

CHAPTER 5-4

ECOPHYSIOLOGY OF DEVELOPMENT: GAMETOPHORE BUDS

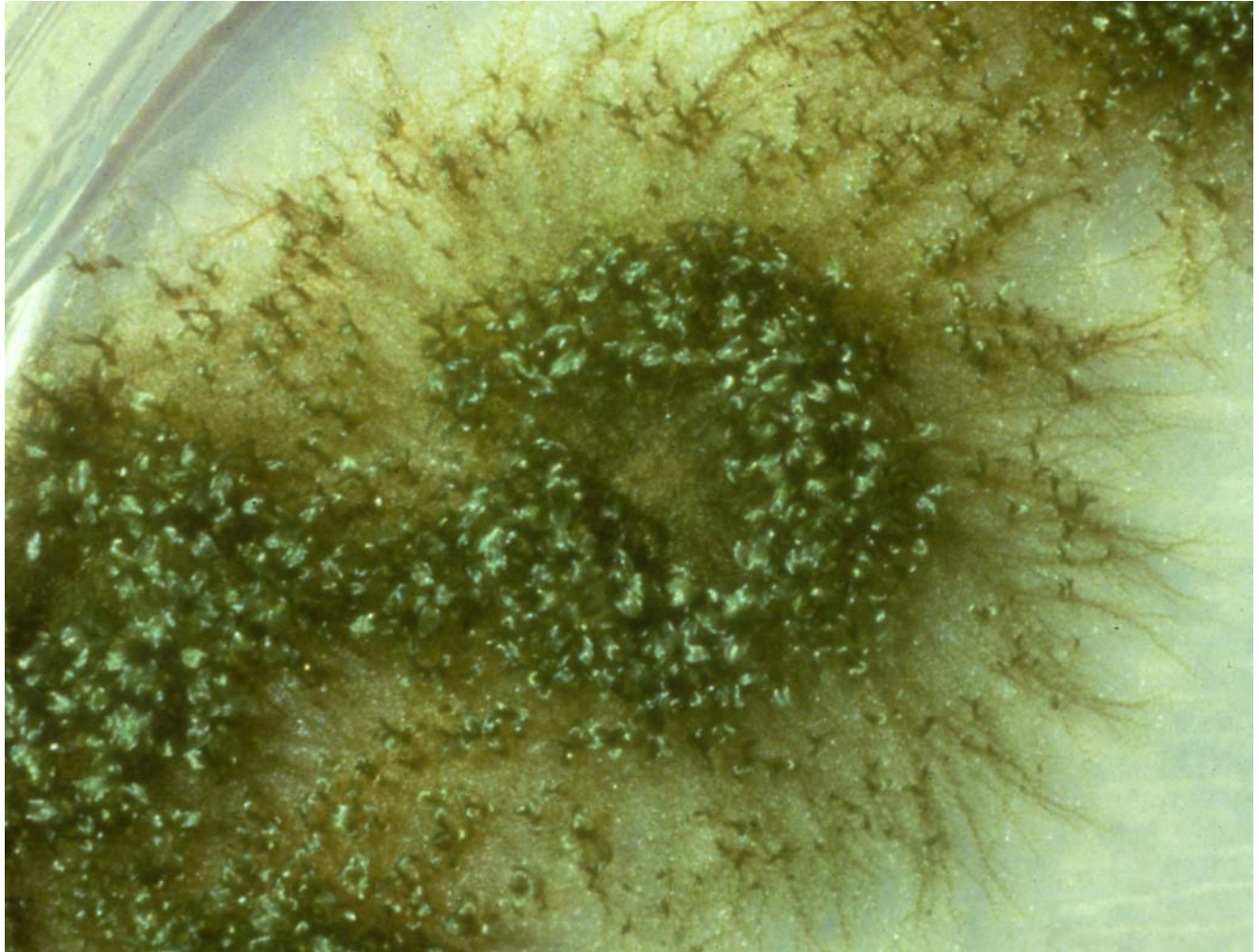


Figure 1. *Funaria hygrometrica* with prolific buds forming a doughnut, all from the protonemata produced by one spore. Photo by Janice Glime.

Establishment Success

The next step in the development of mosses and leafy liverworts is the production of gametophore buds – those forerunners of the upright plant, or gametophore (Figure 1). (That suffix, phore, means a supporting structure, and of course, the leafy gametophyte will ultimately bear the gametangia and gametes.) As protonemata grow, they change the environment, providing shade, leaking hormones and other substances, and changing the moisture retention capability of the population. These may contribute to the developmental changes leading to the growth of the leafy plant. We have learned in *Physcomitrella patens* that going that next step to bud formation requires cytokinins, resulting in a rapid influx of

calcium. This is followed by bud development on the second sub-apical caulonema cells (Gonneau *et al.* 2001). But application of ABA will inhibit bud formation (Christianson 2000a), suggesting a possible adaptation to drought.

Spore density may play a role in the establishment success (Hassel & Söderström 1999). In *Pogonatum dentatum*, young shoots on a new forest road in northern Sweden represented far less than the number of spores sown. Using planting densities of 1/2 capsule, 1 capsule, and 2 capsules in 10x10 cm plots, Hassel and Söderström found the mean establishment rate after one year was 11, 10, and 12 shoots, respectively; in the second year it was

17, 20, and 22. Apparently other factors were far more important to establishment after germination. When planted in Petri plates on nutrient-rich agar in a growth chamber, this species produced a mean of 712,000 spores per capsule and reached 96.6% germination after 21 days.

Light

Mitra and coworkers (1959, 1965) found that protonemal buds in *Pohlia nutans* were produced only in white and red light but never in blue or green light, or in darkness. Furthermore, Pringsheim and Pringsheim (1935) found that dark-grown cultures of *Funaria* produced gametophore buds if exposed to white or red light, but not blue or green light, perhaps explaining its lack of success in the forest. Mitra and Allsopp (1959) found that sugar was important in bud formation in *Pohlia nutans*, but they also concluded that a more specific substance was needed as well. They determined that this unknown substance was one synthesized only in the presence of light, again implicating possible phytochrome mediation.

We also know that in *Funaria hygrometrica* bud initiation is enhanced by red light and reversed by far-red (Simon & Naef 1981). Results in both of these studies are consistent with phytochrome as the light receptor and suggest the possibility of photoperiod control of bud formation. These results could implicate a role for the IAA/cytokinin balance. In fact, Szweykowska (1963), after inducing buds in *Ceratodon purpureus* in the dark with kinetin (a cytokinin), suggested that the kinetin replaced the role of light. This implies that the role of light might be to induce the production of a cytokinin.

Light intensity is also important in development of the normal form of gametophores. Low light results in etiolated stems (Figure 2). The expanding stems also exhibit a strong phototropism (Figure 2).

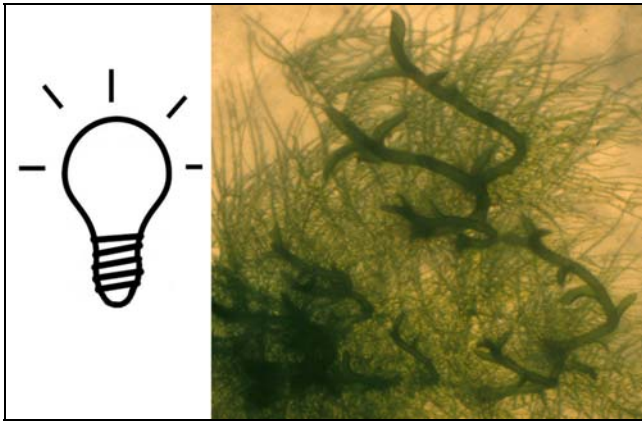


Figure 2. *Funaria hygrometrica* in culture exhibiting strong phototropism. Compare the etiolated stems to the compact ones in Figure 1. The Petri plate is covered with black paper on the right side so light is coming from left side. Photo by Janice Glime.

Growth Regulators

Bopp (1974) found that all **cytokinins** tested produce buds on isolated caulonemata. In fact, the response of *Funaria hygrometrica* (Figure 3) to cytokinin by producing buds is so reliable that it is now the standard bioassay for cytokinin in plant physiology (Christianson 2000b). However, when the protonema is removed from the

cytokinin it loses its bud-producing ability, except at 2°C. This suggests that the cytokinin is quickly broken down, except at low temperatures, and must be continuously produced by an active caulonema to induce bud formation. On the other hand, we also know that IAA inhibits the development of buds (Reski 1998), so that moving it to a new medium should have been expected to enhance the production of buds. It appears that cytokinins and IAA work together in some cases (Cove & Ashton 1984), suggesting that we should look for a habitat role in the selection for these behaviors.

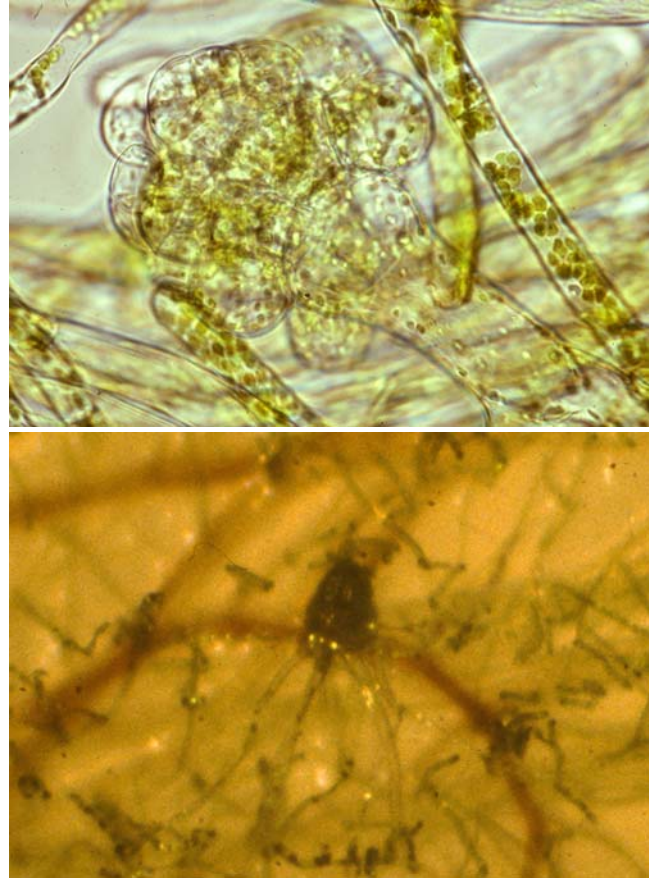


Figure 3. Buds on protonema of *Funaria hygrometrica*. **Upper:** young bud before leaf differentiation. **Lower:** older bud beginning to form leaf shape. Photos by Janice Glime.

Cytokinins have been implicated elsewhere in bud initiation. Szweykowska (1963) found she could get *Ceratodon purpureus* to initiate buds in the dark by adding **kinetin** (a cytokinin), but could get no buds even in light without it. In the moss *Anoetangium thomsonii* exogenous kinetin and auxin act **synergistically** (complement or help each other) to produce buds (Chopra & Rashid 1969). Burkholder (1959) found that *Atrichum undulatum* remained in the protonema stage in 2% sucrose plus IAA, whereas **arginine** and **glycine** (amino acids) favored leafy shoots. (Recall that Factor H is an arginine derivative.) Sood (1975) tried numerous additives and light regimes in an attempt to induce buds in *Pogonatum aloides*; only with a combination of kinetin, IAA, and sucrose could he induce buds. Normal buds grew and produced leafy gametophytes only in a combination of 0.05 ppm IAA, 1 ppm kinetin, and 0.25% sucrose.

Kumra (1985) found that not only cytokinin but also the auxins IAA, **2,4-D** (herbicide that mimics IAA), **NAA** (naphthylacetic acid potassium), and **NOA** (naphthoxyacetic acid, an auxin that inhibits auxin influx into cells) shortened the time to bud initiation and increased the number of buds produced in the moss *Anisothecium molliculum*. *Bryum atrovirens* produced no buds in culture on a basal medium until auxins were added (Chopra & Vashistha 1990). Antiauxins did not induce buds. Furthermore, the auxin concentration influenced the morphology of the leafy plants, with lower concentrations producing more normal-looking plants. The herbicide 2,4-D caused an increase in bud number but did not improve shoot morphology. It appears that in at least some mosses IAA is necessary for bud development.

It appears that this protonemal bud cytokinin system differs from other more familiar branch bud cytokinin systems. Rather, the induction of buds from moss protonemata involves not just one, but two cytokinin-mediated events. The second event controls the number of buds (Christianson & Hornbuckle 1999). Increase in cytokinin subsequently results in the increase in RNA in protonemal bud cells and an increase in the adenine:guanine ratio (Schneider *et al.* 1969). It follows, then, that another factor in controlling bud formation is the DNA replication. In the caulonema, DNA can replicate to 8 copies and even 16 copies in older cells (Knoop 1978). Buds arise irregularly from these older cells, coming instead from the younger apical cells without the DNA duplication (Bopp *et al.* 1980). We now know that ABA can intervene to prevent the second cytokinin event in shoot bud formation, at least in *Funaria hygrometrica* (Christianson 2000b). Since the ability of ABA to inhibit bud formation is concentration dependent, this cytokinin inhibition system is useful as a bioassay for ABA as well.

Could these multiple sets of DNA in the protonema contribute to the known bryophyte resistance to radiation damage during a critical life cycle stage? How does the second cytokinin event relate to these subsequent DNA multiplication events in bud formation? There seems to be so much we can learn about cell function from these one-cell-wide protonemata.

In 1968, Bopp showed that **gibberellins** will increase the number of buds and that **IAA** can in some cases cause a similar effect. On the other hand, Sarla and Chopra (1987) found that cultures of *Bryum pallescens* supplemented with 2,4-D, IAA, and NAA failed to produce buds, unlike the response of *Anisothecium molliculum* (Chopra & Vashistha 1990), whereas NOA induced at least some buds. Later, Duckett *et al.* (1993) found that cytokinin stimulates bud formation in *Ephemerum*, but that IAA instead induces chains of desiccation-tolerant brood cells, similar to those in aging cultures, which are heavily covered with mucilage. This causes one to wonder if in fact the IAA may have induced ethylene production that led to premature aging.

Few experiments have examined the role of ethylene in bryophytes. It appears that it could play a role in the maturation of protonemata and formation of buds. In experiments on *Funaria hygrometrica*, I found that a high concentration of **ACC**, the ethylene precursor (previous compound in chemical pathway), induced buds sooner than did lower concentrations or controls with no ACC (Figure 4; Glime unpublished data). This could be an effective

signalling device to let the moss know that there were sufficient protonemata to form a colony large enough to sustain moisture and could explain the ability of *F. hygrometrica* and other mosses to fill the available space with protonemata before making gametophores. As a gas, ethylene would accumulate and build in concentration around the developing protonemata.

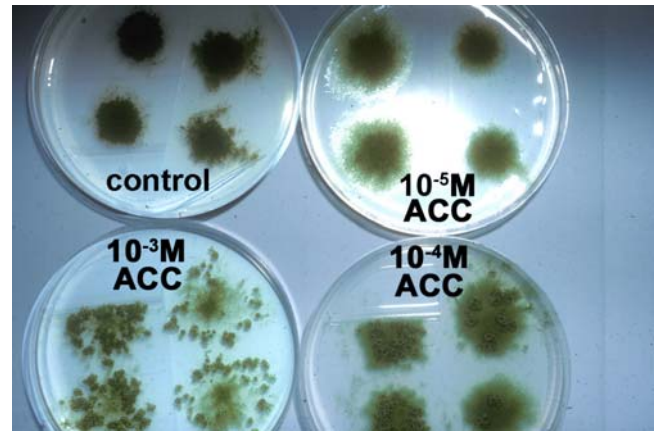


Figure 4. Effects of ACC, the ethylene precursor, on bud formation in *Funaria hygrometrica*. The highest concentration tested caused the earliest bud formation. Photo by Janice Glime.

Bopp and coworkers (1978) found that **caulonema-specific proteins** (CSP) correspond with the ability of the caulonema to respond to cytokinin and produce buds. Isolation of single cells results in the loss of ability to maintain CSP, so regeneration of protonemata occurs. Since a protonema is the first product of regeneration in mosses, it seemed logical that CSP degenerated more rapidly than other protein, causing the reversion to protonemata. However, Bopp *et al.* (1978) showed this to be incorrect. Erichsen, Knoop, and Bopp (1978) found that kinetin is metabolized, primarily to adenine derivatives, immediately upon uptake into the protonema. When adenosine was added, kinetin turnover was reduced. Since adenosine induced bud formation, we can surmise that it is not kinetin, but some product further in a reaction chain that has stimulated bud production.



Figure 5. The ephemeral moss *Ephemerum serratum*. Photo by Michael Lüth.

But how do all of these factors relate to the ability of the moss to complete its normal life cycle in nature? We can only speculate here, and weak speculation it is. It appears that light quality, and probably duration, plays a

role. This could be manifested in a phytochrome-mediated response that stimulates the production of necessary hormones, or in a photosynthetic response that builds stores of sugars, or some balance between these two. Furthermore, the lack of water could reverse the process by causing the protonema to produce ABA, hence preventing the completion of the cytokinin-directed process of bud development.

Moss protonemata seem to differ as widely in their physiology as do their mature gametophores. Cytokinin, IAA, 2,4-D, ethylene, GA, arginine, and glycine have all induced buds in some species. IAA and cytokinin can work synergistically to cause bud formation. But IAA can also inhibit bud formation and in some cases will induce the production of brood cells. ABA can prevent the second cytokinin event, which controls number of buds, and consequently inhibit bud formation. Somehow, all of this ties in with the duplication of DNA, up to 16 sets in some taxa, that seems to keep the distal cells of the protonema from producing many buds. We have no understanding of how these various signals relate to habitat or microclimate.

Interactions with Other Organisms

In the aquatic moss *Fontinalis squamosa*, development of gametophores is difficult to achieve in culture (Glime & Knoop 1986). Only one plate in 113 produced gametophores after 48 days in a variety of culture conditions. Nevertheless, the other protonemata continued to grow. Interestingly, in the plate with gametophores, more than ten were produced, and these occurred on protonemata that had developed from more than one spore. This suggests that either some necessary condition was supplied in that plate or that an induction factor was produced when one moss began to bud. Since one bud occurred in advance of all the others, it is possible that it induced the others.

The low production of buds in *Fontinalis squamosa* cultures suggests that some critical factor may be supplied by its natural habitat (Glime & Knoop 1986). Support for this need for an exogenous substance comes from the fact that the one culture that produced gametophores was contaminated with fungi. Capsules of *Fontinalis* are usually produced in shallow water or above the water, so this might permit spores to lodge on wet rocks. In this thin water layer, any products produced by fungi, bacteria, and **periphyton** (algae and other microorganisms living on plant) would be in relatively high concentration in the film on the rock. Fungi are known to leak gibberellins, and we have seen that these can increase the production of buds.

Another environmental substance is B₁₂, a vitamin produced by green algae (Chlorophyta) and blue-green bacteria (Cyanobacteria). Spiess and coworkers (1971) have shown that in the presence of the bacterium *Agrobacterium tumefaciens*, the moss *Pylaisiella selwynii* forms gametophores, but that little gametophore development is achieved in the absence of the bacteria. Spiess *et al.* (1973) have shown that B₁₂ can probably be supplied by *Rhizobium* or *Agrobacterium*.



Figure 6. *Pylaisiella selwynii* growing on bark. Photo by Janice Glime.

Nutrients or Inhibitors?

It appears that the protonema may have different requirements for nutrients than the mature plant, at least in some taxa. Li and Vitt (1994) found that nitrogen in particular might inhibit the establishment of many peatland species. They felt that the different abilities of these taxa to utilize nutrients over the temporal scale of establishment might be a strong determinant of the bryophyte patterns of the mature peatland.

Many heavy metals are needed by plants in minute quantities. They serve in making enzymes and carriers for electrons. But these same metals soon become toxic in greater quantities. Kapur and Chopra (1989) found that many metal ions (cobalt, cadmium, aluminum, lead, nickel, zinc, copper, mercury) inhibit protonemal growth, increase the time for bud initiation, decrease number of buds, and retard the gametophore growth in the moss *Timmia anomala*. At a concentration of 10⁻⁶ M, nickel increases protonemal growth slightly, but at 10⁻⁵ M it drastically decreases the number of gametophore buds. Cobalt inhibits protonemal growth but seems to have no effect on bud formation. Phillips and Peterson (1982) likewise found heavy metals to be highly toxic to the protonemata. The most toxic was copper, yet copper in small quantities is essential to formation of chlorophyll. Mercury, cadmium, and zinc were likewise toxic, in that order.

Perhaps the most critical nutrient involved in bud formation is calcium. As in germination and protonemal growth, calcium seems to be essential in bud formation. Olarinmoye *et al.* (1981) found this to be true for *Stereophyllum radiculosum*, where a minute quantity of calcium is essential. Saunders and Hepler (1982, 1983), in studying *Funaria*, suggested that control of intracellular calcium may be the means of regulating cytokinin. They indicated that increases of intracellular calcium were most likely essential for bud initiation. Calcium is important in gluing cells together, so it is unlikely that much growth could occur without it. This essential nutrient could surely play a role in determining where mosses are able to get established, with some species being better at facilitating uptake when the element is scarce and others being excluded from such habitats.

Little is known about the effects of nutrients on protonemal bud development. Yet what we know suggests they could be of great importance in controlling the establishment of bryophytes. In particular, heavy metals seem to increase the time required for bud formation and decrease the number of buds, suggesting that the bryophytes would be less competitive and may be unable to establish before tracheophytes arrive to outcompete them. In some cases, a nutrient such as nitrogen, essential for all proteins, may inhibit bud formation if present in quantities sufficient for most tracheophytes, perhaps explaining the dominance of *Sphagnum* in low-nutrient fens and bogs. Calcium is essential for all stages of development because it is part of the glue that holds the cell walls together, but it may also play a role in regulating cytokinin and therefore regulating production of gametophore buds.

Temperature

Although temperature surely plays a role in protonemal development, its effects seem to be poorly known. Kumra and Chopra (1985), in studying *Anisothecium molliculum*, found 25°C to be optimum for bud formation, the same temperature that was optimum for protonemal growth. This temperature, however, would seem a bit high as an optimum for these C₃ plants, but one must consider that the spores must presumably wait to germinate until after danger of frost is gone, or at least infrequent, then must grow a protonema before a bud can form. The bud must then expand into a leafy gametophore (Figure 7). By this time, the rapidly increasing temperatures of spring are giving way to the heat of summer, so there may be no other choice.

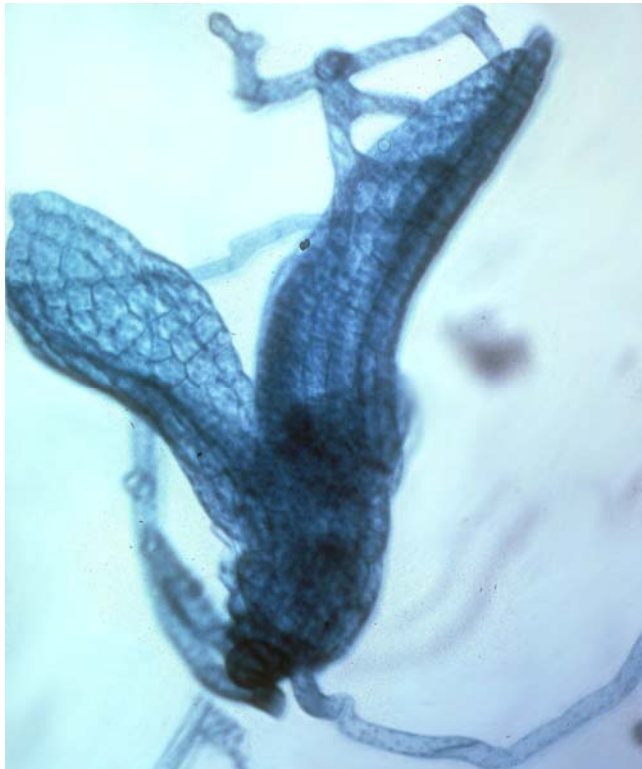


Figure 7. Bud expanding on moss protonema. Photo by Janice Glime.

A surprising effect of temperature is seen in the epiphytic *Macromitrium*. Female protonemata can produce buds at 10°C, whereas male protonemata require a lower temperature for bud formation (Une 1985). Yet, when one considers the rest of the life cycle, and the timing of gametangial formation in males and females, this is not surprising at all. Male plants and male gametangia in general seem to be initiated first, therefore requiring initiation at a lower temperature if both males and females are to be mature at the same time.

There appear to be specific nutrient and time requirements among the bryophytes that determine when the gametophore buds will develop (Giordano *et al.* 2002). In the case of *Pleurochaete squarrosa*, 8-10 months were needed for buds to form, whereas in *Funaria hygrometrica* and *Bryum capillare*, buds formed in young cultures after only a few weeks. Yet it is likely that these time requirements are temperature dependent and will vary among geographic locations.

Summary

Cytokinins seem to be a common need for initiating gametophore buds in mosses, whereas ABA can inhibit them. Density of protonemata seems also to exercise control over the number of buds in some species, most likely through a hormonal exudate. Wavelength of light can also be important, with white and red light stimulating bud formation in *Pohlia nutans*, but blue, green, and darkness failing to do so. A red/far red reversal suggests the involvement of phytochromes and perhaps involves IAA. The balance of amino acids can likewise be important. An increase in the adenine:guanine ratio results from an increase in cytokinin, coupled with a replication of DNA up to 16 copies in older cells. Most of the buds, however, arise from the younger apical cells.

Gibberellins can increase the number of buds, but it is not clear if these are supplied by the moss. GA and other growth substances, such as vitamin B₁₂, can be supplied by co-inhabiting organisms – bacteria, fungi, and algae.

Heavy metals are generally toxic and can inhibit development, but some, such as nickel, can enhance it at low concentrations. Temperature surely plays a role, but we seem to know almost nothing about it.

Acknowledgments

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