

CHAPTER 5-8

ECOPHYSIOLOGY OF DEVELOPMENT: GAMETOGENESIS

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CHAPTER 5-8

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Figure 1. Antheridial splash cups of *Polytrichum juniperinum*. Photo by Janice Glime.

Definition

Gametogenesis – the development of gametes (*genesis* means origin) is the essential process leading to sexual reproduction. In bryophytes, gametes are produced by mitotic division of tissue within multicellular structures, the **antheridia** (male) and **archegonia** (female), collectively known as **gametangia**. The location of these structures on the mosses is the basis for dividing the mosses into two large groups, the **acrocarpous** mosses that produce archegonia at the tips of upright stems, and the **pleurocarpous** mosses that produce archegonia on side branches of a generally horizontal stem. The differences in location of these archegonia can present differences in the ease with which the sperm can reach the archegonium, and hence reach the egg.

Developmental Stages

Lal and Bhandari (1968) described the developmental stages of the sex organs of the moss *Physcomitrium carpathicum*. The archegonium begins its development in a manner similar to that of the antheridium. In these early

stages, it produces a stalk, then the two-sided apical cell gains a third cutting face and the archegonium develops from this cell. The antheridial development is similar to that of other mosses. This chapter will examine the interaction of hormones and the environment as they influence this development.

Environmental Factors

The timing of the induction of gametangia is a critical function in the life cycle of bryophytes. For sexual reproduction to be successful, gametangia must form at a time when they can survive and they must mature at a time when it is safe and sufficient water is present for the sperm to reach the egg. This timing is controlled by external signals in the environment, and this is interpreted internally through such controls as hormones and nutrient levels.

Water Availability

Gametogenesis (development of gametes) must be timed in such a way as to take advantage of the most

critical need in fertilization – water. Because sperm in bryophytes must swim to the archegonium, adequate water is critical, but too much water or rapidly flowing water may dilute or carry off the sperm and make directional movement toward the archegonium all but impossible. In fact, timing of moss reproduction, whether a response to day length or temperature or other environmental stimulus, is often related to the season of proper moisture. Since gametangial initiation can occur several (or many) months prior to the actual time of fertilization, environmental cues other than moisture must trigger the process. It is therefore an expected consequence that different species within a genus respond to different environmental cues for gametogenesis, permitting them to live in different habitats. And even within species, populations can differ widely (Clarke & Greene 1970). But for many bryophytes, water is an important signal for gametangia to develop, perhaps because it permits the gametophyte to be active and produce sugars needed for energy.

Gametangium Developmental Need for Water

Waterfalls can provide continuous moisture sufficient for sperm dispersal and even contribute to dispersal itself. At Churchill Falls, Labrador, Canada, the bryophytes are very fertile within the spray zone, whereas other vegetation expresses retarded phenology (Brassard *et al.* 1971). It could be that the spray itself induces gametangial production. Kumra and Chopra (1983) found that culture in liquid media favors antheridial induction in *Barbula indica* var. *gregaria* (Figure 2) and *Bryum coronatum* (Figure 3) over that in solid gel culture, greatly hastening it in *Barbula indica* var. *gregaria*.



Figure 2. *Barbula indica* var. *gregaria*, a moss where liquid medium favors antheridial production. Photo by Li Zhang, with permission.



Figure 3. *Bryum coronatum*, a moss where liquid culture favors antheridial induction. Photo by Michael Lüth, with permission.

Sphagnum (Figure 4) provides a good example of effect of water on gametangial maturation. Sundberg (2002) studied nine sites in Sweden for six years, during which the nine most abundant species produced capsules. Capsule production related most to moisture regime of the previous summer, with more precipitation resulting in more capsules. This presumably relates to success of gametangial formation. Capsule success in wetter pits related positively to spring precipitation in the same year as capsule production, suggesting it was also important for fertilization success. Further discussion of timing of reproduction with moisture availability is in the phenology chapter.

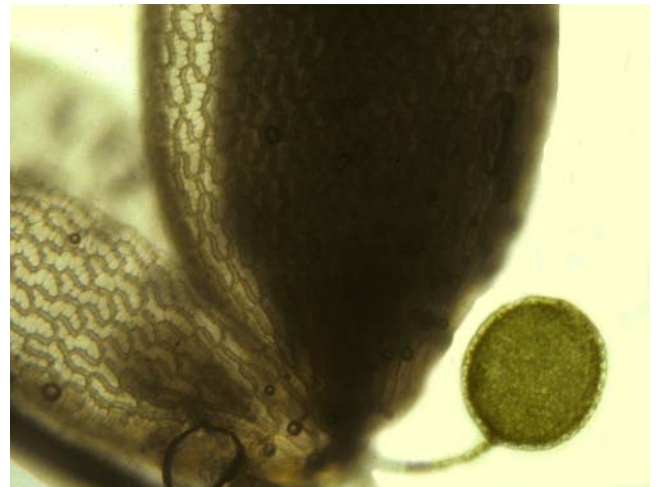


Figure 4. *Sphagnum papillosum* antheridium, a species for which moisture is important for gametangial success. Photo courtesy of Yenhung Li.

Swimming Sperm

For sperm to reach the archegonium, they must swim. But a tiny sperm cell (Figure 5) cannot carry that much energy with it, so the distance is limited. Some mosses maximize the effect of rainwater by producing **splash cups** (Figure 1) or **splash platforms** (Figure 6) that house the antheridia.

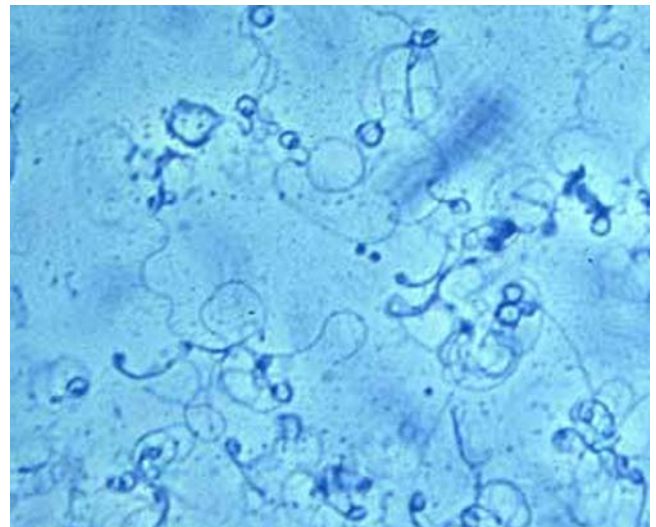


Figure 5. *Marchantia polymorpha* stained sperm. Photo from Botany Website, UBC, with permission.

The study by Andersson (2002) on *Plagiomnium affine* (Figure 6) provides insight into just how this splash works. He is the only one who has published photographs of the arrival and splash of an actual raindrop, eliminating the problem of laboratory tests where the drops do not reach terminal velocity. The splash is somewhat reminiscent of the expulsion of *Sphagnum* (Figure 4) spores from a capsule, both demonstrating fluid dynamics. When the raindrop first hits a hard surface (splash cup or platform), it forms a crater many times the diameter of the drop. A jet of water then rises from the center of the crater (Rayleigh's jet). One or more large drops may be pinched off. In a splash cup, this force is typically sufficient to push all the water out of the cup.



Figure 6. *Plagiomnium affine* showing antheridial platforms and runners. Photo by Janice Glime.

Splash cups and platforms are not flat, so the water angles are oblique (Andersson 2002). As the water flows outward from the point of impact, the edge of the water mass bends upward to form a crown. As the drop collapses, the circle of water widens and the crown bends up more. Wave motions travel both vertically and horizontally; a thick cylinder of water forms around the upper rim of the crown and small jets of water extend outward. As these jets become unstable, they break into many tiny droplets that shoot out from the crown with high velocity. The crown collapse occurs after about 8 ms on a wet surface. Most of the droplets are less than 0.5 mm, and many are less than 0.05 mm. The spermatozooids are only about 1 μm (0.001 mm) in diameter and can therefore easily be carried by the droplets of water.

Most experiments with splash cups have not been at distances that mimic terminal velocity. Based on data from Laws (1941), a 3 mm drop would need to be dropped from about 7 m to reach terminal velocity, a height not available in most labs. Reynolds (1980) considered that distances of 30 cm splash from point of impact would not be uncommon.

But does this splash really disperse the sperm? To be dispersed, sperm must be able to exit the antheridium, and this requires that the antheridium must burst. That criterion is satisfied by the first raindrop to strike a mature antheridium (Andersson 2002). But... members of the *Mniaceae* shrivel when dry and do not rewet easily. *Mnium* (Figure 7-Figure 8) species may require soaking for an hour before they are ready for making a slide (Koponen 1974), indicating that the leaves in a rainstorm

are not ready to make a splash platform in less than an hour. Furthermore, the forest canopy traps many of the raindrops and reduces their velocity (Andersson 2002) or even diverts them so that they run down the trunk instead of striking the forest floor beneath them. Hence, it may take some time before the splash platform is exposed directly to raindrops in a storm, and this might not be achieved at all in a light shower.



Figure 7. *Mnium spinosum* wet. Photo by Michael Luth, with permission.



Figure 8. *Mnium spinosum* dry. In this condition, it is slow to take in water. Photo by Michael Luth, with permission.

To add further to the complications of reaching a female, the sperm are not released directly as individuals from the antheridium. Rather, they are released in a package, a **vesicle** of fluid. This vesicle must be disturbed by water drops before it will break apart. The vesicles become separated from each other by lipid drops and slowly dissolve, freeing the sperm.

Some seed plants have a chemical delay mechanism to prevent seed germination in a short rain shower, with chemical inhibitors being removed in a more significant rainstorm that is sufficient to sustain the young plant. The intervening factors required for a raindrop to splash the bryophyte sperm successfully seems like a mechanical method to delay sperm dispersal until it is certain there will be sufficient water for the sperm to complete their journey after the splash, with the delay in freeing sperm contributing to this mechanism.

The moss *Plagiomnium affine* (Figure 6) is less fortunate than the species with real cups. Its antheridial

platforms succeed only in splashing droplets with sperm about 100 mm (Andersson 2002). Fortunately, most of the females within 80 mm are successfully fertilized, but that does not permit much outcrossing.

In *Polytrichum ohioense* (Figure 9), the 2-3 mm cup permits sperm to be splashed 60 cm or more (Brodie 1951). A similar distance is accomplished by the splash platform of *Marchantia polymorpha* (Figure 10) (Buller 1942). Even greater distances, up to 230 cm, are achieved by antheridial splash cups of *Dawsonia longifolia* (Figure 11-Figure 12) (Clayton-Greene *et al.* 1977; see chapter on sexuality), aided by its greater height (up to 50 cm). These dispersal distances match the observed maximum distances between males and sporophyte-bearing females observed in the field. Very small splashes create an aerosol effect that could permit the sperm to float for considerable distances, and wind can increase the distance downwind.



Figure 9. *Polytrichum ohioense* with spent antheridial splash cups producing new growth. Photo by Janice Glime.



Figure 10. *Marchantia polymorpha* male splash platforms. Photo by David T. Holyoak, with permission.



Figure 11. *Dawsonia longifolia* with perigonia. Photo by Allan Fife, with permission.

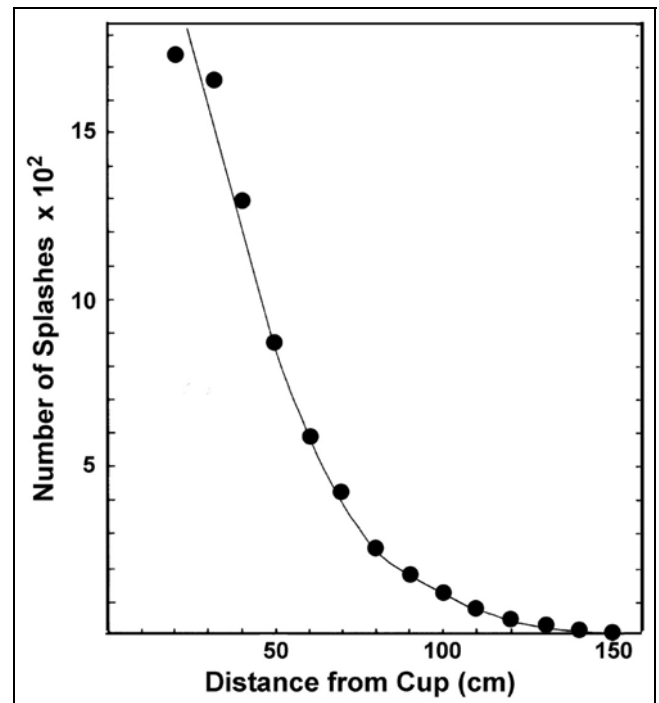


Figure 12. Distance of splashes from 0.055 ml drops dropped from 228 cm and splashed from the splash cup of *Dawsonia longifolia*. Redrawn from Clayton-Greene *et al.* (1977).

Monoicous species (having male and female organs on the same plant) have a greater chance for fertilization than **dioicous** species because there will always be gametangia of the opposite sex nearby. Rohrer (1982) compared the success of dioicous species with and without splash cups in an aspen forest and a swamp forest of Michigan's northern Lower Peninsula. Those with splash cups had significantly higher sporophyte production (Figure 13). Unfortunately,

splash cups are relatively uncommon, but leaves surrounding antheridia can sometimes act as splash cups or platforms by spreading when hit by a raindrop (reference forgotten ☺).

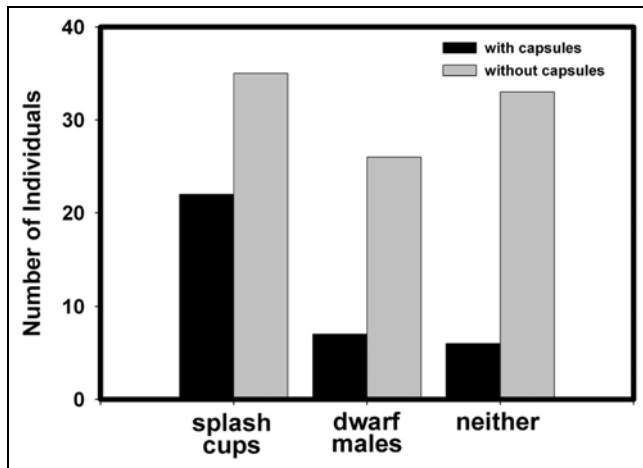


Figure 13. Effect of splash cups and epiphytic dwarf males on number of individuals with sporophytes in dioicous mosses of an aspen forest in the northern lower peninsula of Michigan, USA. Based on data from Rohrer 1982.

Paraphyses

This story is not complete without a discussion of **paraphyses**, those sterile structures, usually filamentous, that accompany most gametangia among the bryophytes. For something to persist this commonly while requiring energy for their development, we usually consider them to have some adaptive function. But little if any testing has been done to show that they make a difference.

Paraphyses usually occur in sufficient density to produce capillary spaces. With this knowledge, we can theorize as to their value. Such spaces would mean that water drops would be drawn between them, providing swimming spaces surrounding the archegonia. For antheridia, these can create water pressure that could aid in the rupture of the antheridium and hence the release of sperm.

But this does not seem to be the only excuse for their continued existence. In the Neckeraceae, structures that can be interpreted as paraphyses develop after fertilization in *Neckeropsis* (Figure 14), forming on the perichaetia (Merced-Alejandrea & Sastre-De Jesús 2009). These researchers found that transitions between uniseriate and multiseriate paraphyses occur at different stages in the developing reproductive branch. In early stages they are more typical of paraphyses in most mosses; this stage is the terminal stage in some *Neckeropsis* species. In other species, these continue to become multiseriate and ligulate to lanceolate. But what could their function be if they do not develop until after fertilization?



Figure 14. *Neckeropsis undulata*, a genus in which paraphyses develop after fertilization. Photo by Michael Luth, with permission.

As discussed earlier, Reese (1955) tested a very different function for these paraphyses. He was able to demonstrate their ability to regenerate plants in *Bryum capillare* (Figure 15-Figure 16), *Aulacomnium palustre* (Figure 17), and *Funaria hygrometrica* (Figure 18-Figure 19). Could this be a back-up plan for unsuccessful sexual reproduction? Most likely it is actually a rare occurrence in nature, and thus its most frequent function is most likely that surmised by the early bryologists who considered them to have both a capillary function to draw in water, but also to retain water among the developing gametangia.



Figure 15. *Bryum capillare* males with antheridia and paraphyses. Photo by Dick Haaksma, with permission.



Figure 16. *Bryum capillare* antheridia, and paraphyses that can regenerate. Photo by Dick Haaksma, with permission.



Figure 17. *Aulacomnium palustre* males, a species in which paraphyses can regenerate new plants. Photo by David T. Holyoak, with permission.



Figure 18. *Funaria hygrometrica* with antheridia. Photo by Barry Stewart, with permission.

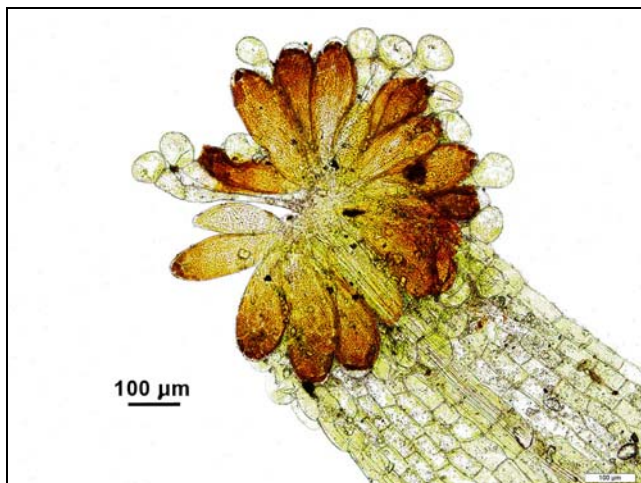


Figure 19. *Funaria hygrometrica* antheridia with paraphyses (white) that can regenerate. Photo by Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

Photoperiod and Light Intensity

Bryophytes, like flowering plants, can be classified into a variety of short-day and long-day types (Benson-Evans 1964; Maravolo 1980; Glime 1984; Li & Glime

1991). Tracheophyte species that occupy a wide latitudinal range, such as *Typha latifolia*, show population differences in response to day length (McNaughton 1966), and Longton (1972) has demonstrated this for the mosses *Polytrichum* (Figure 1, Figure 9) and *Psilopilum* (Figure 20). The physiological response mechanism in these two taxa is unknown, and a large number of substances can induce the same response, depending on the species.

In 1983, Chopra and Bhatla contended that mosses, except for *Sphagnum plumulosum* (= *S. subnitens*; Figure 21), appeared to be independent of photoperiod for the induction of gametangia. On the other hand, they found that all liverworts tested to date, except *Ricciella crystallina* (= *Riccia crystallina*; Figure 22) (Chopra & Sood 1973a), were either long-day or short-day plants. But they clarified this statement – it appears that even in liverworts, the response seems to be quantitative, with greater light intensities increasing the photoperiod response.



Figure 20. *Psilopilum cavifolium*, member of a genus where populations can show differences in response to day length. Photo by Niklas Lonnell, with permission.

In mosses, other factors such as light intensity and temperature modify the response. For example, *Bartramidula bartramoides* [optimum of 3500-4000 continuous light (Chopra & Rahbar 1982)] and *Leptobryum pyriforme* (Figure 23) respond linearly to increasing light intensity for gametangial response (Chopra

& Rawat 1977; Chopra & Bhatla 1983), whereas *Bryum argenteum* (Figure 44-Figure 45), *B. coronatum* (Figure 3), and *Barbula indica* var. *gregaria* (Figure 2) respond to a specific light intensity for their optimal response (Chopra & Bhatla 1983). In *Bryum coronatum* and *Barbula indica* var. *gregaria*, antheridia develop under "ordinary" cultural conditions (Kumra & Chopra 1983), requiring no specific photoperiod for induction, but having a greater response as the photoperiod increases. *Philonotis turneriana*, on the other hand, remains sterile under "ordinary" conditions. Temperature likewise plays a role, but its role is primarily to constrain the photoperiodic effect within certain temperature limits. However, in *Philonotis turneriana* a temperature of 18°C is needed for induction. In *Barbula indica* var. *gregaria* and *Bryum coronatum*, the antheridial induction increases as the temperature increases, up to 24°C.



Figure 21. *Sphagnum plumulosum*, one of the first mosses known to respond to photoperiod for gametangial induction. Photo by J. C. Schou <<http://www.biopix.com/>>, with permission.



Figure 22. *Ricciella* cf. *crystallina* (= *Riccia crystallina*) Bareilly India. Photo by Michael Lüth, with permission.

Knoop (1984), like Chopra and Bhatla (1983), contends that most mosses seem to be day-neutral. Nevertheless, Benson-Evans (1964) examined a large number of bryophyte taxa with varying environmental influences on initiation of gametangia; photoperiod seemed to be the overriding influence in most cases. In ten liverworts (4 Marchantiales, 6 Jungermanniales), the plants were long-day plants. *Riccia glauca* (Figure 24), *Phaeoceros laevis* (Figure 25), and *Sphagnum plumulosum* (Figure 21) are short-day plants. The moss

Pogonatum aloides (Figure 26) (Benson-Evans 1964) and liverwort *Ricciella crystallina* (Figure 22) (Chopra & Sood 1973b) are day-neutral. *Phaeoceros* spp. (hornworts) are predominantly long-day induced, a condition that may be true for most hornworts (Schofield 1985). Temperature and other external factors can modify these responses, and surely energy will play a role. But are most mosses really day-neutral?



Figure 23. *Leptobryum pyriforme* with capsules, a moss that produces more gametangia as light intensity increases. Photo by David T. Holyoak, with permission.



Figure 24. *Riccia glauca*, a long-day liverwort. Photo by Jan-Peter Frahm, with permission.



Figure 25. *Phaeoceros laevis*, a long-day hornwort. Photo by Robert Klips, with permission.



Figure 26. *Pogonatum aloides* with male splash cups. Photo by David T. Holyoak, with permission.

Despite the tendency for liverworts to be controlled by photoperiod, *Lophocolea* (Figure 27) in southern Illinois, USA, is day neutral (Zehr 1979). And the mosses *Diphyscium foliosum* (Figure 28), *Atrichum angustatum* (Figure 29), and liverwort *Trichocolea tomentella* (Figure 31) are long-day plants for gametangial production. *Nowellia curvifolia* (Figure 30) is likewise a long-day liverwort, but only for initiation. They will continue to develop unless the process is halted by desiccation.



Figure 27. *Lophocolea heterophylla* on a log, a day-neutral liverwort, at least in southern Illinois, USA. Photo courtesy of Betsy St. Pierre.



Figure 28. *Diphyscium foliosum* showing female plants with perichaetial leaves and purplish male plants. Photo by Li Zhang, with permission.



Figure 29. *Atrichum angustatum* males, a long-day species for gametangial production. Photo by Bob Klips, with permission.



Figure 30. *Nowellia curvifolia*, a long-day liverwort for gametangial induction. Photo by Michael Lüth, with permission.



Figure 31. *Trichocolea tomentella*, a long-day plant for gametangial production. Photo by Michael Luth, with permission.

Voth and Hamner (1940) found that photoperiod controlled the development of gemma cups vs gametangiophores in *Marchantia polymorpha* (Figure 10). Short days stimulated gemma cup production, whereas long days stimulated more gametangiophores. Miller and Colaiace (1969) found that this species could be grown from gemmae and induced to produce antheridiophores and

archegoniophores in 3-6 weeks under a 24-hour photoperiod at 23°C.

Perhaps *Fontinalis* can again give us insight into these seemingly different results. Members of this genus, like *Fontinalis novae-angliae* (Figure 32), that are common in fast water of mountain streams face the problem of losing their tiny sperm rapidly downstream as soon as they are released. Goebel (1930) suggests that *Fontinalis* can only reproduce when it is in standing water because the water would otherwise wash the sperm away too easily. Hence, it appears that those mosses that live submersed in streams must time their sperm release to coincide with low water levels when the moss is moist, but not in rushing water.



Figure 32. *Fontinalis novae-angliae* in a swift mountain stream in New Hampshire, USA. Photo by Janice Glime.

This need for timing of sperm release suggests that a photoperiod response would be beneficial in those regions where low water level periods are somewhat predictable. Indeed, in *Fontinalis dalecarlica* (Figure 33), photoperiod seems to control production of gametangia quantitatively, rather than being an on-off signal, with short days causing the maximum number of archegonia to be mature when the moss is above water, but wet, during late summer and early autumn (Figure 34; Glime 1984). Longer days seem to lengthen the time for archegonia production, but aeration (from being above water) is also an important factor, resulting in more archegonia compared to those on submersed stems. Maturation of gametangia when the antheridia and archegonia are located above water, but moist, provides moisture for fertilization but protects the sperm from being washed away by fast water (Figure 35). Perhaps initiation of archegonia is more complex in mosses, causing the appearance of being day-neutral when the combination of stimulating factors is not present.

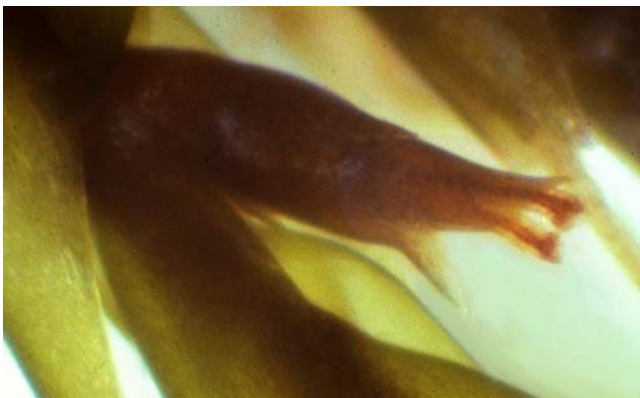


Figure 33. *Fontinalis dalecarlica* archegonia, a genus that responds to day length. Photo by Janice Glime

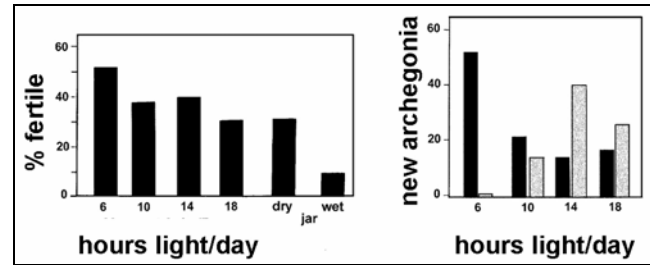


Figure 34. Effects of photoperiod and exposure to air on production of archegonia in *Fontinalis dalecarlica* (Figure 33) after 16 weeks of cultivation in artificial streams. **Left:** Day length effect and effect of submersed (wet) vs emergent (dry) at 14-hr photoperiod. Fertility does not differ significantly among the photoperiods, but emergent mosses produced significantly more than wet ones ($p < 0.01$). **Right:** Effect of photoperiod on development time required for archegonia. Black bars ■ are numbers of archegonia produced during weeks 1-7; gray bars ▒ are numbers produced during weeks 7-16. $n = 40$ plants in each condition. From Glime 1984.

Leitgeb (1868) found *Fontinalis antipyretica* (Figure 35) to produce antheridia from spring until fall, but he did not mention whether the number maturing remained constant. At least for *Fontinalis dalecarlica* (Figure 33) from North Carolina, the fact that production is not perfectly responsive to short days, but rather occurs more slowly during longer days, assures the moss of having at least some gametangia ripe whenever water conditions are right (Glime 1984). It is a bet-hedger in the sense of Stearns' (1976) r and K strategies. *Fontinalis* can afford to be a bet-hedger because its vegetative parts are both persistent and capable of reproducing by fragmentation. Even a series of years when gametangial maturity does not match the right water level would not cause a serious reproductive problem.



Figure 35. *Fontinalis antipyretica* partially above water, providing an opportunity for splashed sperm to locate an archegonium. Photo by Jan-Peter Frahm, with permission.

The suitable photoperiod may be altered by temperature, permitting the plant to be plastic and able to

complete its life cycle in different geographic regions where the photoperiod relationship to temperature is different. For example, *Fossombronina brasiliensis* is a short-day plant at 18°C, requiring 6-12 hours of night, whereas at 10°C its light requirements are more quantitative (Chin *et al.* 1987). Furthermore, photoperiod affected the sex ratio, with more female gametangia being produced at 10°C and more male gametangia at 18°C.

Continuous light can favor some moss gametangial production. For the moss *Microdus brasiliensis* (Figure 36), Chopra and Mehta (1987) found that gametangial production increased with increasing photoperiod, with continuous illumination at 18°C being optimal.



Figure 36. *Microdus brasiliensis*, a moss in which gametangial production increases with increasing photoperiod. Photo by Jan-Peter Frahm, with permission.

Light intensity can also control fertilization success. *Phascum cuspidatum* (Figure 37) has greater fertilization in shade, due to larger antheridia and greater dehiscence, than in sun (Hughes & Wiggin 1969). Since free water is required for fertilization, this mechanism provides a longer period of moisture while the sperm attempts to reach the egg.



Figure 37. *Phascum cuspidatum* with capsules, a moss with greater fertilization in shade. Photo by Michael Lüth, with permission.

Little seems to have been done to understand the relationships of photoperiod in gametangial development in the **Anthocerotophyta**. Benson-Evans (1964) reported that this group is comprised of short-day plants, but I haven't found enough references to justify that assertion. She reported that *Phaeoceros laevis* (Figure 25) is sterile in 18-hour days, but produces gametangia in 8-12 hour days. Ridgeway (1967) found photoperiod to be the critical factor to induce antheridia and *Anthoceros* (Figure 38), *Phaeoceros*, and *Notothylas* (Figure 39), whereas a range of temperatures from 10 to 20°C had almost no effect. However, at 5 and 25°C, the six species studied failed to produce antheridia. At 10°C, none of the species produced antheridia in 18-hour days, whereas all produced them in that photoperiod at 8°C. Most also produced them at 4 and 12°C.



Figure 38. *Anthoceros agrestis*, a hornwort that produces gametangia in response to photoperiod, shown here with sporophytes. Photo by Jan-Peter Frahm, with permission.



Figure 39. *Notothylas orbicularis* with involucres, a species that responds to photoperiod but not temperatures. Photo by Michael Lüth, with permission.

Using single-spore cultures, Lazarenko and Lesniak (1972) found that the long-day (16 hours daylight) *Desmatodon cernuus* is sterile in 24 hours of light. Such requirements from the natural environment could eliminate the sexual reproduction in populations that develop in more northern latitudes and may explain the reliance of some species on asexual reproduction. The sibling species *Desmatodon ucrainicus* is fully self compatible.

In a more recent study, Lee *et al.* (2010) found that it can actually be the change in photoperiod that induces gametangia. In *Pohlia nutans* (Figure 40), changes from long days to short days effected gametangial initiation. It appears we need many more studies before we can assess the importance of photoperiod (and light intensity) on gametangial induction in bryophytes, especially mosses.



Figure 40. *Pohlia nutans* with perigonia, a plant that responds to a change in photoperiod to initiate gametangia. Photo by Michael Lüth, with permission.

But it appears that we know little about the effects of light intensity or light quality on the development of gametangia or the success of fertilization. Could it be that in certain wavelengths the sperm are more likely to die, particularly in the UV range?

Photoperiod response is likely to be one of the most frequent differences seen between populations at different latitudes. Wavelength is also likely to be a selection factor, especially at high altitudes. Selection forces would be strong against those individuals that produced gametangia at times when completion of reproduction was unlikely due to low temperatures and possibly strong UV light. Weitz and Heyn (1981) demonstrated that reaction to day length was one of the traits that differed among populations of the ubiquitous moss *Funaria hygrometrica* (Figure 41) from various geographic-climatic regions.



Figure 41. *Funaria hygrometrica* (Common Cord-moss) male plants with antheridial splash platforms. Photo by Barry Stewart, with permission.

The moss *Bartramidula bartramoides* is unusual in having a high nutrient requirement. Chopra and Rhabar (1982) found that it grew best at full strength Knop's medium plus Nitsch's minor nutrient solution. Gametangial induction (initiation of development) occurred at $25 \pm 2^\circ\text{C}$, 3500-4000 lux continuous light.

Nutrients

Nutrient supply as a control of gametogenesis occurs throughout the plant kingdom, although it is probably best developed in the algae. The green algae *Oedogonium* (Singh & Chaudhary 1990) and *Chlamydomonas* (Figure 42) (Trainor 1959; Matsuda *et al.* 1992) recognize the approach of winter by the diminishing supply of nitrogen in a usable form, developing gametes and creating zygotes (then zygospores) that are able to survive the winter. It is appropriate to ask what role nutrients play in the life cycles for organisms that have quite low nutrient requirements – the bryophytes.



Figure 42. *Chlamydomonas*, a genus that responds to diminishing N supply by producing gametes. Photo by Janice Glime.

Ramina *et al.* (1979) demonstrated the role of nutrients in *Bougainvillea*, where flower production increased in direct relationship to leaf production but decreased in relation to branch production (which used nutrients without making more). In the aquatic moss *Fontinalis dalecarlica* (Figure 43), production of gametangia likewise is inversely related to branch production from 10 August to 14 October (Figure 43), again suggesting an energy limitation (Glime 1984).

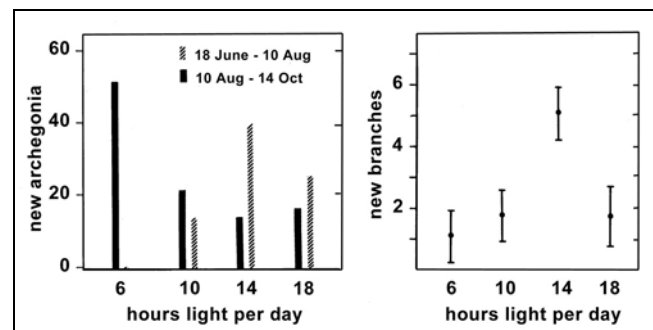


Figure 43. Effect of photoperiod on number of archegonia vs branches in *Fontinalis dalecarlica*. Redrawn from Glime 1984.

Selkirk (1979) has shown that limited nitrates cause gamete production in several species of the liverwort *Riccia* (Figure 24), and Joenje and During (1977) showed that lower nutrients stimulate the production of sex organs

in *Bryum argenteum* (Figure 44-Figure 45). A low N:high C ratio in *Marchantia* (Figure 10) likewise stimulated production of sexual branches (Lockwood 1975). On the other hand, in *Fossombronia brasiliensis* (see Figure 46), N as nitrate caused more gametangial production than when it was supplied as ammonium (Chin *et al.* 1987). Such differences can help to explain differences in habitat preferences among species.



Figure 44. *Bryum argenteum* with several plants showing antheridial apices. Photo by Dick Haaksma, with permission.



Figure 45. *Bryum argenteum* perigonium showing antheridia. Photo by George J. Shepherd, through Creative Commons.



Figure 46. *Fossombronia* sp. *Fossombronia brasiliensis* produces gametangia in response to nitrate nitrogen. Photo by Ken-ichi Uedo, through Creative Commons.

Carbohydrates are important for gametangial formation in at least some bryophytes. Whereas *Bryum argenteum* (Figure 44-Figure 45), *B. coronatum* (Figure 3), and *Barbula indica* var. *gregaria* (Figure 2) produce gametangia in the absence of carbohydrates in culture, *Ricciella crystallina* (Figure 22) and *Bartramidula bartramoides* respond to enhanced carbohydrates (Chopra & Bhatla 1983), and addition of sugar in culture seems to be essential for *Bartramidula bartramoides* (Chopra & Rahbar 1982). But, as discussed above, Chopra and Bhatla (1983) found that a high carbohydrate:nitrogen ratio was more important than carbohydrates alone in the initiation of gametangia. In particular, bryophytes are likely to respond to depletion of nitrate or ammonium (depending on species), whereas organic nitrogen (amino acids, peptone, urea) affects gametangial formation differently among various species of liverworts.

Amino acids and kinetin, both found in the environment, can alter the photoperiodic response of gametangial induction in the leafy liverwort *Cephalozia lunulifolia* (= *C. media*; Figure 56) (Lockwood 1975). Arginine, cysteine, and tryptophan plus kinetin negated photoperiodic control. Those compounds that stimulated asexual reproduction (gemmae) under short photoperiods would also inhibit gametangial activity under long-day conditions. Addition of inorganic nitrogen had no effect on these responses.

Thus, as concluded by Chopra and Bhatla (1983), the importance of the nutrient status varies by species. Generally, however, low nutrient levels seem to be the most important in gametangial induction.

The need for sugar may be an artifact of culture. In their study of the liverwort *Cryptomitrium himalayense*, Awasthi *et al.* (2013) found that sugar was necessary in the lab for gametangial induction, but when cultured on soil, this species produced gametangia under the same temperature of 21°C and long day (16 hours light) regime with colder nights (8 hours darkness at 15°C), but with no added sugar necessary.

Belkengren (1962) had some rather unusual results in *Leptodictyum riparium* (Figure 47). In this species, he was able to induce gametangia by culturing in continuous light, using a CO₂-free period followed by addition of sugar or CO₂. I don't know how this relationship would apply in nature.



Figure 47. *Leptodictyum riparium*, a species that can produce gametangia in continuous light. Photo by David T. Holyoak, with permission.

I find it interesting that the same nutrient status that favors gametangial production also favors vegetative growth in *Bartramidula bartramoides* (Chopra & Rahbar 1982). This was demonstrated using Knop's major nutrients plus Nitsch's minor nutrients at full strength with 1% sucrose. Perhaps the added sucrose gave it the energy it needed to support both.

A low nutrient status in the environment can trigger transport of nutrients from leaves to younger, growing parts in tracheophytes (Salisbury & Ross 1978), and Ogawa and King (1979) have shown that in *Pharbitis nil*, translocation of assimilate is essential for flowering. Perhaps translocation of assimilate accounts for the stimulus to produce gametangia under low nutrient conditions in bryophytes as well, but at present we have no clue that this occurs. By contrast, working with *Bartramidula bartramoides*, Chopra and Rahbar (1982) showed that optimum conditions for induction of gametangia included full strength nutrient solution.

In *Ricciella crystallina* (Figure 22), there was no response in growth of thalli when calcium nitrate concentration was doubled or even quadrupled in Knop's solution (Sood 1974). However, increasing potassium nitrate cause a "considerable" increase in growth. Changing to ammonium nitrate or ammonium sulphate caused the formation of callus tissue. Fe-EDDHA and Fe-EDTA had no effect on thalli, but slightly increased production of archegonia (optimum at 10^{-5} M). Urea as a nitrogen source supported both robust growth and increased archegonial production. Amino acids likewise affected sexuality, with hydroxyproline, serine, threonine, asparagine, glutamic acid, alanine, and leucine causing production of more archegonia. Glycine, tryptophan, aspartic acid, and valine caused production of more antheridia.

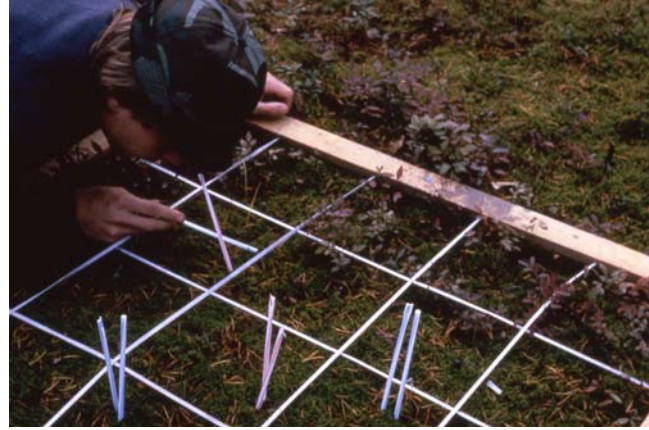


Figure 48. Geert Raeymaekers measuring distances between sporophytes on *Pleurozium schreberi* following simulated acid rain treatment. Photo courtesy of Geert Raeymaekers.

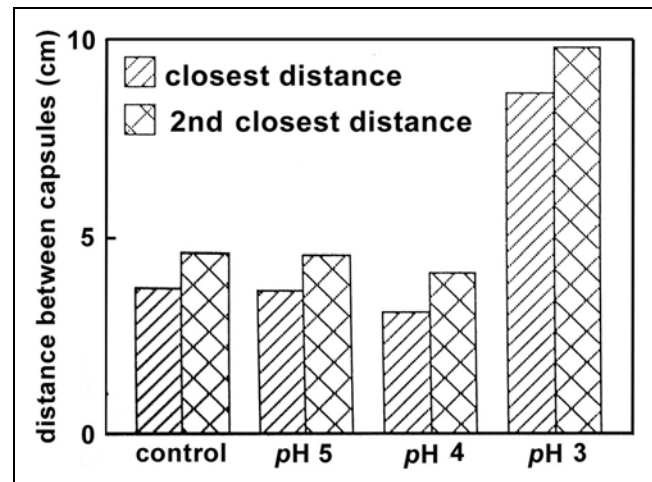


Figure 49. Comparison of distances between sporophytes in *Pleurozium schreberi* under simulated acid rain treatments. Redrawn from Raeymaekers 1986.

pH

Chopra and Bhatla (1983) concluded that bryophytes exhibit maximal gametangial initiation within a specific pH range, and that the pH of the medium changes during gametangial production. Bhatla (1981) found that a pH of 4.5 inhibited sexual induction in the moss *Bryum argenteum* (Figure 44-Figure 45). Raeymaekers (1986) found that a pH of 3.5 inhibited formation of capsules (Figure 48-Figure 49) in the acid-loving *Pleurozium schreberi* (Figure 50), thus indicating a possible connection with gametangia (Figure 51). Whether pH plays a role in induction of gametangia is unknown, but certainly low pH of acid precipitation can be detrimental to some mosses by interfering with sexual reproduction.

Rahbar and Chopra (1982) found that *Bartramidula bartramoides* produced more gametangia in liquid media than on semi-solid media. The two media exhibit different changes in pH, but these changes do not affect the time of gametangial induction. However, increasing pH, up to pH 7.0, increases the percentage of fertile gametophytes.



Figure 50. *Pleurozium schreberi*, a moss whose sexual reproduction is sensitive to low pH. Photo by Bob Klips, with permission.

One interesting correlation in several species of *Splachnum* (Figure 66) is that low pH, along with low light and nutrient concentration, can favor males over females

(Cameron & Wyatt 1990). This results in clumps of one gender, but the changing pH with aging of the dung could favor a change in gender in later populations, ultimately resulting in the presence of both sexes on the same dung. In fact, the ratios on Isle Royale, Michigan, were typically 2:1 females to males.

In the eleven species of bryophytes from a Brazilian Atlantic Rainforest, Maciel-Silva *et al.* (2012) found that monoicous and dioicous species had different responses to pH. At sea level, the monoicous taxa were favored by a lower pH.



Figure 51. Archegonia of *Pleurozium schreberi* showing the loose perichaetial protection they have. Photo by Janice Glime.

Temperature

Temperature induces a variety of responses in flowering plants (Salisbury & Ross 1978), and we might expect even more variety in bryophytes, where some species remain active throughout winter even at high latitudes and altitudes. For example, *Fontinalis hypnoides* (Figure 52) produces more gametangia at 15°C than at 1, 5, 10, or 20°C (Glime 1982). Clarke and Greene (1970) showed that the reproductive response of *Pohlia nutans* (Figure 40) to day length is dependent upon temperature. In *Leptobryum* (Figure 23), low temperature is necessary for induction of antheridia, but once started they are independent of temperature (Chopra & Rawat 1977). On the other hand, for the thallose liverwort *Ricciella crystallina* (Figure 22), it appears that temperature is the overriding factor, provided there was a certain minimum photoperiod provided (Chopra & Sood 1973a).

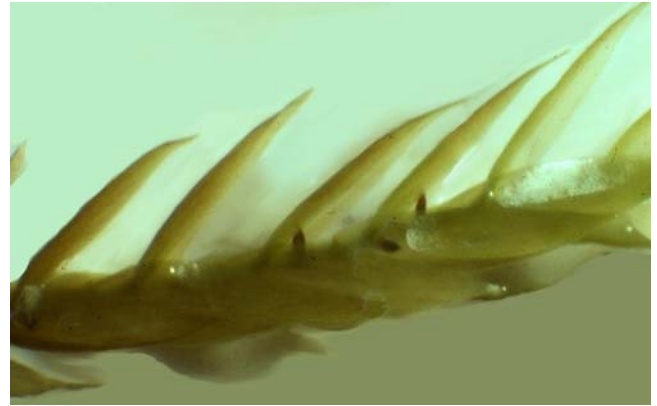


Figure 52. *Fontinalis hypnoides*, a moss that produces maximum gametangia (seen in early development here) at 15°C. Photo by Janice Glime.

Chopra and Bhatla (1983) suggest that bryophytes operate in a range of temperatures, and that responses to light intensity and photoperiod might only operate within a range of temperatures that are broad in some species and narrow in others. Nevertheless, bryophytes do not seem to require any low temperature pretreatment for the induction of gametangia.

Hohe and coworkers (2002) found that both temperature and day length affect the expression of a MADS-box gene in *Physcomitrella patens* (Figure 53). In particular, one gene that was concentrated in the shoot apex and developing sporophytes produced higher RNA under conditions of 15°C, 8 hours light per day, whereas vegetative growth was predominant at 25°C, 16 hours light per day, suggesting that lower temperatures and photoperiod were important in sexual reproduction. This interdependence of temperature and photoperiod is an important way to coordinate gametangial production with the appropriate time for sporophyte development.



Figure 53. *Physcomitrella patens*, a moss that responds to both photoperiod and lower temperatures for gametangial development. Photo by Jan-Peter Frahm, with permission.

Environmental Signalling Interactions

In many cases, perhaps most, the response to photoperiod or temperature or nutrients does not respond to just that one factor. The response is likely to differ in different geographic regions, and this can be the result of selection for a different factor as the trigger, perhaps because one factor cannot be expressed in this

environment. In *Fossombronia brasiliensis* (Figure 46), Chin *et al.* (1987) found that at 18°C the plants were short-day plants, requiring a night length of 6-12 hours. (Short-day plants typically are long night plants, measuring number of hours of darkness). When the temperature was only 10°C, this species became a quantitative short-day plant. But temperature also affected the gender expression, with more male gametangia being produced at 18°C and more female gametangia being produced at 10°C. Furthermore, the type of nitrogen available made a difference, with nitrate nitrogen causing production of more gametangia than did nitrogen in the form of ammonia.

In the dioicous moss *Bryum argenteum* (Figure 44-Figure 45), temperature, light intensity, and photoperiod all play a role in gametangial formation (Chopra & Bhatla 1981b). Both males and females produce the maximum gametangia at 25±2°C and in the light intensity range of 1800-2000 lux. At higher light intensities, vegetative growth occurs instead. If the temperature is lowered to 10±2°C, the response decreases. Chopra and Bhatla consider this species of *Bryum* to be a quantitative day-neutral plant because it is able to produce gametangia in as little as 8 hours of light, increasing production as the day lengthens.

The thallose liverwort *Asterella tenella* (Figure 54) requires the right conditions of both temperature and day length (Bostic 1981). For this species, **archegoniophores** (female reproductive branches) were induced under short days (10 hours) with 15°C daytime and 10°C nighttime temperatures.



Figure 54. *Asterella tenella* with archegoniophores. Gametangia are induced by short days in this species. Photo by Li Zhang, with permission.

Hormones

These physical cues must somehow be translated into biochemical responses. In the fern *Blechnum spicant*, gibberellic acid is known to illicit production of antheridia (Fernandez *et al.* 1997). In flowering plants, it can cause flowering. Since one known function of GA in flowering plants is increased water uptake (Salisbury & Ross 1978), this role might be important in maintaining an adequate internal water supply during gametogenesis of bryophytes.

Induction of **gametogenesis** by gibberellic acid is consistent with the role of GA₃ in increasing alpha-amylase activity, thus facilitating the metabolism of starch to sugar through hydrolysis. We know from the studies on *Marchantia* (Figure 10) (Maravolo 1980) that this starch conversion permits energy-supplying sugars to move to the actively growing regions such as gametangia. This sequel is so consistent with the need for sugar to maintain the sporophyte condition in callus culture (Bauer 1963b) and its requirement for gametophore production (Maravolo 1980), that one is tempted to accept this explanation alone. But how does this relate to photoperiod and temperature? And why do some plants respond to short days and others to long ones? I must conclude, as most flowering plant physiologists have done, that more than one substance is involved. In *Fontinalis dalecarlica* (Figure 33), the quantitative response to short days suggests a two-substance response – one present continuously and one that must accumulate as a function of photoperiod/light (Glime 1984).

Salisbury and Ross (1978) state that high auxin concentrations inhibit flowering and Benson-Evans (1961) found that auxins inhibit development of sexual organs in the thallose liverwort *Conocephalum conicum* (Figure 55). Growth substances such as 2,4-D and NAA induced receptacle formation but not gametangial production. Application of auxin at 16°C caused cell elongation of the archegoniophore, but not production of new cells. Therefore, it seems that gametogenesis might require the suppression of IAA.



Figure 55. *Conocephalum conicum* with antheridia whose development is inhibited by auxins. Photo by Malcolm Storey, through Creative Commons.

IAA seems to have other interesting reproductive functions. For example, in the dioecious hemp, IAA caused predominantly female sex expression (Chailakhyan & Khryanin 1978), but Salisbury and Ross (1978) point out that auxin levels and flowering seldom correlate in any meaningful way. In experiments on the leafy liverwort *Cephalozia lunulifolia* (Figure 56), kinetin + IAA inhibited sexual reproduction (Lockwood 1975). Tremaine and Glime (unpub. data) supplied IAA to *Fontinalis duriaei* (Figure 57) at concentrations of 10⁻⁶ and 10⁻⁸ M on a 12 hr light/12 hr dark cycle and there was no sign of gametangial initiation after 5 weeks. Yet this species usually produces gametangia during short days (personal observations).



Figure 56. *Cephalezia lunulifolia* with perianths (light color) enclosing archegonia. Photo by Michael Lüth, with permission.



Figure 57. *Fontinalis duriaei* archegonia, a species in which they fail to initiate with added IAA. Photo by Janice Glime.

Cytokinins can also play a role in sexual development. In the liverwort *Riccia discolor*, 10^{-4} M kinetin proved to be the best concentration for promoting archegonial development as well as enhancing growth (Chopra & Gupta 1992).

Hormones may not affect the antheridial and archegonial inductions equally, possibly explaining how bryophytes manage to begin antheridial development long before archegonial development in most species. Chopra and Bhatla (1983) demonstrated that gibberellins contribute to the stimulation of antheridial formation in the bryophytes they investigated, whereas cytokinins stimulate archegonial induction while inhibiting antheridial induction in *Ricciella crystallina* (Figure 22) and *Bryum argenteum* (Figure 44-Figure 45). They found that auxins, gibberellins, and cytokinins can interact in controlling the gametangial response – no surprise there.

The hormone **IAA** may likewise have the opposite effects on the two sexes (Chopra & Bhatla 1983). In the thallose liverwort *Ricciella crystallina* (Figure 22), IAA increased archegonial induction, but in the mosses tested [*Bryum coronatum* (Figure 3), *B. argenteum* (Figure 44-Figure 45), *Barbula indica* var. *gregaria* (Figure 2)], it favored antheridial induction.

Bhatla and Chopra (1981; Chopra & Bhatla 1981a) examined hormonal regulation of gametangial induction in *Bryum argenteum* (Figure 44-Figure 45) and found that both IAA and gibberellins (GA3) increase the induction of male gametangial branches while inhibiting the female clones in this dioicous moss. Cytokinins (kinetin, DMAAP) increased gametangial induction in the female

clone while slightly inhibiting it in the male clone. When IAA and kinetin were both present, they were able to nullify the inhibitory capacity of each other. Cyclic AMP prevented kinetin from inhibiting male gametangial induction but stimulated the kinetin effect on females. ABA served as an inhibitor of both growth and gametangial induction in both sexes. Females proved to be more sensitive to ABA than males.

Cyclic AMP is one factor that may help in the control of hormone action and hence in controlling gametangial formation (Chopra & Bhatla 1983). This compound is a common mediator of hormone action in animals and is now known to increase gametangial induction in the moss *Bryum argenteum* (Figure 44-Figure 45). Cyclic AMP also increases antheridial induction in *Bryum coronatum* (Figure 3) and *Barbula indica* var. *gregaria* (Figure 2). To further confuse the investigator, it can overcome the inhibitory effects of ammonium ions and concentrations of sucrose that are too high, hence increasing gametangial formation, as Chopra and Bhatla have shown in *Bryum argenteum*.

Environmental Hormone Interactions

Interactions with the environment can supply bryophytes with hormones, such as yeast extract and sex hormones from animals (Chopra & Bhatla 1983). These can increase the induction of both antheridia and archegonia.

Basile *et al.* (1969) found that the leafy liverwort *Scapania nemorea* (Figure 58) regularly associates with the bacterium *Pseudomonas estorquens*. This association provides it with stimulation for both larger growth and earlier reproductive maturity than sterile cultures.



Figure 58. *Scapania nemorea*, a liverwort that associates with *Pseudomonas estorquens* that stimulates earlier reproductive maturity. Photo by Li Zhang, with permission.

Sugars

Chopra and Rhabar (1982) found that sugar (1%) was necessary for gametangial induction in *Bartramidula bartramoides*. On the other hand, *Bryum argenteum* (Figure 44-Figure 45) has markedly lower gametangial induction in 4% sucrose (Bhatla & Chopra 1979). Adding cyclic 3',5'-AMP neutralized the effects of the sucrose, but the concentrations are different for male (10^{-7}) and female (10^{-5}).

Overall Physiology

In summary, metabolic changes are needed for the initiation of gametangia (Chopra & Bhatla 1983). Liverworts may have an increase in cellular levels of carbohydrates, auxins, RNA, and proteins as the gametangial development begins. Enzymes and their concentrations change. Phenolic compounds change. And new colors develop. Reynolds and Maravolo (1973) found that two of the phenolic compounds inhibited IAA oxidase activity and two enhanced it in *Marchantia polymorpha* (Figure 10). The significance of this interaction in gametangial development seems still to be a mystery.

Both vegetative growth and gametangial development are regulated by and favored by iron and copper chelating agents such as EDTA and EDDHA (Chopra & Bhatla 1983). But it is interesting that in *Riccia* (Figure 24) these chelates favor archegonial development more than antheridial formation, whereas the opposite is true in *Bryum argenteum* (Figure 44-Figure 45) (Chopra & Bhatla 1983). Salicylic acid (the effective compound in aspirin) inhibits gametangial formation in most bryophytes, probably by chelating iron and copper or other metals involved in needed enzymes. We know that in *Bryum argenteum* there are marked changes in iron and copper levels. Iron seems to induce the reproductive phase, but copper inhibits it. In *Bartramidula bartramoides*, on the other hand, salicylic acid enhances both vegetative growth and gametangial formation.

Cyclic AMP enhances antheridial production in the moss *Bryum coronatum* (Figure 3) and *Barbula indica* var. *gregaria* (Figure 2) and overcomes the inhibitory effects of ammonium ions and high levels of sucrose on gametangial development in *Bryum argenteum* (Figure 44-Figure 45) (Chopra & Bhatla 1983).

In an attempt to understand the physiological changes leading to development of gametangia in liverworts, Rao and Das (1968) studied *Exormotheca tuberifera*, *Plagiochasma articulatum*, *Reboulia hemisphaerica* (Figure 59), *Fimbriaria angustata*, and *Pallavicinia canara*. In *Fimbriaria angustata*, a sharp rise in respiration and a doubling of the C:N ratio accompanied the transition from vegetative to reproductive state in females. Formation of archegoniophores occurred with an increase in the plant's own IAA, RNA, and protein. Carbohydrates accumulated in the archegoniophore at the expense of the gametophyte as the sporangia developed. By contrast, the antheridial production was correlated with a decrease in levels of IAA, RNA, and protein, and unlike the females, there was no notable increase in the C:N ratio.

Color Changes

Both antheridia and archegonia are often recognizable first by the addition of red coloration as they develop. In archegonia, this is often present in the neck canal cells (Figure 33, Figure 57). In antheridia, the color can be so intense that it is visible through the surrounding leaves, making branch tips red in some species of *Sphagnum* (Figure 60). In *Marchantia berteroana* (Figure 61), production of the flavone acacetin stops and instead 8-hydroxyapigenin and 8-hydroxyluteolin glycosiduronic acids (previously absent) become the predominant

flavonoids (Markham *et al.* 1978). Acacetin seems instead to be important during the asexual phase.



Figure 59. *Reboulia hemisphaerica* male & female gametangiophores. Photo by Bob Klips, with permission.



Figure 60. *Sphagnum* with red antheridial branches. Photo by Janice Glime.



Figure 61. *Marchantia berteroana* antheridial heads showing red color. Photo by Clive Shirley, Hidden Forest, with permission.

Delay of Gametogenesis

But suppose that gametogenesis is **not** a process to be initiated, but rather it is a natural process that **must be stopped**. Sexual reproduction is ancient. It no doubt began with like cells bumping into each other and managing to stay together long enough to fuse. No special

male and female existed; no special inducers were needed. Perhaps something was needed to cause the two membranes to lose their integrity at the region of contact. Then the process became more sophisticated. Attracting substances drew cells together; different strains arose, some repelling and others attracting. Ultimately, special structures housed these one-celled gametes, and then some control was possible. As this scenario continued, the process became more complex and more controlled. The joining and dividing cycle of primitive cells was then subject to controlled delays. Whole sequences of differentiation were interjected to delay the sexual process. These sequences are the ramifications by which we identify species, genera, even phyla of plants. Therefore, it is reasonable that gametogenesis is controlled by inhibitors, factors of the surrounding tissues that retard gamete production and allow productivity of the organism to increase.

It follows that the multitudinous environments for the many species have caused this problem to be solved in multitudinous ways (see Stebbins & Hill 1980). Thus in one species a high concentration of IAA prevents gametogenesis, whereas in another the lack of alpha-amylase or GA deprives the prospective gametangia of the necessary energy source. As long as the raw ingredients (*e.g.* energy, nitrates, amino acids) are being diverted to other sources, gametogenesis is retarded. Such a multitude of ways can accomplish this that surely no consistent pattern could be recognized or even expected. The possibilities of combinations of concentrations and mobilities necessary to override the limits caused by the parent plant are almost limitless.

Male vs. Female

It is often considered a paradox that bryophytes tend to have female-biased sex ratios, whereas flowering plants usually have male biased sex ratios (Rydgren *et al.* 2010). Early control over gender was most likely simple. Internal environment may have been important. For example, Bhandari and Lal (1968) observed abnormal archegonia in *Physcomitrium immersum* that behaved as antheridia. Each had an egg, ventral canal cell, and neck canal cells as would be found in a normal archegonium, but in some these divided repeatedly, forming instead a mass of antheridial cells. They suggested that this is evidence of common origin of the two sexual organs.

Such behavior is somewhat suggestive of sex determination in maple (*Acer*) flowers. In these plants, the concentration of plants affects the ethylene concentration as the flower develops and determines the sex ratio by abortion of one of the parts. Factors related to sex ratio in bryophytes have been discussed in the chapter on sexuality. Therefore, they will be covered only briefly here.

We have noted that bryophytes, or at least many of them, do have sex chromosomes, a phenomenon known for plants first in the liverwort genus *Sphaerocarpos* (Figure 62) (Allen 1930; Anderson 2000). The gender is expressed only in the gametophyte generation by having either a small Y chromosome (male) or an X chromosome (female). This determination is made at meiosis, providing two male and two female spores. The monoicous (bisexual) taxa seem to have been derived mostly from polyploidy in

which the chromosome number is duplicated and both X and Y chromosomes are present.



Figure 62. *Sphaerocarpos michelii*, member of the genus where X and Y sex chromosomes were first discovered. Photo by Jan-Peter Frahm, with permission.

When the sexes are separate, *i.e.* dioicous/unisexual taxa, it is not unusual to find all male or all female populations, derived from a single spore carrying genes for only one gender. In other cases, one gender may outcompete and overgrow the other. Such is the case with *Marchantia papillata* subsp. *inflexa* (Figure 63), a dioicous thallose liverwort that lives on rock and bark surfaces (McLetchie *et al.* 2001). In this case, the females seemed to benefit from light to moderate disturbance and gradually eliminated the males. However, at high disturbance levels, the males dominated. This change in dominance seemed to result from dispersal of gemmae within the patch. We have seen in the brood body chapter that females typically produce fewer gemmae, instead spending energy to support the female reproductive organs and developing sporophyte.



Figure 63. *Marchantia papillata* subsp. *inflexa*, a species in which females can outcompete males in disturbed areas. Photo by Scott Zona, with permission.

McLetchie *et al.* (2001) found that in *Marchantia papillata* subsp. *inflexa* (Figure 63) spores were needed to colonize large areas following disturbance, and that sexual reproduction predominated. However, as the population grew and the space became fully occupied, reproductive effort shifted to less sexual and more asexual means. Does this strategy predominate? It would seem more advantageous to reproduce asexually to fill the area, then reproduce by more widely dispersed spores when it gets crowded.

Maintaining the sexual specificity can get complicated in regenerants. Bauer (1963a) explained that sex determination in regenerated tissue can take two forms:

1. Sex determination is restored following de-differentiation, as in *Funaria hygrometrica* (Figure 18-Figure 19).
2. Sex determination is disturbed, causing the sexual balance to remain permanent or to slowly return to normal, as seen in members of *Splachnaceae* (Figure 64).

In the *Splachnaceae*, as the tendency toward femaleness is weakened, the male expression becomes more common until eventually only male plants can arise (Bauer 1963a). Surprisingly, this can occur even in species such as *Splachnum rubrum* (Figure 64) wherein sex determination is genetic. This species produces dwarf males, but these are usually sterile. The change in gender from vegetative offspring could be from cytoplasmic or genetic changes. However, Bauer reasoned that the constant changes among intermediate kinds of sex determination provides evidence against gene mutation.

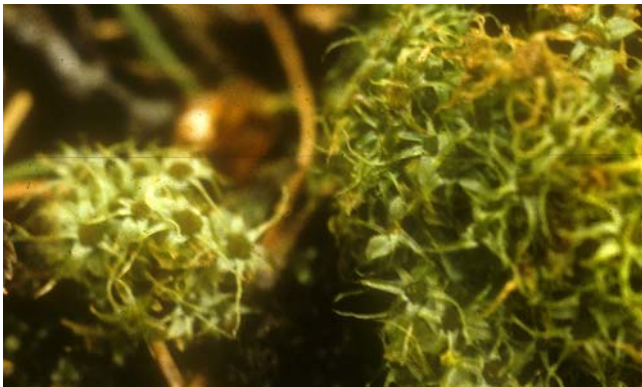


Figure 64. *Splachnum rubrum* with antheridial splash platforms, a species where gender is genetically determined. Photo by Janice Glime.

Rydgren *et al.* (2010) explored the ability of maintaining a female-biased sex ratio by testing it in *Hylocomium splendens* (Figure 68), a dioicous pleurocarpous moss that is common on the boreal forest floor. They found that males had a slightly lower production and survival of vegetative offspring than did the non-sporophytic females. This bias is important in a species such as this where sporophytes are uncommon. The slightly better success of males permitted them to expand into female clones, thus facilitating reproduction.

Differential Survival

Not all sex ratio differences are the result of adult competition. Shaw and Gaughan (1993) studied eleven populations of the moss *Ceratodon purpureus* (Figure 65) and found that at the time of germination female gametophytes outnumbered males 3:2, suggesting differential survival rates of spores or germlings. Furthermore, female clones formed much more biomass than did male clones, further increasing the bias. Nevertheless, male clones produced more stems, permitting them to provide additional gametangia and sperm.



Figure 65. Prolific production of capsules exhibited by *Ceratodon purpureus*, suggesting a predominance of females. Photo by Michael Lüth, with permission.

Sex ratio can often change dependent upon growing conditions, even in species where gender of an individual is genetically predetermined. Shaw and Beer (1999) observed that despite chromosomal sex determination in *Ceratodon purpureus* (Figure 65) that would produce equal numbers of male and female cells at meiosis, the sex ratio varied considerably among families of offspring. Some genetically identical individuals (*i.e.*, grown from a single spore) that maintained a nearly 1:1 gender ratio had progeny that produced either predominately male or predominately female offspring.

This discrepancy between offspring sex ratios of two families of siblings suggests that there is a differential germination of spores, most likely related to environmental factors. Additional factors that may be relevant are the differences in size, maturation rates, and reproductive output of the male and female gametophytes in this species.

One factor that can account for highly biased sex ratios is simply the gender of the spore that lands there. Generally, one spore will produce multiple gametophores of one gender. However, Cameron and Wyatt (1990) rejected this as an explanation of the highly biased sex ratio in *Splachnum*. They concluded that the unbiased and abundant dispersal by flies precluded such a bias by ensuring that both genders would arrive on the substrate. But even more interesting is the fact that in *Splachnum ampullaceum* (Figure 66), a single spore can give rise to both male and female gametophores. Instead, it is low light, pH, and nutrients that favor production of males over females.



Figure 66. Massive number of capsules of the dung moss *Splachnum ampullaceum* resulting from the guaranteed close proximity of males. Photo by Michael Lüth, with permission.

There is some evidence that at least in some bryophytes gender may be determined like that of crocodile eggs – by temperature. For the liverwort *Sphaerocarpos texanus* (Figure 67), sex ratios showed female bias among spores that broke dormancy after treatment at 25/15°C for 1-8 weeks (McLetchie 2001), despite a 1:1 ratio of male:female among spores produced (McLetchie 1992). In both field and laboratory-grown cultures, pure female clones were most common, followed by mixed sex, and least frequently, pure male (McLetchie 1992). It appears that the male spore has a lower survival and germination rate that continues into the gametophyte stage.

There seems also to be a physiological gender bias that depends in part on ecological conditions. In *Mnium hornum* (Figure 77) and *Plagiomnium undulatum* (Figure 76), only female regenerants from fragments survived desiccation (77%) (Newton 1972b). Such a strategy could soon create a population of predominantly females.

McLetchie and coworkers (2001) demonstrated that competitive interactions between genders could account for some sex differences at gametophyte maturity in the dioicous thallose liverwort *Marchantia papillata* subsp. *inflexa* (Figure 63) in Trinidad. Using differential equations, they modelled interactions of the two genders under various disturbance regimes. They found no way to stabilize the sex ratio, but rather, under conditions of low to moderate disturbance, females would gradually eliminate males. Under high disturbance conditions, males would eliminate females. Successful germination of gemmae dispersed within the patch played an important role. Since females of this species have only a narrow window in which to produce gemmae without interfering with energy needed for sexual reproduction, they would have less opportunity for successful gemma dispersal and establishment under large disturbance, but under conditions of small disturbance, already established female thalli might be able to outgrow male thalli. Although gemmae appear to be the most important means of maintaining replacement due to disturbances within patches, spores are the primary means for colonizing areas of major disturbances. Production of spores among initial colonizers when the patch becomes fully occupied is maximal, but that production subsequently declines as the sex ratio drifts toward one or the other gender.

Additional information on the costs and tradeoffs of producing archegonia vs antheridia is covered in Chapter 3 of this volume.

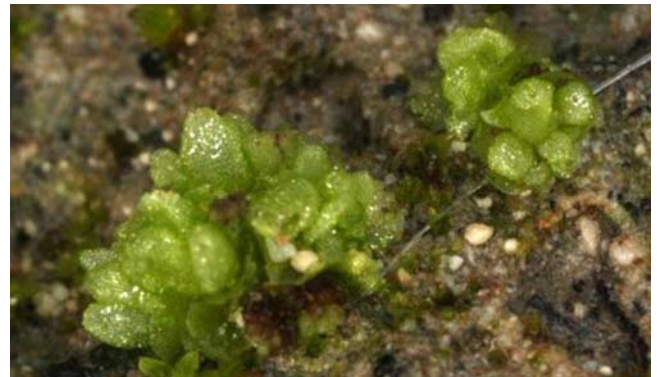


Figure 67. *Sphaerocarpos texanus*, a species in which females seem to outcompete males. Photo by Jan-Peter Frahm, with permission.

Bisexual Gametangial Differentiation

Differentiation of a single cell such as a spore ultimately into an organism with cells of many functional types is always intriguing. Differentiation of parts of an organism into male and other parts into female is no less intriguing. What determines which branch, which gametangial cluster, will become male and which female? If we can understand these processes in plants, perhaps we can begin to understand the complexities that contribute to cross-gender behavior in humans.

Using the dioicous *Hylocomium splendens* (Figure 68) as a model, Rydgren and Økland (2002) examined a Norwegian population for five years. During that time, the tissue devoted to gametangia differed. That population had a 4:1 ratio of male to female branches. Of those females, ~30% produced sporophytes. Production of sporophytes varied three-fold during the five years, relating to weather favorability for growth and development. Large segments with high relative growth rates were more likely to produce sporophytes, with a distinct lower size threshold. Although the size limit increased in years with low sporophyte production, the lowest limit was ~2 mg segment dry weight. Furthermore, production of sporophytes was much greater in upper parts of plants, regardless of size differences, suggesting a role for light in initiation of sexual branches.



Figure 68. *Hylocomium splendens* bearing sporophytes. Photo by Janice Glime.

Hormonal Regulation of Gender

In some trees, such as *Acer*, ethylene concentration affects the male:female ratio. But in the small space of a bryophyte mat, could such a high concentration accumulate? There seems to be no evidence that packing of gametophytes, hence higher ethylene production, is a sex determinant. Nevertheless, lab evidence demonstrates that ethylene control is a possibility. Location of sexual structures on the bryophyte could result from a balance among IAA, ethylene, and GA₃. Chopra and Sood (1973b) showed that GA₃ plus ethrel (which produces ethylene in water) enhanced antheridia production, whereas IAA + cyclocel (CCC) enhanced archegonia production in *Ricciella crystallina* (Figure 22). This is consistent with the role of IAA in favoring femaleness in flowers (Salisbury & Ross 1978). If this relationship holds true, a strong apical dominance, concomitant with apical production of IAA, should produce archegonia at the apex. This is exactly the correlation seen in acrocarpous mosses. Conversely, lack of apical dominance should result in archegonia on side branches, as we see in pleurocarpous mosses. However, Schofield (1985) reminds us that IAA is not involved in sex determination in the same way in all taxa, inducing female sex organs in the liverwort *Riccia* (Figure 24) and male organs in the mosses *Barbula* (Figure 2) and *Bryum* (Figure 45). Because it is common in the environment, IAA could serve as an environmental control, interfering with sexual coordination and hence sporophyte production for some taxa in some habitats. It is likely that hormones interact and that concentrations or relative concentrations are important in gender determination.

Dwarf Males

Dwarf males present an interesting modification to sexual differentiation. In theory, the presence of dwarf males should increase the success of fertilization for a species, particularly among dioicous taxa. However, in two habitats in Michigan, USA, the presence of dwarf males had no significant impact on sporophyte production of dioicous mosses (Rohrer 1982). Dwarf males have been discussed in detail in Chapter 3; this chapter will concentrate on physiological relationships.

In the moss *Trachybryum megaptilum* (= *Homalothecium megaptilum*; Figure 69), males are typically dwarf, but this is a function of being on a female plant (Wallace 1970). Occasional full-sized males are found growing alone, but dwarf males never occur on these full-size males. Despite differences in gametophore appearance, there is no morphological difference between male and female spores. Wallace suggested that some substance released from the female plant might inhibit growth of the male plant.

In *Dicranum* (Figure 70), it appears that female plants present a growth-inhibiting substance that keeps their epiphytic males small (Loveland 1956). On the other hand, in *Macromitrium* (Figure 71) it is genetically determined in those taxa that are truly **anisoporous** (having a bimodal distribution of spore sizes with smaller spores generally producing males), whereas isoporous taxa again seem to be affected by hormones from females (Une 1985). Auxin, applied as 2,4-d, results in dwarf males, suggesting again a role for IAA.



Figure 69. *Trachybryum megaptilum*, where dwarf males form on female plants. Photo through Creative Commons.

Another puzzle that has physiological implications suggesting hormonal concentration gradients is development of morphs among gametangia of a single reproductive head. In *Plagiomnium medium* (Figure 72), antheridia typically surround archegonia. In the border zone between the two sexes, Bryan (1927) always found at least one abnormal gametangium in each of the 100's of heads examined, from nearly perfect to possessing a combination of antheridial and archegonial cells. This likewise suggests some sort of hormonal control that involves concentrations or interaction – or both.

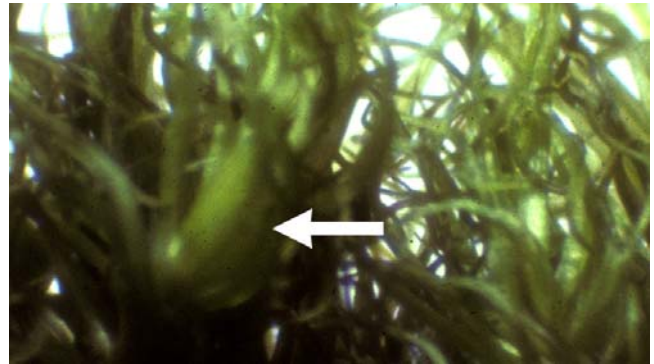


Figure 70. Dwarf male (arrow) of *Dicranum polysetum* growing on a female plant. Photo by Janice Glime.



Figure 71. *Macromitrium piliferum* with capsule, an autoicous moss in a genus where isoporous spores may form dwarf males in the presence of auxin. Photo by Jan-Peter Frahm, with permission.



Figure 72. *Plagiomnium medium*, a moss in which antheridia usually surround the archegonia. Photo by Jan-Peter Frahm, with permission.

Different Controls

One consequence of sexual differences is that antheridia and archegonia can be under different controls. This can result in maturation of males and females at different times, perhaps accounting for sterility in many populations. Allsopp (1964) suggested that nutritional factors cause male and female production at different times on monoicous species. Lockwood (1975) found that amino acid additives promoted maleness and inhibited femaleness in *Cephalozia lunulifolia* (Figure 56); ammonium nitrate plus citrate also inhibited female gametangia. Machlis (1962) found that males of *Sphaerocarpos donnellii* (Figure 73) dropped the pH of their media from 5.3 and 7.1 to 4.1 in 15 days, whereas females raised the pH, suggesting physiological and possibly nutritional differences. Riemann (1972) suggested that mild, humid winters may result in maturation of the male and female of *Rhytidiadelphus triquetrus* (Figure 74) at different times, whereas harsh winters regulate their timing. Berthier (1966) has shown that antheridial production in *Fontinalis* (Figure 75) is greater under conditions of minimal growth and greater dominance by the main axis; fewer antheridia occurred in high light at 15°C, whereas 8°C and 90% light produced the most antheridia. It is likely that a wide variety of these mechanisms play a role in **protandry** (male gametangia mature first) and **protogynandry** (female gametangia mature first) among bryophyte species.



Figure 73. *Sphaerocarpos* sp. *Sphaerocarpos donnellii* can lower the pH of its medium to 4.1. Photo by Belinda, through Creative Commons.



Figure 74. *Rhytidiadelphus triquetrus*, a moss that may have males and females mature at different times when winters are mild but mature together when they are harsh. Photo by Janice Glime.

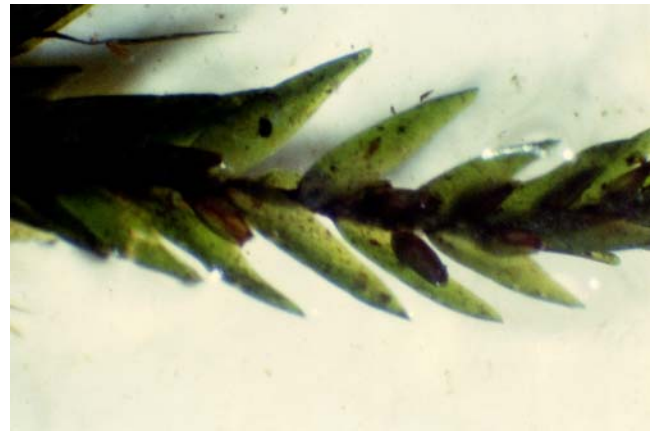


Figure 75. *Fontinalis antipyretica* var *gigantea* with perigonia (male). Antheridia are produced during times of minimal growth. Photo by Janice Glime.

One selective factor at work is that antheridia generally require a longer time to mature than do archegonia, as discussed earlier regarding phenology. Therefore, they must begin development sooner to mature when the population of female structures is receptive. *Plagiomnium undulatum* (Figure 76) has met this challenge with very different signals to initiate gametangia. Males require long days and diurnal temperature fluctuations to produce gametangia, whereas females require a short 7.25- to 12-hour day at 10°C or warmer (Newton 1972a). We have already seen that more male gametangia are produced by the liverwort *Fossombronia brasiliensis* at 18°C and more female gametangia at 10°C. These differences in temperature and/or photoperiod can permit male gametangia to start development sooner and be ready when female gametangia are ready. But such timing differences meet new challenges when spores are dispersed to new locations where timing of day length and temperature can be different from that of the parent location, so that males and females are no longer synchronized. If only vegetative reproduction follows after introduction by a single spore, no opportunity arises for selection of synchronized variants; instead the species may persist sterile for centuries.



Figure 76. Vegetative branches of *Plagiomnium undulatum*. Photo by Michael Lüth, with permission.

Newton (1972a, b) found that male and female plants themselves differed in photoperiod response in *Mnium hornum* (Figure 77). In short days, the number of males and females was about equal, but in intermediate and long days significantly more female plants arose from buds and produced mature plants. Even regeneration favored females. Thus, in northern areas where sufficiently warm temperatures may occur only during long days, a disproportionate number of females can result. This shifts the population toward dependence on regeneration, due to insufficient fertilization, further promoting females. Similarly, Longton and Greene (1969a,b) found that *Pleurozium schreberi* (Figure 50-Figure 51) produced capsules predominantly in coniferous forests due to lack of males elsewhere.



Figure 77. Male plants of *Mnium hornum*, exhibiting a splash platform. Photo by Michael Lüth, with permission.

The problem of separate stimuli for the two sexes is further complicated by non-sex-related selection pressures after dispersal. Van Zanten and Pocs (1981) concluded that monoicous species of Jungermanniidae have better dispersal than dioicous species because the percentage of monoicous species with a bipolar distribution is greater than that of dioicous species. This is reasonable since production of spores is more likely to occur in monoicous taxa, and these are dispersed more easily than asexual propagules due to the smaller size of spores. Since selection pressures related to sporophyte development are absent in isolated dioicous individuals, selection pressures would revert to gametophyte benefits. These could easily be different in male and female plants because of different amounts of time required for development of male and female gametangia.

Day length and temperature influence the onset of gametogenesis, and we have good evidence that responses to these variables vary within a species throughout the world (Monroe 1965, Clarke & Greene 1970). In dioicous species, vegetative reproduction is the only mechanism for reproduction until a second spore arrives. By that time environmental selection pressures and genetic drift in these small populations have had ample time to make the two sexes uncoordinated. If the signal for gametogenesis is different in the two sexes, there might never be an opportunity for the two gametes to meet. I would predict what van Zanten and Pocs (1981) have already illustrated, that long range dispersal of dioicous species would result in a large number of physiological species with low or no sexual reproduction.

All of these controlling factors suggest that Dan Norris may have been right in his comments to Bryonet on 2 May 2003 – the conditions of monoicy and dioicy and all their subsets may not be as distinct as we present them. The expression of gender may be under control of the environment and not any predetermined genetic distinction.

Numbers of Gametangia

Although each female branch typically produces only one sporophyte, archegonia occur in clusters within **perigonia**. One might ask why all this wasted energy to produce multiple archegonia if only one is successful. Even if all get fertilized, only one embryo succeeds in emerging from its archegonium. Could it be that multiple archegonia are needed to produce sufficient attractant for the sperm to find the location? Or might there be dangers lurking as sperm enter the archegonia, making backups necessary? Have we examined them closely enough to know that all eggs are simultaneously receptive, or might it be that this is a way to insure that one of the eggs is ready at the time of successful sperm dispersal?

The male gametangia generally outnumber female gametangia, but not always (Table 1). Since males must disperse the sperm, with nearly all of them being unsuccessful in fertilizing an egg, large numbers are necessary to provide enough chances for a few to succeed. Note in Table 1 that the ratio of male to female gametangia is considerably higher in the dioicous taxa.

Table 1. Mean number of gametangia per inflorescence, based on data for inflorescences that had gametangia in immature to dehiscent stages. From Une & Tateishi (1996).

<i>Physcomitrella patens</i>	$\frac{\text{♂}}{\text{♀}}$	2.0	Paroicous	Une & Tateishi 1996
subsp. <i>californica</i>	$\frac{\text{♂}}{\text{♀}}$	7.2		
<i>Astomum crispum</i>	$\frac{\text{♂}}{\text{♀}}$	3.3	Autoicous	Deguchi & Hidaka 1987
	$\frac{\text{♂}}{\text{♀}}$	14.1		
<i>Aulacopilum japonicum</i>	$\frac{\text{♂}}{\text{♀}}$	2.2	Autoicous	Deguchi & Hidaka 1987
	$\frac{\text{♂}}{\text{♀}}$	3.3		
<i>Venturiella sinensis</i>	$\frac{\text{♂}}{\text{♀}}$	3.6	Autoicous	Deguchi & Hidaka 1987
	$\frac{\text{♂}}{\text{♀}}$	5.1		
<i>Fabronia matsumurae</i>	$\frac{\text{♂}}{\text{♀}}$	2.7	Autoicous	Deguchi & Hidaka 1987
	$\frac{\text{♂}}{\text{♀}}$	5.8		
<i>Entodon challengerii</i>	$\frac{\text{♂}}{\text{♀}}$	5.5	Autoicous	Deguchi & Hidaka 1987
	$\frac{\text{♂}}{\text{♀}}$	8.0		
<i>Pogonatum inflexum</i>	$\frac{\text{♂}}{\text{♀}}$	3.4	Dioicous	Imura 1994
	$\frac{\text{♂}}{\text{♀}}$	64.4		
<i>Atrichum rhystophyllum</i>	$\frac{\text{♂}}{\text{♀}}$	4.6	Dioicous	Imura 1994
	$\frac{\text{♂}}{\text{♀}}$	76.4		
<i>Trachycystis microphylla</i>	$\frac{\text{♂}}{\text{♀}}$	9.8	Dioicous	Imura & Iwatsuki 1989
	$\frac{\text{♂}}{\text{♀}}$	43.1		
<i>Bryum argenteum</i>	$\frac{\text{♂}}{\text{♀}}$	5.5	Dioicous	Miles <i>et al.</i> 1989
	$\frac{\text{♂}}{\text{♀}}$	10.6		
<i>Pleurozium schreberi</i>	$\frac{\text{♂}}{\text{♀}}$	8.2	Dioicous	Longton & Greene 1969a
	$\frac{\text{♂}}{\text{♀}}$	6.1		

In the survey of literature presented by Une and Tateishi (1996), *Pleurozium schreberi* (Figure 50-Figure 51) had more female than male gametangia per inflorescence, and *Bryum argenteum* (Figure 44-Figure 45) had little difference between them. Perhaps this is possible because these species are so successful at vegetative reproduction. In Canada, large geographic areas have only one gender of *Pleurozium schreberi*, yet the species is still quite successful. *Bryum argenteum* is easily spread by broken tips.

Gender Recognition

Recognizing the gender of a bryophyte is often difficult if reproductive structures are absent. For mosses like *Polytrichum*, old splash cups may be present, with new growth proceeding from the center (Figure 78). But even these can eventually change sex and thus determination of the sex of the moment may be less convincing. Size often plays a role, but this is affected by growing conditions as well, so one must assess it for each population. In *Marchantia polymorpha* (Figure 10), the male plants are narrow compared to females if one examines the thallus ~1 cm back from the tip, but then one needs both genders at hand to make the assessment (Voth 1941). Voth has observed another difference that I have not confirmed – the female plants have a smoother upper surface and reflect more light than male plants, at least in culture, but again, one really needs the male plants for comparison.



Figure 78. *Polytrichum juniperinum* with new growth from the antheridial splash cups. Photo by Janice Glime.

Yet, somehow, through biochemical means, a sperm is able to recognize a female of its own species, be it on a separate plant or the same one, and travel in that direction. As discussed in the chapters on life cycles of bryophytes, this recognition is facilitated by a concentration gradient from the disintegrated neck canal cells of the archegonium. But the nature of that exudate, and particularly what makes it specific for that species, remains a mystery.

Fertilization

Success of fertilization varies widely from very successful monoicous annual taxa to poorly successful dioicous perennials (Rohrer 1982). Rohrer found that success varied by habitat, with only 19.3% of the populations of the dioicous, vs 75.9% of monoicous taxa

producing sporophytes in a dry aspen (*Populus*) forest. In a wet coniferous forest, the success of monoicous taxa increased to 84.1%, whereas that of dioicous taxa decreased to 12.3%. Surprisingly, having dwarf males epiphytic on female plants did not significantly increase the production of sporophytes in dioicous taxa.

Although several archegonia are typically present on a branch or stem tip, in most species only one sporophyte develops. Stark and Castetter examined the archegonia of *Trichostomum planifolium* (= *T. perligulatum*) at the end of the fertilization season and found that 8% of the archegonia and 7% of the antheridia were abortive. In 13 of the 47 fertilized perichaetia they examined, there was at least one aborted embryo in addition to the developing embryo. Only two had more than two fertilized archegonia. There were no cases where more than one embryo developed. The abortions were all in early developmental stages. Hughes (1979) found that in *Phascum cuspidatum* (Figure 37) archegonial initiation ceases when one of the archegonia has been fertilized. The archegonial abortion raises the question of causes of this abortion. Is there an inhibitory substance produced by the first developing embryo that stops the others? Is there insufficient energy for more than one to continue? Could the hybrid status enter into the success or failure?

A more in depth discussion of fertilization is in Chapter 3.

Self-incompatibility

Fertilization is the termination of the gametogenesis development phase. Successful fertilization must be followed by successful development of the embryo to the mature sporophyte. We know that seed plants have a variety of mechanisms that prevent self-fertilization, either as prezygotic mechanisms that prevent the sperm from reaching and penetrating the egg or from postzygotic mechanisms that interfere with development of the embryo or mature sporophyte. This self-incompatibility has barely been explored in bryophytes.

We have suggestive evidence that self-compatibility exists among bryophytes. Boisselier-Dubayle *et al.* (1996) found the monoicous leafy liverwort *Plagiochasma rupestre* (Figure 79) to be self-compatible based isozyme markers of progeny. Lazarenko and Lesniak (1972) cultured two species of *Desmatodon* to determine their self-compatibility. *Desmatodon cernuus* was sterile in 24 hours of light, being a long-day plant at 16 hours of illumination and requiring low temperatures in the dark for normal sporophyte development. On the other hand, *Desmatodon ucrainicus* was completely self compatible in 24 hours of light, successfully producing sporophytes in single-spore cultures. However, this study raises a caution. One must reproduce the conditions of gametangial development, fertilization, and sporophyte development to test self-compatibility or other conditions involving reproduction.

When a spore travels to a new geographic area, it can encounter changes to the environmental signals needed for its normal development. Failing these signals, the reproductive state might never be initiated. Absence of such developmental signals seems to interfere with sexual reproduction in *Desmatodon cernuus* (Lazarenko & Lesniak 1972).

Jesson *et al.* (2011) considered that both polyploidy and monoicism could strongly depress inbreeding. They tested this in 21 populations of *Atrichum undulatum* (Figure 80). In one population, using allozyme markers, they found that the rates of selfing were greater than zero, despite the population having only one-third monoicous individuals. Lazarenko (1974) found that an inbred clone of *Tortula cernua* (= *Desmatodon randii*; Figure 81) was able to persist through 15 generations. This clone also gave rise to a sterile line that thus forth reproduced vegetatively, but also by producing apogamous capsules through 14 generations because the few spores, despite lacking an exosporium, were able to germinate. These studies suggest that self-incompatibility is not strong among bryophytes and that self-fertilization is possible.



Figure 79. *Plagiochasma rupestre*, a self-compatible monoicous liverwort. Photo by Michael Lüth, with permission.



Figure 80. *Atrichum undulatum* males with splash cups and antheridia. This is a long-day plant. Photo by Janice Glime.



Figure 81. *Tortula cernua* with capsules, a species that can survive 15 generations of inbreeding. Photo by Lars Hedenäs, with permission.

Stark (1983) reported that the autoicous *Entodon cladorrhizans* (Figure 82) was self-fertile and protandrous on a given stem. He found that approximately 90% of the perichaetia developed sporophytes and that this was independent of the number of perichaetia per stem, attesting to a high success rate for fertilization. Since only one archegonium typically develops a mature sporophyte in any given perichaetium, this is a good percentage. Self-fertilization is evidenced by significantly higher frequency of fertilization on bisexual stems than on those with only perichaetia, by the tendency for unfertilized perichaetia to be near the end of the stem away from perigonia, and by the highest fertilizations occurring on stems with perigonia.



Figure 82. *Entodon cladorrhizans*, an autoicous moss with abundant sporophytes. Photo by Bob Klips, with permission.

Trichostomum planifolium is a protogynous monoicous desert moss, but it has a period of gametangial overlap, ending with a period of only ripe male gametangia

(Stark & Castetter 1995). Based on their observations of the population in southern New Mexico, USA, Stark and Castetter concluded that this moss is self-compatible, with common occurrences of fertilization from gametangia on the same stem. They supported this conclusion by the fact that stems that lacked a sporophyte had fewer antheridia and had no perigonia ($n=3$) and that all stems that produced sporophytes had at least one perigonium. The evidence is circumstantial and not definitive, but does suggest self-compatibility.

Zieliński (1986) used two peroxidase alleles to indicate presence of self-fertilization. He found that 38 of the 40 progeny examined in *Pellia epiphylla* (Figure 83) subsp. *borealis* were monomorphic for one of the two alleles involved and interpreted this to mean that self-fertilization had occurred. But we really need to know more than just the constancy of two alleles. Logic would suggest that in many cases the heterozygosity resulting from cross-fertilization would make those individuals more fit, consequently selecting against those individuals lacking a mechanism to prevent self-fertilization. But does this exist among bryophytes?



Figure 83. *Pellia epiphylla*, a species wherein identity of alleles suggests selfing. Photo by Li Zhang, with permission.

We know that seed plants often (usually?) are self-sterile. They have several mechanisms during and following pollination/fertilization to prevent success of self-fertilization, and these can provide suggestions for possible mechanisms in bryophytes:

- different maturation times of male and female parts
- dispersal vector behavior – moving from mature females to mature males (several animal vectors are now known)
- sperm unable to swim in neck of archegonium
- failure of self-fertilized embryo to develop
- rejection of self-fertilized embryos by plant
- better competition by hybrid embryos
- failure of next generation to reproduce

But do we know that any of these mechanisms occur in bryophytes? Gemmell (1950) suggested that all monoicous species were obligate inbreeders. This seems unlikely since evolution from dioicous to monoicous is a common

direction in bryophytes. Lazarenko and Lesnyak (1972) disproved the suggestion of Gemmell by demonstrating cross breeding in *Desmatodon* (Figure 84), including cross breeding between two different species in the genus. Now we are raising the question whether monoicous bryophytes actually have mechanisms to ensure outbreeding in at least a portion of the population.



Figure 84. *Desmatodon latifolius* with abundant capsules, a species in which hybrids among species in the genus are known. Photo by Michael Lüth, with permission.

Just in time for this writing, Stark and Brinda (2013) published their study on *Aloina bifrons* (Figure 85), a dioicous moss living in the dry Mojave Desert, USA. Despite being dioicous in an environment unfriendly toward fertilization by water, this moss had frequent sporophyte production, leading the researchers to question its dioicous status (Stark & Delgadillo M. 2001). They found that it could, at least occasionally, be **rhizautoicous**. They found **ramets** (individuals in clone of genetically identical individuals that have grown in given location, originating vegetatively from single plant), connected by single rhizoids, that produced both **perichaetia** (archegonial groupings) and **perigonia** (antheridial groupings).



Figure 85. *Aloina bifrons*, a moss that is apparently facultatively autoicous. Photo from Proyecto Musgo, through Creative Commons.

But all is not well for self-fertilization because it leads to all those dangers of inbreeding that make the offspring less fit. Rather, Stark and Brinda (2013) found that *Aloina bifrons* (Figure 85) actually practices self-incompatibility. First, it practices **protandry** – a condition wherein the male reproductive structures mature before the female structures. There was some overlap in maturity times between archegonia and antheridia, and self-fertilization did occur within single clones. However, sporophytes aborted during the embryonic development. Stark and Brinda did allow for the possibility that these cultures might require a resting phase to continue their sporophyte development, so we are still left wondering.

It appears that we know little about incompatibility mechanisms in bryophytes. Let's recall that the monoicous condition in bryophytes is apparently derived from the dioicous condition. Hence, the mechanisms had to arise anew after the monoicous taxa arose. We should perhaps expect that self incompatibility is an imperfect condition that is still evolving. But for now, there are no studies to determine if more embryos abort from self-fertilizations than from outbreeding. There is no evidence to determine the effect of self-fertilization on future generations. There is no study that has examined the success of sperm from the same plant vs different plants in reaching and penetrating the egg. Hence, we have no idea how extensive or important self-incompatibility is in bryophytes.

Geographic and Habitat Relationships

Certainly physiological evolution has occurred as species have broadened their ranges to more and more distant locations. *Pleurozium schreberi* (Figure 50-Figure 51) often is without capsules because no male plants are present. Longton and Greene (1969a,b) found that females are more abundant worldwide, causing us to ponder on the cause. Could it be that male expression requires a temperature and photoperiod combination that is not available in their more cosmopolitan distribution?

Working with *Macromitrium* (Figure 71), Une (1985) found a possible explanation for the absence of mature males in some species. In isosporous *Macromitrium*, female protonemata developed buds at 10°C, but after 160 days the males had failed to produce buds, making it impossible for them to complete a life cycle in a short growing season.

Two *Pohlia* (Figure 86) species provide evidence to suggest that changes in the reproductive response are possible mechanisms for survival in widespread locations, and this plasticity may explain the abundant capsules seen on some *Pohlia* species. Clarke and Greene (1970) found that gametangial maturation was faster in the Arctic and sub-Arctic than in Britain, permitting these species to complete their maturation in the shorter Arctic summer. Lewis Smith and Convey (2002) indicated that in the Antarctic sexual reproduction likewise was highly successful, suggesting that the severe climate with its low temperatures and short growing season is not a severe detriment to successful gametangial production. They consider that microhabitats make this reproduction possible. Most of the fertile species are monoicous, short acrocarpous species on rather calcareous soils. Could it be that calcium is an important part of the reproduction story?



Figure 86. *Pohlia filum* growing in an alpine area and producing abundant sporophytes. Photo by Michael Lüth, with permission.

In the Brazilian Atlantic Rainforest, an altitudinal cline permits us to compare reproductive performance. Maciel-Silva *et al.* (2012) monitored eleven species for fifteen months at sea level and a montane site to compare reproductive performance. The highest level of reproduction was among monoicous taxa, especially for sexual branches and fertilized gametangia. At sea level, there were more females and more sexual branches than at the montane site. But these differences seemed only to compensate for other factors because the sporophyte frequency was similar in both sites. Microhabitats like decaying wood were important in maintaining sufficient water levels for good gametangial production. Water availability and maintenance may have been the major factor influencing the success of sporophyte production.

Another geographic problem is that timing that is ideal in one locality may be all wrong in another. Signals for production of gametangia may come from photoperiod, signalling an upcoming rainy season, but in another, the rainy season may be during a different part of the year. For example, *Octoblepharum albidum* (Figure 87) in Brazil times its reproductive maturity to coincide with the rainy season (Pôrto & Oliveira 2002). The capsules begin their development during the rainy season, but complete it during the subsequent dry season when they disperse their spores. In this case, the rainfall seems actually to enhance development of gametangia, hence ensuring the correct timing. The behavior of *Sematophyllum subpinnatum* (Figure 88) in these tropical lowland forests is similar (Oliveira & Pôrto 2001). Although both antheridia and archegonia develop and mature throughout the year, they increase in number during the rainy season. Subsequent appearance of sporophytes primarily from June to September indicates that most fertilization events occur during the rainy season.

Odu (1981) found similar timing in tropical Africa. The perennial *Racopilum africanum* (Figure 89), *Fissidens weirii*, and *Thuidium gratum*, and an annual *Stereophyllum* sp. (Figure 90) all develop their gametangia at the onset of the rainy season, complete fertilization during that season, and produce mature capsules ready for spore dispersal at the onset of the dry season.



Figure 87. *Octoblepharum albidum*, a moss in which rainfall seems to enhance gametangial production. Photo by Niels Klazenga, with permission.



Figure 88. *Sematophyllum subpinnatum*, a species in which antheridia and archegonia are produced throughout the year, but increase in the rainy season. Photo by Michael Lüth, with permission.



Figure 89. *Racopilum africanum* with young sporophytes that are initiated near the beginning of the rainy season and mature at the beginning of the dry season. Photo by Jan-Peter Frahm, with permission.



Figure 90. *Stereophyllum radiculosum*, a moss that develops its gametangia at the beginning of the rainy season. Photo by Scott Zona, with permission.

For the mosses *Bryum coronatum* (Figure 3), *Hyophila involuta* (Figure 91), and *Barbula indica* (Figure 2) in southwestern Nigeria, gametangia development starts at the onset of the rainy season (March), providing them sufficient water to mature (Fatoba 1998). But their maturation requires 8-10 months (ending November – January), whereas the rainy season ends in mid October. The southwestern Nigerian rainy season has a "little dry season" (mid-July to mid-September, but mostly in August) (Adejuwon & Odekunle 2006), although the length decreases away from the coast. This little dry season might influence the persistence of the long developmental period for these gametangia. Temperatures typically range 26-28°C annually, so they have little influence on the bryophyte timing. This 8-10 months for maturation of gametangia places time of fertilization so that it permits the capsules to mature and spores to be dispersed in October – November, early in the regular dry season.



Figure 91. *Hyophila involuta*, a moss that begins gametangial development at the beginning of the rainy season in Nigeria. Photo by Niels Klazenga, with permission.

In desert habitats, even timing can fail to provide an opportunity for gametangial production. The desert moss *Syntrichia caninervis* (Figure 92) had 85% non-sexual ramets in a 10-hectare study area in the southern Mojave

Desert of Nevada, USA (Bowker *et al.* 2000). Those that had sexual expression were associated mostly with shaded microsites, higher soil moisture content, and taller ramets. The taller ramet may have been a result of the greater moisture available, but it also may have been the size that had reached the required threshold for available energy as discussed earlier in this chapter.

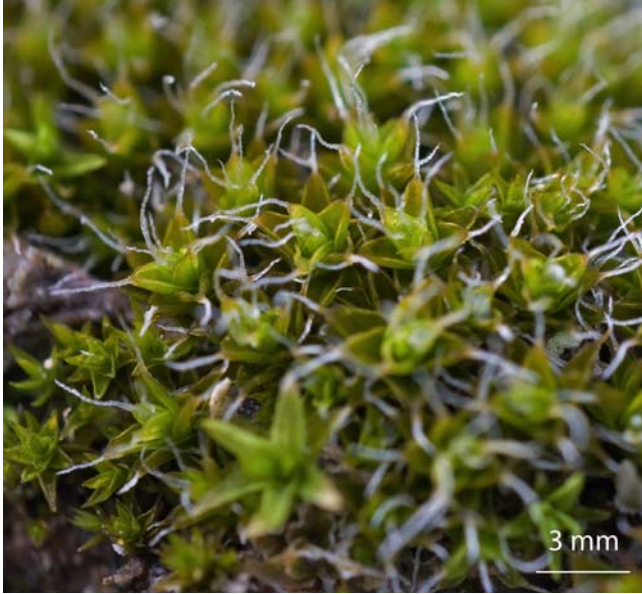


Figure 92. *Syntrichia caninervis*, a moss with 85% non-sexual ramets in the Mojave Desert. Photo from Proyecto Musgo, through Creative Commons.

In another desert moss, *Syntrichia inermis* (Figure 93), also from the Mojave Desert, more than 90% of the plants are monoicous (Stark 1997). In this species archegonia are initiated and receptive in the same winter, whereas antheridia require 1-3 years to reach maturity. Abortion is only 3-4% for both gametangia, but only 50% of the current cycle of perichaetia become fertilized. The slowest growth rates known, an 18-month dormancy period during sporophyte maturation, and the longest known period for antheridial maturation attest to limitations placed on reproduction in this moss by its desert habitat.



Figure 93. *Syntrichia inermis* with capsules, showing high sporophyte production of this monoicous moss. Photo from Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

Tradeoffs – Cost of Reproduction

Reproduction of any type comes at a price. Sexual reproduction requires considerable energy, and it benefits a plant to maximize success of its gametes in achieving fertilization. Actual measures of energy costs for any process in bryophytes are rare. The cost of reproduction can be indicated indirectly by its apparent effect on production of other structures and growth. For example, in *Marchantia polymorpha* (Figure 10), gemmae cups are generally not produced on the same portions of a colony as are the sexual structures (Figure 94) (Une 1984). But Une suggested that this might actually be due to age of the thallus, or to available nutrients, assuming that the interior of the colony where the gametangial branches occurred was the older and hence may have used up more of the available nutrients.

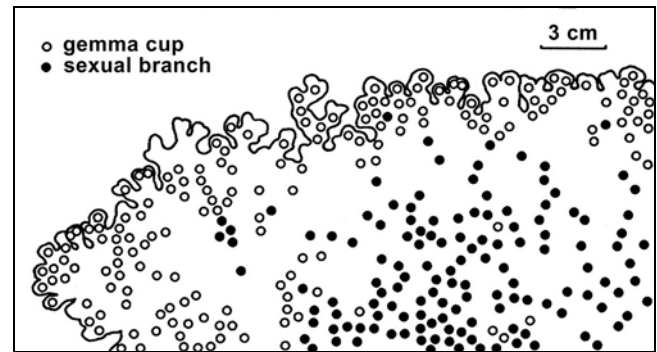


Figure 94. Location of gemma cups and archegoniophores in a colony of female *Marchantia polymorpha*. Modified from Une 1984.

The example of the leafy liverwort *Lophozia ventricosa* (Figure 95) var. *silvicola* demonstrates the high cost of being female (Laaka-Lindberg 2001). Female plants allocated 24% of their biomass to sexual reproduction whereas their male counterparts expended only 2.3%. The cost to the female was reduced stem length and both genders exhibited modified branching of gametangial shoots. When compared with asexual shoots, both genders had reduced stem length. Predictably, asexual plants produced the most gemmae (mean 2100), males next (1360), and females least (800).



Figure 95. *Lophozia ventricosa* with gemmae, a species with a high cost for gametangia. Photo by Michael Lüth, with permission.

The desert moss *Syntrichia inermis* (Figure 93) seems to tell a different story. In that species, it is more costly, by an order of magnitude, to produce male sexual organs than female ones (Stark *et al.* 2000). Stark *et al.* attributed this extra cost to the longer time required for development, greater number of male gametangia per perigonium than for archegonia per perichaetium, and presence of paraphyses among antheridia in that species. It would be interesting to see if this sex ratio could be modified by providing the limiting resources, presumably sugar.

Stark and coworkers (1998, 2001) found other indications of tradeoffs resulting from sexual reproduction in *Syntrichia caninervis* (Figure 92). Interestingly, non-sex-expressing individuals exhibited lower biomass, shorter total stem length, fewer branches, and shorter ramets than sex-expressing individuals; all individuals weighing more than 2 mg dry weight were sexually expressing, suggesting a threshold size needed for reproduction in order to provide sufficient energy. Furthermore, when inflorescence number was considered, the biomass of males and females did not differ.

McLetchie (1996) found that distance between male and female plants, as expected, decreased sexual success of the plants, but he also found that smaller males were less successful in accomplishing successful fertilization in the dioicous, thallose *Sphaerocarpos texanus* (Figure 67). From this he concluded that successful fertilization is sperm-limited. One might also argue that these could represent maturity differences.

For the epiphyte *Neckera pennata* (Figure 96), Wiklund and Rydin (2004) found a similar indication of minimum size. The first reproduction occurred at a colony size of 12-79 cm², requiring an estimated 19-29 years until the plants were sexually active! These apparent thresholds suggest that a critical size is important for sex expression. This implies that an energy threshold is required, and thus there must be a tradeoff between stored energy and sexual productivity.

Not only is production of gametangia expensive, but the ensuing production of sporophytes likewise is costly. It is therefore not surprising that Stark and coworkers (2001) found that 63% of the fertilized perichaetia of *Syntrichia caninervis* (Figure 92) had abortive sporophytes. This need for energy to produce the sporophyte seems to be subject to high selection pressure, as most bryophytes produce only one sporophyte per apex despite having multiple archegonia.

Relative fitness of sexual and asexual individuals can depend on the environmental conditions. In *Marchantia papillata* subsp. *inflexa* (Figure 63), Fuselier and McLetchie (2002) found that light intensities can shift sexual fitness and alter the timing of asexual reproduction. There were negative tradeoffs between the asexual and sexual fitness of females at some light intensities. In high light intensities, female plants suffer a sex-specific cost for their plasticity in timing, and asexual fitness shifts the population toward monomorphism of sexes. Fuselier and McLetchie concluded that opposing selective forces on sexual vs asexual expression could explain persistence of sexual dimorphism despite selection against dimorphism in the pre-adult phase.

Bisang and Ehrlén (2002) clearly demonstrated costs of sexual reproduction in female plants of the polysetous

Dicranum polysetum (Figure 97). They used a retrospective method to estimate photosynthetically active gametophyte biomass present at the onset of the sporophyte cycle and determined that reproductive effort, that is the proportional investment into reproductive structures, was 16% when sporophytes were successfully produced and only 1.3% when no fertilization occurred. The reproductive output of capsule number and dry weight were positively correlated with vegetative apical growth, whereas the reproductive effort was inversely related to dry mass of the annual segment preceding sporophyte initiation, indicating that energy was evidently shunted from that apical gametophyte tissue into the sporophyte. But even the next growth cycle paid the price of that reproduction; the probability of initiation of subsequent perichaetia was reduced as a result of sporophyte development, and when new perichaetia did develop, they were reduced in mass. In plants with sporophytes, investments in innovations were negatively correlated with reproductive structures. And, more sporophytes per plant resulted in reduced mass per sporophyte.



Figure 96. *Neckera pennata*, a moss that requires 19-29 years before plants are sexually active. Photo by Jan-Peter Frahm, with permission.



Figure 97. *Dicranum polysetum* showing multiple sporophytes from a single stem. Photo by Janice Glime.

Summary

Gametes in bryophytes are produced in **antheridia** (sperm) and **archegonia** (eggs). The location of these structures divides mosses into **acrocarpous** mosses with terminal gametangia and **pleurocarpous** with side-branch gametangia. Water is needed for dispersal of sperm and in some cases this is aided by the presence of splash cups or splash platforms. Once released the sperm swims to the archegonium, attracted by some factor released when the neck canal cells of the archegonium disintegrate.

Both **monoicous** and **dioicous** taxa of bryophytes exist, and chromosome numbers suggest that monoicous taxa are derived through **polyploidy**. Sex determination is under genetic control in at least some bryophytes, with either an X or a small Y chromosome programming females vs males, respectively. There are implications that expression of these genetic differences is manifest in **IAA** differences, but it appears that **ethylene** could interact with IAA or that concentrations or relative concentrations may be important.

Some *Macromitrium* taxa have two spore sizes that translate into dwarf males from small spores, but generally dwarf males seem to be determined by some factor from the female upon which they land. Gender survival ratios, already discussed in the chapter on sexuality, are altered by spore survival, protonemal survival, competition, and survival of the gametophores. It may furthermore be altered by the environment to express one or the other sex.

Initiation of gametangia may be an ancient event that must be controlled by inhibition rather than initiation. The apparent initiation could instead be a set of conditions that override or immobilize inhibitors. Initiation of gametangia can be triggered by light intensity, photoperiod, temperature, and water availability, but it appears that many bryophytes, especially mosses, may respond to some combination of these. Liverworts seem to be more dependent on photoperiod. Other factors that influence gametangial development and gender expression include pH and form and availability of N. There may be a minimum size, at least for some taxa, before gametangia will develop, implying need for sufficient energy supply. Antheridia typically initiate before archegonia and take longer to develop. Because these two gametangia are initiated at different times, they are often under different controls that can cause a mismatch in maturity times. This can be particularly problematic when they disperse to a new geographic region and may account for absence of sporophytes on particular species in some geographic regions.

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