. In several xerophytic species we find that at maturity these capsules are often shed in their entirety, including *Pleuridium* (Figure 20; Claudio Delgadillo, Terry Hedderson on Bryonet 26 May 2006) and some species of *Physcomitrella* (Jerry Jenkins on Bryonet 26 May 2006).

All of these factors are hardly sufficient to explain the marked differences between the sporophyte and gametophyte. A major difference arises as a result of the number of cutting faces of the apical cell, and Bauer (1963) feels that this is a major key to the difference between the gametophyte and sporophyte. However, we have no physiological explanation for the change in number of cutting faces. We must now look into the cell for changes in polarity and cellular organization and trace the biochemical pathway that signals them.



Figure 20. Capsules of *Pleuridium subulatum*, a moss in which entire capsules may be dispersed. Photo by Michael Lüth.

Spore Production

Spores are produced in the capsule as a result of meiosis. Each sporocyte divides to produce four meiospores, each with only one set of chromosomes. In dioicous taxa, the spore will be either male or female, but in other taxa it can produce protonemata that may give rise to partly males and partly females or to monoicous gametophores. Spore dispersal is facilitated in most mosses by the movement of hygroscopic teeth that often trap the spores in spaces among the degenerate cells (Figure 21). These cells resorb their walls in such a way as to produce chambers along the teeth (Figure 22). The unequal binding of the walls creates a hygroscopic response to changes in moisture. Ingold (1959) changed the humidity levels 171 times in one moss with two rows of teeth, causing the dispersal of 15,647 spores! In *Fissidens*, unequal patterns of cellulose and hemicellulose cause peristome movement (Mueller 1973); in others, unequal suberization contributes (Schnepf *et al* 1978).

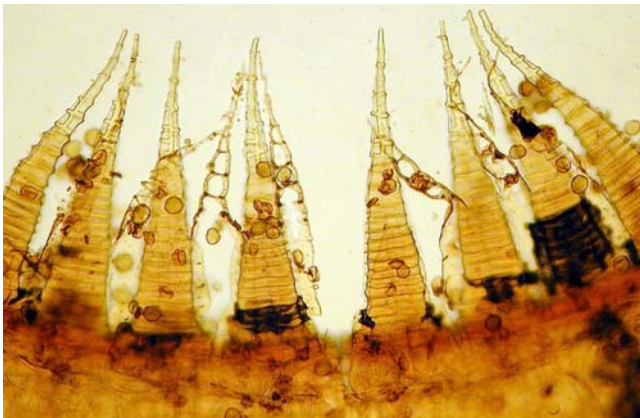


Figure 21. Peristome teeth of *Bryum inclinatum* with spores among them. Photo by Michael Lüth.

Spore number can vary considerably among bryophyte taxa, with mosses generally having a higher number than liverworts (Patidar *et al.* 1987). Capsule size is one factor in determining that number. However, spore size also determines spore number, with fewer large spores than small ones at the same capsule size – simple physics. This is somewhat true with liverwort spores in the Marchantiopsida, but the correlation is certainly not perfect (Table 1).

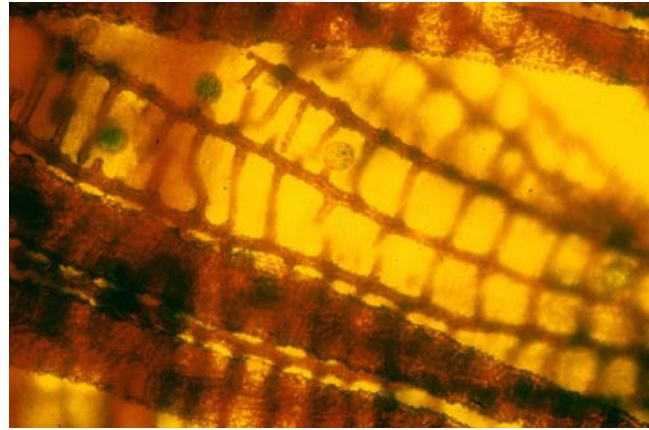


Figure 22. Peristome with trapped spores of *Fontinalis squamosa*. Photo by Janice Glime.

Perennial mosses typically have small spores, less than 24 μm , permitting them to travel greater distances, whereas they can expand locally by vegetative means more easily than annual mosses (cf. spores sizes for Michigan mosses in Crum 1973 as discussed earlier under spore germination). *Buxbaumia aphylla* has the smallest spores (6.5-8 μm) among Michigan mosses, perhaps accounting for its ability to colonize disturbed sites. Many (most?) acrocarpous mosses are annual; approximately 40% of these in Michigan have spores larger than 24 μm and range up to 68 μm . Larger spore size provides more food reserves that ensure greater success of establishment for these species that depend on spores for their year-to-year existence. Short-lived Antarctic mosses likewise have large spores, which Convey and Smith (1993) considered would help them in local colonization. The species in Michigan with the largest spores is the epiphyte *Drummondia prorepens*, which has multicellular spores measuring 60-100 μm .

Table 1. Mean numbers and sizes of spores in fifteen liverwort species of the Marchantiopsida. From Patidar *et al.* 1987.

Species	Number	Size (μm)
<i>Riccia fluitans</i>	180	60-75
<i>Riccia billardieri</i>	190	150-180
<i>Riccia gangetica</i>	196	130-140
<i>Riccia discolor</i>	210	120-160
<i>Riccia huebneriana</i>	320	50-60
<i>Cyathodium barode</i>	490	40-50
<i>Targionia hypophylla</i>	1,200	30-40
<i>Plagiochasma appendiculatum</i>	2,200	60-70
<i>Reboulia hemispherica</i>	2,700	60-90
<i>Asterella blumeana</i>	2,900	60-75
<i>Plagiochasma intermedium</i>	3,200	60-70
<i>Asterella angustata</i>	3,300	60-65
<i>Marchantia nepalensis</i>	19,700	20-30
<i>Marchantia palmata</i>	20,100	20-30
<i>Dumortiera hirsuta</i>	21,200	22-26

Sundberg and Rydin (1998) found a positive correlation between capitulum size and capsule size, suggesting one could estimate number of spores from capsule size. *Sphagnum tenellum* had a mean number of 18,500 spores per capsule, whereas the larger capitulum of *S. squarrosum* had a mean of 243,000. Fenton and

Bergeron (2006) found a similar relationship in *Sphagnum capillifolium*, where capsule-bearing colonies were significantly larger and taller than those without capsules, most likely related to an energy threshold. However, spore sizes among Michigan *Sphagnum* species suggest no correlation of spore size with plant size, with diameters ranging from 17 μm in *S. warnstorffii* and the relatively large *S. squarrosum* to 42 μm in *S. cuspidatum*.

Capsules in Polytrichopsida are generally considerably larger than those of Bryopsida. In *Pogonatum dentatum* mean spore number per capsule was 712,000 in a Fennoscandian study (Hassel & Söderström 1999). The largest moss with one of the largest capsules is *Dawsonia*, with an estimated 5,000,000 spores per capsule (Kreulen 1972). At the other extreme is *Gigaspermum* (Figure 23) with only four spores reaching up to 200 μm in diameter, contributing to the success of this moss in colonizing disturbed habitats of deserts and soil cracks. More general trends are indicated by Longton and Schuster (1983) of 50,000-600,000 spores per capsule for 17 mosses in their study. Further discussion of spore sizes can be found in the earlier chapter on ecophysiology of spore development.



Figure 23. Capsules of *Gigaspermum repens* on a floodplain in southern Australia. Photo by Janice Glime.

Dehiscence

The loss of the **operculum**, or lid, of the capsule is generally under control of weather. Warm, sunny days dry the capsule, causing it to shrink (Figure 24). This often results in breakage of the **annulus** cells that are specially designed for this purpose. In some mosses, like *Sphagnum* (Figure 25), the operculum is expelled explosively, making a small "poof" as it exits and propelling the majority of spores out of the capsule in a single event.



Figure 24. Shrunken capsule of *Funaria hygrometrica* with peristome teeth that have been exposed when the operculum was shed. Photo by Michael Lüth.



Figure 25. Mature capsules of *Sphagnum rubellum* with missing opercula. Photo by Janice Glime.

In some genera, the capsule is cleistocarpous, *i.e.*, it does not split or open and has no operculum. This morphology is typical of the desert-adapted mosses in the Gigaspermaceae (Figure 23) and genera such as *Acaulon* (Figure 26), *Archidium*, *Astomum*, *Bruchia*, *Ephemerella*, *Micromitrium*, *Phascum* (Figure 27), *Physcomitrella*, *Pleuridium* (Jerry Jenkins on Bryonet 26 May 2006), *Aschisma carniolicum*, and *A. cuynetii* (Patxi Heras & Marta Infante on Bryonet 28 May 2006). These are typically short-lived mosses of ephemeral habitats.



Figure 26. Cleistocarpous capsules of *Acaulon triquetrum*. Photo by Michael Lüth.



Figure 27. Cleistocarpous capsules of *Phascum cuspidatum*. Photo by Michael Lüth.

Tradeoffs

The cost of sexual reproduction for the female continues into the cost incurred by the sporophyte generation. At this point, it seems the cost is even higher than that of the production of archegonia and eggs. In the case of *Dicranum polysetum* (Figure 28), the total allocation of carbon to sexual reproduction and sporophyte production was ~75% (Ehrlén *et al.* 2000). When sporophytes were aborted, the top shoots accrued considerably more biomass than those shoots where sporophytes were allowed to complete development, resulting from greater elongation. This large allocation is probably unusual because this species is one of the few acrocarpous mosses to produce more than one capsule per gametophyte stem. Like some flowering plants (*e.g.* Jack-in-the-pulpit) that change gender or become sterile in the year following "fruit" production, the probability of gametangial production of these *D. polysetum* plants in the following years was reduced by sporophyte production (Bisang & Ehrlén 2002). Furthermore, annual shoot segments and size of new branches were negatively correlated with the development of mature sporophytes. Stark *et al.* (2000) supported this high cost for sporophytes. They found that the desert moss *Syntrichia inermis* accrued only 8% as much mass in aborted sporophytes as it did in those that matured. Apical sinks of these plants compete for resources needed to produce sporophytes vs producing new shoots or sexual reproductive structures.



Figure 28. Multiple setae per stem on *Dicranum polysetum*. Photo by Janice Glime.

Rydgren and Økland (2002, 2003) found that in *Hylocomium splendens*, the production of sporophytes likewise reduces the frequency of branching, causes lower mature segment survival and inferior size development to the next maturity stage, results in fewer immature branches developing into the first stage of maturity, and fewer plants produce new annual segments. Furthermore, the larger, sporophyte-producing branches had significantly less growth than their archegonia-bearing but non-sporophyte bearing counterparts. The most expensive stage in the sporophyte development is the late phase when the capsule expands, develops its mature color and shape, and the

spores are produced (Rydgren & Økland 2003). Rydgren and Økland (2002) point out that there is no evidence of a spore bank or of establishment of new gametophytes from spores in this species, suggesting that sexual reproduction comes at a high cost with little benefit. Nevertheless, spores apparently do germinate in new locations following disturbance, providing an ecological benefit for the species.

The cost of being a reproductive female appears to affect not only size, but also fitness. In *Marchantia inflexa*, females are less fit as a result of their narrow window for suitable timing of the production of gemmae, at least in high light (Fuselier & McLetchie 2002). This competitive energy drain must necessarily be timed so as not to compete with energy required for sexual reproduction and sporophyte maturation. Furthermore, selection pressures that favor the asexual plants and gemma production may not coincide with those that favor the sexually mature female.

Not only does being female reduce the number of gemmae produced and affect the production of the gametophyte plant, but it can actually be lethal. Following production of capsules, there is a high mortality in the leafy liverwort *Lophozia silvicola* (Laaka-Lindberg 2000). In numerous other taxa, having a sporophyte at the apex means the end of growth. In the thallose liverwort *Blasia pusilla* (Figure 29), the parent gametophyte actually dies before the sporophyte is mature and the immature sporophyte overwinters within the dead tissues (Duckett & Renzaglia 1993).

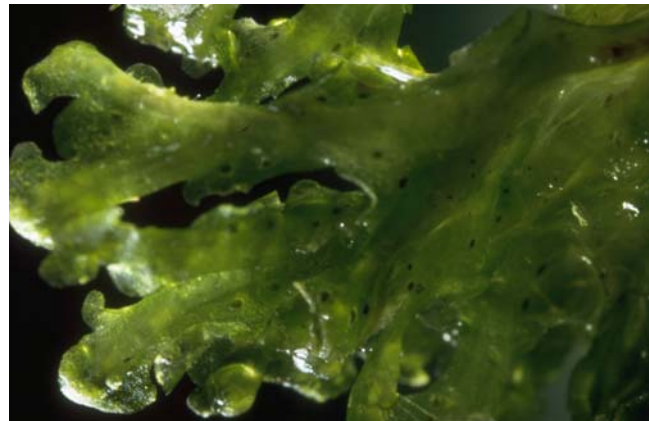


Figure 29. Thallus of *Blasia pusilla*. Photo by Jan-Peter Frahm.

Other tradeoffs are less drastic. In the Pottiales, there is a negative correlation between life expectancy and probability of producing sporophytes, but that does not necessarily imply cause and effect (Hedderson 1995). On the other hand, their negative correlation of sporophyte production with production of asexual propagules can be the result of competition for energy reserves.

Habitat Adaptations

It is easy to think of the gametophyte in terms of adaptations to its habitat, but the sporophyte is often neglected in such considerations. As a generation dependent on the gametophyte, it has no choice where to develop and must therefore cope with the microhabitat provided for it. Nevertheless, different capsule shapes, sizes, and exposures seem to relate to habitat adaptations. If the sporophyte is adapted for a habitat different from that

of the gametophyte, it may not be successful in producing spores. Therefore, selection pressures will favor those genotypes in which the gametophyte is adapted for the habitat in which the sporophyte is also successful.

Vitt (1981) contends that reduction of sporophyte characters is an adaptation to xeric habitats. These are manifest in shorter setae, reduced peristomes, and broader, erect capsules. Capsules of mosses in epiphytic habitats, which are typically xeric, are nearly all erect (Grout 1908). Reduction of the peristome can result from fusion or reduction of parts (Figure 30). This reaches its epitome in some ephemeral taxa, where the seta is virtually absent and there not only is no peristome, but there is no operculum; spores are large. Such reduction permits these taxa to reach maturity more quickly. In the saxicolous/epiphytic genus *Orthotrichum* (Figure 31), Vitt found that mesophytic taxa produced longer setae and capsules than more xerophytic taxa. More mesic members of the family, occurring in the tropics (e.g. *Macromitrium*; Figure 32), have longer setae, albeit shorter than in most non-epiphytic taxa.

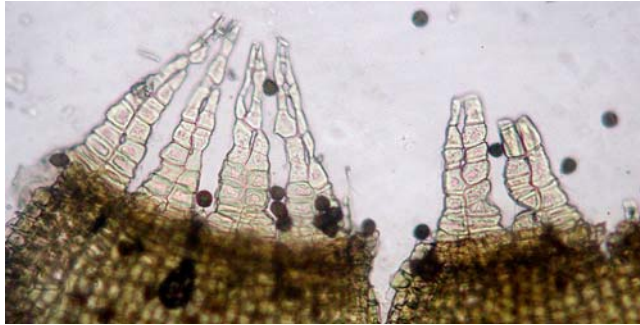


Figure 30. Reduced peristome teeth of *Orthotrichum acuminatum*. Photo by Michael Lüth.



Figure 31. Capsules with short setae on the epiphytic *Orthotrichum consimile*. Photo by Michael Lüth.

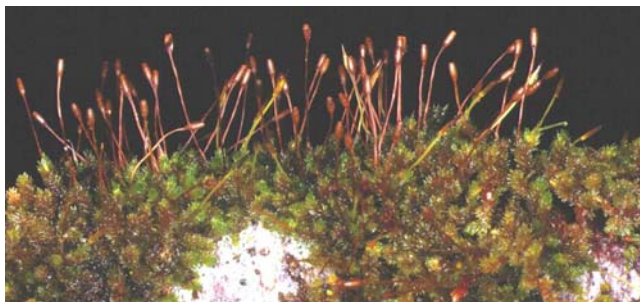


Figure 32. Capsules with long setae on *Macromitrium longipes*. Photo by Jan-Peter Frahm.

Vitt (1981) observed that species occurring on mesic forest floors are more likely to have long, straight setae with curved, smooth, cylindrical capsules that are horizontal to pendent and have well-developed peristomes (Figure 33).

Sporophytes on the aquatic taxa seem to be the most reduced, more closely resembling those of xeric taxa than of mesic taxa. These often have reduced or absent peristomes, smooth, oblong, immersed capsules, and enlarged perichaetial leaves (Vitt 1981). In *Fontinalis* it appears that the absence of a seta is an adaptation to the fast-flowing water that often submerges it. While this genus has an operculum and peristome (Figure 22), it often fails to dehisce.



Figure 33. Curved, horizontal capsules of *Rhizomnium punctatum*, a species of moist or mesic woods. Photo by Michael Lüth.

Bryophyte Senescence

Senescence is the process in which the cell reaches a state wherein it cannot undergo either progressive or regressive development and its only future change will lead toward death of the cell (Giles 1971).

Only in bryophytes can the lower part of the plant be completely dead while the upper part is still very much alive. *Sphagnum* is a classic example, exhibiting healthy, reproductive tops and dead bases, decades old. In mosses such as *Hylocomium splendens* (Figure 34), one might find 4-7 years of live growth atop several more years of senescent or dead plant.



Figure 34. Living plants of *Hylocomium splendens* forming a turf on top of their own senesced branches (arrow). Photo by Michael Lüth.

At least in some taxa, the initiation for senescence results from the production of male gametangia or capsules. In many acrocarpous mosses, these structures can effectively prevent further growth of the plant by occupying what would have been the region of apical growth, as shown for *Tetraphis pellucida* (Kimmerer 1991; Figure 35). In this species, high density increases sexual reproduction, which increases capsule production and proportion of males, which in turn initiate senescence for the population. Some mosses overcome this apical growth termination by producing innovations – side branches near the tip that become new tips and continue the growth upward (see chapter on gametophore development).

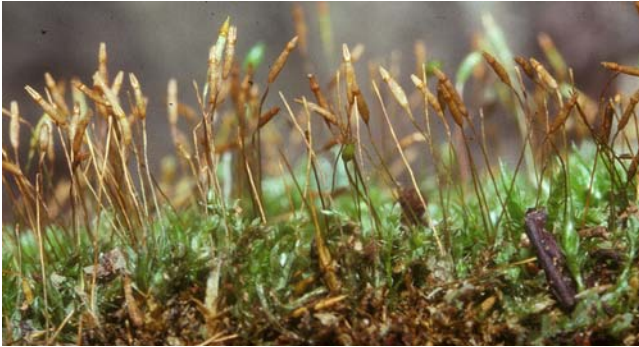


Figure 35. Mature capsules that mark the onset of senescence in *Tetraphis pellucida*. Photo by Janice Glime.

As in higher plants, it appears that ethylene induces senescence, as shown in *Marchantia* (Stanislaus & Maravalo 1994). Spermine, spermidine, and putrescine can reverse it. If we dare to generalize from this meager example, the story makes sense. As the moss grows and the cushion or mat (or whatever) becomes more dense, there is less and less air movement in the lower part of the growth form (see Figure 36). This permits gases to accumulate, so if ethylene is being produced, this surely is a place for it to reach higher concentrations. Now all we need to do is show that indeed there is ethylene given off here, that it accumulates, that it reaches high enough concentration, and that it indeed induces senescence in most (all?) bryophytes!



Figure 36. Senescence in lower, brown portion of *Dicranum scoparium*. Photo by Janice Glime.

Summary

The sporophyte of a bryophyte is composed of a foot, seta, and capsule. The seta typically has hydroids and may have leptoids. The sporophyte gains its nutrition from the gametophyte, although up to 50% of its energy may come from photosynthesis of the capsule prior to maturity. Transfer between the generations is accomplished by transfer cells with extensive wall labyrinths in the sporophyte foot. These cells are the site of extensive phosphatase activity that activates ATP. The gametophyte tissues influence/determine the morphology of the sporophyte, and zygotes cultured outside the gametophyte develop into gametophyte morphology.

In liverworts the seta elongates after the capsule is mature, whereas in mosses the seta elongates first. IAA has a role in seta growth and gravitropism. Temperature, photoperiod, light intensity, and wavelength can all play a role in initiation and rate of development of the sporophyte. Water plays a major role in the elongation of the seta.

Capsule development requires a huge investment of energy and there is a tradeoff between capsule production and growth, branching, and gemma formation in the gametophyte. This energy need is most likely responsible for the threshold size requirement for sexual reproduction observed in a number of bryophytes. The form of N available seems to play a role in capsule formation in at least some bryophytes.

A few bryophytes are neotenous, producing capsules directly from the protonema or having extremely reduced gametophores. The shape of the capsule is influenced by the calyptra, and its removal will generally cause failure of capsule development, at least in mosses.

Spores are dispersed in most mosses by action of the peristome teeth that respond to changes in moisture. These responses are due to unequal thickenings of cell walls, cellulose distribution, eroded cell walls and chambers, and uneven distribution of suberin.

Xerophytic mosses tend to have short setae, upright capsules, and reduced peristomes, with aquatic mosses having similar characters. Mesic mosses are more likely to have nodding capsules and well developed peristomes.

Bryophytes are the only plants where the lower portion of the plant can be senescent or dead and still maintain a healthy upper portion.

Acknowledgments

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