

CHAPTER 3-4

SEXUALITY: REPRODUCTIVE BARRIERS AND TRADEOFFS

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CHAPTER 3-4

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Figure 1. *Funaria hygrometrica*, a monoicous species showing numerous capsules. Photo by Michael Lüth, with permission.

Reproductive Barriers: Selfing and Hybrids

Bryophytes are fundamentally different from tracheophytes by having a dominant haploid generation. Since many bryophytes can produce both antheridia and archegonia on the same plant (Figure 1), self fertilization (**selfing**) is likely to occur. Reproductive barriers to prevent selfing are important components of speciation. As long as genes are able to mix and appear in new offspring, the populations involved will be unable to become distinct species (Anderson & Snider 1982). When two species reside within centimeters of each other, they may receive sperm from the other species. We might expect some of the same mechanisms to prevent both selfing and hybridization.

Linley Jesson (pers. comm. 25 January 2014) used allozyme markers and successive innovations to measure selfing rates between individuals expressing one sex (in one year) and individuals expressing both sexes. Her (unpublished) work has shown extensive hybridization in the *Atrichum* (Figure 2-Figure 3) complex.

Selfing and Inbreeding Depression

Selfing in bryophytes can happen in two ways: intragametophytic and intergametophytic. **Intragametophytic selfing** is self-explanatory, where the crossing occurs between antheridia and archegonia on the same ramet (branch/gametophore), and can thus occur only

in unisexual bryophytes. Being **gametophyte** (haploid) and **monoicous** (having male and female reproductive organs on same gametophyte plant) means that all gametes are produced by mitosis, hence are identical. Therefore, any result of intragametophytic self-fertilization (sometimes also referred to as 'true self fertilization' or **autogamy**) results in a sporophyte that is homozygous for every trait!



Figure 2. Female *Atrichum undulatum* showing perichaetial leaves. Photo by Janice Glime.



Figure 3. Male *Atrichum undulatum* showing male splash cups. Photo by Janice Glime.

Intergametophytic selfing, therefore, is a specific type of inbreeding where mating occurs between separate gametophytes produced by the same sporophyte (Klekowski 1969; Krueger-Hadfield 2013). This is the only form of selfing that is possible in **dioicous** (male and female reproductive organs on separate gametophyte plants) bryophytes, where the two sexes are, by definition, on different plants. It is genetically comparable to selfing as the term is applied in heterosporous seed plants (see, e.g., Shaw 2000). When meiosis occurs in a dioicous bryophyte sporophyte, some spores will give rise to female plants and some to male plants. Those will not be identical, due to independent assortment during meiosis, but will be siblings. When those siblings mate (**inbreeding**), those events in bryophytes are considered to be **selfing**. If one considers the event in flowering plants, meiosis occurs in separate male and female sporangia, and makes separate gametophytes, so the gametes, even from the same plant, are not identical and are no more closely related than bryophyte gametophytes developed from separate spores. Hence, whether spores develop enclosed within the sporophyte (flowering plants) or on the substrate

(bryophytes), if they came from the same sporophyte and they cross, it is selfing.

Since **inbreeding** results from fertilization by close relatives such as siblings or in bryophytes between ramets of the same gametophyte, this may imply duplicating deficient genes or inheriting absence of genes. In tracheophytes, this typically results in decreased fitness. Some organisms are protected from this wasted energy and decreased fitness by having mechanisms to suppress inbreeding, such as different maturation times of male and female parts on the same individual. Others express the inbreeding depression in the offspring, typically by reduced fitness. But based on tracheophytes, we are accustomed to evaluating the effects of inbreeding in diploid organisms, not haploid generations such as the leafy bryophyte gametophyte. Nevertheless, inbreeding is an expected consequence of monoicous bryophytes with limited capacity for sperm dispersal.

Fortunately, at least some bryophytes have mechanisms to prevent self-fertilization (Ashton & Cove 1976), but Crum (2001) assumed that most were self-fertilized because the sperm and eggs mature at the same time on the same plant (but see Chapter 3-2 on Protogyny and Protandry in this volume). Ashton and Cove (1976) showed experimentally that among six strains of *Physcomitrella patens* all were self-sterile, but certain combinations were cross-fertile. In this case, the self-sterility resulted from mutations to the nicotinic acid and p-aminobenzoic acid genes. Hence, incompatibility can result from missing but necessary genes, and these are supplied by having an outside partner. Does this mean the incompatibility affects the embryo? But abortion later in sporophyte development is also possible. Nevertheless, Maciel-Silva and Válio (2011), examining bryophyte sexual expression in Brazilian tropical rainforests, found that monoicous species used strategies that increased their chances for out-crossing. For example, they produce unisexual branches as well as bisexual ones. It is further possible that self-infertility is widespread; we simply have not gathered the data needed to understand the extent of its distribution, as proposed by Stark and Brinda (2013). These authors suggest incompatibility after self-fertilization in a clonal line of the monoicous *Aloina bifrons* (Figure 4). They also refer to reports of self-incompatibility in *Desmatodon cernuus* and mutants of *Physcomitrella patens* (Figure 5). Modern DNA techniques should make it relatively easy to determine this.



Figure 4. *Aloina bifrons*, a monoicous species that seems to be self-incompatible. Photo by Martin Hutten, with permission.



Figure 5. *Physcomitrella patens* on soil. Photo by Michael Lüth, with permission.

Although truly self-fertilizing monoicous taxa pass on the full complement of genes to all their offspring, each sporophyte is in fact a separate **genet** (group of genetically identical individuals) that results from a single fertilization (Eppley *et al.* 2007). The sporophyte has no normal means of spreading vegetatively, so that genet cannot spread. Hedrick (1987) suggested that the complete homozygosity that results from intragametophytic selfing in monoicous bryophytes should select for extremely high inbreeding depression, but Eppley *et al.* (2007) considered that elimination of those (spores?) with deleterious alleles resulting from the inbreeding would remove those genotypes from the population and remove the inbreeding depression in future generations, hence favoring selfing. But dioicous species predominate, so we must examine the situation further.

Eppley *et al.* (2007) suggest that it is the level of intergametophytic selfing that maintains dioicy. If the level of selfing is low in dioicous bryophytes, accumulating deleterious alleles in the diploid stage would create a high cost for selfing through such effects as sporophyte abortion. Hence, the cost of selfing may maintain separate sexes. On the other hand, if selfing is high in both mating systems, deleterious genes would cause selection against both sexual strategies and select for monoicy due to higher fertilization rates. Eppley and coworkers found low or non-existent selfing in a mean of 41% of the sampled five dioicous species. If their reasoning is correct, this could explain the high level of dioicy in bryophytes when compared to flowering plants.

Selfing in bisexual bryophytes is evidenced by high F_{is} values (*i.e.*, a measure of heterozygote deficiency) observed in the sporophytic phase of all bisexual species investigated so far (Eppley *et al.* 2007; Hutsemekers *et al.* 2013; Johnson & Shaw 2015; Klips 2015; Rosengren *et al.* 2016). Using allozyme electrophoresis to estimate the deviations from expected heterozygosity, *i.e.* to estimate inbreeding, Eppley *et al.* (2007) estimated **selfing rates** for 10 species of New Zealand mosses. As one might expect, monoicous species had significantly higher levels of heterozygote deficiency (more selfing) than did dioicous species (inbreeding coefficient = 0.89 ± 0.12 and 0.41 ± 0.11 , respectively). An unexpected result, however, was to find that in two dioicous species [*Polytrichadelphus*

magellanicus (Figure 6-Figure 7) and *Breutelia pendula* (Figure 8)], there were significant indications of mixed mating or biparental inbreeding in a handful of populations.



Figure 6. *Polytrichadelphus magellanicus* females. Photo by Tom Thekathyl, with permission.



Figure 7. *Polytrichadelphus magellanicus* males with splash cups. One appears to be a female, possibly from the same clone. Photo by David Tng, with permission.



Figure 8. *Breutelia pendula*. Photo by Tom Thekathyl, with permission.

The classical explanation for the success of dioicous plants, based on tracheophyte literature, is that inbreeding, a product of having both sexes on the same plant, decreases fitness. In that case, one might assume that bryophytes, like other plants, have some mechanism of inbreeding depression (Beatriz Itten, Bryonet 26 May 2005). That is, they have some lethal or deleterious allele that gets expressed, leading to death or greatly reduced success. If such a gene is expressed in the haploid gametophyte, it is eliminated, rather than depressed, due to death of the individual.

In an attempt to remedy the absence of experimental data, Taylor *et al.* (2007) tested inbreeding depression in a monoicous and a dioicous moss species. Somewhat contrary to expectations, inbreeding depression occurred in the dioicous *Ceratodon purpureus* (Figure 9); crossing between siblings of the opposite sex significantly reduced fitness in both seta length and capsule length out of the four traits they examined. By contrast, the monoicous *Funaria hygrometrica* (Figure 10) exhibited no evidence of inbreeding depression in seta length, spore number, capsule mass, or capsule length. Jesson *et al.* (2011) found that hermaphroditism (monoicy) increased selfing rates rather than depressing them in *Atrichum undulatum* (Figure 2-Figure 3). Furthermore, they failed to demonstrate significant inbreeding depression in monoicous individuals of this species.

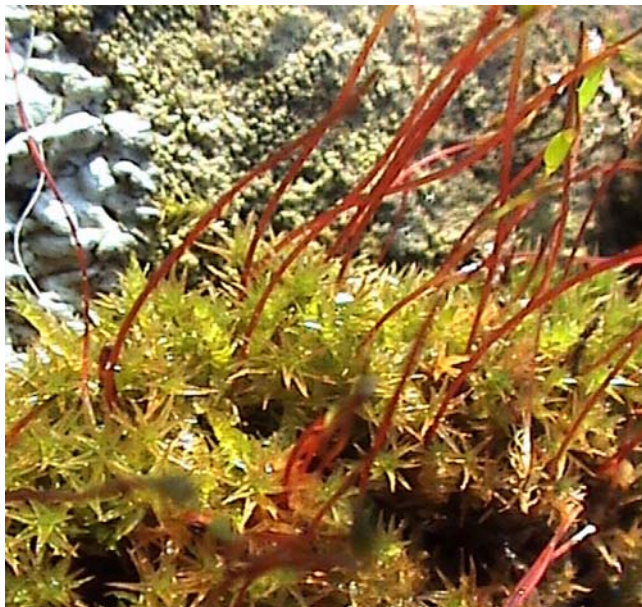


Figure 9. *Ceratodon purpureus* with sporophytes in a mixed population of males and females. Photo by Christian Hummert through Creative Commons.

Szöveni *et al.* (2009) noted in dioicous *Sphagnum lescurii* (Figure 11) that sporophyte size was correlated with the level of heterozygosity, in line with the prediction of inbreeding depression. This species experienced multiple paternity among sporophytes of a single female, enabling preferential maternal support of the more heterozygous embryos, which suggested active inbreeding avoidance and a possible post-fertilization selection. In contrast, inbreeding depression did not appear to be common in either dioicous or monoicous species in a multi-population study of 14 *Sphagnum* species (Johnson &

Shaw 2015), despite that monoicous species exhibited higher levels of inbreeding than dioicous ones.



Figure 10. *Funaria hygrometrica* in southern Europe. Photo by Michael Lüth, with permission.



Figure 11. *Sphagnum lescurii* with *Thuidium delicatulum*. Photo by Bob Klips, with permission.

Although further research on inbreeding depression in bryophytes is necessary, the evidence above suggests that the effects of bryophyte inbreeding are mitigated by the rapid purge of deleterious mutations during the gametophytic stage (Taylor *et al.* 2007; Jesson *et al.* 2011; Johnson & Shaw 2015). In particular, bisexual species are thought to rapidly purge recessive deleterious mutations through intra-gametophytic selfing (*i.e.* merging of gametes produced by shoots from the same protonema and hence, originating from the same spore. (See also below, Hybrid Success.)

Flowering plants frequently have mechanisms to prevent selfing. Could it be that monoicy in bryophytes is so recent that bryophytes have not yet evolved mechanisms to discourage it, or is it that they don't need to depress selfing, as implied by some of the above-cited studies? The former seems unlikely in view of evidence of many reversals indicated above (see also Chapter 3-1 in this volume).

We can suggest possible mechanisms to prevent selfing. As mentioned above and in Chapter 3-2, these might include timing (antheridia and archegonia mature at different times), as well as mechanisms of self incompatibility during fertilization or development. Hypotheses for possible mechanisms include:

1. rejection of sperm with same genotype (reminiscent of autoimmune diseases)
2. need for gene complementation to develop
3. embryo abortion
4. failure at meiosis

However, it would seem that any post-fertilization mechanism (2-4) would be wasteful (but see Szövényi *et al.* 2009, above), so selection should be greater for those species that can reject their own sperm, hence still allowing for subsequent outcrossing.

Could it be, then, that bryophytes are different from other major plant groups? Patiño *et al.* (2013) consider that **Baker's law** – as the loss of dispersal power and the bias toward self-compatibility after immigration to islands – applies to bryophytes. To defend this assertion, they cite evidence that the proportion of monoicous taxa was significantly higher on islands, and that a significant proportion of continental species that are monoicous or dioicous are represented on oceanic islands only by monoicous populations. This argument assumes a Founder Principle in which few colonists arrived and contact with the opposite sex was impossible. But it is also true that monoicous populations from the continent would have a greater chance of arriving on the island due to the greater ease of fertilization and spore production on the mainland. The shifts in life history traits toward a greater proportion of species producing asexual propagules and smaller proportion of species producing spores point to the loss of long-distance dispersal ability of bryophytes on oceanic islands.

Reduced Fitness

One consequence of selfing can be reduced fitness. This is illustrated in *Atrichum undulatum*. Populations in the *Atrichum undulatum* complex (Figure 2-Figure 3) contain females, males, and hermaphrodites, and hermaphrodites can have sex organs in close proximity or spatially separated across branches. In their experiments Jesson *et al.* (2012) found that there was significant selfing within gametophytes, whereas there was no significant selfing between siblings, supporting the importance of proximity for fertilization. But what is the price for this selfing? They found that sporophyte size did not differ between sibling (**intergametophytic**) and **intragametophytic** selfing, but other factors suggest reduced fitness for products of selfing. Sporophytes from females contained 29% more spores than those from monoicous (~30% selfed on same branch) individuals. When the cultures were stressed by supplying only tap water instead of a nutrient medium, only the progeny from females (*i.e.* non-selfed) survived on tap water after 6 months (Figure 12). Progeny of females transplanted onto tap water media had a greater photosynthetic capacity but higher non-photochemical quenching than did the

monoicous individuals, causing these females to have photosynthetic rates similar to those of the monoicous progeny. These are weak effects of partial selfing, but under certain stressful conditions may result in lower survival among progeny that are the product of selfing.

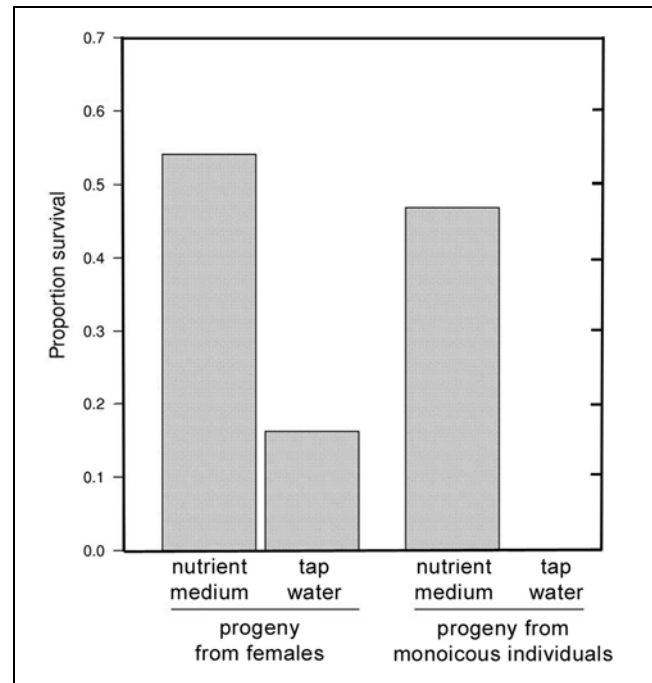


Figure 12. Proportion of culture plates with spores from females ($n=39$) compared to progeny of monoicous individuals ($n=30$) of *Atrichum undulatum* s.l. (Figure 2-Figure 3) germinating after 6 months on nutrient medium (Bold's basic media) vs tap water (stressful condition). Modified from Jesson *et al.* 2012.

Hybridization

Hybridization is the opposite of reproductive isolation. In the past, bryologists tended to consider hybridization in bryophytes to be unimportant (Andrews 1942; Vitt 1971; Smith 1978, 1979; Anderson 1980). But in fact, it seems to be widespread among bryophytes (Ruthe 1891; Nyholm 1958; Andrews & Hermann 1959; Crundwell & Nyholm 1964; Proskauer 1967; Ochi 1971; Delgadillo 1989; Schuster 1991; Ros *et al.* 1994; Natcheva & Cronberg 2004), often confounding attempts at cladistics when hybrids are among the data sets (Xu 2000).

It is interesting that among the bryophytes gametophyte hybrids seem only to exist in mosses, at least based on genetic information (Natcheva & Cronberg 2004). A number of hybrid liverwort species have been suggested, based on morphology, but so far few have been supported by genetic/molecular data – see, for example *Targionia hypophylla* (Figure 13) (Boisselier-Dubayle & Bischler 1999). Summarizing data, Natcheva and Cronberg concluded that moss hybrids usually occur among the "weedy" species with life history strategies of fugitive, annual, and short-lived shuttle or colonist, *i.e.*, species with life spans of only a few years.



Figure 13. *Targionia hypophylla*. Photo by Ken-Ichi Ueda through Creative Commons.

Intergeneric Hybrids

Intergeneric Hybrids – It is even more interesting that within the **Polytrichaceae** there are apparent **intergeneric hybrids**. *Polytrichastrum pallidisetum* (Figure 14-Figure 15) and *Polytrichastrum ohioense* (Figure 16) both appear to have had one progenitor in *Polytrichastrum* and one in *Polytrichum* (Figure 43) (Derda & Wyatt 2000). *Polytrichastrum sexangulare* (Figure 17) appears to have had a species of *Pogonatum* (Figure 18) as one of its progenitors (but then, the mosses may classify themselves differently from the way we currently do and place themselves all in *Polytrichum*).



Figure 14. *Polytrichastrum pallidisetum* with capsule. Photo by Štěpán Koval, with permission.



Figure 15. *Polytrichastrum pallidisetum* with capsules from Europe. Photo by Michael Lüth, with permission.



Figure 16. *Polytrichastrum ohioense* females. Photo by Janice Glime.



Figure 17. *Polytrichastrum sexangulare*, a species produced by hybridization. Photo by Michael Lüth, with permission.



Figure 18. *Pogonatum urnigerum* with numerous capsules at Swallow Falls, Wales. Photo by Janice Glime.

Hybrid Success

Sphagnum (Figure 19-Figure 21) is a genus where polyploids are common (see also 3.1., Genome Doubling). Ricca *et al.* (2011) point out that we might expect all occurrences of polyploidization to result in instant sympatric speciation. But they cite several cases, *e.g.* *S. lescurii* (Figure 11), in which the resulting hybrid produces triploid sporophytes that are larger than those of the parents, but most of the spores are not viable. Furthermore, the spores that do germinate develop their sporelings more slowly. But such species are able to persist because of the pervasive vegetative reproduction. And some day in the future, some genetic error might enable successful spore reproduction.

Shaw *et al.* (2012) demonstrated **homoploid hybridization** (no change in chromosome number) and allopolyploidy in multiple species of *Sphagnum*. In the *S. fimbriatum* (Figure 19) complex they found one species with diploid gametophytes. Based on plastid DNA sequences, all samples of the polyploid *S. tescorum* (Figure 20) share an identical haplotype with most samples of *S. girgensohnii* (Figure 21). Fixed or nearly fixed heterozygosity at ten microsatellite loci show that *S. tescorum* is an allopolyploid. Many other examples indicating the role of hybridization in creating species differences are known in this genus.



Figure 19. *Sphagnum fimbriatum* with capsules. Photo by David Holyoak, with permission.



Figure 20. *Sphagnum tescorum* in Alaska. Photo by Vita Plasek, with permission.



Figure 21. *Sphagnum girgensohnii* with open capsules. Photo by Janice Glime.

Flatberg *et al.* (2006) studied natural hybrids between haploid female *Sphagnum girgensohnii* (Figure 21) and diploid male *S. russowii* (Figure 22). These hybrids were discovered because when *S. girgensohnii* was in the presence of *S. russowii*, large capsules formed. The spores from these crosses yielded viable spores that produced triploid protonemata and juvenile gametophores in culture. *Sphagnum russowii* is itself a hybrid of *Sphagnum girgensohnii* and *S. rubellum* (Figure 23). Not only were the capsules larger in the *S. girgensohnii* x *S. russowii* cross, but spores were larger as well. Nevertheless, spore germination from this hybrid was less than 5%, which is much less than when *S. girgensohnii* is crossed with others of its own species. Hence, while these hybrids may make a few super plants, the numbers of offspring are greatly reduced. Even so, through vegetative reproduction such a population could expand and grow.



Figure 22. *Sphagnum russowii*. Photo by Blanka Shaw, with permission.



Figure 23. *Sphagnum rubellum*. Photo by Jan-Peter Frahm, with permission.

It is fitting, then, to conclude that barriers to cross breeding among species are incomplete in the bryophytes and that evolution of new species through hybridization may occur somewhat frequently in this group. This suggestion is supported by the apparent lack of external barriers to cross fertilization and the nearly total absence of sperm vectors to help enforce same species selection.

When Barriers Are Needed – or Not

Eppley *et al.* (2007) conclude that for taxa that are colonizers and must be able to self-fertilize in repeated colonization events, being self-compatible is an evolutionary advantage. This permits them to establish and spread rapidly in a new location. This is also suggested by Baker's law, which was recently found to apply for bryophytes (Patiño *et al.* 2013; see above).

In seed plants, elaborate modifications help to ensure that the male gametophyte (pollen grain) will disperse and reach the appropriate female gametophyte, where it will release sperm and effect fertilization. Specialized behaviors of pollinators also ensure that self-pollination is minimal. Such specialized facilitators (**external isolating mechanisms**) are rare in bryophytes, but other environmental mechanisms exist. As in seed plants, reproductive isolation that prevents hybrids in bryophytes may also result from various **internal isolating mechanisms** or a combination of internal and external isolating mechanisms (Natcheva & Cronberg 2004).

In bryophytes, the spore is needed for dispersal, and being small permits a greater distance for that dispersal

than that of many seed plant pollen grains. On the other hand, dispersal of the sperm of the bryophyte to the female reproductive organ lacks the protection and carrier capability of a pollen grain in tracheophytes and must get there by other means. As already discussed (Cross Fertilization in Chapter 3-1 of this volume), these gametes are motile and most of them must be transported in water or swim through a film of water. Thus, gene flow in bryophytes is affected by both gamete flow distances and spore dispersal distances. Anderson and Snider (1982) further contend that bryophyte establishment is more hazardous than that of seed and seedling establishment (see also Wiklund & Rydin 2004; Cleavitt 2005; Söderström & During 2005). These limitations make it advantageous to be bet-hedgers (having more than one strategy; see below) and permit at least some self-fertilization.

Effects of different reproductive barriers might be seen in the lack or scarcity of sporophyte formation. Bisang and Hedenäs (2008) transplanted males of the dioicous fen moss *Drepanocladus trifarius* (Figure 24) into the center of female patches. They could not observe any sporophytes in archegonia in the 'swollen venter stage.' Rather, the archegonia were withered or dehiscent. Using a similar experimentation in forest habitats, the dioicous *Rhytidiadelphus triquetrus* (Figure 25) produced capsules freely, with 100% of the plots exhibiting sporophytes (Bisang *et al.* 2004). *Abietinella abietina* (Figure 26), on the other hand, had sporophytes in only 41% of the plots. Furthermore, these *A. abietina* sporophytes maintained their calyptrae and did not dehisce when they should have; 36% of the capsules aborted. These examples demonstrate that not only lack of one sex or spatial segregation of the sexes are responsible for lack of capsules in dioicous bryophytes, but multiple factors may have an influence and probably interact. *Hamatocaulis vernicosus* (Figure 27) fails to produce capsules in France; only embryonic sporophytes were observed in more than 12,000 studied archegonia from 45 localities (Pépin *et al.* 2013). A combination of factors related to sexual phenology and environment is required for sporophytes to be produced: sex expression of mixed-sex colonies, short distance between sexes, light grazing, and high water table.



Figure 24. *Drepanocladus trifarius*. Photo by Andrew Hodgson, with permission.



Figure 25. *Rhytidiadelphus triquetrus*. Photo courtesy of Eric Schneider.



Figure 26. *Abietinella abietina*. Photo by Janice Glime.



Figure 27. *Hamatocaulis vernicosus*, a species that requires a limiting combination of environmental and sexual conditions to produce sporophytes. Photo by Des Callaghan, with permission.

Anderson and Snider (1982) summarized these differences and presented the reproductive barriers "used" by bryophytes. Bryophyte reproductive barriers can, as in seed plants, be divided into external and internal barriers (Anderson & Snider 1982).

External Barriers

Spatial or Geographic Isolation

For spatial or geographic isolation to occur, the distance between biotypes must be greater than the spore dispersal distance. That is more a theoretical limit than a practical one because spores can occasionally travel great distances through the atmosphere. Nevertheless, the greater the distance, the smaller the chance for genetically compatible biotypes to join. This same external barrier applies to sperm, which rarely travel more than a meter. However, as Anderson and Snider (1982) and much earlier Gayet (1897) suggested, it has by now been demonstrated that **mites**, **springtails**, and other small invertebrates can not only carry the sperm from male **perigonia** to female **perichaetia**, but in some cases facilitate much greater fertilization than in their absence (Cronberg *et al.* 2006; Rosenstiel *et al.* 2012; Bisang *et al.* 2016). Furthermore, we now know that some small portion of sperm are likely to survive even desiccation (Shortlidge *et al.* 2012), permitting survival during a much greater dispersal distance. Nevertheless, short-distance spatial separation is much more effective as an isolating mechanism among bryophytes than among tracheophytes.

Bryophytes, like tracheophytes, often exhibit incomplete isolation (Natcheva & Cronberg 2004). For example, some geographic races of the liverwort *Sphaerocarpos texanus* (Figure 28-Figure 29) are partly reproductively isolated whereas others are fully interfertile (Allen 1937). The hornwort *Phaeoceros* (Figure 30) has good reproductive isolation among species, but under some circumstances geographic races of *Phaeoceros laevis* (Figure 30) are able to interbreed (Proskauer 1969).



Figure 28. *Sphaerocarpos texanus* involucres of male plants, looking very much like archegonia! Photo by Paul Davison, with permission.



Figure 29. *Sphaerocarpos texanus* female. Photo by Martin Hutten, with permission.



Figure 30. *Phaeoceros laevis* with sporophytes. Photo by Bob Klips, with permission.

Ecological Isolation

A second external barrier is ecological isolation. In this case, the biotypes are confined to different habitats, making crossing unlikely. These differences were difficult to identify until recently because one had to do common garden or transplant studies to determine if perceived morphological differences were environmentally induced or genetically based. Such environmental plasticity differences have been especially noticeable for species that occur both in and out of water. And often transplanted populations did not succeed or looked different from any established field population. Now advances in the use of genetic markers permit us to identify different variants of a species. These may eventually be expressed as races, cryptic species, or microspecies, and if isolated long enough may evolve into separate species.

Ecological isolation in bryophytes is closely tied with spatial isolation because of the typical short distance of sperm dispersal. If they are close enough for the sperm to reach the archegonium, the microhabitat is not likely to differ much.

Seasonal Isolation – Gametangial Timing

In some locations, timing or climate can make one gender unable to complete its task. Seasonal isolation, as in pollination, can cause male and female gametangia to mature at different times (see Protogyny and Protandry in Chapter 3-2). Species that arrive by long distance travel may lack the necessary environmental triggers at the appropriate time to ensure that gametangia are coordinated. New arrivals may not be coordinated with established populations. Hence, if male and female propagules arrive at different times or from different places, they may be seasonally incompatible, a factor that can also isolate wind-pollinated members of the same genus among seed plants. This mechanism may be incomplete, working as an isolating mechanism in some years but not in others, depending on the weather.

We seem to have little verification of seasonal isolation in bryophytes. We do know that timing of male and female gametangial maturation can differ in monoicous bryophytes (Anderson & Lemmon 1973, 1974; Longton & Miles 1982; Shaw 1991). This mechanism can successfully isolate the eggs from being fertilized by sperm from the same plant (See Protogyny and Protandry in Chapter 3-2).

Speculation suggests that seasonal isolation is effective among several species of *Sphagnum* (Natcheva & Cronberg 2004). Other speculations include *Weissia* (Khanna 1960; Williams 1966), and the geographic races of *Anthoceros* (Proskauer 1969). A combination of phenology studies and genetic information revealing closely related **sympatric taxa** (having overlapping distributions) should reveal some examples.

Internal Barriers

In addition to external barriers, internal barriers may exist. Natcheva and Cronberg (2004) referred to these as reproductive isolation.

Gametic Isolation

Gametic isolation is a mechanism known from algae, animals, and tracheophytes, but it appears to be lacking, or perhaps simply unknown, in bryophytes. Wiese and Wiese (1977) define it in the green alga *Chlamydomonas* as nonoccurrence of initial contact between non-compatible gamete types. In other words, the opposite gene types such as sperm and egg cannot find or attract each other. In *Chlamydomonas*, gamete contact depends on molecular complementarity between **glycoproteinaceous** components. Parihar (1970) suggested that in bryophytes attractive substances such as sugars or proteins might help to guide the sperm to the archegonium and hence to the egg, but the importance and exact identity of such substances remains to be studied. (See Sperm Dispersal by the Bryophyte in Chapter 3-1.)

Natcheva and Cronberg (2004) found no studies to support the hypothesis that bryophytes produce substances to hamper or prohibit foreign sperm from entering the neck of an archegonium or to prevent penetration of the egg. In fact, Showalter (1926) showed that both moss and liverwort sperm [*Aneura* (Figure 31), *Sphaerocarpos* (Figure 29-

Figure 28), *Asterella* (Figure 80), and *Funaria* (Figure 38-Figure 39)] were able to penetrate the egg cells of the liverwort *Fossombronia* (Figure 81). Duckett (1979; Duckett *et al.* 1983) even reported that sperm of *Mnium hornum* (Figure 32) were able to penetrate the egg cells of the tracheophyte *Equisetum* (Figure 33).



Figure 31. *Aneura pinguis*, a possible sperm donor for the liverwort *Fossombronia*. Photo by Li Zhang, with permission.



Figure 32. *Mnium hornum* males, potential sperm donors for such different taxa as *Equisetum*. Photo by David T. Holyoak, with permission.

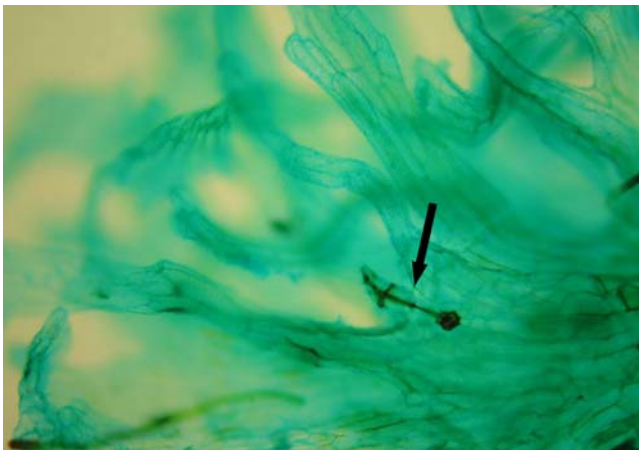


Figure 33. *Equisetum* prothallus with archegonium (arrow). Note the neck projecting from the gametophyte. Eggs of this species can be penetrated by sperm of other phyla, including the moss *Mnium hornum*. Photo by Ross Koning, with permission.

Nevertheless, it does appear possible that the archegonium may attract and perhaps trap the sperm. In most cases, when the archegonium is mature and ready to receive the sperm, the neck canal cells and ventral canal cell disintegrate and exude a gelatinous matrix from the opening of the archegonial neck (Watson 1964). This has been considered the attracting substance, but others consider it a means of entrapment.

Since we know little about this entrapment in bryophytes, let's consider a well-known fern example. In the fern *Marsilea*, sperm reach the gelatinous matrix extruded by the archegonium when the neck canal opens. Once "entrapped" by the matrix, sperm are all directed toward the neck of the archegonium, which they enter, albeit slowly. Although Machlis and Rawitscher-Kunkel (1967) argue that these *Marsilea* sperm are trapped rather than attracted, it is significant that all sperm are clearly pointed toward the archegonial neck. Machlis and Rawitscher-Kunkel cite Strasburger (1869-1870) for a description of the massing of sperm at the opening of the neck canal in *Marchantia polymorpha* (Figure 34-Figure 35), suggesting that this likewise was entrapment in a gelatinous matrix surrounding the opening of the neck canal. Machlis and Rawitscher-Kunkel further cite Pfeffer (1884) as confirming observations of chemotactic responses of sperm to archegonia in the liverworts *Marchantia polymorpha* (Figure 34-Figure 35) and *Radula complanata* (Figure 36) and mosses *Brachythecium rivulare* (Figure 37), *Funaria hygrometrica* (Figure 38-Figure 39), and *Leptobryum pyriforme* (Figure 40). Alas, no substance he tested attracted the two liverwort sperm. Sperm of *Brachythecium rivulare*, *Funaria hygrometrica*, and *Leptobryum pyriforme* responded to sucrose, whereas the pteridophytes examined responded to malate. Parihar (1970) reported that sperm of the thallose liverwort *Riccia* (Figure 41) were attracted by proteins and inorganic sources of potassium.

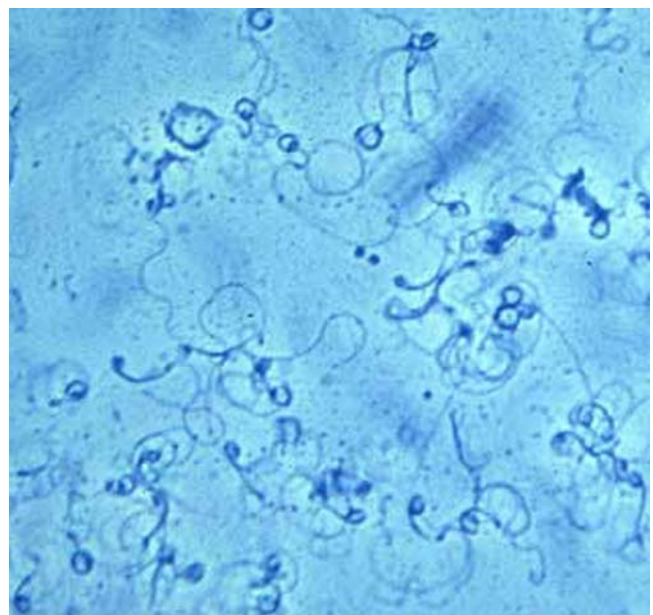


Figure 34. *Marchantia polymorpha* sperm swarming. Photo from Botany 321 website at the University of British Columbia, with permission.

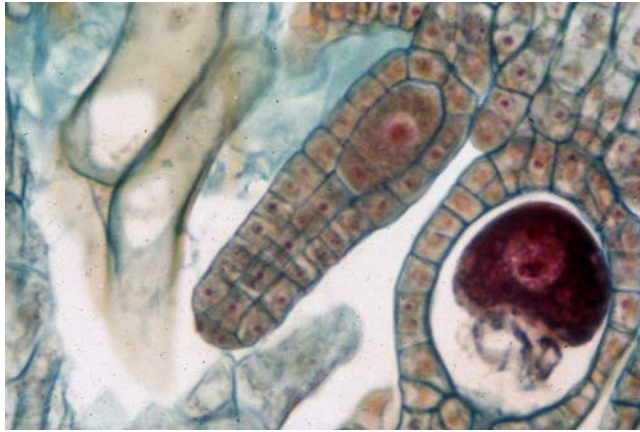


Figure 35. *Marchantia polymorpha* with immature archegonium with neck canal cells intact (left) and archegonium venter with large purplish egg and sperm attached, penetrating the egg. When the neck canal cells break down, they exude a mucilage that attracts the sperm. Photo by Janice Glime.



Figure 38. *Funaria hygrometrica* males with splash cups. Photo by James K. Lindsey, with permission.



Figure 36. *Radula complanata* with dehiscent sporophytes. Photo by Michael Lüth, with permission.



Figure 39. *Funaria hygrometrica* archegonia with emerging sporophytes covered by calyptrae. Photo by Andrew Spink, with permission.



Figure 37. *Brachythecium rivulare*. Photo by David T. Holyoak, with permission.



Figure 40. *Leptobryum pyriforme* with capsules in Sweden. Photo by Michael Lüth, with permission.



Figure 41. *Riccia sorocarpa*, a thallose liverwort that attracts its sperm by proteins and inorganic sources of potassium. Photo by Michael Lüth, with permission.

These early observations were somewhat hit or miss and did not clarify what substances in the archegonia had attractive powers. Furthermore, Showalter (1928) reported that in the thallose liverwort *Riccardia* (Figure 42) the collapsed cells of the archegonial neck played no role in attraction.



Figure 42. *Riccardia latifrons* with emerging capsules. This species does not seem to produce a sperm attractant when the archegonial neck cells disintegrate. Photo by Michael Lüth, with permission.

More recent compendia ignore the topic completely (Chopra & Bhatla 1990; Crum 2001; Vanderpoorten & Goffinet 2009). We find it hard to believe that the archegonia of bryophytes lack such attractants. But are they able to attract only specific sperm? Perhaps it is the clonal nature of bryophytes that decreases the likelihood of a foreign sperm finding the egg. Nevertheless, specialization occurs, as demonstrated by studies where invertebrates carry the sperm to archegonia that attract those insects (Rosenstiel *et al.* 2012).

Genetic Incompatibility

Stenøien and Sæstad (2001) contend that bryophytes might experience inbreeding depression through genes that are silenced in the gametophyte phase but expressed in the sporophyte phase. Experimental evidence for this is beginning to emerge.

McLetchie (1996) found that in the dioicous liverwort *Sphaerocarpos texanus* (Figure 29-Figure 28), increasing inter-mate distance and decreasing male size reduced sporophyte production, thus suggesting sperm limitation. On the other hand, when three males and three females were mated in a factorial design resulting in nine unique crosses, sporophyte production was very low in some pairs of genotypes known to be fecund in other combinations. McLetchie suggested that genetic interactions may be responsible for some of the low levels of sexual reproduction in dioicous bryophytes. This would suggest that genes from a different population might be less compatible.

Genetic incompatibility was also suggested as one potential explanation for rare and incomplete sporophyte formation in dioicous *Abietinella abietina* (Figure 26) in a transplantation experiment (Bisang *et al.* 2004). But detailed data on the mechanisms in bryophytes are lacking. Natcheva and Cronberg (2004) could find no data indicating abortion of embryos in bryophytes and we are unaware of anything more recent. Nevertheless, Van Der Velde and Bijlsma (2004) found that up to 90% of the hybrid sporophytes from the crossing of *Polytrichum commune* (Figure 43) x *Polytrichum uliginosum* (= *Polytrichum commune* var. *uliginosum*; Figure 44) were aborted. Despite this poor reproductive performance, *P. uliginosum* has been considered to be a synonym of *P. commune* var. *commune* (Kew 2014), but Kew currently lists it as *Pogonatum uliginosum*.



Figure 43. *Polytrichum commune* 2-year growth. Photo by Michael Lüth, with permission.



Figure 44. *Pogonatum uliginosum* male plants with antheridial splash cups. Photo by Hermann Schachner, through Creative Commons.

Hybrid Sterility

Internal isolation among bryophytes is usually manifested by sterility of the hybrid sporophyte (Natcheva & Cronberg 2004). Nevertheless, hybrid sterility seems to be less important in bryophytes than in tracheophytes. There are numerous examples of presumed hybrids in mosses, in many cases being the mechanism of becoming monoicous. One consequence of fertilization from the wrong species is that the reproduction following that cross is unsuccessful. For example, sporophytes from these individuals typically produce many non-viable spores. But, since bryophytes are clonal, vegetative reproduction can lead to populations of ramets that are compatible with each other because all have the same number and type of chromosomes. There have also been a number of presumed interspecific hybrids noted in natural populations. Wettstein (1923) experimented with hybridization in the **Funariaceae** and was able to produce phenotypes that could also be observed in the field.

Bryophytes have two known types of **sterility barriers**: chromosomal sterility and developmental sterility. **Chromosomal (segregational) sterility** results from structural differences in chromosomes of the two parental species, causing disruption of pairing during meiosis and ultimately resulting in spores with incomplete chromosome sets or extra chromosomes. This type of sterility is known in pairing between *Ditrichum pallidum* males (Figure 45) and *Pleuridium acuminatum* (Figure 46, Figure 47), a case in which few spores formed and those that did aborted (Anderson & Snider 1982). The hybrid has intermediate characters of seta length, differentiated but indehiscent operculum, and spores of variable size (Andrews & Hermann 1959). Finally, Anderson and Snider (1982) reported almost a complete lack of chromosome pairing in hybrids between *Pleuridium subulatum* (Figure 48-Figure 49) ($n=26$) and *P. acuminatum* (Figure 47) ($n=13$).



Figure 45. *Ditrichum pallidum* with capsules, a species that hybridizes with *Pleuridium subulatum*, producing hybrid sporophytes with intermediate characters but that do not function normally. Photo by Michael Lüth, with permission.



Figure 46. *Pleuridium acuminatum* with sporophytes. Photo by Jonathan Sleath, with permission.



Figure 47. *Pleuridium acuminatum* with capsules. Photo by Jan-Peter Frahm, with permission.

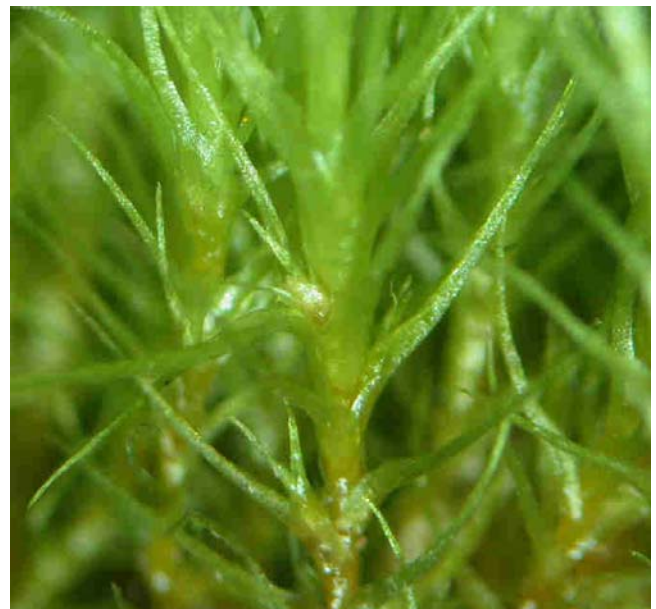


Figure 48. *Pleuridium subulatum* with axillary buds with antheridia. Photo by David Holyoak, with permission.



Figure 49. *Pleuridium subulatum* with capsules, a species that hybridizes with *P. acuminatum* but hybrids subsequently exhibit failure of chromosome pairing. Photo by Kristian Peters, with permission.

Consider that of the numerous spores formed in some species, it seems likely that there will be the occasional spore that gets the right set of chromosomes during pairing of meiosis. But wait, spores normally are protected by other spores, and as we have seen, those other spores die slowly as some continue to enlarge and reach maturity. Those other spores help to maintain moisture and may even provide nutrients as needed in the maturing capsule, so this massive abortion could explain why those normal spores generally are not able to reach maturity in a capsule lacking protection by other spores due to abortion during or immediately following meiosis.

Developmental sterility occurs when hybridization successfully produces a new plant, but it is developmentally different from its parents. Typically, these plants are sterile, producing what appeared to be normal tetrads of meiospores, but lacking viability. Wettstein (1923) suggested that one explanation was that the paternal set of chromosomes was unable to function in the maternal cytoplasm. There are other possibilities of incompatibility between the two sets of chromosomes – chromosomes that led to production of incompatible or lethal substances or that interfered with timing mechanisms.

These hybridization phenomena occur in nature as well as in the lab, as in the well known examples of hybrids between *Astomum* (Figure 50) and *Weissia* (Figure 51) (Nicholson 1905; Andrews 1920, 1922; Reese & Lemmon 1965; Williams 1966; Anderson & Lemmon 1972). These hybrids between *Astomum* (Figure 50) and *Weissia* (Figure 51) resulted in sporophytes that were intermediate in seta length, capsule shape, operculum being present but non-dehiscent, and presence of a rudimentary peristome (Nicholson 1905; Andrews 1920, 1922; Reese & Lemmon 1965; Williams 1966; Anderson & Lemmon 1972).

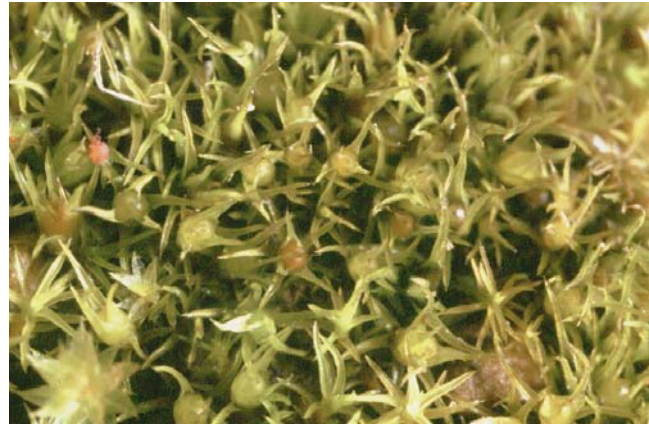


Figure 50. *Astomum crispum* with capsules, member of a genus that is able to produce hybrids. Photo by Jan-Peter Frahm, with permission.



Figure 51. *Weissia muhlenbergianum* with capsules, a species with chromosome numbers of $n=13$ and $n=26$. Photo by Bob Klips, with permission.

But if one tracks chromosome numbers in bryophytes, it becomes clear that some of these hybrids have succeeded in making new species (see 3.1, Genome Doubling in Mosses). Hence, from the basic chromosome number of 10 in bryophytes, we find that *Weissia* (= *Astomum*) *muhlenbergianum* (Figure 51) has a basic number of $n=13$ and $n=26$ (Reese & Lemmon 1965; Anderson & Lemmon 1972). It is interesting that all hybrids in these two genera occur with *Astomum* as the gametophyte female parent. Is that merely a problem of human perception of what constitutes the two genera?

In the cross of *Weissia ludoviciana* with *W. controversa* (Figure 52) and of *W. muhlenbergiana* (formerly placed in *Astomum*) (Figure 51) with *W. controversa*, meiosis proceeded normally (Anderson & Lemmon 1972). But during the maturation and differentiation of the spores, abnormalities occurred, including spore abortion, failure to enlarge, retention in tetrads, and failure to develop chlorophyll.



Figure 52. *Weissia controversa* var. *densifolia* with capsules. Photo by Barry Stewart, with permission.

It is likely that many species experience both selfing and cross fertilization. These species necessarily either lack reproductive barriers or have barriers with incomplete effectiveness. For example, in the polyploid ($n=18$) monoicous liverwort *Plagiochasma rupestre* (Figure 53), both self fertilization and cross fertilization occur (Boisselier-Dubayle *et al.* 1996). Using two isozyme markers, Boisselier-Dubayle and coworkers determined that the two chromosome sets behave independently.



Figure 53. *Plagiochasma rupestre* with two archegoniophores. Photo by Michael Lüth, with permission.

Apomixis?

Ozlem Yayintas asked me if mosses have apomixis. That stopped me short. I understand it in seed plants – seeds are produced without fertilization due to a failure in meiosis. Dandelions have apomixis. But do bryophytes? If so, what would define it?

Hans Winkler (1908) defined **apomixis** as replacement of the normal sexual reproduction by asexual reproduction, without fertilization. Bryophytes certainly have lots of forms of asexual reproduction that fit his original definition. But as time passed, the definition narrowed and is often restricted to production of seeds without fertilization, a definition that cannot fit bryophytes. If we stay with Winkler's original definition, bryophytes have exhibited chromosome doubling through autopolyploidy, but they also have created sporophytes from gametophytes,

fitting more closely with the seed definition (see Sporophytes from Fragments in Chapter 3-1 of this volume).

We turned to Google to see what others have said about apomixis in bryophytes. We found a 2013 study in which the researchers removed the KNOX2 gene and caused apomixis in a bryophyte (Elder 2013)! Sakakibara *et al.* (2013) deleted the KNOX2 gene in the moss *Physcomitrella patens*, the bryophyte version of a lab rat, and caused it to develop gametophyte bodies from diploid embryos without meiosis. It may sound easy, but it is a lengthy process. The next step for the food world is to knock out that gene in hybrid food plants, create apomictic offspring, and have reliable seeds with the hybrid characters they want, representing two sets from the mother.

Vegetative Apomixis?

Terminology evolves as our knowledge evolves, and we find that some bryologists use the broader definition of Winkler (1908). This confuses those familiar with the seed plant definition. As suggested by Katja Reichel (Bryonet 21 February 2014), perhaps it is best not to define it for bryophytes, i.e., don't use it. She cites the ambiguity of the earlier definition by Åke Gustafsson (1946) that includes every form of asexual reproduction in plants, compared to Gustafsson's later definition as **agamospermy**, which means seed formation without fertilization. But Täckholm (1922) had already clearly defined apomixis in higher plants as being divided into two groups of phenomena: agamospermy and vegetative multiplication. Richards (1997) removed the vegetative form of apomixis in the chapter Agamospermy in his 2nd edition of Plant Breeding Systems, arguing that it is not a breeding system. No matter how we choose to define it, the damage has been done and confusion will continue to reign.

Reichel refers us to Goffinet and Shaw (2009) for a discussion of apogamy and apospory: a life cycle without sex and meiosis, where the term is avoided in a discussion where its use would be appropriate with the broader definition. Similarly, information on apomixis can be found in the discussion of asexual reproduction in mosses by Newton and Mishler (1994).

"But who knows," Reichel continues, "perhaps we just do not have enough data to find sporophytes producing $2n$ spores after a failed attempt at meiosis (this would, I think, be equivalent to 'diplospory' in seed plants) etc!" We agree with Reichel: "Since the frequency and importance of all this in nature appears to be largely in the dark and/or controversial, perhaps it's still more important to describe what is seen than to try to find the right box and label."

Reproductive Tradeoffs

When conditions are constant, we can expect either sexual or asexual reproduction to dominate, ultimately to the loss of the other (Brzyski *et al.* 2014). But conditions are not constant, and year-to-year or habitat variations can favor one reproductive system in some years and the other system in other years (Bengtsson & Ceplitis 2000; Bowker *et al.* 2000). That is, the relative fitness varies among years and habitats. For example, in *Marchantia inflexa* (Figure

54), females invested more in asexual reproduction in man-made environments relative to females in natural habitats, and relative to males in similar habitats (Brzyski *et al.* 2014).



Figure 54. *Marchantia inflexa*. Photo by Scott Zona, with permission.

Cost of Sexual Reproduction

But what is the cost of producing a sporophyte, or more generally, of reproducing sexually? The basic assumption is that reproduction is costly, *i.e.* that a tradeoff exists between present reproduction and future performance (cost of reproduction) (Bell 1980; Williams 1996). Ehrlén *et al.* (2000) provided the first estimates of cost of sporophyte production, using the moss *Dicranum polysetum* (Figure 55) by experimentally manipulating sexual reproduction. They estimated that 74.8% of the total carbon allocation into top shoots during the study interval of about one year went to sexual structures in sporophyte-producing shoots. Shoots that aborted all sporophytes had significantly higher growth rates in the top shoots than did those with sporophytes. The difference in the mass of vegetative apical growth between control shoots and shoots in which sexual reproduction was manipulated was mainly because of different length increments. Mass per unit length was similar between groups.

In the same species, *Dicranum polysetum* (Figure 55), Bisang and Ehrlén (2002) found by examining patterns of growth and reproduction in shoots that females invest 16% of their productivity, as measured by photosynthetically active gametophyte biomass, into reproduction leading to sporophytes, but only 1.3% when eggs remain unfertilized, providing evidence of reproductive cost. Consequently, there is a negative correlation between development of mature sporophytes and annual shoot segment and innovation size. Sporophyte development further reduced the probability of future perichaetial development and mass of new perichaetia. It appears that the gametophyte and sporophyte must compete for limited resources within the plant.



Figure 55. *Dicranum polysetum*, one of the few bryophytes producing multiple sporophytes from one gametophyte apex. Photo by Janice Glime.

Laaka-Lindberg (2001) explored biomass allocation in the leafy liverwort *Lophozia ventricosa* var. *silvicola* (Figure 65). She found that females allocated an average of 24% of their biomass to sexual reproduction whereas males allocated only 2.3%. Gametangial shoots had shorter stem length and modified branching patterns.

Costs for sporophyte formation were also demonstrated in other species, measurable as lower shoot elongation in *Entodon cladorrhizans* (Figure 56) (Stark & Stephenson 1983), less favorable size development and branching patterns in *Hylocomium splendens* (Figure 57) (Rydgren & Økland 2002, 2003), and decreased regeneration capacities in *Pterygoneurum ovatum* (Figure 58), *Tortula inermis* (Figure 59) and *Microbryum starckeanum* (Figure 60) (McLetchie & Stark 2006; Stark *et al.* 2007, 2009, and references therein). Stark *et al.* (2009) induced sporophytic abortion in *Pterygoneurum ovatum*, and subjected plants to upper leaf removal and nutrition amendment treatments. The sexually reproducing plants were less likely or were slower to regenerate tissues or parts (protonemata or shoots). Nutrient amendment had no effect on ability or time of sexual reproduction or on the ability to regenerate clonally. Removal of leaves around the sporophyte base made the sporophytes slower to mature, less likely to mature, and smaller than those with their normal leaves remaining. Hence, there appears to be a cost in future development due to sexual reproduction.



Figure 56. *Entodon cladorrhizans*, a species in which shoot elongation is affected by sporophyte production. Photo by Janice Glime.



Figure 57. *Hylocomium splendens*. Photo by Amadej Trnkoczy through Creative Commons.



Figure 58. *Pterygoneurum ovatum* with capsules. Photo by Hermann Schachner, through Creative Commons.



Figure 59. *Tortula inermis* with young sporophytes. Photo by Michael Lüth, with permission.

Stark *et al.* (2000) also found that males in *Syntrichia caninervis* (Figure 61) seem to invest more in antheridia than do females in archegonia. They made two assumptions and suggested that these may apply to other female-biased populations: 1) that male sex expression is more expensive than female; 2) that sexual reproduction is resource limited. This would give support to the "cost of sex" hypothesis, which predicts that the sex that is more expensive should be the rarer sex (Stark *et al.* 2000).



Figure 60. *Microbryum starckeanum* with sporophytes. Photo by Jonathan Sleath, with permission.



Figure 61. *Syntrichia caninervis*. Photo by Proyecto Musgo, through Creative Commons.

Using the dioicous moss *Drepanocladus trifarius* (Figure 62), Bisang *et al.* (2006) asked whether the formation of sexual structures indeed incurred a cost in terms of reduced growth or future sexual reproduction. This species is female dominant but rarely produces sporophytes. The annual vegetative segment mass was the same among male, female, and non-sexual individuals, suggesting there was no threshold size for sexual expression. On the other hand, sexual branches in females exhibited higher mean and annual mass than did those in males, while branch number per segment did not differ from that of males. Females thus had a higher prefertilization reproductive effort (11.2%) than did males (8.6%). Nevertheless, these investments had no effect on vegetative growth or on reproductive effort in consecutive years. Therefore, a higher realized reproductive cost in males, suggested to occur in the desert moss *Syntrichia caninervis* (Figure 61) (Stark *et al.* 2000), cannot explain the unbalanced sex ratio in *Drepanocladus trifarius* (Figure 62).



Figure 62. *Drepanocladus trifarius*. Photo by Andrew Hodgson, with permission.

Spore Size and Number

Spore size matters as well. During (1992) points out that when spores are small, bryophytes have the problem of juvenile mortality risk, but when they are large, the species has reduced dispersal potential. So it is not only a tradeoff in expenditure of parental energy vs providing offspring energy, or having many offspring vs few, but one of expanding the species to new areas vs staying put.

It would seem that having lots of large spores would overtax the female, whereas producing lots of small spores would provide ample opportunity to reach a suitable location for development of progeny. A compromise might be reached, but apparently has rarely been achieved by bryophytes, by having small male and large female spores. But is there further tradeoff to having lots of small spores? Noguchi and Miyata (1957) think there is. Their data indicate that mosses that produce abundant spores (implying mostly small ones) have a wide geographic range – the result of improved dispersal for tiny objects borne by wind, but the trade-off is reduced establishment success that restricts their habitats.

Where animals have had the evolutionary choice of producing many small offspring or few large ones and seed plants of producing many small seeds or few large ones, the bryophyte has a choice between producing spores of a small size in great numbers, larger spores but few in number, or producing no spores at all. For those taxa that produce no spores at all, we must assume that for most, either one sex is missing, or that they have spread beyond the range in which the proper signals and conditions permit them to produce spores. This usually means that fertilization cannot be accomplished. In these cases, vegetative means maintain the population and even permit it to spread to new localities, an option not available to most other groups of organisms.

Sexual vs Asexual Strategies

Sexual vs asexual strategies affect **metacommunity** (set of interacting communities which are linked or potentially linked by the dispersal of multiple, potentially interacting species) diversity (Löbel *et al.* 2009). In a study of Swedish obligate epiphytic bryophytes, forest patch size affected the species richness of monoicous species that reproduced sexually, whereas it did not affect the dioicous species that reproduced asexually. Löbel *et al.* found that it could take several decades for monoicous species to reach sexual maturity and produce spores. The researchers indicated that population connectivity in the past was more important for species richness in monoicous taxa than present connectivity. The difference in reproductive potential creates a tradeoff between dispersal distance and age of first reproduction. They suggested that this may explain the parallel evolution of asexual reproduction (primarily dioicous taxa) and monoicy for species that are able to live in patchy, transient habitats. Success in these conditions implies that relatively small changes in the habitat conditions could lead to distinct changes in the diversity of the metacommunity, wherein species using asexual reproduction may drastically decline as distances among patches increase, whereas those sexually reproducing species may decline as patch dynamics increase. (Sexual vs asexual strategies are discussed

further in Chapter 4-7, Adaptive Strategies: Vegetative vs Sexual Diaspores, in this volume.)

Bet Hedgers

Bet hedgers are those species that use multiple strategies, often making each of those strategies less successful than they might be if all energy were concentrated on one of them. They are beneficial in unpredictable environments where one strategy is best in some years and another in different years or where disturbance may occur.

Specialized asexual reproductive structures such as gemmae require energy and thus compete with productions of sexual structures. But it seems that at least some, perhaps most, of the bryophytes are bet hedgers by maintaining both vegetative and sexual reproduction. They may reduce this competition for energy by temporal separation of the programmed asexual and sexual reproductive stages. For example, in the thallose liverwort *Marchantia polymorpha* (Figure 34-Figure 35), in which large archegoniophores and antheridiophores require considerable tissue production, the production of gemma cups and their asexual gemmae is timed so it does not coincide with development leading to sexual activity (Une 1984). In the moss *Tetraphis pellucida* (Figure 63-Figure 64), the terminal position of the gemmae and their splash cups precludes the simultaneous production of the likewise terminal reproductive structures.

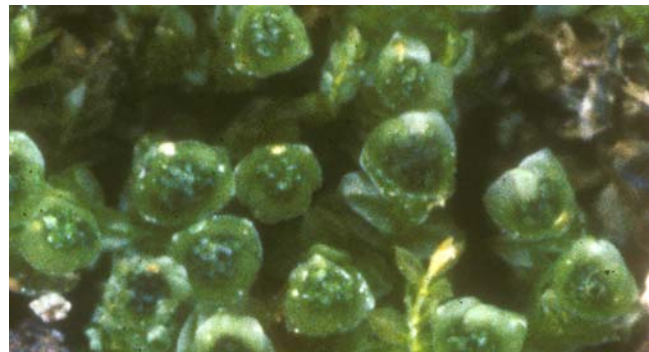


Figure 63. Top view of *Tetraphis pellucida* showing terminal gemma cups that prevent simultaneous development of reproductive structures. Photo by Janice Glime.



Figure 64. Side view of *Tetraphis pellucida* showing terminal gemma cups (and clusters that have lost their cup leaves) that prevent simultaneous development of reproductive structures. Photos by Michael Lüth, with permission.

Nevertheless, distinct tradeoffs between sexual and asexual reproduction have been detected. In studying biomass allocation of the leafy liverwort *Lophozia ventricosa* var. *silvicola* (Figure 65) Laaka-Lindberg (2001) found that sexual reproduction affected gemmae production. Female shoots averaged 800 gemmae, males 1360, and asexual shoots 2100, revealing a trade-off between sporophyte production (female sexual reproduction) and number of gemmae (asexual reproduction). In *Marchantia inflexa* (Figure 54), female sex expression was negatively associated with gemmae production under certain light conditions (Fuselier & McLetchie 2002). In agreement, *Marchantia polymorpha* ceases gemmae cup production during the period of producing sexual reproductive structures (Terui 1981). Pereira *et al.* (2016) reported a trade-off between prezygotic investment into gametangia and asexual reproduction, in terms of fewer gametangia in gemmae-producing shoots compared to barren shoots. Both the formation of gametangia and gemmae were in their turn positively associated with monthly precipitation. In contrast, Holá *et al.* (2014) suggested a minimal trade-off between sexual and asexual reproduction to occur in the aquatic liverwort *Scapania undulata* (Figure 66-Figure 67) as they found high gemmae production on male and female sex-expressing shoots.



Figure 65. *Lophozia ventricosa* showing gemmae on leaf tips. Photo by Jan-Peter Frahm, with permission.



Figure 66. *Scapania undulata*, a male-biased dioicous liverwort. Photo by Hermann Schachner, through Creative Commons.



Figure 67. *Scapania undulata* gemmae. This species produces numerous gemmae at the leaf margins on both males and female plants. Photo by Paul Davison, with permission.

Whereas tracheophytes may often reproduce by bulbs, rhizomes, stolons, or other specialized bulky organs, bryophytes have the advantage that most can reproduce by tiny fragments (Figure 68) from any part of the gametophyte, and under the right conditions, sometimes even sporophyte parts, all of which can travel more easily than the bulky organs of a tracheophyte. This strategy is an effective fallback even for many successive years of spore production failure.

The Japanese and others have taken advantage of fragmentation to propagate their moss gardens, pulverizing mosses, then broadcasting them like grass seed (Shaw 1986; Glime pers. obs.). For some mosses, like *Fontinalis* species (Figure 69) (Glime *et al.* 1979) or *Bryum argenteum* (Figure 70) (Clare & Terry 1960), fragmentation may be the dominant reproductive strategy, and for those dioicous taxa where only one sex arrived at a location, or one or the other sex is not expressed, or sexes are spatially segregated, it is the only means.



Figure 68. *Syntrichia caninervis* protonemata produced from a leaf fragment. Photo courtesy of Llo Stark.



Figure 69. A clump of *Fontinalis novae-angliae* that has been scoured and broken loose from its substrate. Photo by Janice Glime.



Figure 70. *Bryum argenteum* showing large terminal buds that break off and disperse the plant. Photo by Janice Glime.

Growth vs Asexual Reproduction

Gemma cup number was negatively related to vegetative meristematic tips in *Marchantia inflexa* (Figure 54) (McLetchie & Puterbaugh 2000). Gemma production in *Anastrophyllum hellerianum* (Figure 71), on the other hand, did not affect shoot mortality (Pohjamo & Laaka-Lindberg 2004).



Figure 71. *Anastrophyllum hellerianum* with gemmae in Europe. Photo by Michael Lüth, with permission.

To test the tradeoffs in growth rate, asexual and sexual reproduction, and allocation to above and below-ground regenerative biomass, Horsley *et al.* (2011) cloned *Bryum argenteum* (Figure 70) for a growth period of 92 days, replicating each genotype 16 times, to remove environmental effects. There appeared to be three distinct ecotypes among the populations tested (representing 12 genotypes). It appears that the degree of sexual vs asexual reproductive investment is under genetic control. Furthermore, growth of the protonemata was positively correlated with both asexual and sexual reproduction. Asexual reproduction (Figure 72) was negatively correlated with shoot density, suggesting an energetic trade-off. None of these relationships appeared to be sex-specific. The sexes did not differ in growth traits, asexual traits, sexual induction times, or above- and below-ground biomass, but female sexual branches (Figure 73-Figure 75) were longer than those of males (Figure 76-Figure 77). Males produced many more perigonia (Figure 76) per unit area of culture media than the perichaetia produced by females, giving males 24 times the prezygotic investment. Horsley *et al.* considered that this strong sex bias in energy investment in male perigonia could account for the strongly female-biased sex ratio.



Figure 72. *Bryum argenteum* with terminal (1) and lateral (2) shootlets. Photo from Horsley *et al.* 2011.

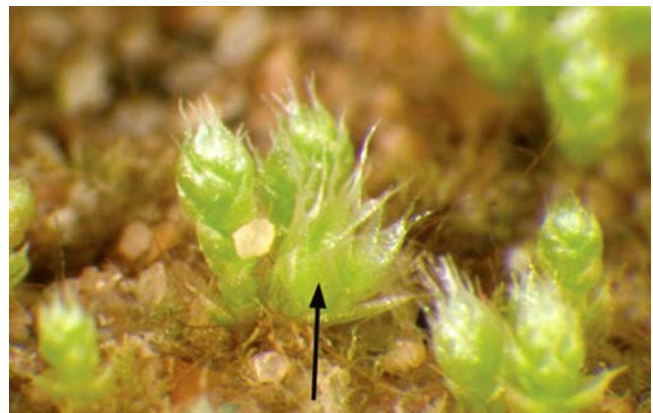


Figure 73. *Bryum argenteum* female plants. Photo from Horsley *et al.* 2011.



Figure 74. *Bryum argenteum* female plant with excised perichaetial leaves and archegonia. Photo modified from Horsley *et al.* 2011.

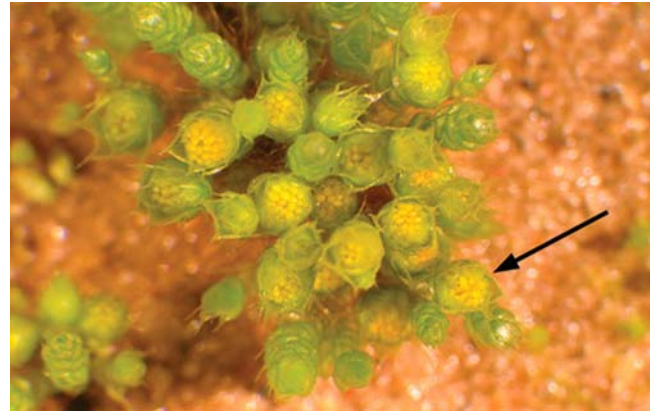


Figure 76. *Bryum argenteum* male plants, illustrating the numerous perigonia and antheridia present. Photo from Horsley *et al.* 2011.



Figure 77. *Bryum argenteum* male plant with excised perigonial leaves and antheridia. Photo modified from Horsley *et al.* 2011.

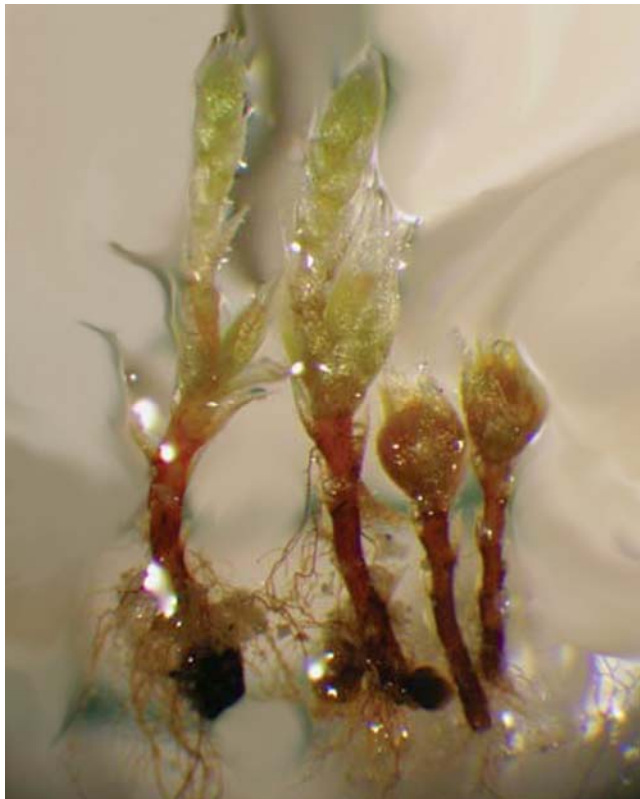


Figure 75. *Bryum argenteum* female (left) and male (right) plants, illustrating sexual dimorphism. Photo modified from Horsley *et al.* 2011.

Significance of a Dominant Haploid Cycle

Longton (2006) provided evidence that dispersal of a spore is an extremely important aspect of bryophyte success in establishing new populations, whereas vegetative reproduction is more important for colony expansion and maintenance. Spores are $1n$ (**haploid**), and to be effective as a dispersal propagule, that body derived from the spore must have the characters needed for survival of the environment. This contrasts with those plants where it is a $2n$ (diploid) seed that gets dispersed. In the latter case, the $2n$ plant provides the needed environment for the development of the gametophyte, and the gametophyte is greatly reduced and resides mostly within the tissues of the

$2n$ plant. Hence, those plants (bryophytes and non-seed tracheophytes) that disperse largely by spores must find a suitable habitat for their gametophytes (See chapter on Dispersal).

In bryophytes, the diploid stage is forever attached to the haploid stage and dependent at least partially upon it. Haig and Wilczek (2006) point out that the diploid stage has one set of nuclear genes in common with its haploid mother, in addition to obtaining resources from that mother; the paternal haploid genes are not in common with those of the mother. They explain that all of the "offspring's maternal genome will be transmitted in its entirety to all other sexual and asexual offspring that the mother may produce," but not all will have the genes of the father. Haig and Wilczek suggest that this will favor genomic imprinting and predict that a "strong sexual conflict over allocation to sporophytes" will occur. Furthermore, chloroplast genes are inherited from the mother, but there has been little or no assessment of the effect this has on physiological behavior or environmental needs of bryophyte species as they relate to sexual bias.

Ricklefs (1990) reminds us that, just as in the algae, the haploid ($1n$) plant has the ability to express its alleles in the generation where they first occur, whereas the diploid ($2n$) plants have the ability to mask deleterious recessive alleles. The haploid ($1n$) generation possesses "immediate fitness" if a favorable change occurs among the alleles, but is immediately selected against if the change is unfavorable, unless, of course, the trait is one not expressed in the gametophyte. This immediate expression is a tradeoff with the ability to mask genes that may be retained and beneficial in a different location or different point in time.

Zeyl *et al.* (2003) used yeast, with both haploid and diploid generations, to test the question of whether there is any advantage to being haploid. Based on their experiments, they argued that being haploid permits an organism to accumulate beneficial mutations rather than to avoid the effects of those that are deleterious. This is founded on the premise that even beneficial genes are masked in diploid organisms and thus provide no immediate advantage, if ever. Rather, the rate at which a beneficial gene increases in frequency in a haploid organism is far greater than in a diploid organism (Greig & Travisano 2003). Of course it is never the case that all genes are expressed simultaneously, or even that all genes are expressed during the lifetime of an organism. They are there to be turned on when the physiological state of the organism calls for them.

Zeyl *et al.* (2003) hypothesized that in small populations, the haploid organisms would lose their advantage. They reasoned that by having twice as many of each gene, diploid organisms may have an increased rate at which adaptive mutations are produced. Hence the supply of adaptive mutations would be reduced, rather than any reduction in the time required to fix them. By doubling the adaptive mutation rates (diploidy) the adaptive mutations become more important in small populations. When adaptive mutations are rare the rate of adaptation by diploid populations approaches a doubling of that found in haploid populations. In small populations, having two sets of chromosomes is an advantage if the adaptive mutations are dominant because they will be expressed and gain

prominence through natural selection. But when the mutations are recessive, diploidy is a disadvantage because the mutations are not often expressed. In large populations, the extra genes (of the $2n$ state) would gain little advantage over the increased rate of expression of mutated genes.

Their experiments with haploid and diploid yeast (*Saccharomyces cerevisiae*) supported their hypothesis; in large populations, haploid populations adapted faster than diploid populations, but this was not the case when both populations were small (Figure 78) (Zeyl *et al.* 2003). They reasoned that a greater adaptation rate is not a general consequence of diploidy and does not, by itself, explain the prominence of diploidy in plants or animals. However, in their experiments they did not permit the yeast to mate, thus reducing the advantage of mixing in diploid organisms with chance mating of two beneficial or complementary mutations.

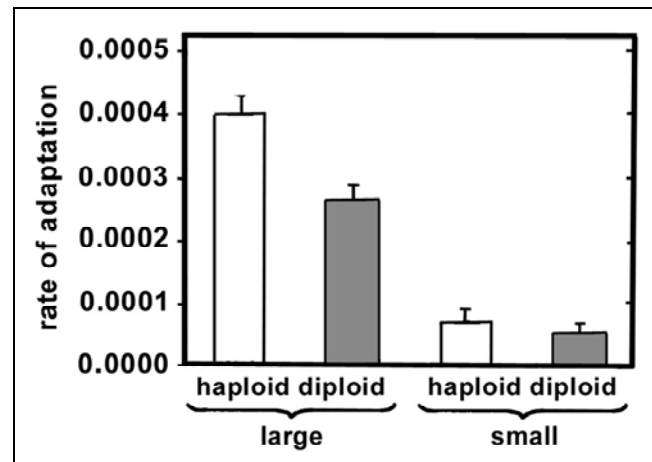


Figure 78. Rates of adaptation in large and small haploid and diploid populations of yeast. Bar length and 95% confidence interval was determined by slopes using linear regression of fitness on the generation number ($n=5$ pooled for 4 regressions). Ploidy was highly significant for large populations ($p<0.001$), but not for small populations ($p=0.35$). (2-tailed heteroscedastic t tests). Modified from Zeyl *et al.* 2003.

Would these experiments on one-celled yeast produce the same results if tried on multicellular bryophytes? There are genera, for example in the **Mniaceae**, in which some monocious taxa possess a double set of chromosomes, apparently derived from a dioicous taxon with a single set. These would seemingly make appropriate experimental organisms for such testing. Our current molecular methods should make such an evaluation possible.

Having a dominant gametophyte has its limits, however. Longton and Schuster (1983) remind us that, unlike tracheophytes, once having achieved fertilization, the bryophyte is able to produce only a single sporangium that subsequently produces spores all at one time (except in **Anthocerotophyta**). On the other hand, tracheophytes (polysporangiate plants) produce many branches, hence many sporangia, and these may be produced on the same plant year after year, all resulting from a single fertilization. The closest behavior to this among the bryophytes is in **Anthocerotophyta**, a dubious bryophyte as noted earlier, where meiosis occurs on the same sporophyte over a period of time, with older spores at the apex and new ones produced at the base of the sporophyte (Schofield 1985).

But bryophytes are more '**polysporangiate**' than they might seem. Whereas they cannot produce multiple sporophytes from a single fertilization, pleurocarpous species do have multiple sporangia produced on a single gametophyte plant (Figure 79), each potentially with a different combination of genes. And most bryophytes are **perennial** (persisting for multiple years), thus in most cases sequentially accomplishing multiple fertilizations under multiple conditions and selection pressures. Furthermore, the meiotic events in multiple cells of sporogenous tissue, even though all in one sporophyte, result in different sortings of chromosomes, thus different combinations among the many spores produced.



Figure 79. *Callicladium haldanianum* showing multiple capsules from one plant. Photo by Misha Ignatov, with permission.

In diploid plants, on the other hand, the number of recessive alleles continues to increase until the effect of their expression is the same in the **homozygous diploid state** (both alleles for a trait are the same) as it is in their haploid state (Ricklefs 1990). This provides the diploid organism with a short-term advantage of maintaining steady state while sequestering alleles that may at a later date become advantageous due to changing environmental conditions. A further advantage to diploid plants is that heterozygous organisms (those having two different alleles for the trait) frequently are the most fit, in some cases due to complementation (two traits that complement or help each other), in others due to having more possibilities of possessing fit alleles. On the other hand, presence of two alleles can mask somatic mutations (*i.e.*, mutations in non-reproductive cells) that ultimately could result in a lack of coordination between cells. Perhaps this lack of masked genes is only a disadvantage for a large (complex) organism that must keep all its parts working together, whereas in organisms where there are few cell types to coordinate, the condition is less likely to be problematic, particularly in an organism where vegetative reproduction is often the rule and little other specialization occurs.

Immediate fitness of haploid organisms permits the few individuals possessing a trait to exploit a new situation, whereas the delayed fitness of diploid organisms that require a like partner is unlikely to permit these species to respond quickly to environmental change.

One complication to this scenario of haploid and diploid is that often haploid organisms are not pure haploids. In fact, it appears that **autopolyploidy** (having more than 1 set of homologous chromosomes in the gametophyte) has been a significant factor in bryophyte evolution (Newton 1984). Many, probably most, genes are identical in the two sets, but some differ, and possibly in rarer cases, an entire chromosome may differ. These cases of autopolyploidy result in **functional haploidy** (Cove 1983), albeit with twice as many alleles as were present in the parent species. But does meiosis subsequently separate them into the same identical sets after fertilization has joined these with a new doubled set? Wouldn't this be an opportunity for new combinations of alleles to have different homozygosity and heterozygosity?

Do Bryophyte Sexual Systems Affect Genetic Diversity?

Where do these strategies leave bryophytes in their genetic variation? Bryonettors questioned the lack of diversity in bryophytes (see also Glime 2011). Do their mating systems, and in some cases lack of them, affect their genetic diversity?

Most people think of diversity in terms of morphology. But genetic diversity may not be expressed as morphological diversity. Rather, differences in biochemistry may occur without our recognition. Recent studies using molecular and phylogenetic methods support the conclusion that bryophytes in fact have greater diversity than we have supposed, as evidenced by the genetic differences between geographically different populations (Shaw *et al.* 2011).

Although differences in form among closely related species of small organisms such as bryophytes are limited because of their small number of cells and small size, we are beginning to find that physiological variety is great. Stenøien and Sæstad (2001) suggest that the mating system does not really matter in bryophytes in this respect. Rather, inbreeding can profoundly influence variation in the haploid generation. Furthermore, high levels of selfing are not a necessary consequence of being monoicous, as outbreeding is still possible, and even likely in some cases (see Reproductive Barriers above). Such mechanisms as different male and female gametangial maturation times would force outbreeding. Rather, the monoicous condition provides many other individuals nearby with whom genes can be exchanged, and it is possible that some of these have come from spores that represent a new combination of genes.

Whereas seed plants spend most of their lives with two sets of chromosomes ($2n$), they seldom express the mutations that arise because a second allele is present that still retains the old trait. For example, the absence of a gene to code for making a red pigment in the leaf might result in a green leaf in a species that would normally have a red leaf. Organisms with such hidden traits therefore have hidden changes that are retained in the population and that might at some future time be an advantage when conditions change. The ability to retain traits provides the plants with variability that might mean future success, but that do little for immediate fitness. In our pigment example, red pigment could protect the leaf against strong UV light, but if greenhouse gases and atmospheric exhaust

were to shield the Earth from UV light and reduce the light available for photosynthesis, being red might be less advantageous and a green leaf might then become beneficial for trapping more of the photosynthetically active portion of the spectrum.

Haploid bryophytes, on the other hand, cannot carry adaptive genes in a second set of chromosomes, but rather have immediate fitness or lack of fitness with the advent of a new gene. If these beneficial mutations occur in vegetative cells, they can be carried forward in clones or established in new colonies through fragmentation with no masking effects. Hence, if the bryophyte has a red pigment to protect it against strong UV light, it might not succeed in the shade, but those microspecies with no red pigments are immediately ready for the lower light levels. The individuals that do not have suitable genes may die, but those that have them are immediately fit.

Perhaps the answer to the paradox of genetic variation without cross fertilization does lie in asexual reproduction. It seems that asexual reproduction in bryophytes, unlike that of tracheophytes, may be a source of considerable variation (Mishler 1988, Newton & Mishler 1994). In addition to fragmentation, we know that bryophytes produce a variety of asexual propagules or gemmae (see Gemma-bearing Dioicous Taxa above and Chapter 4-10 of this volume) both above- and below-ground.

Clearly, producing gemmae or other propagules has served the dioicous taxa well. Growth by divisions of a single apical cell (instead of a meristematic region as in higher plants) can provide considerable genetic variation, with the fitness being determined almost immediately (Newton & Mishler 1994). Subsequent branches from this new growth, including gemmae and other propagules, and fragments that form new plants, would spread this new genetic variant. In some taxa, for example *Lophozia ventricosa* var. *silvicola* (Figure 65), the number of gemmae produced annually seems to outnumber the number of spores (Laaka-Lindberg 2000). Mishler (1988) suggested that sexuality is regressing in bryophytes with a concomitant increase in asexual reproduction, as later supported by During (2007) and others, particularly for dioicous bryophytes with high propagule production. Mishler feels that genetic variability is being maintained through somatic mutation, a suggestion by Shaw (1991) to explain variability in *Funaria hygrometrica* (Figure 10, Figure 38-Figure 39). The loss of sexuality is in sharp contrast to the suggestion of Longton (1997, 1998) that the monoicous condition will increase and with it the success of sexual reproduction.

If bryophytes can truly accomplish somatic mutations and make new plants, and they can derive new combinations from mating of autopolyploid plants, why then, are bryophytes still seemingly so primitive? Have they had a particularly slow evolution, with mutations providing little or no advantage? Some researchers have defended the position of slow evolution by referring to their small chromosome number (base = 9 or 10 in most, but 4 or 5 in some). Speculation suggests that their lack of structural support places severe limitations on the size bryophytes can support and the efficiency of water movement internally. This, in turn, limits the structural complexity they can support. However, recent biochemical evidence supports a genetic evolution as rapid as that of

lignified plants (Asakawa 1982, 1988, 2004; Asakawa *et al.* 1979a, b, c, 1980a, b, 1981, 1990, 1991, 2012; Mishler 1988; Stoneburner 1990; Newton & Mishler 1994). That is to say, the rate of allele change and the number of isozyme differences found among species is as great as in their more complicated lignified relatives.

So where have all these genetic changes been expressed? One explanation is that the bryophytes harbor a tremendous variety of secondary compounds (Asakawa 1982, 1988, 2004; Asakawa *et al.* 1979a, b, c, 1980a, b, 1981, 1990, 1991, 2012), *i.e.* compounds that do not seem to have any direct role in any metabolic pathway. Their apparent role in antiherbivory, antibiotics, and protection from desiccation and light damage may be the secret to the continuing success of the bryophytes.

With an understanding of the life cycle, we can begin to understand the conditions that are required for the survival of an individual species. Yet, few studies have examined the requirements and responses of individual species throughout all the stages of their lives. Their absence on a given site may relate to climatic events during their juvenile life when they must bridge the stage between spore and leafy plant, when they are a one-cell wide protonema and fully exposed with no protection from desiccation or blazing sun, or when they arrive as other forms of propagules (Cleavitt, 2000, 2002a, b). In the coming chapters we will examine their growth patterns, the effects of their habitats on their phenology, and their ability to adjust to habitat variability.

The Red Queen Hypothesis

Nothing in the life of a species plays a more important evolutionary role than reproduction. The ability to retain non-expressed genes that may later be expressed and be beneficial permits organisms to be pre-adapted to sudden or gradual changes in their environment.

The terminology **Red Queen** derives from Lewis Carroll's *Through the looking-Glass*. The Red Queen explained to Alice the nature of Looking-Glass Land: "Now, here, you see, it takes all the running you can do, to keep in the same place." Van Valen (1973) saw coevolution as running to keep in the same place.

The **Red Queen Hypothesis** was first proposed by Van Valen (1973) as an evolutionary hypothesis that proposes that organisms must "constantly adapt, evolve, and proliferate not merely to gain reproductive advantage, but also simply to survive while pitted against ever-evolving opposing organisms in an ever-changing environment." Van Valen devised the hypothesis to explain constant extinction rates exhibited in the palaeontological record as a result of competing species on the one hand and the advantage of sexual reproduction by individuals on the other. The theory was developed to explain predator-prey and host-parasite interactions in the evolution of animals. If the prey developed more skill in avoiding the predator, the predator subsequently developed more skill in catching the prey. If a host developed immunity to a parasite, the parasite that survived was a more virulent or aggressive one. The theory expanded to explain other evolutionary drivers. In our context here, it emphasizes the importance of sexual reproduction in maintaining protection against changes in the environment, including predators and parasites.

An example of the workings of this concept can be illustrated by the snail *Potamopyrgus antipodarum* (Jokela *et al.* 2009). When mixed asexual and sexual populations of this snail were cultured, the parasite population increased. The asexual snails were quickly reduced by the parasites, with some clones going extinct. Sexual populations, on the other hand, remained nearly stable over time, apparently adapting through genetic selection for the resistant genotypes that had been carried as a result of sexual mixing. Kerfoot and Weider (2004) supported the Red Queen Hypothesis by demonstrating a genetic relationship between changing predators and prey (*Daphnia*) through time using diapausing eggs of *Daphnia*, a parthenogenetic cladoceran. These eggs were derived from cores of sediment in Portage Lake from 1850-1997 and the eggs subsequently cultured to assess changes in characters. Clay and Kover (1996) tested the hypothesis in plant host-parasite interactions. They found that portions of the theory are supported, but not all.

At first this may not seem to apply to bryophytes, but consider the wide array of secondary compounds present among them. These compounds are known for their ability to protect the bryophytes from bacteria, fungi, and herbivores. This consideration can be considered as a parallel to the predator-prey or host-parasite relationships. As more herbivores evolved to attack the bryophytes, those bryophytes with the most protective array of secondary compounds were most likely to survive. But can it help to explain the persistence and re-introduction of the dioicous condition in bryophytes, as demonstrated for some animals (Morran *et al.* 2011)?

Sexual reproduction at the gene level permits sexually reproducing organisms to preserve genes that may be disadvantageous at present, but that may become advantageous under future conditions. This is somewhat complicated in bryophytes because of the dominance of the haploid gametophyte. But if the gene is not disadvantageous, or it is expressed only in the sporophyte, it could remain in the genetic line for centuries. If these genes code for secondary compounds that have been effective against predators, bacteria, fungi, or other dangers, they may be conserved in the genotype even if the danger is no longer present. And as new dangers arose, different secondary compounds would have been preserved in the genome, with the surviving bryophytes changing as the dangers changed. If the Red Queen Hypothesis applies, we should be able to see changes in the secondary compounds or the genome that relate to changes in the dangers. We can argue that the variability provided by the dioicous condition makes such changes possible to a greater extent than does the monoicous condition.

To our knowledge, there has been no test of the Red Queen hypothesis in bryophytes. Suitable fossils are scarce, but we should be able to test these ideas in ice cores that provide living organisms as much as 1500 years old (Roads *et al.* 2014)! By growing new organisms from fragments (see La Farge *et al.* 2013; Roads *et al.* 2014), we can compare the genes and also the potential responses to bacteria, fungi, or predators by looking at concentrations of secondary compounds using methods similar to those of Kerfoot and Weider (2004) for *Daphnia*.

Surviving in the Absence of Sexual Reproduction

Surviving unfavorable conditions is often a sexual function. In algae, **zygospores** (resting, resistant stage following fertilization) are the most common means of survival. In many invertebrate animals, including those living among bryophytes, the fertilized egg is likewise often the survival stage. Bryophytes do not use the fertilized egg to survive unfavorable conditions because that stage is dependent on the leafy haploid stage. Rather, many can produce sexual spores (meiospores) that survive during periods of drought and other unfavorable conditions. Spores are known to survive for long periods (See Chapter on Dispersal). Some species form persistent sporebanks that allow them to bridge unfavorable periods, then become active following disturbance. But bryophytes have many physiological means that permit them to survive without sexual reproduction.

As an alternative to spore survival, bud survival is important to some species. Haupt (1929) found that the thallose liverwort *Asterella californica* (Figure 80) survives hot, dry summers on banks and canyon sides in southern California as a leafy plant, but that only the ends of branches remain alive, starting new plants in autumn when sufficient moisture returns. In southern Illinois, *Fossombronina foveolata* (Figure 81) produces capsules in spring, but likewise survives the dry summer by means of its terminal bud, resuming growth in autumn and producing capsules a second time that year on the same plant (James Bray, pers. comm.).

These physiological mechanisms permit bryophytes to survive through vegetative reproduction for many years in the absence of sexual reproduction. And bryophyte species, unlike most tracheophytes, can survive for centuries without the intervening genetic mixing and resting stages afforded by sexual reproduction.



Figure 80. *Asterella californica* with archegoniophores and terminal buds that are able to survive drought. Photo by David Hofmann, through Flickr Creative Commons.



Figure 81. *Fossombronia foveolata* with young sporophytes and resistant terminal buds that can survive drought. Photo by Des Callaghan, with permission.

Bryophytes vs Seed Plants

The higher percentage of dioecy in bryophytes than in seed plants still begs explanation, and we have discussed possible explanations above and especially in Chapter 3.1. Could it in addition be that fragmentation, generally only available in poorly dispersed underground structures in seed plants, but available and easily dispersed from any part of the plant in bryophytes, might account for greater success of the dioicous condition among bryophytes?

Furthermore, since bryophytes are haploid-dominant, being dioicous provides immediate production of new genotypes as soon as sexual reproduction occurs, thus making selection for this strategy more rapid than in seed plants. Does this explain the high degree of dioecy among the early-diverging bryophyte group, where there has been considerable time to develop the best of the two strategies?

One answer may lie in short-distance dispersal of the male gametes, coupled with ease of vegetative reproduction in bryophytes. In seed plants, the male gametophyte (pollen grain) is more easily dispersed with less danger to its viability. There has been an enormous amount of evolution perfecting transfer by vectors, especially insects, among seed plants. While this would seem to improve dioecy fertilization success, it also provides for considerable outcrossing success for monoecy. It may also be the case that seed plants have more effective mechanisms for preventing successful self-fertilization. On the other hand, the vegetative ability to reach new locations is extremely limited in seed plants, although it can be quite effective over the short distance. For seed plants, long distance dispersal is almost entirely dependent on sexual reproduction. By contrast, many bryophytes can be dispersed considerable distances by both specialized vegetative diaspores and fragments (see for example Laenen *et al.* 2015), thus compensating for any lack of spores.

Summary

Monoecy (both sexes on same individual) frequently has arisen through **hybridization** and **polyploidy** (multiple sets of chromosomes). Barriers to hybridization and to **selfing** in bryophytes are poorly

known. These include **external barriers** such as **spatial/geographic isolation**, **ecological isolation**, and **seasonal isolation**. **Internal barriers** include **gametic isolation**, **genetic incompatibility**, **hybrid sterility**, and **reduced fitness**. Nevertheless, hybridization seems to have played a major role in the evolution of monoecy due to lack of these barriers in many species.

Formation of gametangia and especially sporophyte formation incur reproductive costs measurable in reduced future vegetative and reproductive performance. Overall investment in sexual reproduction may vary among species, in some cases being greater in males and in others greater in females, depending on if assessed at the pre- or postfertilization stage.

Tradeoffs occur between dispersal ability of small spores and success of establishment of large spores. Fragments and vegetative diaspores are most successful at colonizing over short distances and are more likely to succeed than spores. Asexual reproduction can keep the species going for many years in the absence of sexual reproduction. Tradeoffs occur also among asexual reproduction, sexual reproduction, and vegetative performance. These tradeoffs vary among species.

The dominant haploid state of bryophytes limits their ability to store **recessive alleles**, but **autopolyploidy**, **somatic mutations**, **vegetative reproduction**, and **independent assortment** at meiosis contribute to genetic diversity. Despite their clonal nature, bryophytes still exhibit considerable genetic variation. This may be explained in part by the **Red Queen hypothesis**, a hypothesis that also might explain the persistence of evolution to a dioicous condition despite the difficulty of accomplishing sexual reproduction. Inbreeding depression may occur in monoicous bryophytes, but limited data suggest that it may be to a lesser degree compared to that of tracheophytes.

Bryophytes may lack the morphological diversity expressed by sporophytes in higher plants, but there is evidence that haploid plants and their diaspores can contain as much diversity as tracheophytes, often expressed in their biochemistry as a variety of secondary compounds rather than in morphology. They have life strategies that have survived since the beginning of land plants.

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