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3-1-1

CHAPTER 3-1
SEXUALITY: SEXUAL STRATEGIES

JANICE M. GLIME AND IRENE BISANG

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CHAPTER 3-1
SEXUALITY: SEXUAL STRATEGIES

Figure 1. Depiction of the *Marchantia* sexual life cycle in a chalk drawing by Gerald W. Prescott. Photo by Janice Glime.

Expression of Sex

Understanding sexuality is fundamental to understanding evolution, and by extension, to understanding the ecology of the species. The topic of sexual expression has led to interesting discussions for many centuries and still remains to perplex us. On Bryonet, 14 February 2016, Ken Kellman asked several pertinent questions that remain with incomplete answers. His questions included the role of auxins or other hormones in the **perigonium** (leaves surrounding male reproductive structures) and **perichaetia** formation (leaves surrounding female reproductive structures). How does **polyploidy** (multiple sets of genes) relate to separate sexes? How many totally asexual species are there? (In California Kellman is aware that only *Dacryophyllum falcifolium* is never known to form gametangia. And some species are sexual in Europe, but not in North America, *e.g.* ***Hennediella stanfordensis*** (Figure 2), ***Tortula pagorum*** (Figure 3).

Figure 2. *Hennediella stanfordensis*, a species that is sexual in Europe but not in North America. Photo by Martin Hutten, with permission.

Figure 3. ***Tortula pagorum***, a species that is sexual in Europe, but not in North America. Photo by Michael Lüth, with permission.

But it turns out that Ken Kellman's example from California is only a drop in the bucket – many species in Europe are not known to reproduce sexually, including ***Bryoerythrophyllum caledonicum***, ***B. ferruginascens*** (Figure 4), ***Bryum dixonii***, ***Campylopus gracilis*** (Figure 5), ***Didymodon maximus*** (Figure 6), ***Ditrichum plumbicola*** (Figure 7), ***Leptodontium gemmascens*** (Figure 8), ***Pohlia scotica***, ***Thamnobryum cataractarum*** (possibly a form of ***T. alopecurum***), and ***Tortella limosella*** (Christopher Preston, Bryonet 15 February 2016). To these, Misha Ignatov (Bryonet 15 February 2016) added ***Limnohypnum muzushimae***, a rare pleurocarpous species in Kamchatka, Kurils, and Japan. Johannes Enroth, Bryonet 16 February 2016) added ***Caduciella mariei***, a species that occurs in eastern Africa, SE Asia, Queensland, and New Britain; it seems to reproduce only by caducous branch leaves. Liverworts include ***Mastigophora woodsii*** (Figure 9), ***Plagiochila norvegica***, ***Riccia rhenana*** (Figure 10), ***Scapania nimbosa*** (Figure 11), ***Herbertus borealis*** (Figure 12), ***H. norenus***, ***Lophozia wenzelii*** (Figure 13-Figure 14), ***Protolophozia herzogiana***, ***Anastrophyllum alpinum*** (Figure 15), and ***Marsupella arctica*** (Jeff Duckett, Bryonet 15 February 2016). But as Nick Hodgetts pointed out (Bryonet 16 February 2016), some may reproduce by sexual union only rarely and "bryologists are unfortunately likely to miss the event!"

Figure 5. ***Campylopus gracilis*** showing caducous tips by which it reproduces. Sexual plants are unknown. Photo by Michael Lüth, with permission.

Figure 6. ***Didymodon maximus***, a species for which sexual structures are unknown. Photo by Rory Hodd, with permission.

Figure 4. ***Bryoerythrophyllum ferruginascens***, a species not known to reproduce sexually. Photo by David T. Holyoak, with permission.
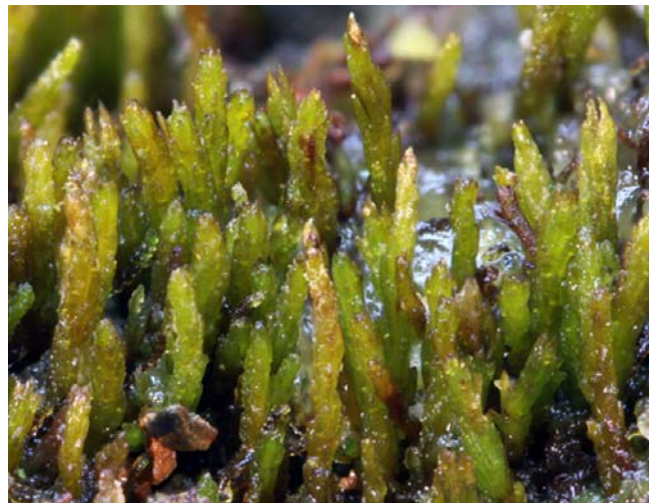

Figure 7. ***Ditrichum plumbicola***, a species for which sexual plants are unknown. Photo by Des Callaghan, with permission.

Figure 8. *Leptodontium gemmascens* with gemmae, a species with no known sexual plants. Photo by Michael Lüth, with permission.

Figure 9. *Mastigophora woodsii*, a species for which sexual structures are unknown. Photo by Des Callaghan, with permission.

Figure 10. *Riccia rhenana*, a liverwort for which there are no known sexual plants. Photo by Štěpán Koval, with permission.

Figure 11. *Scapania nimbosa*, a species that is unknown in a sexual state. Photo by Michael Lüth, with permission.

Figure 12. *Herbertus borealis*, a species with no known sexual plants. Photo by Jan-Peter Frahm, with permission.

Figure 13. *Lophozia wenzelii*, a species with no known sexual plants. Photo by Michael Lüth, with permission.

Figure 14. *Lophozia wenzelii* with water trapped in leaves. No sexual plants are known in this species. Photo by Des Callaghan, with permission.

Figure 15. *Anastrophyllum alpinum*, a species in which sex organs are unknown. Photo by Michael Lüth, with permission.

Unisexual and Bisexual Taxa

Jesson and Garnock-Jones (2012) attempted to provide a single classification of functional sex that could be used for all land plants. They divided the strategies into three categories: sporophyte (and gametophyte) **dimorphic** (having two forms); sporophyte-**cosexual** and gametophyte-dimorphic; gametophyte (and sporophyte) cosexual (having both sexes). Bryophytes exhibit only the latter two of these, always having sporophytes that are cosexual and never dimorphic. The gametophyte is always dimorphic in seed plants. [Note that in seed plants, the female (♀) gametophyte is embedded in the sporophyte tissue and the male (♂) gametophyte is a pollen grain; hence the gametophyte sexes are always on separate gametophyte individuals.] Despite this simplistic approach, Jesson and Garnock-Jones consider that there are many variations within these three categories and that closer examination should reveal that bryophytes have as many variations in strategy as do the more complex seed plants.

In bryophytes, it is the gametophyte (1*n*, **haploid**) plant that exhibits the **bisexual** (**monoicous**) trait. To the seed-plant botanist, the terms monoecious and dioecious are familiar, referring to having male and female organs on one sporophytic individual or on separate individuals, respectively, but the terms are legitimately restricted to sporophytes (Magill 1990). The counterpart to these terms for bryophytes, applied to the gametophyte, are **monoicous**

and **dioicous**. Nevertheless, the sporophyte terms are often applied, as are the terms leaf and stem, but the oicy terms emphasize important differences in bryophyte sexuality (Zander 1984; Allen & Magill 1987; Magill 1990). Their root words are the same, derived from the Greek μόνος (mónos), single, or δι- (di-), twice, double, and οἶκος (oîkos) or οἰκία (oikía), house. In other words, one house for sperm and egg on one plant (**monoicous**) or two houses for sperm and egg on different plants (**dioicous**).

Bryophytes have an unusually high number of **dioicous** taxa (male and female gametangia on separate individuals) among green land plants, roughly 60% (Hedenäs & Bisang 2011) (57% estimated by Villarreal & Renner 2013a) in mosses and somewhat higher in liverworts (68% estimated by Villarreal & Renner 2013a), although McDaniel and Perroud (2012) consider them to be about equal. This may differ somewhat by geographic distribution, but more careful analysis is needed. By contrast, in seed plants only 4-6% of the species are dioecious (Renner & Ricklefs 1995; de Jong & Klinkhamer 2005) and the sex ratio is more likely to be male-biased (Sutherland 1986; Delph 1999; Barrett *et al.* 2010).

Bryophytes exhibit all sorts of arrangements of sexual organs on their **monoicous** species (having male and female gametangia on the same individual), providing them with various strategies for outbreeding. When male and female organs are on separate individuals (Figure 1), outbreeding is ensured whenever sexual reproduction occurs; the opportunities for fertilization decrease and the opportunities for genetic variation increase.

One of the major problems for dioicous species is that one sex may arrive in a new location without the other, as in the case of *Didymodon nevadensis* (Figure 16). On the gypsiferous ridges of Nevada, only female plants are known (Zander *et al.* 1995). Nevertheless, with a variety of vegetative reproductive means, the species can persist.
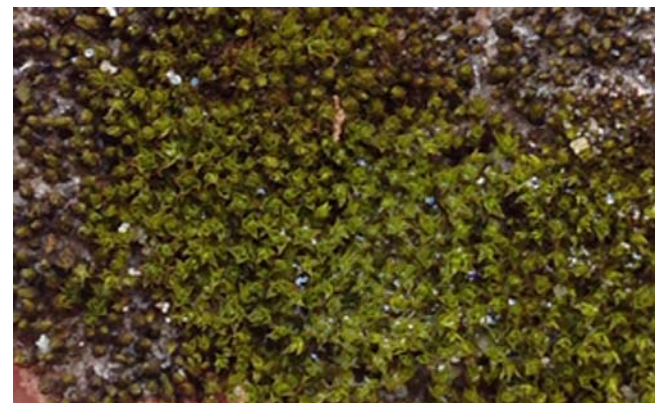

Figure 16. *Didymodon nevadensis*. Photo by Theresa Clark, with permission.

Among the bryophytes, it is well known that many taxa with separate sexes never produce capsules [*e.g. Sphagnum* (Cronberg 1991)], presumably due to absence of the opposite sex or to inability of the sperm to reach the female plant and its reproductive structures successfully. For example, in a population of *Cyathophorum bulbosum* (Figure 17) in New Zealand, where male plants were located nearly a meter above the females, sporophytes existed in several developmental states, but on a nearby bank the entirely female population was completely barren

(Burr 1939). In studies by Grebe (1917) on 207 German mosses and Arnell (1875) on 177 Scandinavian mosses, 200 of the 220 taxa that seldom produced capsules were dioicous. So one must ask what is the genetic mechanism that underlies the sexual differences in these unisexual taxa (taxa having only one sex on an individual; **dioicous**) and just what permits these unisexual taxa to persist?

Sex Chromosomes

Bryologists are the proud discoverers of **X** and **Y** sex chromosomes (Figure 18) in plants (Anderson 2000), first discovered in the liverwort genus *Sphaerocarpos* (Figure 19) (Allen 1917, 1919, 1930). And it is fitting that one of the first sex markers in bryophytes was likewise found in *Sphaerocarpos* (McLetchie & Collins 2001), although this was predated by identifying the tiny X and Y chromosomes in the female and male liverwort *Marchantia polymorpha* (Figure 20-Figure 21) (Okada *et al.* 2000; Fujisawa *et al.* 2001). These researchers have determined that the Y chromosome of the dioicous *Marchantia polymorpha* has unique sequences that are not present on the X chromosome or on any autosomes. Note that these individual haploid plants each have only one sex chromosome. To emphasize differences between **haploid** and **diploid** sex determination, the haploid single sex chromosomes have recently been distinguished as **U** (female) and **V** (male) chromosomes (Bachtrog *et al.* 2011; Olsson *et al.* 2013).

Figure 17. *Cyathophorum bulbosum*, a species that can readily be fertilized when males are above females, but not when females are isolated on a nearby substrate. Photo by Niels Klazenga, with permission.
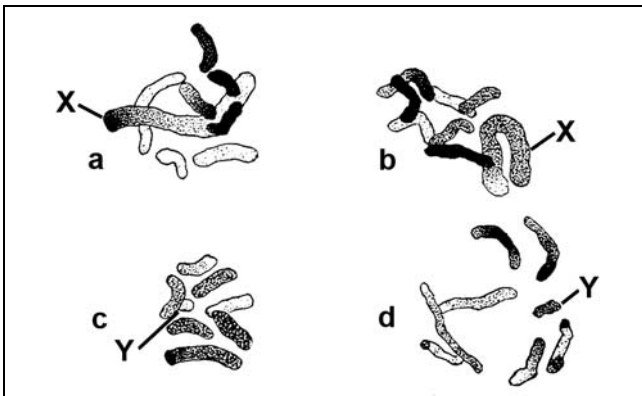

Figure 18. Chromosomes of *Sphaerocarpos donnellii*. **a** & **b**: Chromosomes from female gametophyte. **c** & **d**: Chromosomes from male gametophyte. From Allen 1919.

Figure 19. *Sphaerocarpos michelii*, member of the genus where X and Y sex chromosomes were first discovered. Photo by Jan-Peter Frahm, with permission.

Figure 20. *Marchantia polymorpha* male with antheridiophore, first bryophyte species in which sex markers were found and unique sequences found on males that were not present on females. Photo by David T. Holyoak, with permission.

Figure 21. *Marchantia polymorpha* females with archegoniophores, the first bryophyte species in which sex markers were found. Photo by Janice Glime.

But the presence of sex chromosomes does not mean that all bryophytes have separate sexes, or even that all bryophytes have sex chromosomes, so we must ask what determines the sexual differentiation. Ramsay and Berrie (1982) discussed the mechanisms of sex determination in bryophytes, including physiological and genetic regulation of sexuality. They considered that genetic sex is determined at the spore stage, but Bachtrog *et al.* (2011) consider that it is determined at meiosis. Even within the same genus, some bryophytes may be **unisexual** (Figure 22-Figure 25), others **bisexual** (having both sexes on the same individual; monoicous) (Figure 26-Figure 27). Clearly we need more research to discover how some of these determinations are made.

Figure 22. Clonal colony of male ***Philonotis calcarea***. Note innovation branches below the male splash cups. Photo by Michael Lüth, with permission.

Figure 23. A dioicous species, ***Philonotis calcarea***, showing antheridial splash cups. Photo by David T. Holyoak, with permission.

Figure 24. Female plants of the dioicous ***Philonotis calcarea***, distinguishable by their sporophytes. Photo by David T. Holyoak, with permission.

Figure 25. Colony of non-expressing or female plants of the dioicous ***Philonotis calcarea***. Archegonia are hidden among **perichaetial leaves** at the tip of the plant and are often difficult to distinguish without destroying the tip of the plant. Photo by David T. Holyoak, with permission.
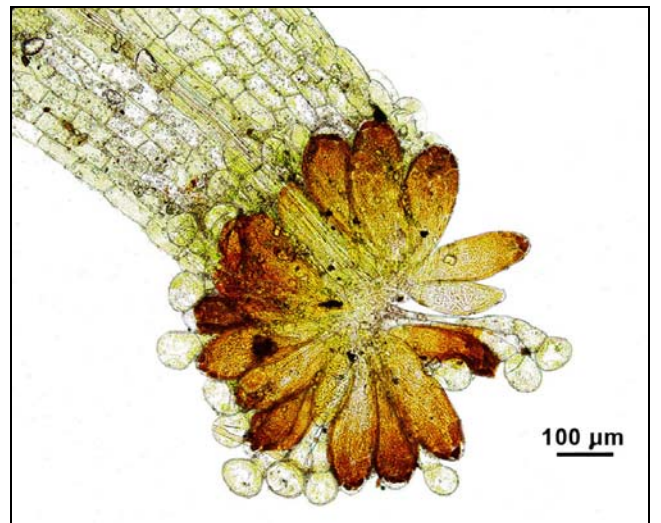

Figure 26. **Antheridia** of ***Funaria hygrometrica***. This is a special case of monoicous termed **autoicous**. Both male and female gametangia are on the same plant, but in separate places. Here the antheridia are at the base of a leaf. The white-knobbed structures with them are **paraphyses**. Photo from Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

An Unusual Y Chromosome

An active "Y"-chromosome-specific gene has been unknown in plants, although mammals such as humans do have specific genes on the Y chromosomes (Okada *et al.* 2001). But Okada *et al.* found that the bryophytes, or at least ***Marchantia polymorpha*** (Figure 20-Figure 21), have at least one such gene. This gene is unique and is expressed specifically in the male sex organs.

Since that earlier discovery, Yamato *et al.* (2007) have identified 64 genes on the Y chromosome of ***Marchantia polymorpha*** (Figure 20-Figure 21). Of these, 14 occur only in the male genome and have been linked exclusively

to expression in reproductive organs. Although their individual functions are still not known, this relationship suggests that they participate in the reproductive functions of the male. Additional genes (40 genes) on the Y chromosome are expressed in both male sexual organs and male thalli, suggesting that they have cellular functions unrelated to reproduction.

Figure 27. *Funaria hygrometrica* undeveloped and nearly mature capsules on female plant portions. Photo by Robert Klips, with permission.

Gametangial Arrangement

There are multiple configurations of gametangia among the various bryophytes. The monoicous condition of sexuality among mosses can be further divided into **autoicous**, **paroicous**, and **synoicous**. In the **autoicous** condition, the male and female gametangia are in separate clusters, as in *Orthotrichum pusillum* (Figure 28-Figure 30). In the **paroicous** condition, the male and female gametangia are in separate groupings but in a single cluster, as they are in a number of species of the liverwort *Lophozia* (Figure 31) (Frisvoll 1982). The **synoicous** condition is one in which the male and female gametangia occur intermixed in the same cluster, as in *Micromitrium synoicum* (Figure 32), a condition unusual enough to be used in the specific name. Whereas archegonia in acrocarpous mosses are always terminal, pleurocarpous mosses grow horizontally, and the female and male sex organs occur at the apex of specialized short branches, **perichaetia** and **perigonia**, respectively. In dioicous taxa, antheridia of acrocarpous mosses are in various positions, whereas archegonia are terminal. The same arrangements into perichaetia and perigonia is true for both monoicous and dioicous species.

Figure 28. *Orthotrichum pusillum*, an **autoicous** species with capsules. Photo by Robert Klips, with permission.
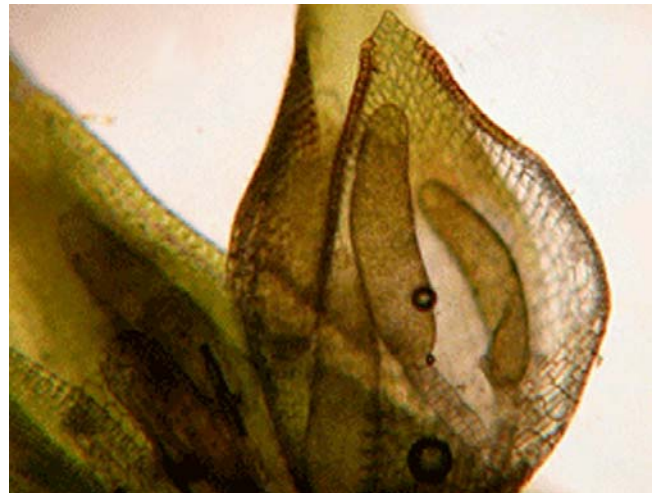

Figure 29. *Orthotrichum pusillum*, an autoicous species showing antheridia. Photo by Robert Klips, with permission.

Figure 30. *Orthotrichum pusillum*, an **autoicous** species showing archegonia. Photo by Robert Klips, with permission.

Figure 31. *Lophozia excisa*, a **paroicous** species. Photo by Michael Lüth, with permission.
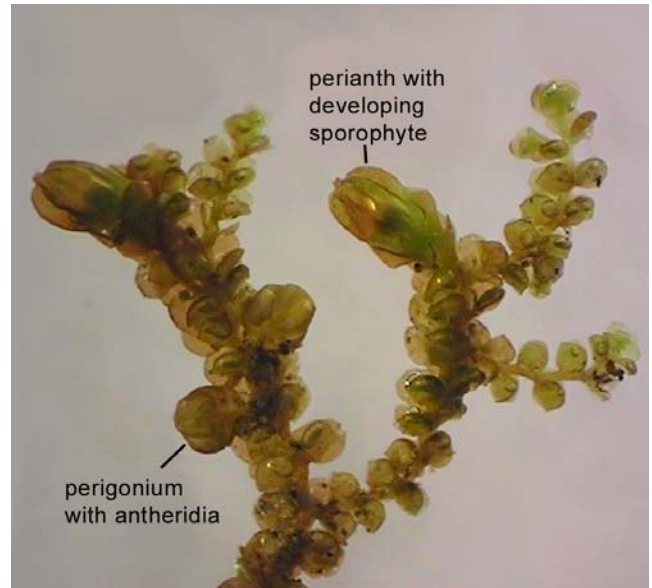

Figure 33. Arrangement of **perianth** with archegonia and **perigonium** with antheridia in the monoicous leafy liverwort *Frullania oakesiana*. Photo by Paul Davison, with permission.
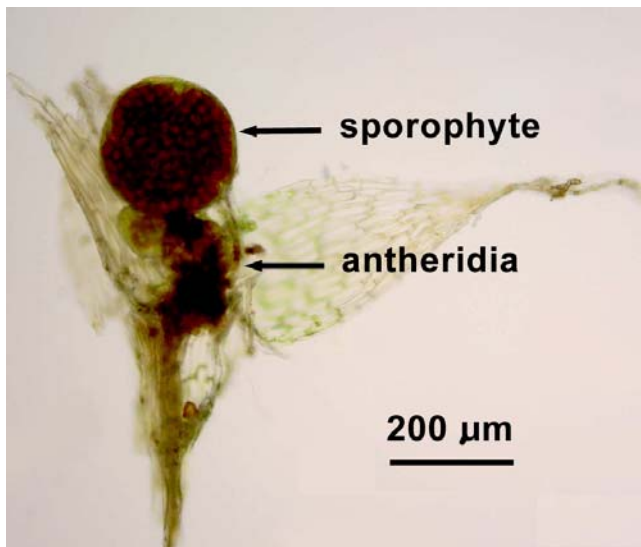

Figure 32. *Micromitrium synoicum* with male and female gametangia among the same bracts (synoicous). Photo from Duke University, through Creative Commons.

Figure 34. Antheridial arrangement on the leafy liverwort *Kurzia*. Photo by Tom Thekathyil, with permission.

In **Jungermanniopsida**, the antheridia are arranged behind the growing point (Figure 33-Figure 35). In most of the leafy **Jungermanniopsida** the archegonia occur in perianths (Figure 33, Figure 36) that may be terminal on stems and branches or located along these. In the **Metzgeriales** (**Jungermanniopsida**), the archegonia appear along the midrib of the thallus, thus permitting continued apical growth (Figure 37). In the **Marchantiopsida** the antheridia occur in clusters on the thallus (Figure 38) or elevated on a stalk (Figure 39), with similar arrangements for archegonia (Figure 39-Figure 40). In **Anthocerotopsida** the antheridia are imbedded in the thallus (Figure 41-Figure 42) and archegonia are single and surrounded by involucres (Figure 41).
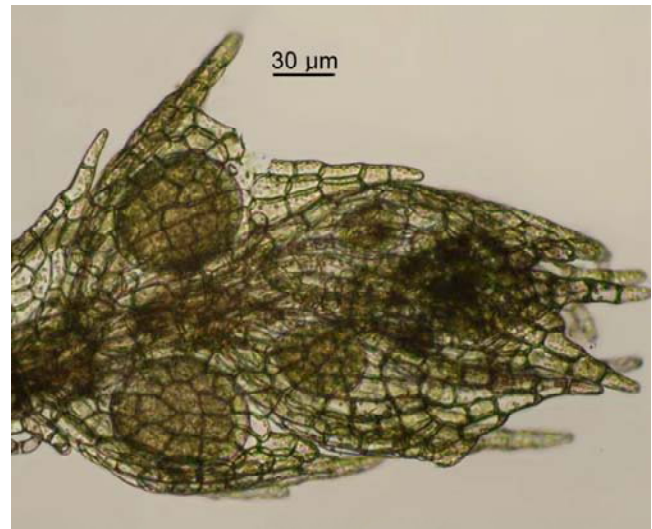

Figure 35. *Pellia endiviifolia* with antheridia on the thallus in positions not at the apex. Photo by Ralf Wagner <www.dr-ralf-wagner.de>, with permission.

Figure 36. **Perianth** of the leafy liverwort *Frullania* (**Jungermanniopsida**) in its terminal position. Photo by George Shepherd, through Creative Commons.

Figure 39. *Marchantia polymorpha* showing flat-topped antheridiophores with antheridia embedded in them and archegoniophores with fingerlike arms with archegonia on the undersides. Photo by Robert Klips, with permission.

Figure 37. *Symphogyna brasiliensis* (**Metzgeriales**) showing subapical position of archegonia, hidden in this case by fimbriate scales. Photo by George J. Shepherd through Creative Commons.
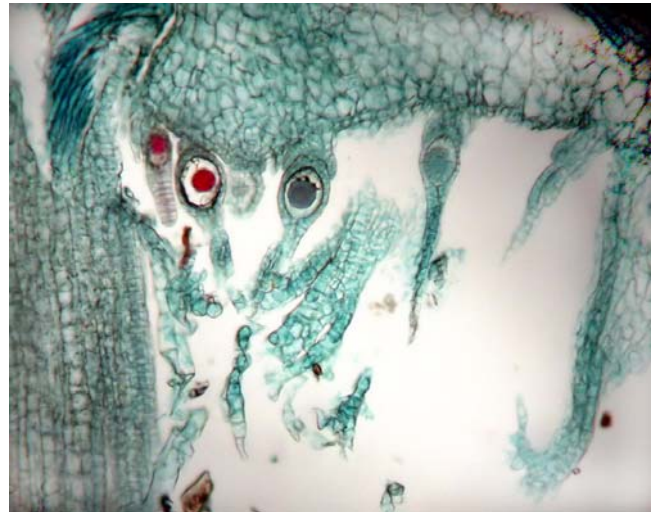
Figure 40. Arm of archegoniophore head of *Marchantia polymorpha* with archegonia hanging down. Photo by George Shepherd, through Creative Commons.

Figure 38. *Conocephalum conicum* antheridia in clusters on the thallus (arrow). Photo by Malcolm Storey, through Creative Commons.
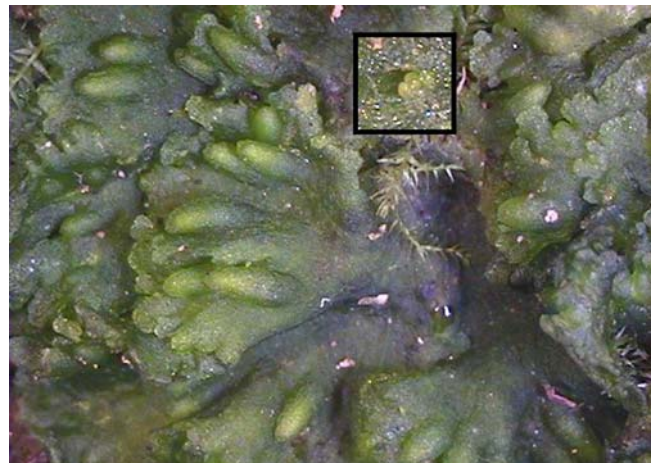
Figure 41. *Notothylas orbicularis* (**Anthocerotopsida**) with involucres that surround archegonia and pouches that contain antheridia (see insert). Photo by Paul Davison, with permission.

Figure 42. **Antheridia** in the pocket of a hornwort (**Anthocerotopsida**), expelling sperm. Photo by Hatice Ozenoglue Kiremit, with permission.

Origin of Bisexuality in Bryophytes

As already noted, the number of dioicous species of bryophytes is greater than the number of monoicous species (Hedenäs & Bisang 2011), with 68% of liverworts, 57% of mosses, and 40% of hornworts being dioicous (Villarreal & Renner 2013a). Longton and Schuster (1983) recognized 205 liverwort taxa as dioicous, 112 as monoicous in New Zealand. In Guatemala, 161 taxa are dioicous compared to 145 monoicous. Une (1986) found 613 (62.2%) of the bryophyte species in Japan were dioicous and 356 (36.2%) were monoicous. This prevalence of dioicous taxa is an unusual situation among plants and raises questions about its significance. The switch to monoicy has previously been suggested to be a derived character in bryophytes (but see below under Monoicy as a Derived/Advanced Character?), and in many genera it drives speciation through doubling of some or all of the chromosomes. One must then ask, how do so many dioicous taxa survive and spread?

Monoicy as a Derived/Advanced Character?

Ando (1980) suggested seven reasons to consider monoicy as advanced over dioicy in bryophytes, based on concurrent knowledge on bryophyte systematics, distributions, and life histories:

1. Frequently the strain with the haploid chromosome number is dioicous and the monoicous one is diploid.

2. Monoicous taxa seem to have more limited distribution, despite their higher frequency of producing sporophytes and spores.

3. Bryophytes of specialized, more recent habitats such as on decaying wood or living leaves of tracheophytes include many monoicous taxa.

4. Taxa with small gametophytes are more commonly monoicous.

5. Most annual bryophytes are monoicous, e.g. Ephemeraceae, Funariaceae, and Splachnaceae.

6. More advanced groups such as Marchantiales and Anthocerotophyta include many monoicous taxa. [This statement does not fit with 2016 thinking about the phylogenetic position of these groups.]

7. Monoicous taxa have several means to prevent self-fertilization and may have evolved by hybridization.

This suggested direction of evolution is in line with the recent study in hornworts, discussed below, which revealed a transition rate from dioicy to monoicy that was twice as high as in the opposite direction (Villarreal & Renner 2013a, b). Devos and coworkers (2011) consider genetic history in their treatise on the evolution of sexual systems in the mostly epiphytic liverwort genus **Radula** (Figure 43). They also found that shifts from dioicy to monoicy in that genus occurred multiple times, with some epiphytes having facultative shifts.

Figure 43. **Radula complanata** growing epiphytically and exhibiting numerous sporophytes. Photo by David Holyoak, with permission.

However, recent studies using modern comparative phylogenetic analyses and large scale phylogenies of mosses (McDaniel *et al*. 2013) and liverworts (Laenen *et al*. 2016) found that transitions in sexuality are biased towards dioicy. Furthermore, they found that there seem to be higher rates of diversification among the monoicous moss taxa than among the dioicous ones. In liverworts, bisexuality evolved multiple times. It is nonetheless

associated with certain clades in the liverwort tree, which suggests that it might be a response to environmental conditions (Laenen *et al.* 2016). The distinct phylogenetic signal in sexual systems across the liverwort phylogeny contrasts with the high lability of sexual systems in mosses and hornworts. McDaniel and coworkers (2013) suggest that dioicy works best when separate sexes derive some advantage in their different morphologies.

One might look for these dioicy advantages in genera such as *Diphyscium* (Figure 44) where males and females have very different morphologies, or in those taxa with **dwarf males** (See Dwarf Males in Chapter 3-3). But even more likely are sexual differences in physiology – phenomena that have barely been explored (see discussions for *Syntrichia caninervis* and *Marchantia inflexa* in section on Environmental and Geographic Differences in Chapter 3-2).

Figure 44. *Diphyscium foliosum* females with capsules surrounded by perichaetial leaves and photosynthetic males (green leaves in foreground). Photo by David T. Holyoak, with permission.

It seems that it still remains for us to unravel the selection pressures and evolutionary processes behind this dioicous phenomenon, but this unravelling is promising with current molecular techniques. It is likely that further phylogenetic analyses as well as the thorough study of genome evolution will shed light on the evolution of sexual systems in bryophytes (Crawford *et al.* 2009; Laenen *et al.* 2016; McDaniel & Perroud 2012; McDaniel *et al.* 2013; Villarreal & Renner 2013a, b).

Multiple Reversals

The hornworts (**Anthocerotophyta**) are unique in many ways, and among these are their sexual systems. Villarreal and Renner (2013a, b) contend that hornworts underwent numerous transitions between dioicy and monoicy, with a transition rate from dioicy to monoicy that was twice that from monoicy to dioicy. But a seemingly strange occurrence is that monoicous groups of hornworts have higher extinction rates. This might be explained by the fact that in the hornworts, diversification rates do not correlate with higher ploidy levels as they do in some mosses (*e.g.* Lowry 1948 for **Mniaceae**). Rather, in hornworts polyploidy in monoicous taxa is rare, occurring

in only one (*Anthoceros punctatus*, Figure 45-Figure 46) of 20 species that have been assessed (Villarreal & Renner 2013a). Crawford *et al.* (2009) consider the evidence for simultaneous transitions in chromosome ploidy numbers and sexual systems to be inconclusive in mosses as well. And in liverworts, only about 5% of the species are polyploid whereas 30-40% of the species are monoicous (Fritsch 1991 in Laenen *et al.* 2016).

Figure 45. *Anthoceros punctatus* with sporophytes. Photo by Des Callaghan, with permission.
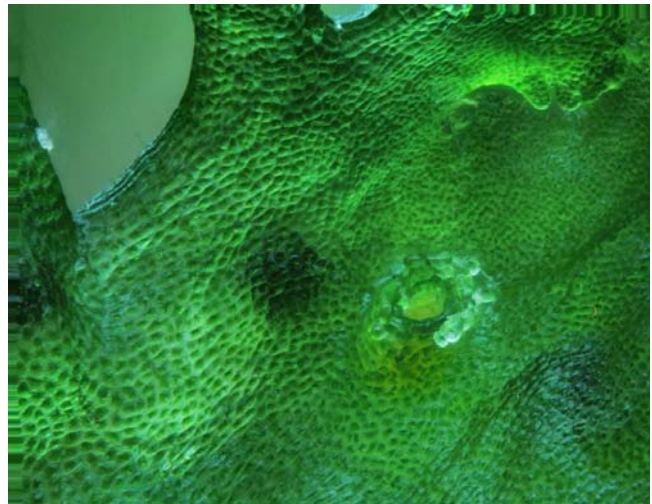
Figure 46. *Anthoceros punctatus* antheridial pit. Note the bluish *Nostoc* colony to the left of the antheridial pit. Photo by Des Callaghan, with permission.

Villarreal and Renner (2013a) examined the sexual systems of 98 of the 200 known species of hornworts. Knowing that a relationship between dioicy and small spores exists in mosses, they looked for a similar relationship in hornworts. Using Bayesian techniques, they found at least a weak support for this correlation in hornworts. More to the point, they showed that the sexual system depends on spore size, but that the reverse relationship is not true. They reasoned that dioicous species would be more successful with small spores by providing dense carpets of gametophytes for reproduction. It would seem that this character also permits them to occupy their disturbed and ephemeral habitats where they can thrive without competition.

The Monoicous Advantage

The effects of these oicy differences on bryophyte ecology and biology are impressive for this gametophyte-dominant group. As in other plants, monoicous species might appear at a competitive advantage, as all individuals in a population can potentially produce offspring. Moreover, monoicous species in general also reproduce by spores more frequently than do dioicous taxa (Longton & Schuster 1983), although this is not always the case. In 1950, Gemmell published vice-county records for the sexual condition of British mosses, using Dixon's The Student's Handbook of British Mosses, and supported the concept that mosses with the monoicous condition are more successful at producing capsules than those of the dioicous condition (Figure 47). Although a much higher percentage (97% compared to 58% in dioicous taxa) of the monoicous group has capsules frequently (Figure 47), presumably because of greater opportunity for fertilization, the dioicous group occupies a greater proportion of the vice-county observations compared to the number of monoicous species (Figure 48).
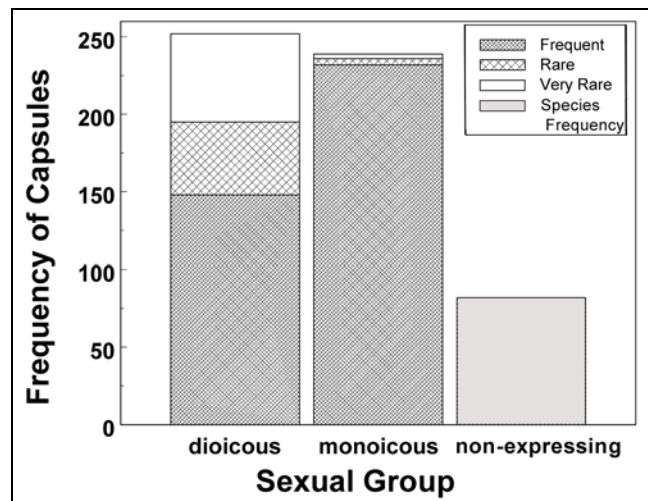

Figure 47. Frequency of producing capsules in dioicous and monoicous mosses and frequency of non-expressing species in vice-counties of Great Britain. The total number of species is 573, and the bars represent the relative frequency of the three types. Based on table in Gemmell 1950.

Heegaard (2001) illustrates the problem of dioicy in *Andreaea* (Figure 49-Figure 52). Both monoicous and dioicous species occur in western Norway, permitting us to compare genetically similar sibling taxa from a limited geographic range. The only dioicous species, *Andreaea blyttii* (Figure 49), had a lower percent (38%) of sporophytes on cushions bearing **perichaetia** (leaves surrounding archegonia) than did the three monoicous taxa (60-86%). Nevertheless, even among monoicous taxa, *A. nivalis* (Figure 50) and *A. obovata* var. *hartmannii* (Figure 51) rarely produced capsules. The production of capsules in monoicous *A. rupestris* var. *rupestris* (Figure 52) was highly correlated with the environment, with one group having capsule production that was strongly correlated with altitude and slope, corresponding with **perichaetial** development, and a second group where there was no correlation with perichaetial development, but sporophyte

production correlated with gradients of flushing and snow cover. Yet another group produced sporophytes throughout its environmental range. Coordination between the sexes for timing of formation and maturation of the sexual structures, influenced by the environment, could add to the problems of both monoicous and dioicous taxa.
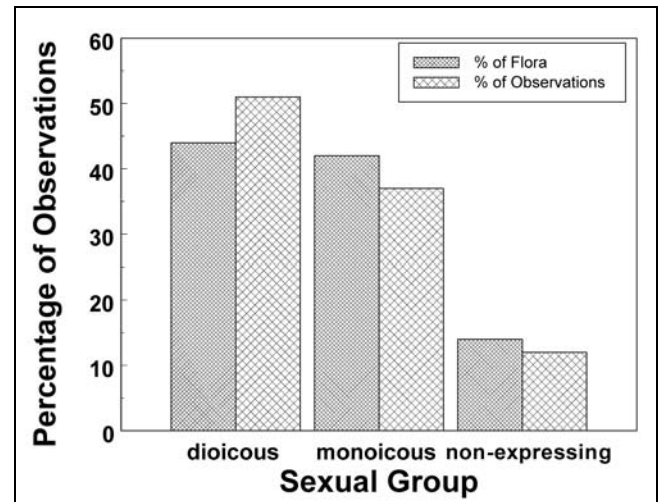
Figure 48. Effect of sexual group on relative frequency of bryophytes in British vice-counties. Total number of species is 573. Percent of flora was obtained by dividing number of species in the category by total number of species. Percent of observations was obtained by dividing total number of vice-county observations by number of species in the category and converting to percent. Based on table in Gemmell (1950).

Figure 49. *Andreaea blytii* at Khibiny Mountains, Apatity, Murmansk. Photo by Michael Lüth, with permission.

Figure 50. *Andreaea nivalis* in Europe. Photo by Michael Lüth, with permission.
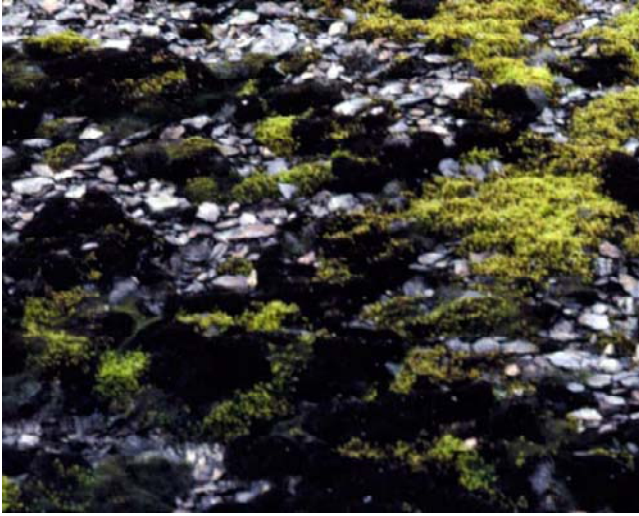
Figure 51. *Andreaea obovata* (the dark-colored moss) at Akisko, Sweden. This population lacks capsules, as indicated by the smooth black color. Photo by Dale Vitt, with permission.

Figure 52. *Andreaea rupestris* with capsules. Photo by Michael Lüth, with permission.

In comparing taxa that commonly produce capsules, Longton and Schuster (1983) reported only 22 British dioicous mosses, compared to 134 monoicous taxa, commonly have capsules; 154 dioicous taxa rarely or very rarely have capsules, compared to 12 monoicous taxa. It is apparent, then, that factors other than sexual reproduction contribute to the success of dioicous taxa.

Nishimura and Une (1989) examined sporophyte production in **pleurocarpous** mosses (horizontally growing taxa with reproductive organs on short side branches; Figure 53) of the Hiruzen Highlands in Japan. Out of 22 **autoicous** (monoicous with antheridia and archegonia in different clusters) species, 20 produced sporophytes (91%). However, out of 49 dioicous species, including 5 with **dwarf males** (**phyllodioicous** – see Dwarf Males in Chapter 3-2), only 27 produced sporophytes (55%). Studies like this suggest that there is a sexual reproductive advantage to being monoicous. But they still beg the question of better survival.

One possible consequence of being dioicous and spreading to new locations is the total absence of sporophytes for some species in part of their geographic range. This appears to be the case for the entire genus of *Sphagnum* in California, USA (Carl Wishner, Bryonet 14 August 2012; Norris & Shevock 2004). McQueen and Andrus (2007), in Flora of North America vol. 27, report

that most, if not all, of the species known from California are dioicous. Yet, for the typically dioicous *Sphagnum russowii* (Figure 54), Shaw *et al.* (2012) report that some specimens are apparently monoicous.   The common presence of sporophytes for some California species [*e.g.* *S. capillifolium* (Figure 55), *S. angustifolium* (Figure 56)] when they occur elsewhere suggests that there may be a **founder principle** at work (Carl Wishner, Bryonet 14 August 2012) wherein only one sex arrived to colonize a particular location. This was also suggested for *S. palustre* (Figure 57) in Hawaii where sporophytes are not known to occur (Karlin *et al.* 2012). But without genetic evidence, we cannot rule out the possibility of a climate that is not suitable for expression of one of the sexes or that makes the two sexes mature at different times.

Figure 53. *Plagiothecium denticulatum*.   Photo by Bob Klips, with permission.

Figure 54. *Sphagnum russowii* in Europe. Photo by Des Callaghan, with permission.

Figure 55. *Sphagnum capillifolium* in Chile, showing capsules. Photo by Juan Larrain, with permission.

Figure 56. *Sphagnum angustifolium* in Europe. Photo by Michael Lüth, with permission.

Figure 57. *Sphagnum palustre* in Europe. Photo by Michael Lüth, with permission.

Herbarium records are frequently the basis for descriptions of bryophytes and frequency of sporophytes. One must view herbarium collection records for such factors a male:female ratios and sporophyte production with caution, however, due to collection bias. As Harpel (2002) demonstrated for bryophytes collections for the western U.S., bryologists are more likely to collect the unusual, creating a bias toward over-collecting the rarer species and those with capsules, while ignoring the common.

Or the Dioicous Advantage?

To their potential detriment, monoicous taxa frequently experience **selfing** (being fertilized by sperm from the same plant; see Reproductive Barriers in Chapter 3-4), despite having neighbors that can produce gametes of the opposite sex (Eppley *et al.* 2007). This results in significantly fewer heterozygous fertilizations than that found in dioicous taxa. Furthermore, these monoicous near-neighbors typically belong to the same clone, produced through vegetative reproduction, or have developed from spores from the same parent. This results in a deficiency of heterozygous sporophytes among monoicous taxa. Could it be that the heterozygous condition might itself drive the "mistakes" that result in having two sex chromosomes in one spore, resulting from a misalignment of chromosomes during meiosis? This would drive the bryophytes toward monoicy.

As suggested for the California *Sphagnum* species (see The Monoicous Advantage above), total absence of the opposite sex in dioicous taxa can force species to survive vegetatively in many isolated regions and margins of distribution. Because of the success of **vegetative propagation** (reproduction by asexually produced pieces or branches of the plant) (Figure 58-Figure 59), entire single-sex populations of dioicous taxa may exist and expand over large areas without ever producing capsules. Such is often the case with aquatic taxa like *Fontinalis* (Figure 60) and in parts of its distribution for *Pleurozium schreberi* (Figure 61).

Figure 58. *Syntrichia laevipila* exhibiting **gemmae**. These are one means of asexual reproduction. Photo by Paul Davison, with permission.
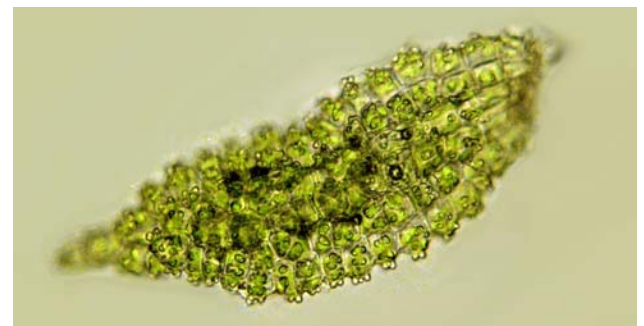

Figure 59. **Gemma** of *Syntrichia laevipila* (=*Tortula pagorum*), illustrating its very papillose cells. Photo by Bob Klips, with permission.

Figure 60. *Fontinalis duriaei* showing its flowing growth of a single clone. It is unlikely a female in this position would ever get fertilized and produce capsules unless a male clone became intermixed. Photo by Janice Glime.

Figure 61. *Pleurozium schreberi*, seen here with capsules in Baraga County, Michigan, USA, is barren northward in Ontario where apparently only one sex exists. Photo by Janice Glime.

As a result of being dioicous it may be possible to harbor more genetic variation than that of monoicous species. Both mating systems permit species to reproduce asexually by **ramets** (individual members of clone, arising vegetatively), but the greater percentage of species with asexual diaspores permits those dioicous species to carry non-functional or non-lethal genes as potential pre-adaptations without the selection step that often occurs during failed pairing in meiosis.

Shaw (1991) found that the monoicous moss *Funaria hygrometrica* (Figure 27) never had heterozygous sporophytes for 14 allozyme loci, *i.e.*, it had a high level of heterozygote deficiency. The dioicous moss *Polytrichum juniperinum* (62), on the other hand, had extremely high levels of heterozygosity based on six allozyme loci (Innes (1990).

In short, monoicous taxa do not always gain the advantages of cross-breeding, although their chances for cross-breeding may in some cases be equal to or greater than that of dioicous taxa. This cross-breeding opportunity assumes that spores of another genotype of a monoicous taxon have equal chances of germinating and growing near that taxon compared to spores of a dioicous taxon growing close enough for fertilization of a plant of the opposite sex of that taxon.

62. *Polytrichum juniperinum*, a dioicous moss shown here with prolific capsule production. Photo by Daniel Mosquin, through Creative Commons.

In fact, the opportunities for cross fertilization in monoicous taxa should be greater than those of dioicous taxa because any spore of the species that germinates near another of the same species should be able to cross with it, whereas the dioicous taxon must have a pair of sexes. On the other hand, if the archegonia of a monoicous taxon lack any protection against self-fertilization, their own sperm have the greater chance of reaching them due to the shorter distances. Thus, taxa of both mating systems have opportunities for different individuals nearby to fertilize them. At present we do not have enough data to generalize about the numbers of cross-fertilizations that occur in monoicous taxa. Due to the higher number of total successful fertilizations, monoicous taxa have much better dispersal through spores, increasing the possibility of a different genotype nearby and providing it a source of cross-fertilization. The likelihood of cross-fertilization with a different genotype in both sexual strategies is complicated by arrival times, competition, leakage of inhibitory substances, and the degree of self-incompatibility (See Chapter 3-4 in this volume). But dioicous taxa have the advantage of more frequent asexual reproduction and guaranteed mixing of genes when they do reproduce sexually, creating the variability for the species to survive throughout environmental changes.

Origins of Polyploidy

The monoicous condition in mosses may be the result of **polyploidy** (in bryophyte gametophytes, having more than one complete set of chromosomes). Polyploidy is a common occurrence among plants, being rare only among the gymnosperms (Ahuja 2005). Bryophytes seem to have multiple avenues by which to become polyploids. This increase in ploidy is often considered to make the monoicous condition possible by providing an extra set of chromosomes. But in this group where sex chromosomes have been identified in at least some species, the understanding of how all of these possible origins work is complex. See Monoicy as a Derived/Advanced Character? above and examples below.

Sporophytes from Fragments

It is still unclear how the majority of monoicous taxa arose. We know that it is possible in the lab to grow 2*n* (**diploid**) protonemata and leafy gametophores from bits of sporophyte tissue, producing monoicous plants (Crum 2001). Marchal and Marchal (1907, 1909, 1911) grew nineteen species of diploid moss gametophytes from setae in the lab. Since then, many others have succeeded in producing diploid moss gametophytes without spores (Crum 2001). Lorbeer (1934) induced diploid gametophytes from capsules and setae in 52 species of liverworts. But this development of sporophyte tissue into a gametophyte has been observed only once (*Funaria hygrometrica*, Figure 26-Figure 27) in nature (Brizi 1892; Crum 2001).

Sporophytes have also been developed from gametophyte tissues. The first was produced as outgrowths from 2*n* leaves and stem tips of *Tortula acaulon* (=*Phascum cuspidatum*) (Marchal & Marchal 1911; Springer 1935). These were initially misinterpreted by Marchal and Marchal as asexual reproductive structures, but later Springer (1935) interpreted them as apogamous sporophytes. These seemed to be the result of altered, mostly dry, conditions. However, these pseudosporophytes failed to produce normal capsules and never produced spores. More recently El-Saadawi *et al.* (2012) discovered what appears to be an apogamous sporophyte – one that lacks any evidence of an archegonium at its base, in *Fissidens crassipes* subsp. *warnstorfii* (Figure 63). It likewise never produced spores. It originated at the base of the stem, whereas this species normally produces its sporophytes at the apex.
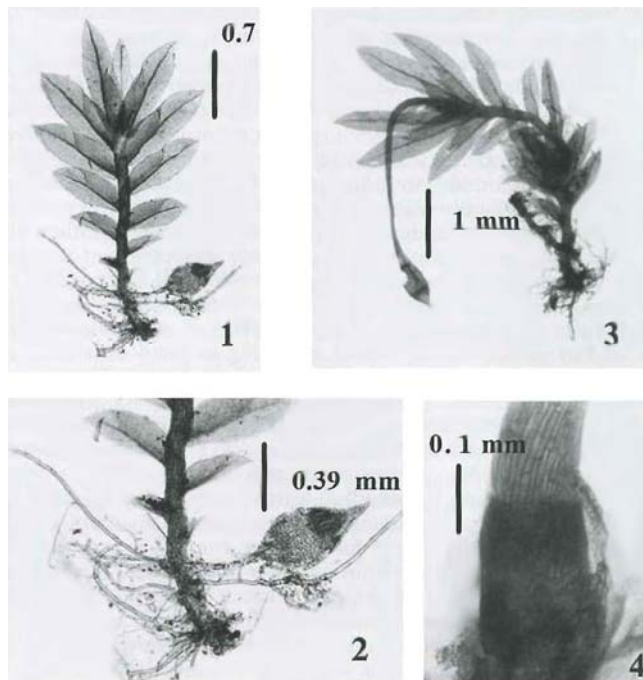
It is unlikely that these vegetative anomalies have contributed much, if any, to the creation of monoicous taxa. On the other hand, the accidental fusion of haploid gametophyte cells other than gametes can result in capsules with at least some viable spores. This suggests that cases might exist where cells join but remain as gametophyte, possibly becoming polyploid monoicous plants.

Genome Doubling in Mosses

Genome doubling seems to occur commonly in mosses [76% polyploidy (Przywara & Kuta 1995)], but seemingly less often so in hornworts (Villarreal & Renner 2013a) and liverworts [10% (Newton 1983); 5% (Fritsch 1991 in Laenen *et al.* 2016) (this can include ancient polyploidism and subsequent chromosome loss). Polyploidy might be coupled with a change in sexual system from dioicous to monoicous, but not necessarily so (Jesson *et al.* 2011). Both **autopolyploidy** (self-doubling of chromosomes within a single bryophyte) and **allopolyploidy** (**hybridization**) are known to be present among bryophytes in nature (Natcheva & Cronberg 2004; see also 3.4, Hybridization).

Autopolyploids – Although **autopolyploidy** was once considered the primary source of polyploidy in mosses (Boisselier-Dubayle & Bischler 1999), this may not be the case. Košnar *et al.* (2012) were able to use genetic markers to demonstrate autopolyploid origin of several lineages in the *Tortula muralis* (Figure 64) complex, making them the first group of mosses in which autopolyploidy was demonstrated with molecular markers. Google Scholar, when searched for bryophyte autopolyploidy, listed mostly allopolyploidy references. In one species that does exhibit autopolyploidy, *Targionia hypophylla* (Figure 65), its triploidy seems to actually be a combination of autopolyploidy and allopolyploidy (Boisselier-Dubayle & Bischler 1999).

Figure 63. *Fissidens crassipes* showing an apogamous sporophyte (**1 & 2**) compared to a normal sporophyte (**3**) and the vaginula at the base of the normal sporophyte (**4**), but absent on the apogamous sporophyte. Photo from El-Saadawi *et al.* (2012). Permission pending

Figure 64. *Tortula muralis* with capsules. Photo by Derek Christie, with permission.

Figure 65. *Targionia hypophylla* showing black marsupial pouches that house the archegonia and sporophytes. Photo by Martin Hutten, with permission.

Allopolyploids – allopolyploids can be achieved by **hybridization** (crossing of non-identical genomes, as in a different strain or species) and has been demonstrated in a number of bryophyte species. For example, Wyatt *et al*. (1988, 1992) showed that *Plagiomnium medium* (**Mniaceae**; Figure 66) arose from a cross between *Plagiomnium ellipticum* (Figure 67) and *Plagiomnium insigne* (Figure 68-Figure 69), resulting in **allopolyploids** (having two or more complete sets of chromosomes that derive from more than one species). Not only did it happen, but it happened multiple times! *Plagiomnium cuspidatum* (Figure 70-Figure 72) is likewise an allopolyploid, but one of its parent species is unknown (Wyatt & Odrzykoski 1998). *Cinclidium stygium* (Figure 73) (n=14), also a member of **Mniaceae**, is a monoicous polyploid closely related to *C. arcticum* (Figure 74) and *C. latifolium* (Figure 75), both having n=7 (Wyatt *et al.* 2013). *Cinclidium stygium* appears to have an **allopolyploid** origin from these two close relatives. Also *Cinclidium subrotundum* (Figure 76) is a monoicous polyploid (n=14) that exhibits strong evidence for allopolyploidy, having 7 fixed heterozygous loci out of 17 scored (Mogensen 1973).

Figure 67. *Plagiomnium ellipticum* Khibiny Mountains, Apatity, Murmansk. Photo by Michael Lüth, with permission.

Figure 68. *Plagiomnium insigne* male splash cup. Photo from Botany 321 website, UBC, with permission.

Figure 66. *Plagiomnium medium*. Photo by Jan-Peter Frahm, with permission.

Figure 69. *Plagiomnium insigne* female with sporophytes. Photo from Botany 321 website UBC, with permission.

Figure 70. *Plagiomnium cuspidatum* females with sporophytes. Photo by Michael Lüth, with permission.

Figure 73. *Cinclidium stygium* with capsules, a species with *n*=14. Photo by Misha Ignatov, with permission.

Figure 71. *Plagiomnium cuspidatum* showing males with black centers containing antheridia and females with green centers. This arrangement fits the dioicous condition discussed by Andrews (1959), not the more typical synoicous condition known for the species. Photo by Betsy St. Pierre, with permission.

Figure 74. *Cinclidium arcticum* (*n*=7), a close relative of *C. stygium* (*n*=14). Photo by Michael Lüth, with permission.

Figure 72. *Plagiomnium cuspidatum* showing female reproductive structures on left and male splash cup on right. Photo by Betsy St. Pierre, with permission.

Figure 75. *Cinclidium latifolium* from Spitzbergen, a species with *n*=7. Photo by Michael Lüth, with permission.

Figure 76.   *Cinclidium subrotundum* from Spitzbergen. Photo by Michael Lüth, with permission.

In cases when monoicous taxa are polyploids developed from dioicous taxa, we could hypothesize that the monoicous taxa should have more variability and thus better survival. Natcheva and Cronberg (2004) report that the spontaneous hybridization among bryophytes is sufficient to have a significant evolutionary significance, with the many allopolyploid taxa supporting this contention. (See Chapter 3-4, Sexuality: Reproductive Barriers and Tradeoffs).

Relationship of Polyploidy and Monoicy in *Atrichum*

In an *Atrichum undulatum* (**Polytrichaceae**, Figure 79) complex from a study in New Brunswick, Canada, monoicous plants were either diploid or triploid, with the number of monoicous individuals increasing as the number of triploids increased (Figure 77; Jesson *et al*. 2011). Many diploid populations, on the other hand, were dioicous (Figure 78). Jesson and coworkers found that male and female gametophytes were represented by haploid, diploid, and triploid individuals (Figure 78). Perley and Jesson (2015) examined the association between polyploidy and sexual system further in the genus, including species of different ploidy-levels. In the haploid state, this genus has either a female **U** chromosome or a male **V** chromosome. Using genetic markers, they determined that certain gene sequences are consistent with independent **allopolyploid** origins of **diploid** (2 sets of chromosomes) and triploid (3 sets of chromosomes) species. In the **triploid *Atrichum undulatum*** (Figure 79-Figure 81), and possibly the diploid *A. altecristatum* (Figure 82-Figure 83) as well, hermaphroditism appears to be a result of allopolyploidy. However, in the diploid *A. crispulum* (Figure 84), this allopolyploid event did not result in the hermaphrodite condition. This tells us again that the creation of monoicy (hermaphroditism) is more complex than simply doubling the chromosome number. (See more in Chapter 3-4, Reproductive Barriers: Selfing and Hybrids.)
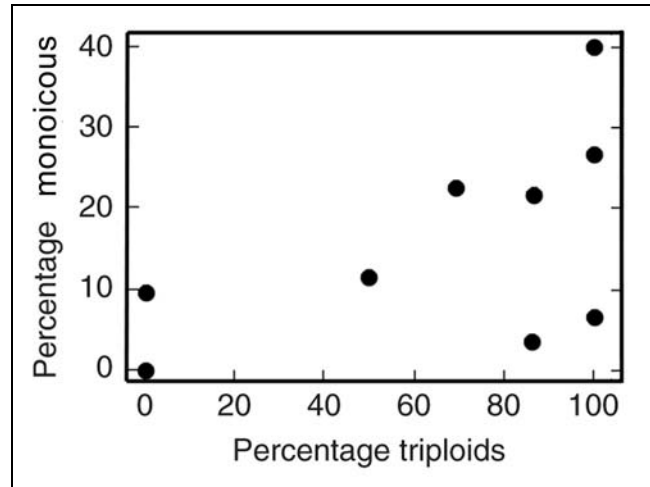
Figure 77.   Relationship between percentage of triploid individuals and monoicism in 21 randomly sampled populations of the *Atrichum undulatum* complex in New Brunswick, Canada. Five populations were not sampled for ploidy determination. Eight populations exhibited no hermaphrodites and no triploids. Modified from Jesson *et al.* 2011.
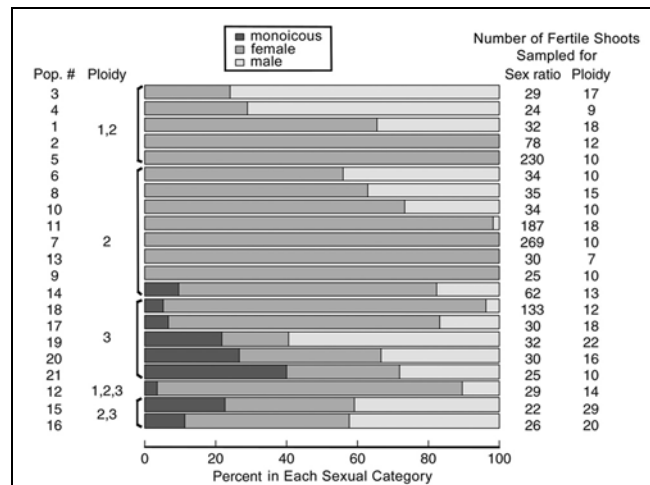
Figure 78.   Percentage of individuals in each of three sexual categories in 21 populations of the *Atrichum undulatum* complex in New Brunswick, Canada.   Column on right indicates the number of individuals in random samples for determining sex ratio and those used for determining ploidy.   Modified from Jesson *et al.* 2011.

Figure 79.   Female plants representative of the *Atrichum undulatum* complex, a group of taxa that may be monoicous or dioicous. Photo by Janice Glime.

Figure 80. *Atrichum undulatum* males. Photo by Janice Glime.

Figure 81. *Atrichum undulatum* females with capsules. Photo by Andrew Hodgson, with permission.

Figure 82. *Atrichum altecristatum* males. Photo by Bob Klips, with permission.
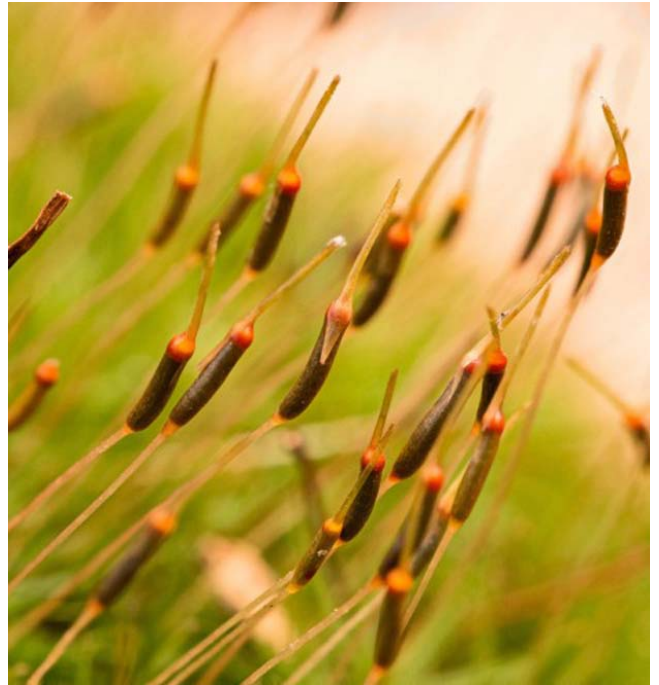
Figure 83. *Atrichum altecristatum* capsules. Photo by Bob Klips, with permission.

Figure 84. *Atrichum crispulum* capsules. Photo by Robert Klips, with permission.

Cross Fertilization

Certain mixing of genes results from cross fertilization, a condition widely accepted among botanists as providing genetic variability and greater chances for the species to survive changing conditions. For sexual reproduction to be successful, the sperm must reach the egg. For bryophytes, this could be an easy task in **synoicous** taxa (monoicous with antheridia and archegonia in same cluster), but quite difficult in dioicous taxa. (See above in The Monoicous Advantage.)

Sperm Dispersal by the Bryophyte

Sperm transfer is a problematic aspect of fertilization for bryophytes. A good release mechanism can start the sperm on their journeys.

The release of sperm in bryophytes is not a simple bursting of the antheridial wall with swimming sperm free to travel their own way. Rather, it typically occurs as the release of **spermatocytes** as a mass (Muggoch & Walton 1942). Meanwhile, as water accumulates at the base of the antheridium, it pushes this mass outward and away from the antheridium. As the spermatocytes reach the air-water interface, they spread apart rapidly to form a regular spaced arrangement on the surface. Muggoch and Walton concluded that this spreading and spacing correlated with the presence of fat from the spermatocyte mass. As the fat lowers the surface tension, the spermatocytes gain their freedom and spread. In some bryophytes, such as *Sphagnum* and some liverworts, fats seem to be absent and surface spreading likewise is absent. Muggoch and Walton further concluded that it is the surface spreading that makes the sperm susceptible to dispersal by invertebrates in dioicous taxa.

Once freed, the sperm are able to swim rapidly, and if they are near enough they may be attracted to the female gamete chemotactically. Pfeffer (1884) found chemotaxis involved in sperm locating archegonia of *Marchantia polymorpha* (Figure 40) and *Radula complanata* (Figure 43). Lidforss (1904) found that the proteins albumin, hemoglobin, and diastase were each able to attract sperm of *Marchantia polymorpha* to a capillary tube that contained them. Chemotaxis of sperm still needs clear verification and some studies suggest there is no chemotaxis (Showalter 1928).

Walton (1943) observed the spreading of sperm in the monoicous thallose liverwort *Pellia epiphylla* (Figure 85-Figure 86). In his observations, the archegonia were only 5-10 mm from the antheridia. Whereas freed sperm in the liverwort *Aneura* (Figure 87) took several hours to travel only 10 mm, those in many moss and liverwort taxa spread rapidly by surface tension over free water at a rate of ~20 mm per minute. *Pellia epiphylla* behaved like these mosses and liverworts, extruding in grey masses into water, breaking apart when they reached the surface, and dispersing over the wet surface rapidly. Once released, they were able to reach the archegonial involucres in only ~15 seconds. The more lengthy process was emergence of the sperm from the spermatocytes, which required ~15 minutes. Walton concluded that if the sperm had to swim it would require several hours, but that the surface tension carried them rapidly to their destination.

Figure 86. *Pellia epiphylla* with sporophyte. Photo by Malcolm Storey, through Creative Commons.

Figure 87. *Aneura pinguis* with capsules, indicating successful sperm transfer. Photo by Jan-Peter Frahm, with permission.

Sperm Travel Distances

One reason for the observed genetic variability in bryophytes is that cross-fertilization may extend greater distances than we had supposed (Table 1). Anderson and Lemmon (1974) considered the maximum distance for sperm to travel in acrocarpous mosses to be 40 mm, with a median dispersal distance of about 5 mm. Pleurocarpous mosses were assumed to have even shorter dispersal distances due to the total lack of splash cups or platforms (see below under Splash Mechanisms) (Anderson & Snider 1982). But as seen in Table 1, known (implied?) distances range up to 230 cm.

Reynolds (1980) found that splashing water on the platforms of the moss *Plagiomnium ciliare* (Figure 88) indicated greater travel distance (50+ cm) than that to the nearest male (5.3 cm). In the thallose liverwort *Marchantia chenopoda* (Figure 89), fertilization distances seem to range 0.7-65 cm (Moyá 1992), a range that suggests microhabitat factors may play a role in dispersal distance. Differences in dispersal mechanisms can account for wide ranges. Earlier chapters on **Marchantiophyta** and **Bryophyta** have discussed these mechanisms, including splash cups and platforms, flowing water, and arthropods.

Figure 85. *Pellia epiphylla* with antheridia (brown). Photo by Des Callaghan, with permission.

Table 1. Maximum known distances for sperm dispersal. Those in bold have splash cups or splash platforms. Distances mostly from Crum 2001.

Splachnum ampullaceum	5-15 mm	Cameron & Wyatt 1986
Breutelia arcuata	2.5 cm	Bedford 1940
Weissia controversa	4 cm	Anderson & Lemmon 1974
Climacium dendroides	7 cm	Bedford 1938
Pleurozium schreberi	10 cm	Longton 1976
Plagiomnium affine	10 cm	Andersson 2002
Atrichum angustatum	11 cm	Wyatt 1977
Abietinella abietina	12 cm	Bisang *et al.* 2004
Anomodon viticulosus	25 cm	Granzow de la Cerda 1989
Rhytidiadelphus triquetrus	34 cm	Bisang *et al.* 2004
Plagiomnium ciliare	50 cm	Crum 2001
Polytrichastrum ohioense	60 cm	Brodie 1951
Marchantia chenopoda	65 cm	Moyá 1992
Polytrichum juniperinum	75 cm	Longton 1976
Ptychostomum (=*Bryum*)		
capillare	200 cm	Gayet 1897
Dawsonia longifolia	230 cm	Crum 2001
epiphytes	2-5 m	Longton & Schuster 1983

Figure 88. *Plagiomnium ciliare* showing male splash cups and horizontal (plagiotropic) branches. Photo by Robert Klips, with permission.

Figure 89. *Marchantia chenopoda*, with males on **left** and females on **right**. Female archegoniophores elongate after fertilization. Photos by Janice Glime.

Maggot and Walton (1942) demonstrated experimentally that some bryophyte sperm can move 0.1-0.2 mm per second and continue movement for several hours, suggesting they could swim for 35 cm. Rosenstiel and Eppley (2009) and Shortlidge *et al.* (2012) provided further evidence of the possibility of greater sperm dispersal distances based on longevity (see below under Sperm Longevity).

Explosive Help in Thallose Liverworts

As discussed in Chapter 2-3 on **Marchantiophyta**, *Conocephalum conicum* (Figure 90) releases its sperm into a mist that makes them airborne (Benson-Evans 1950; Shimamura *et al.* 2008; see Chapter 2-3), suggesting that this could result in greater dispersal distances. Benson-Evans (1950) describes her experience with dried males of this species in the lab, the result of a hot week-end. Upon rewetting, the plants emitted a fine mist. She paid little attention to this until she noticed that "the mist was being emitted from the antheridial heads in regular puffs. Removal into direct sunlight increased the activity and the particles which were being ejected were visible to the naked eye, so that the puffs were obviously composed of distinct granules." A similar "explosion" is known from a number of other Marchantiales taxa (Peirce, 1902; Cavers 1903, 1904a, 1904b; Andersen 1931; Benson-Evans 1950).

Figure 90. *Conocephalum conicum* antheridia. Photo by Janice Glime.

Sperm Dispersal Vectors – After Release

Water has been presumed to be the primary dispersal vector in bryophytes. But interesting mechanisms accompany this water dispersal and still others rely on other organisms to accomplish the task.

Splash Mechanisms

Bryologists have been interested in the use of splash mechanisms in bryophytes for dispersal of sperm. Clayton-Greene *et al.* (1977) found that both field studies and lab tests support the hypothesis that antherozoids of ***Dawsonia longifolia*** (= *D. superba*; Figure 91) are dispersed by a splash mechanism. They found that females up to 1.5 m from males were fertilized, a distance only slightly less than the distance travelled by water drops released at 3.3 m above the splash cups.

Figure 91. *Dawsonia longifolia* male plant with splash cup. Photo by Allan Fife, with permission.

Andersson (2002) used a more sophisticated approach by making a video of splashes of rain on the splash cups of the moss *Plagiomnium affine* (Figure 92). He discovered that a crown forms upon impact of water. Small droplets are propelled away from the rim of this crown. For this to be effective, the diameter of the drop should be 1 mm or less to permit the crown to form, a size common in most showers. Upon impact of the drop, the antheridia rupture. Water fills the capillary spaces between the antheridia and the paraphyses, permitting the spermatozoids to reach the bottom of the splash cup. When the crown forms, it incorporates water from the bottom of the splash cup and hence includes the spermatozoids. These entrapped spermatozoids are ultimately released from the splash cups as the small droplets propel away from the splash cups. Such droplets are known to travel more than 100 mm, fertilizing most of the females within an 80 mm radius.

Figure 92. *Plagiomnium affine*. Photo by Janice Glime.

Among the best known splash platforms among bryophytes is that of *Marchantia polymorpha* (Figure 20-Figure 21). But Duckett and Pressel (2009) inform us that the widely told dispersal story is not entirely correct. Traditional description since the accounting by Goebel (1905) has been that fertilization occurs when the

archegoniophore stalks are still young and short, at which time the archegonial necks still point upward. The antheridiophores, developing first, tower over these, permitting sperm to travel downward by splashing or dripping during rainfall. But it is likely that the sperm actually disperse as they do in *Conocephalum conicum* (Figure 90), discharging into the air up to 15 cm above the antheridial heads (see Sperm Dispersal by the Bryophyte above). This can explain why both Parihar (1970) and Crum (2001) reported that the archegonia continued to be fertilized after the stalk elongated. Furthermore, when female thalli were placed in dye, the coloring reached archegonial heads in 30-60 minutes (Duckett & Pressel 2009), suggesting that capillary action and surface tension movement could carry the water and accompanying sperm from the antheridial splash cups upward to the archegonial heads and archegonia.

The splash mechanism in the dioicous *Fontinalis* (Figure 93) requires a suitable location within a rapid stream. When female plants are elevated above the water and male plants or their rock substrate are obstructing flow to create splash, sperm may be able to go about 2 m (personal estimate based on distance between male plants and females with capsules) in a rocky stream. This takes advantage of the splashing of rapid water, whereas when the antheridia and archegonia are under water, the highly diluted sperm will be swept away, most likely never being able to enter the neck of an archegonium (Goebel 1905).

Figure 93. *Fontinalis dalecarlica* with capsules. This clump is located in a rapid stream where rocks are covered with this species. Photo by Janice Glime.

Invertebrate Dispersal

Clayton-Greene *et al.* (1977) reported on the use by Gayet (1897) of netting over *Rosulabryum capillare* to demonstrate that some outside force was needed for fertilization. With fine nets over the plants, fertilization failed, but when the netting was removed, fertilization occurred over distances of 2 m. Although this may suggest that invertebrates were denied access, hence being prevented from fertilizing the females, it does not eliminate the possibility of the netting affecting the splashing of raindrops.

As early as 1927, Harvey-Gibson and Miller-Brown found that the paraphyses (Figure 26) of both males and females in *Polytrichum commune* (Figure 94) exuded a mucilage, but that mucilage did not contain any sugars.

Nevertheless, **oribatid mites**, **springtails** (**Collembola**), **midges** (**Diptera**), **leaf hoppers** (**Cicadellidae**), **aphids**, and **spiders** visited these structures and lapped up the mucilage. Their body parts carried the mucilage, and thus they might easily have carried the sperm. But this possibility seemed to be ignored by most bryologists until recently.

Figure 94. *Polytrichum commune* with capsules. Photo by David Holyoak, with permission.

Cronberg *et al.* (2006) experimentally demonstrated that **springtails** and **mites** were able to transport sperm over distances of up to 4 cm. Rosenstiel *et al.* (2012) also described one of the more remarkable cases of sperm dispersal in the mosses *Bryum argenteum* (Figure 95-Figure 96) and *Ceratodon purpureus* (Figure 97). These species can have their sperm dispersed from male to female by the springtail *Folsomia candida* (Figure 97). Rosenstiel and coworkers showed that the springtails chose significantly more female mosses than male mosses in *Ceratodon purpureus* (Figure 98) and that their presence facilitated fertilization (Figure 99). This preference was supported by verifying that the volatile compounds differed between the two sexes in *C. purpureus* (Figure 100-Figure 101).

Figure 96. *Bryum argenteum* perigonium, a collection of antheridia that attract invertebrates to facilitate transfer of sperm. Photo by George J. Shepherd, through Creative Commons.
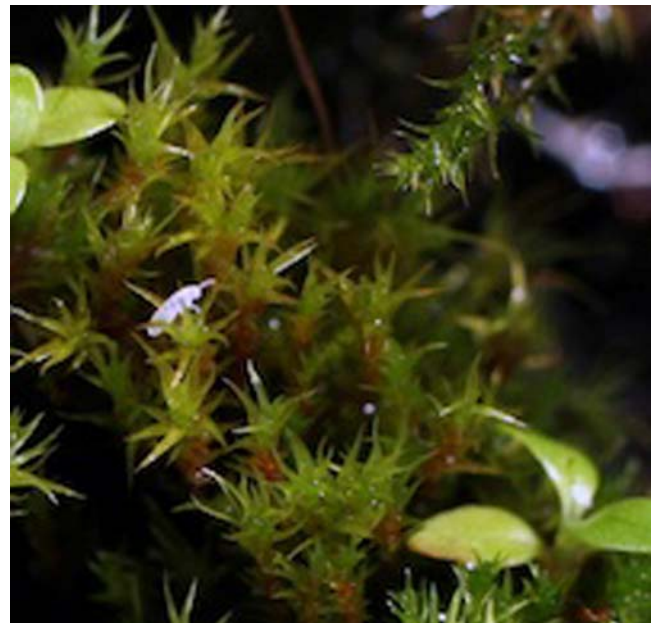
Figure 97. Springtail *Folsomia candida* on *Ceratodon purpureus*, possibly bringing sperm to the archegonia. Photo by Erin Shortlidge, with permission.
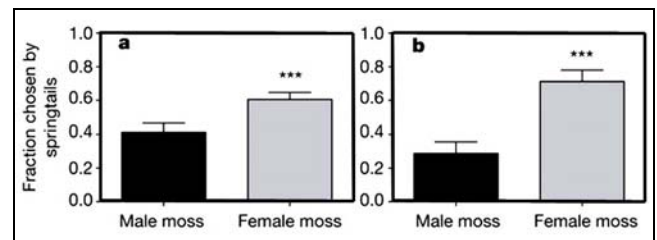
Figure 98. Visits to *Ceratodon purpureus* males and females by the springtail *Folsomia candida*, a showing mean and error bars. n=24 assays, 491 springtails. b represents male vs female samples in an olfactometer; n=10 assays; ***P<0.0001. Redrawn from Rosenstiel *et al.* 2012.

Figure 95. *Bryum argenteum* mixed females and males. Note the open brown tips where antheridia are located. Photo by George Shepherd, through Creative Commons.

Figure 100.   Comparison of profiles (two-dimensional GC3GC–TOFMS chromatograms) of volatile compounds in a male and a female shoot of reproductive ***Ceratodon purpureus***. Colors indicate relative measures of compound abundance; red indicates compounds that are greater than 50% of the largest individual peak area.   Modified from Rosenstiel *et al.* 2012.

Figure 99.   Effect of spray and the springtail ***Folsomia candida*** on sporophyte production in ***Ceratodon purpureus*** / ***Bryum argenteum*** microcosms, showing mean and error bars. + and – represent presence or absence of **springtails** or water spray; n=108 microcosms; *P<0.05.   Redrawn from Rosenstiel *et al.* 2012.
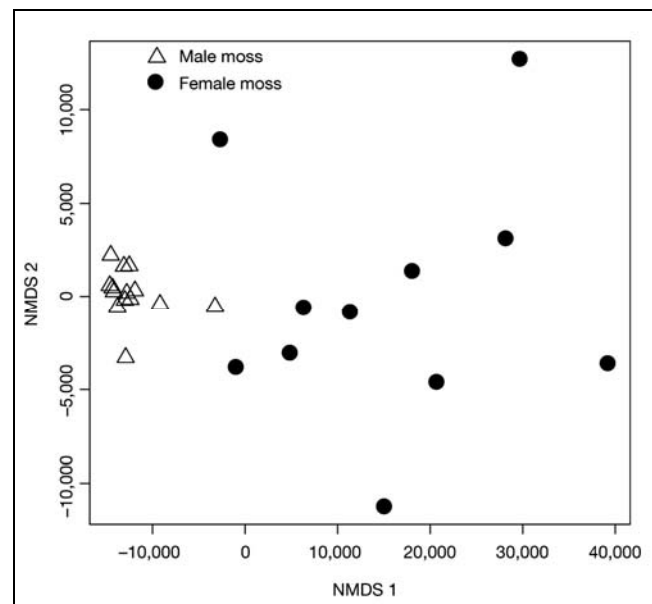
Figure 101.   Differences in volatile gas composition from reproductive male and female individuals of the moss ***Ceratodon purpureus*** using non-metric multidimensional scaling (NMDS). This graph shows that there are significant sex-specific differences in VOC composition (P=0.001). Each symbol represents one individual moss plant.   Modified from Rosenstiel *et al.* 2012.

Sperm Longevity

Few studies have included the life of the sperm or experimented with conditions necessary for their survival. It has always been assumed that sperm had a short life span and were unable to survive desiccation. However, Rosenstiel and Eppley (2009) experimented with sperm from the geothermal moss *Pohlia nutans* (Figure 102) and found this is not the case, at least for this ubiquitous species. Sperm in this species were not affected by temperatures between 22 and 60°C and only showed temperature effects above 75°C. Dilution contributed to their mortality (Figure 103). Moreover, within their safe temperature range 20% survived for more than 200 hours (Figure 104).

Figure 104. The percent of motile (live) *Pohlia nutans* sperm in 40 µl DI or rainwater as they diminish through time. Rainwater created a series of dilutions. Open circles, 1x DI H2O; filled squares, 1x rain; filled triangles, 10x rain; inverted triangles, 100x rain. Redrawn from Rosenstiel and Eppley 2009.

Figure 102. *Pohlia nutans* in the Khibiny Mountains, Apatity, Murmansk. Photo by Michael Lüth, with permission.
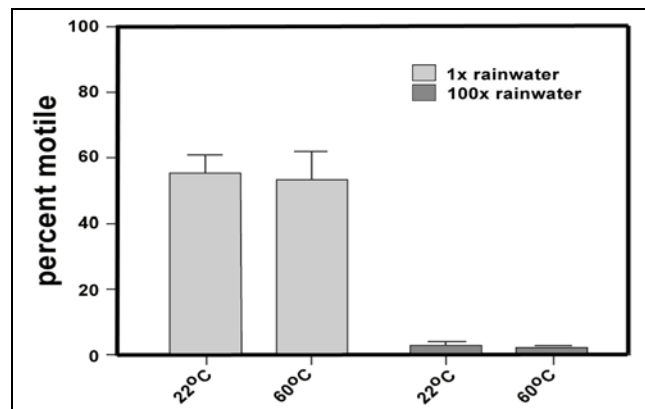

Figure 103. The mean percent of motile (live) *Pohlia nutans* sperm vs dilution in rainwater for 96 hours at 1x (no dilution) and 100x dilution at 22°C and 60°C. Redrawn from Rosenstiel and Eppley 2009.

Shortlidge *et al*. (2012) demonstrated that in *Bryum argenteum* (Figure 95-Figure 96), *Campylopus introflexus* (Figure 105), and *Ceratodon purpureus* (Figure 97) some of the sperm were able to survive environmental desiccation for extended periods of time. The tolerance seemed to be independent of both species and dehydration conditions. Furthermore, the addition of sucrose during drying improved their recovery. Despite the lack of variation among species, there was considerable variability among individuals within a species.

Figure 105. *Campylopus introflexus* with water drops. Such drops can greatly aid fertilization. Photo by Michael Lüth, with permission.

Factors for Successful Fertilization

Multiple factors contribute to the successful fertilization of bryophytes, including sex expression of both sexes, distance to nearest mate, suitable sperm dispersal mechanism (see above), and appropriate weather conditions, especially temperature and water availability. But assessing the relative importance of multiple factors in a single study has rarely been done. Rydgren *et al*. (2006) used generalized linear modelling (GLM) to assess three factors for the dioicous perennial moss *Hylocomium splendens* (Figure 106). They found that most sporophytes (up to 85%) were located within 5 cm of a male, with the longest distance measured being 11.6 cm. But year was an even better predictor of success than distance, attesting to the importance of weather and probably past history, although female segment size as well as distance to closest male were both highly significant. They emphasized the importance of using multiple factors as predictors of reproductive success.

Figure 106. *Hylocomium splendens* with capsules. This dioicous species forms colonies with only one sex, hence not producing sporophytes. Photo by Janice Glime.

Bisang *et al.* (2004) took the distance question further to see if increasing the availability of mates would increase the success of fertilization. They selected two dioicous pleurocarpous mosses, *Rhytidiadelphus triquetrus* (Figure 107) and *Abietinella abietina* (Figure 108) and transplanted individual male shoots into non-sporophyte-bearing female colonies.
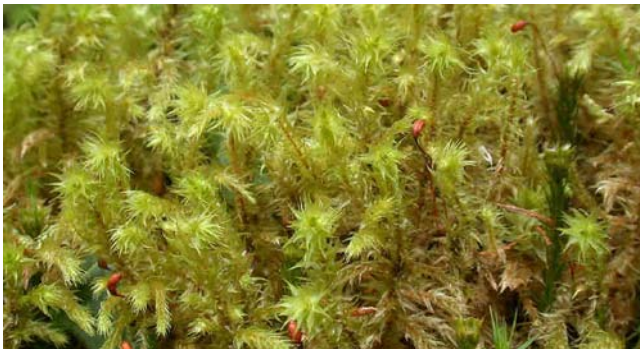

Figure 107. *Rhytidiadelphus triquetrus* with capsules. Photo by David T. Holyoak, with permission.

Figure 108. *Abietinella abietina* in Europe. Photo by Michael Lüth, with permission.

They determined that the number of sporophytes produced depended on the distance from the male mate, *i.e.* spermatozoid source. Furthermore, differences between species were evident, with *R. triquetrus* being more successful than *A. abietina*. They estimated that in *R. triquetrus* the maximum fertilization distance was 34 cm, considerably more than the 3-6 cm previously reported

(Riemann 1972). Bisang *et al.* (2004) found that in *R. triquetrus* the number of female reproductive branches significantly affected the number of sporophytes. By contrast, in *A. abietina* the number of female reproductive branches per plot did not affect sporophyte production. Both species were mate limited.

As one might expect, for both species, when male plants were uphill from female branches, the number of sporophytes was significantly greater than when their positions were reversed, presumably because the sperm were able to travel farther, possibly carried or splashed down the slope by rain (Bisang *et al.* 2004). In *Rhytidiadelphus triquetrus* (Figure 107), a mean of 40 sporophytes per plot (n=25 plots) occurred on sloping substrata compared to 22 on horizontal surfaces. Upslope distances for this species had a mean of 6.2 cm above transplanted males (max=16 cm) and 10.2 cm downslope (max=34 cm). In *Abietinella abietina* (Figure 108), the downslope distances (mean=3.3 cm) were also significantly greater than upslope (mean=1.9 cm) distances, but in this species both the distance travelled and the successful production of sporophytes (mean=2.4 per plot) were considerably less than in *R. triquetrus*. Genes matter.

Granzow de la Cerda (1989) demonstrated movement of sperm in seepage water by transplanting male *Anomodon viticulosus* (Figure 109) to a position at least 25 cm above female plants, a move that resulted in production of sporophytes.

Figure 109. *Anomodon viticulosus* in a seepage area of England. Photo by Janice Glime.

Summary

The liverwort genus *Sphaerocarpos* was the first genus in which sex chromosomes were known in plants. Many bryophytes possess sex chromosomes (**X** & **Y** chromosomes, or designated **U** & **V** to refer to their haploid condition) which may play a role in sex determination. Bryophytes can be **monoicous** (bisexual) or **dioicous** (unisexual). Gametangia in monoicous bryophytes can be **autoicous** (♂ & ♀ gametangia in separate clusters), **paroicous** (♂ & ♀ gametangia in separate groupings but one cluster), or **synoicous** (♂ & ♀ gametangia intermixed in same cluster). **Monoicy** may have arisen through **hybridization** and **polyploidy**. Transitions from monoicy to dioicy and vice versa have happened multiple times. There have been more changes from

monoicy to dioicy than the reverse in mosses, whereas the opposite was the case in hornworts. McDaniel *et al.* suggested that dioicy works best when there are advantages to both sexes for being separate.

Sperm dispersal begins with bursting of the antheridium, often accompanied by movement with surface tension of water drops. In thallose liverworts, sperm are often expelled explosively into the air. Sperm dispersal is usually accomplished by movement through a water film or by splashing and is sometimes aided by gravity. But some species have their sperm dispersed by invertebrates, including insects and mites. Dispersing sperm are known to survive as much as 200 hours and travel distance is known up to 230 cm. Travel distance and weather seem to be the most important factors in determining the success of fertilization in bryophytes.

Acknowledgments

Literature Cited

Ahuja, M. R. 2005. Polyploidy in gymnosperms: Revisited. Silvae Genet. 54(2): 59-68.

Allen, B. H. and Magill, R. E. 1987. In support of a distinct terminology for bryophyte sexuality. Taxon 36: 57-58.

Allen, C. E. 1917. A chromosome difference correlated with sex in *Sphaerocarpos*. Science 46: 466-467.

Allen, C. E. 1919. The basis of sex inheritance in *Sphærocarpos*. Proc. Amer. Philosoph. Soc. 58: 289-316.

Allen, C. E. 1930. Inheritance in a hepatic. Science 71: 197-204.

Andersen, E. N. 1931. Discharge of sperms in *Marchantia domingensis*. Bot. Gaz. 92: 66-84.

Anderson, L. E. 2000. Great discoveries in bryology and lichenology. Charles E. Allen and sex chromosomes. Bryologist 103: 442-448.

Anderson, L. E. and Lemmon, B. E. 1974. Gene flow distances in the moss, *Weissia controversa*. J. Hattori Bot. Lab. 38: 67-90.

Anderson, L. E. and Snider, J. A. 1982. Cytological and genetic barriers in mosses. J. Hattori Bot. Lab. 52: 241-254.

Andersson, K. 2002. Dispersal of spermatozoids from splash-cups of the moss *Plagiomnium affine*. Lindbergia 27: 90-96.

Ando, H. 1980. Evolution of bryophytes in relation to their sexuality. Proc. Bryol. Soc. Japan 9: 129-130.

Andrews, A. L. 1959. Taxonomic notes. XIV. The dioicous form of *Mnium cuspidatum*. Bryologist 62: 230-233.

Arnell, H. W. 1875. Die Skandinaviska löfmossornas kalendarium. Uppsala Univ. Årsskr. Math.-Nat. 4: 1-129.

Bachtrog, D., Kirkpatrick, M., Mank, J. E., McDaniel, S. F., Pires, J. C., Rice, W., and Valenzuela, N. 2011. Are all sex chromosomes created equal? Trends Gen. 27: 350-357.

Barrett, S. C. H., Yakimowski, S. B., Field, D. L., and Pickup, M. 2010. Ecological genetics of sex ratios in plant populations. Philosoph. Trans. Royal Soc. B 365: 2549-2557.

Bedford, T. H. B. 1938. The fruiting of *Climacium dendroides* W. & M. Naturalist 1938: 189-195.

Bedford, T. H. B. 1940. The fruiting of *Breutelia arcuata* Schp. Naturalist 1940: 113-115.

Benson-Evans, K. 1950. Dispersal of antherozoids in *Fegatella*. Nature (London) 165: 324-325.

Bisang, I., Ehrlén, J., and Hedenäs, L. 2004. Mate limited reproductive success in two dioicous mosses. Oikos 104: 291-298.

Boisselier-Dubayle, M. C. and Bischler, H. 1999. Genetic relationships between haploid and triploid *Targionia* (Targioniaceae, Hepaticae). Internat. J. Plant Sci. 160: 1163-1169.

Brizi, U. 1892. Appunti di teratologia briologica. Annuario Reale Ist. Bot. Roma 5: 52-57.

Brodie, H. J. 1951. The splash-cup dispersal mechanism in plants. Can. J. Bot. 29: 224-230.

Burr, I. L. 1939. Morphology of *Cyathophorum bulbosum*. Trans. Roy. Soc. New Zealand 68: 437-456, pls. 44-51.

Callaghan, D. A. and Ashton, P. A. 2008. Attributes of rarity in a regional bryophyte assemblage. J. Bryol. 30: 101-107.

Cameron, R. G. and Wyatt, R. 1986. Substrate restriction in entomophilous Splachnaceae: Role of spore dispersal. Bryologist 89: 279-284.

Cavers, F. 1903. Explosive discharge of antherozoids in Hepaticae. Torreya 3: 179-182.

Cavers, F. 1904a. Contributions to the Biology of the Hepaticae. Part I – *Targionia*, *Reboulia*, *Preissia*, *Monoclea*. Published by Author, Leeds/London.

Cavers, F. 1904b. On the structure and biology of *Fegatella conica*. Ann. Bot. 18: 87-120.

Clayton-Greene, K. A., Green, T. G. A., and Staples, B. 1977. Studies of *Dawsonia superba*. 1. Antherozoid dispersal. Bryologist 80: 439-444.

Crawford, M., Jesson, L. K., and Garnock-Jones, P. J. 2009. Correlated evolution of sexual system and life-history traits in mosses. Evolution 63: 1129-1142.

Cronberg, N. 1991. Reproductive biology of *Sphagnum*. Lindbergia 17: 69-82.

Cronberg, N., Natcheva, R., and Hedlund, K. 2006. Microarthropods mediate sperm transfer in mosses. Science 313: 1255.

Crum, H. 2001. Structural Diversity of Bryophytes. University of Michigan Herbarium, Ann Arbor, 379 pp.

Delph, L. F. 1999. Sexual dimorphism in life history. In: Gender and sexual dimorphism in flowering plants. Springer, Berlin, Germany, pp. 149-174.

Devos, N., Renner, M. A. M., Gradstein, R., Shaw, A. J., Laenen, B., and Vanderpoorten, A. 2011. Evolution of sexual systems, dispersal strategies and habitat selection in the liverwort genus *Radula*. New Phytol. 192: 225-236.

Duckett, J. G. and Pressel, S. 2009. Extraordinary features of the reproductive biology of *Marchantia* at Thursley Common. Field Bryol. 97: 2-11.

El-Saadawi, W., Shabbara, H., and El-Faramawi, M. 2012. The second record of a natural apogamous moss sporophyte worldwide. Cryptog. Bryol. 33: 185-190.

Eppley, S. M., Taylor, P. J., and Jesson, L. K. 2007. Self-fertilization in mosses: A comparison of heterozygote deficiency between species with combined versus separate sexes. Heredity 98: 38-44.

Frisvoll, A. 1982. The status of *Lophozia kiaerii* Jørg. Bryologist 85: 142-144.

Fujisawa, M., Hayashi, K., Nishio, T., Bndo, T., Okada, S., Yamato, K. T., Fukuzawa, H., and Ohyama, K. 2001. Isolation of X and Y chromosome-specific DNA markers from a liverwort, *Marchantia polymorpha*, by Representational Difference Analysis. Genetics 159: 981-985.

Gayet, L. A. 1897. Recherches sur le développement de l'archegone chez les muscinées. Ann. Sci. Nat. Bot. sér. 8 3: 161-258.

Gemmell, A. R. 1950. Studies in the Bryophyta: 1. The influence of sexual mechanism on varietal production and distribution of British Musci. New Phytol. 49: 64-71.

Goebel, K. 1905. Organography of Plants. Translated by I. B. Balfour. Clarenden Press, Oxford.

Granzow de la Cerda, I. 1989. Flujo gamético en poblaciones de un musgo pleurocárpico dioico, *Anomodon viticulosus* (Hedw.) Hook. & Tayl., mediante un experimento de trasplante. Bot. Complutensis 15: 91-100.

Grebe, C. 1917. Studien zur Biologie und Geographie der Laubmoose. Hedwigia 59: 1-108.

Harpel, J. A. 2002. The Northwest forest plan, "Survey and Manage" Bryophytes. Presented at the Annual Meeting of the American Bryological and Lichenological Society, Storrs, Conn., 26-27 July 2002.

Harvey-Gibson, R. J. and Miller-Brown, D. 1927. Fertilization of Bryophyta. *Polytrichum commune* (Preliminary note). Ann. Bot. Lond. 41: 190-191.

Hedenäs, L. and Bisang, I. 2011. The overlooked dwarf males in mosses – Unique among green land plants. Perspect. Plant Ecol. Evol. Syst. 13: 121-135.

Heegaard, E. 2001. Environmental relationships of perichaetial and sporophyte production in *Andreaea* spp in western Norway. J. Bryol. 23: 97-108.

Innes, D. J. 1990. Microgeographic genetic variation in the haploid and diploid stages of the moss *Polytrichum juniperinum* Hedw. Heredity 64: 331-340.

Jesson, L. K. and Garnock-Jones, P. J. 2012. Can classifications of functional gender be extended to all land plants? Perspect. Plant Ecol. Evol. Syst. 14: 153-160.

Jesson, L. K., Cavanagh, A. P., and Perley, D. S. 2011. Polyploidy influences sexual system and mating patterns in the moss *Atrichum undulatum* sensu lato. Ann. Bot. 107: 135-143.

Jong, T. J. de and Klinkhamer, G. L. 2005. Evolutionary ecology of plant reproductive strategies. Cambridge University Press, Cambridge, UK.

Karlin, E. F., Hotchkiss, S. C., Boles, S. B., Stenøien, H. K., Hassel, K., Flatberg, K. I., and Shaw, A. J. 2012. High genetic diversity in a remote island population system: Sans sex. New Phytol. 193: 1088-1097.

Košnar, J., Herbstová, M., Kolář, F., Koutecký, P., and Kučera, J. 2012. A case of intragenomic ITS variation in bryophytes: Assessment of gene flow and role of polyploidy in the origin of European taxa of the *Tortula muralis* (Musci: Pottiaceae) complex. Taxon 61: 709-720.

Laenen, B., Machac, A., Gradstein, S. R., Shaw, B., Patiño, J., Désamoré, A., Goffinet, B., Cox, C. J., Shaw, A. J., and Vanderpoorten, A. 2016. Increased diversification rates follow shifts to bisexuality in liverworts. New Phytol. 210: 1121-1129.

Lidforss, B. 1904. Über die Reizbewegungen der *Marchantia*-Spermatozoiden. Jahrb. Wiss. Bot. 41: 65-87.

Longton, R. E. 1976. Reproductive biology and evolutionary potential in bryophytes. J. Hattori Bot. Lab. 41: 205-223.

Longton, R. E. and Schuster, R. M. 1983. Reproductive biology. In: Schuster, R. M. (ed.). New Manual of Bryology, Vol. 1, Hattori Botanical Laboratory, Nichinan, Japan, pp. 386-462.

Lorbeer, G. 1934. Die Zytologie der Lebermoose mit besonderer Berücksichtigung allgemeiner Chromosomenfragen. I. Jahrb. Wiss. Bot. 80: 567-818.

Lowry, R. J. 1948. A cytotaxonomic study of the genus Mnium. Mem. Torrey Bot. Club 20(2): 1-42.

Maggot, H. and Walton, J. 1942. On the dehiscence of the antheridium and the part played by surface tension in the dispersal of spermatocytes in Bryophyta. Proc. Royal Soc. London B Biol. Sci. 130: 448-461.

Magill, R. E. (ed.). 1990. Glossarium Polyglottum Bryologiae. A Multilingual Glossary for Bryology. Missouri Botanical Garden, St. Louis, MO, 297 pp.

Marchal, É. and Marchal, É. 1907. Aposporie et sexualité chez les mousses. Bull. Acad. Roy. Sci. Belgique Cl. Sci. 1907: 765-789.

Marchal, É. and Marchal, É. 1909. Aposporie et sexualité chez les mousses. Bull. Acad. Roy. Sci. Belgique Cl. Sci. 1909: 1249-1288.

Marchal, É. and Marchal, É. 1911. Aposporie et sexualité chez les mousses. Bull. Acad. Roy. Sci. Belgique Cl. Sci. 1911: 750-756.

McDaniel, S. F. and Perroud, P.-F. 2012. Invited perspective: Bryophytes as models for understanding the evolution of sexual systems. Bryologist 115: 1-11.

McDaniel, S. F., Atwood, J., and Burleigh, J. G. 2013. Recurrent evolution of dioecy in bryophytes. Evolution 67: 567-572.

McLetchie, D. N. and Collins, A. L. 2001. Identification of DNA regions specific to the X and Y chromosomes in *Sphaerocarpos texanus*. Bryologist 104: 543-547.

McQueen, C. B. and Andrus, R. E. 2007. Sphagnaceae. In: Flora of North America Editorial Committee (eds.). Flora of North America North of Mexico. Vol. 27, Bryophytes. Oxford University Press, New York & Oxford, pp. 45-101.

Mogensen, G. S. 1973. A revision of the moss genus *Cinclidium* Sw. (Mniaceae Mitt.). Lindbergia 2: 49-80.

Moyá, M. T. 1992. Phenological observations and sex ratios in *Marchantia chenopoda* L. (Hepaticae: Marchantiaceae). Trop. Bryol. 6: 161-170.

Muggoch, H. and Walton, J. 1942. On the dehiscence of the antheridium and the part played by surface tension in the dispersal of spermatocytes in Bryophyta. Proc. Roy. Soc. London Sec. B Biol. Sci. 130: 448-461.

Natcheva, R. and Cronberg, N. 2004. What do we know about hybridization among bryophytes in nature? Can. J. Bot. 82: 1687-1704.

Newton, M. E. 1983. Cytology of the Hepaticae and Anthocerotae. In: Schuster, R. M. (ed.). New Manual of Bryology Vol. 1, pp. 117-148.

Nishimura, N. and Une, K. 1989. Bryophytes of the Hiruzen Highlands 4. Sexuality and sporophyte-production of

pleurocarpous mosses. Bull. Hiruzen Research Inst., Okayama University of Science 15: 77-81.

Norris, D. H. and Shevock, J. R. 2004. Contributions toward a bryoflora of California. I. A specimen-based catalogue of mosses. Madroño 51: 1-131.

Okada, S., Fujisawa, M., Sone, T., Nakayama, S., Nishiyama, R., Takenaka, M., Yamaoka, S., Sakaida, M., Kono, K., Takahama, M., Yamato, K. T., Fukuzawa, H., Brennicke, A., and Ohyama, K. 2000. Construction of male and female PAC genomic libraries suitable for identification of Y-chromosome-specific clones from the liverwort, *Marchantia polymorpha*. Plant J. 24: 421-428.

Okada, S., Sone, T., Fujisawa, M., Nakayama, S., Takenaka, M., Ishizaki, K., Kono, K., Shimizu-Ueda, Y., Hanajiri, T., Yamato, K. T., Fukuzawa, H., Brennicke, A., and Ohyama, K. 2001. The Y chromosome in the liverwort *Marchantia polymorpha* has accumulated unique repeat sequences harboring a male-specific gene. Proc. Natl. Acad. Sci. 98: 9454-9459.

Olsson, S., Huttunen, S., Sävilammi, T., and Leder, E. 2013. Sex chromosome evolution in mosses. Conference of the International Association of Bryologists, 15-19 July 2013 at Natural History Museum, London, UK.

Parihar, N. S. 1970. An Introduction to Embryophyta. Vol. I. Bryophyta, 4th edn. Central Book Depot, Allahabad.

Peirce, G. J. 1902. Forcible discharge of the antherozoids in *Asterella californica*. Bull. Torrey Bot. Club 29: 374-382.

Perley, D. S. and Jesson, L. K. 2015. Hybridization is associated with changes in sexual system in the bryophyte genus *Atrichum*. Amer. J. Bot. 102: 555-565.

Pfeffer, W. F. P. 1884. Locomotorische richtungsbewegungen durch chemische reize. Unter. Bot. Tübingen 1: 364-482.

Przywara, L. and Kuta, E. 1995. Karyology of bryophytes. Polish Bot. Stud. 9: 1-83.

Ramsay, H. P. and Berrie, G. K. 1982. Sex determination in bryophytes. J. Hattori Bot. Lab. 52: 255-274.

Renner, S. and Ricklefs, R. E. 1995. Dioecy and its correlates in the flowering plants. Amer. J. Bot. 82: 596-606.

Reynolds, D. N. 1980. Gamete dispersal in *Mnium ciliare*. Bryologist 83: 73-77.

Riemann, B. 1972. On the sex-distribution and the occurrence of sporophytes in *Rhytidiadelphus triquetrus* (Hedw.) Warnst. in Scandinavia. Lindbergia 1: 219-224.

Rosenstiel, T. N. and Eppley, S. M. 2009. Long-lived sperm in the geothermal bryophyte *Pohlia nutans*. Biol. Lett. 5: 857-860.

Rosenstiel, T. N., Shortlidge, E. E., Melnychenko, A. N., Pankow, J. F., and Eppley, S. M. 2012. Sex-specific volatile compounds influence microarthropod-mediated fertilization of moss. Nature 489: 431-433.

Rydgren, K., Cronberg, N., and Økland, R. H. 2006. Factors influencing reproductive success in the clonal moss, *Hylocomium splendens*. Oecologia 147: 445-454.

Shaw, A. J. 1991. The genetic structure of sporophytic and gametophytic populations of the moss, *Funaria hygrometrica* Hedw. Evolution 45: 1260-1274.

Shaw, A. J., Flatberg, K. I., Szövényi, P., Ricca, M., Johnson, M. G., Stenøien, H. K., and Shaw, B. 2012. Systematics of the *Sphagnum fimbriatum* complex: Phylogenetic relationships, morphological variation, and allopolyploidy. Syst. Bot. 37: 15-30.

Shimamura, M., Yamaguchi, T., and Deguchi, H. 2008. Airborne sperm of *Conocephalum conicum* (Conocephalaceae). J. Plant Res. 121: 69-71.

Shortlidge, E. E., Rosenstiel, T. N., and Eppley, S. M. 2012. Tolerance to environmental desiccation in moss sperm. New Phytol. 194: 741-750.

Showalter, A. M. 1928. Studies in the cytology of the Anacrogynae-V. Hybrid fertilization in *Riccardia pinguis*. Cellule 38: 295-348.

Springer, E. 1935. Über apogame (vegetativ enstandene) Sporogone an der bivalenten Rasse des Laubmooses *Phascum cuspidatum*. Zeit. Abstam. Vererbung. 69: 249-262.

Sutherland, S. 1986. Floral sex-ratios, fruit-set, and resource allocation in plants. Ecology 67: 991-1001.

Une, K. 1986. Sexuality of the Japanese mosses. Hikobia 9: 339-344.

Villarreal, J. C. and Renner, S. S. 2013a. Correlates of monoicy and dioicy in hornworts, the apparent sister group to vascular plants. Evol. Biol. 13: 1-8.

Villarreal, J. C. and Renner, S. S. 2013b. Transitions from monoicy to dioicy are more likely in hornwort species with small spores, supporting findings from mosses, but with no role for polyploidy. Conference of the International Association of Bryologists, 15-19 July 2013 at Natural History Museum, London, UK.

Walton, J. 1943. How the sperm reaches the archegonium in *Pellia epiphylla*. Nature (London) 152: 51.

Wyatt, R. 1977. Spatial pattern and gamete dispersal distances in *Atrichum angustatum*, a dioecious moss. Bryologist 80: 284-291.

Wyatt, R. and Odrzykoski, I. J. 1998. On the origins of the allopolyploid moss *Plagiomnium cuspidatum*. Bryologist 101: 263-271.

Wyatt, R., Odrzykoski, I. J., and Stoneburner, A. 1992. Isozyme evidence of reticulate evolution in mosses: *Plagiomnium medium* is an allopolyploid of *P. ellipticum* x *P. insigne*. Syst. Bot. 17: 532-550.

Wyatt, R., Odrzykoski, I. J., Stoneburner, A., Bass, H. W., and Galau, G. A. 1988. Allopolyploidy in bryophytes: Multiple origins of *Plagiomnium medium*. Proc. Natl. Acad. Sci. 85: 5601-5604.

Wyatt, R., Odrzykoski, I. J., and Stoneburner, A. 2013. Isozyme evidence regarding the nature of polyploidy in the moss genus *Cinclidium* (Mniaceae). Bryologist 116: 229-237.

Yamato, K. T., Ishizaki, K., Fujisawa, M., Okada, S., Nakayama, S., Fujishita, M., Bando, H., Yodoya, K., Hayashi, K., Bando, T., Hasumi, A., Nishio, T., Sakata, R., Yamamoto, M., Yamaki, A., Kajikawa, M., Yamano, T., Nishide, T., Choi, S.-H., Shimizu-Ueda, Y., Hanajiri, T., Sakaida, M., Kono, K., Takenaka, M., Yamaoka, S., Kuriyama, C., Kohzu, Y., Nishida, H., Brennicke, A., Shin-i, T., Kohara, Y., Kohchi, T., Fukuzawa, H., and Ohyama, K. 2007. Gene organization of the liverwort Y chromosome reveals distinct sex chromosome evolution in a haploid system. Proc. Natl. Acad. Sci. USA 104: 6472-6477.

Zander, R. H. 1984. Bryophyte sexual systems: -oicous versus -oecious. Bryol. Beitr. 3: 4-51.

Zander, R. H., Stark, L. R., and Marrs-Smith, G. 1995. *Didymodon nevadensis*, a new species for North America, with comments on phenology. Bryologist 98: 590-595.

Glime, J. M. and Bisang, I. 2017. Sexuality: Sex Ratio and Sex Expression. Chapt. 3-2. In: Glime, J. M. Bryophyte Ecology. 3-2-1
Volume 1. Physiological Ecology. Ebook sponsored by Michigan Technological University and the International Association of
Bryologists. Last updated 6 March 2017 and available at <http://digitalcommons.mtu.edu/bryophyte-ecology/>.

CHAPTER 3-2
SEXUALITY: SEX RATIO
AND SEX EXPRESSION

JANICE M. GLIME AND IRENE BISANG

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SEXUALITY: SEX RATIO
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Figure 1. *Marchantia polymorpha* developing archegoniophores and antheridiophores on separate thalli. Photo by Bob Klips, with permission.

Sex Ratio

We tend to expect the number of males and females to be about equal (Figure 1), as they are in humans, but many plants and animals have not evolved that way. In bryophytes, it has seemed that mature populations of dioicous species were typically female-biased (Bisang & Hedenäs 2005), and this bias is often huge (but see When Males Are Dominant below). Sex ratios are likely to affect fertilization and thus sporophyte frequency. For example, in *Syrrhopodon texanus* (Figure 2) in the USA central plains, males are very rare (Reese 1984). However, wherever males are found, there are also females bearing sporophytes. These sex ratio imbalances can result from a number of factors, including developmental factors, age, environment, weather, neighbors, and genetic factors.

Figure 2. *Syrrhopodon texanus* in North Carolina, USA. Photo by Janice Glime.

The Bryophyte Female Bias

Stark (2002) reports, based on literature values, that 20 out of 30 species of dioicous bryophytes in those reports have a female bias; 5 have a male bias, and the other 5 seem to have no bias. Bisang and Hedenäs (2005; Bisang, pers. comm. December 2014) reviewed the expressed sex ratios in 143 taxa of dioicous bryophytes (89 mosses, 54 liverworts) based on their own studies and literature data. They used both herbarium specimens and field patches as one category (1) and field studies of individual shoots or thalli (2) as a second. Their study provides us with a cautionary warning that methods can skew the study. They found that for category 1, 85% had a female bias, whereas for category 2, 82% had a female bias. In herbarium studies, the exact bias may be slightly obscured by the tendency of bryologists to collect plants with capsules whenever possible. This is further complicated by the clonal nature of bryophytes, so that it is likely that one small patch is all one clone.

Spore Sex Ratios

In a **dioicous** species, the expectation for a **sporogenous** (giving rise to spores) cell at the onset of meiosis is that it will have one set of chromosomes containing a male chromosome and one set containing a female chromosome. If all proceeds normally during meiosis, a sporogenous cell will produce 4 daughter cells, 2 female and 2 male. But often things do not proceed "normally."

Spore sex ratio has been examined in only a few species so far, by means of cytological evidence (Allen 1919; Newton 1972) or by cultivating plants from spores to sexual maturity (Allen 1919; McLetchie 1992; Shaw & Gaughan 1993; Shaw & Beer 1999; Stark *et al.* 2010). Newton (1972) and Allen (1919) argued for unbiased spore sex ratios in *Plagiomnium undulatum* (Figure 4) and *Sphaerocarpos donnelli*, based on segregation patterns. Also Stark *et al.* (2010) recounted a 1:1 spore sex ratio in *Bryum argenteum*, while ignoring the portion of late-germinating spores. Large fractions of non-germinated spores are also reported for the species investigated in the other cultivation studies, which makes it difficult to assess the actual spore sex ratios in these. This also holds true for the study of the meiotic sex ratio variation in the moss *Ceratodon purpureus* (Figure 3), using a PCR method (Norrell *et al*. 2014). Spore viability varied strongly among sporophytes (0.04 to 0.69) in 9 subsamples each from 11 sporophytes; overall, 63% of the spores did not germinate. Among the germinated spores, the sex ratio at the completion of meiosis was variable, more often female-skewed (proportion of males 0.17-0.72, overall mean 0.41), but not related to spore viability. In contrast, McDaniel *et al.* (2007) found that the EC-NY population cross of *Ceratodon purpureus* had a male-biased sex ratio. They suggested that this was caused by lethal genetic interactions between the sex-linked loci and those of the pseudo-autosomal loci. Nothing like having your genes fight with each other!

While most of the attempts to reveal spore sex ratio so far included easily cultivated ruderals that rapidly express sex in the laboratory, Bisang *et al.* (2017) recently investigated the rarely sexually reproducing perennial dioicous moss *Drepanocladus lycopodioides* (Figure 9).

They used single-spore cultures from field-collected sporophytes, and a molecular sex-associated marker to determine the sex of individual sporelings. They achieved a near-complete or complete spore germinability. In line with cytological evidence in the species mentioned above, spore sex ratio was balanced. However, it differed strongly from the female-skewed adult genetic sex ratios observed in the regional natural populations where the sporophytes were collected, as well as from the sex ratio in the European population established on the basis of a herbarium collection survey (Bisang *et al.* 2013; see also below, Genetic vs Expressed Adult Sex Ratio).

Provided that the observed sex ratios in *Ceratodon purpureus* (Figure 3) correspond to the actual sex ratios in the entire spore population, Norrell *et al.* (2014) may conclude that the noted variability in viability and sex ratio is due to genetic variations within populations. As spore viability and sex ratio were not related, factors other than **sex ratio distorters** (cytoplasmic element such as infection may replace nuclear gene as sex-determination mechanism; see Taylor 1990) may account for sex ratio variation. In this case, and in the case of even spore sex ratios as in *Drepanocladus lycopodioides* (Figure 9) that differ from adult sex ratio biases, other possible causes need to be explored, for example sexual dimorphism in life histories or in eco-physiological requirements, which selectively favor females. Norrell *et al.* (2014) further suggested that the sex ratio might be affected by genetic conflict over meiotic segregation and that this affects the fitness variation in the species.

Figure 3. *Ceratodon purpureus*, a species in which the sex ratio differs among populations. Photo by Michael Lüth, with permission.

Genetic vs Expressed Adult Sex Ratio

Are females truly more abundant, as suggested when counting populations or individuals with sexual structures? Even in female-biased populations male bryophyte plants can be more abundant among non-sex-expressing plants than many counts of plants forming sexual organs would indicate. It is crucial to separate an observed sex ratio pattern into its two elements, namely 1) genetic sex ratio, and 2) differential sex expression among sexes. Knowledge of both components is necessary to understand the underlying mechanisms of sex ratio variation, and to determine when and how observed sex ratio biases are established during the life cycle.

Genetic sex ratios have been studied in relatively few bryophytes to date, and both agreement and differences exist between phenotypically expressed and genetic sex ratios. Newton (1971) pioneered the genetic approach by comparing plants of ***Plagiomnium undulatum*** (Figure 4) with large **heterochromatin bodies** with those having smaller bodies, using heterochromatin body size as a sexual marker. She found that among 239 non-expressing plants examined (34 gatherings) the ratio was 6.5 females to 1 male, but when only the 156 sex-expressing plants in bisexual populations were considered, the ratio was only 3.9♀:1♂. Newton concluded that using only fertile plants underestimates the abundance of male plants. She determined that the non-expressing males of ***Plagiomnium undulatum*** were rarer than non-expressing females, but not as rare as in the expressing male to female ratio. Using this ratio change, Newton suggested a lower sex expression rate for males than for females. This could also suggest a narrower range of environmental conditions in which sex expression is able to occur. Newton (1972) demonstrated in **P. undulatum** that the environmental conditions for production of antheridia were more restricted than those needed for production of archegonia. However, she could find few differences between the sexes for the environmental parameters she tested. In at least some taxa male plants may be less fit, surviving in a narrower range of conditions than do females. The balance of conditions is complicated in bryophytes by the fact that antheridia typically take longer to develop than do archegonia, thus requiring different conditions to initiate them and needing to survive for a longer time under a greater range of conditions.

Figure 4. ***Plagiomnium undulatum***, a species where antheridial expression requires a narrower set of environmental conditions than those required for archegonial expression. Photo by David T. Holyoak, with permission.

Cronberg *et al.* (2003) used electrophoresis to identify **genets** [genetic individual that develops from the zygote and produces **ramets** (any physically and physiologically independent individual plants, whether sexually produced or derived by v**egetative reproduction**) of the same genotype vegetatively] in ***Plagiomnium affine*** (Figure 5), reducing the number of plants with unknown sex to 10%. At the ramet level, the overall sex ratio had a slight female bias, but at the genet level it was close to 1:1. Cronberg *et*

al. (2006) found a sex ratio in five plots of ***Hylocomium splendens*** (Figure 27) to be female biased at the ramet level (2.6 female to 1 male), but it was male biased at the genet level (1 female to 3 males).

Figure 5. ***Plagiomnium affine***, a species that invaded European forests multiple times. Photo by Hermann Schachner, through Creative Commons.

More recently, cultivation approaches and novel techniques of molecular sex markers have been used to reveal genetic sex ratios (see below, Sex Expression and the Shy Male Hypothesis).

Causes of Female Bias

There have been many efforts to explain this female bias (Longton & Schuster 1983; McLetchie & Puterbaugh 2000; Crowley *et al.* 2005; Bisang *et al.* 2006; Rydgren *et al.* 2010; Stark *et al.* 2010; Horsley *et al.* 2011, and many more), to date usually the expressed female bias. In fewer cases the underlying genetic sex ratio has been approached. We ask, if it is real, what evolutionary forces drive a female bias? Henceforth we present a number of studies that have examined bryophyte sex ratios, its variation, and discuss possible explanations for the observed patterns.

Sex Expression and the Shy Male Hypothesis

As already mentioned, our methods so far are usually indirect, such as using capsules, perichaetia, and perigonia to assess sex, and few studies involve a direct count that provides a ratio under field conditions. In most cases to date, we are unable to determine the sex of plants not producing sexual organs. Several factors could cause a disproportionate phenotypically expressed sex ratio. Might males take more time to develop and express sexual maturity? Or is the unbalanced observed sex ratio merely a consequence of differential sex expression, as Newton (1971) suggested for ***Plagiomnium undulatum*** (see above; Figure 4)?

Hedenäs *et al.* (2010) examined the question of sex ratio in non-expressing females of ***Drepanocladus trifarius*** (=*Pseudocalliergon trifarium*) (Figure 6) using a new technique of genetic sex-targetting markers. They estimated the European population sex ratio to be 1.93:1 (female:male) (Hedenäs *et al.* 2010). There were no

significant differences among expressed, non-expressed, and population sex ratios, and thus no differences in expression rates between the sexes.

Figure 6. *Drepanocladus trifarius*. Photo by Andrew Hodgson, with permission.

The "**shy male hypothesis**" suggests that males express sex less frequently than do females (Stark *et al.* 2010). Those individuals that have no sexual structures are often referred to as "sterile," but sterile implies that they are incapable of producing sexual organs. The preferable terminology, therefore, is "non-expressing" or "non-sex-expressing" (Bowker *et al.* 2000).

Using *Bryum argenteum* (Figure 7), Stark *et al.* (2010) tested both the "shy male" hypothesis and the hypothesis that sex ratios of sporelings are biased (for the latter, see above, Spore Sex Ratios). They used both sex-expressing and non-expressing collections from the field and shoots grown from spores in the lab. The field collections revealed a greater than 80% female bias among 154 field collections in the USA, with male expressions being even more rare in arid habitats of the Mojave Desert and California chaparral. They grew non-expressing shoots from mixed-sex populations until they reached sexual expression and found that the ratio of males to females did not differ significantly from that of the sexually expressing field populations. Hence, the "shy male hypothesis" lacks support in *Bryum argenteum*. Populations grown from spores, on the other hand, had a 1:1 sex ratio. This leads us to the conclusion that in these species there are factors between sporeling and mature gametophyte that differentially affect the two sexes.

Brzyski *et al.* (2013) cultivated *Marchantia inflexa* (Figure 8) from different environments. In contrast to *B. argenteum* (Figure 7), they found that in the roadside habitat the males were 4.7 times more likely to express sex than were females, despite the better growth for females in that habitat.

Using herbarium samples from a wide geographic range, Bisang and Hedenäs (2013) assessed the sex ratio in expressing and non-expressing *Drepanocladus* (= *Pseudocalliergon*) *lycopodioides* (Figure 9), using a sex-associated molecular marker to identify the sex of non-expressing plants. They determined that the true genetic population sex ratio (non-expressing plants included) was the same (2.6:1 female bias) as that when non-expressing plants were not included, thus refuting the "shy male" (non-

expressing male) hypothesis also in this species. This distinct female genetic sex ratio bias in the adult population differs from the balanced spore sex ratio (see above, Spore Sex Ratios; Bisang *et al.* 2017). In accordance with the situation in *Bryum argenteum* (Figure 7), biased population sex ratios in this species seem to arise at life cycle stages after spore germination. In any case, simply refuting the "shy male" hypothesis in a species does not answer our question regarding the unequal adult sex ratio.

Figure 7. *Bryum argenteum*, a species with 80% females in the Mojave desert, USA, but with a 1:1 ratio of plants grown in the lab from spores. Photo from India Biodiversity Portal, through Creative Commons.

Figure 8. *Marchantia inflexa* thallus, a liverwort where males and females have different environmental stressors. Photo by Scott Zona, with permission.

Germination Patterns and Spore Mortality

But if we examine what might explain such a biased expressed ratio, we know that meiosis in a dioicous plant such as *Sphaerocarpos texanus* (Figure 10), known to have X and Y chromosomes (now called **U** and **V**), should result in an equal number of male and female spores, as found in *Bryum argenteum* (see above). Nevertheless, also McLetchie (1992) found numbers that support female dominance in sex expression of the liverwort *Sphaerocarpos texanus*. In both the field and in culture,

Sphaerocarpos texanus produces a greater number of pure female clumps, followed by mixed sexes and then pure males. McLetchie interpreted this to mean that males have a lower survival rate than females, both before germination and while growing. The first loss of males, leading to an unequal germination rate, assumedly results from unequal survival and germination capability of spores. This abortion can start immediately after meiosis (Figure 11). These differences can result from a difference in allocation of resources to male and female spores, leading to reduced viability and germination success in the males (McLetchie 1992). McLetchie (1992, 2001) also found that there was a sex-specific determination at germination in *Sphaerocarpos texanus*, with more female than male germinations. Could it be, as suggested by Schuster (1983) for *Sphaerocarpos* (Figure 10), that small spores become male plants and that their poor nutrient conditions as spores give them an inferior start in life, causing them to die soon after producing sperm?

Figure 11. SEM image of spores of *Fontinalis squamosa* showing abortion of two spores in the tetrad. Photo by Janice Glime.

Even where spores are retained in tetrads and thus travel together, as in *Riccia* (Figure 12), females of *Riccia frostii* (Figure 13) outnumber males (Pettet 1967). In this case, at least one factor is greater mortality of males under conditions of rapid desiccation. In *Cryptothallus* (Figure 14), where sex is determined by sex chromosomes, females outnumber the males 5:1 (Shaw 2000). It appears in this genus that some spores of the tetrad are inviable.

Figure 9. *Pseudocalliergon lycopodioides*, a moss with a 2.6:1 female-biased sex ratio among both non-expressing and fertile plants. Photo by David T. Holyoak, with permission.

Figure 10. *Sphaerocarpos texanus* showing female population. Photo by Michael Lüth, with permission.

McLetchie (1992) suggests that even after germination, males may have inferior competitive ability or be more susceptible to unfavorable environmental conditions. In mixed clumps, females may provide added protection that permits more males to survive, and both benefit from the increased sexual reproductive success.

Figure 12. *Riccia sorocarpa* spore tetrads ready for dispersal. Photo by Des Callaghan, with permission.

It would be helpful to know the number of males and females at all life cycle stages to elucidate further the causes of biased sex ratios. Modern molecular techniques (see *e.g.* Pedersen *et al.* 2006; Bisang *et al.* 2010; Bisang & Hedenäs 2013) or cultivation methods (*e.g.* Stark *et al.* 2010) make this possible, albeit very time-consuming.

Figure 13. *Riccia frostii*. Photo by Rosemary Taylor, with permission.

Figure 14. *Cryptothallus mirabilis* producing sporophytes from its subterranean mycorrhizal thallus. Photo by Michael Lüth, with permission.

Environmental and Geographic Differences

Bell (1980) stressed the importance of life history in understanding evolutionary theory. Although he focussed on animals, his principles can apply to bryophytes. He posited that "once reproductive costs are introduced, reproduction will be optimized rather than being merely maximized. The 'survival cost' is the decrease in the rate of adult survival which accompanies a given increase in fecundity." Sex ratio can be influenced by these life history principles.

In the Bisang and Hedenäs (2005) study, expressed sex ratio variation not only occurred among species, but also within species. The latter variation was related to geographic region, elevation, year, substratum, and plant/clone maturity. It was interesting that Bisang and Hedenäs did not find a direct relationship between the sex ratio and the proportion of sporophytic samples or shoots across species. This suggests that the bryophytes may have evolved to optimize the sex ratio for the conditions where they grow. But Bisang and Hedenäs contend that the "data do not support a generalization that the most strongly

female-biased sex ratios among dioicous bryophytes occur in 'extreme environs.'" Rather, they suggest that phylogenetic history may explain at least some of the species-wide sex ratios better than current habitat conditions (Bisang *et al.* 2014).

In *Marchantia inflexa* (Figure 8), habitat seems to play a strong role in the performance of the sexes (Brzyski *et al.* 2013). Females had both higher growth rates and more asexual reproduction among road-collected plants whereas males tended to have better growth and asexual reproduction (but not significantly) in river-collected plants.

Environmental differences can occur even within short distances. Although the sexes of *Marchantia inflexa* (Figure 8) are spatially separated within populations, they overlap in habitat use and their distributions are not correlated with an environmental gradient (Fuselier & McLetchie 2004). Males collected on the island of Trinidad tend to occur in a wider range of light conditions than do females (Fuselier & McLetchie 2002, 2004). Groen *et al.* (2010a), using five locations in Trinidad, found that males in *M. inflexa* occur where there is more tree-canopy openness than that found in locations where females occur. Groen and coworkers (2010a, b) also found that males of this species had lower chlorophyll *a* to *b* ratios compared to females, the opposite of what one would predict for plants in more open areas. On the other hand, in populations from Grangier County, Tennessee, USA, Fuselier (2004) found that laboratory-grown and field-grown males showed little difference in their responses to moisture and light levels.

Fuselier and McLetchie (2002) tested the influence of selection on asexual and sexual fitness components in *Marchantia inflexa* (Figure 8) using a field study on natural selection. They grew replicates of female and male genotypes from Trinidad under two different light environments in a greenhouse. Not only did they find that the timing for the onset of asexual reproduction and the determination of size of the plant during early development were under sex-specific selection in low light, but for females, there was also an apparent cost for plasticity in the timing of their asexual reproduction in high light. Selection pressures favoring asexual fitness tended to favor monomorphism (both sexes looked the same) rather than sexual dimorphism. But if the female morphology was expressed, then selection acted on sexual fitness rather than on morphology, hence favoring females.

McLetchie and Puterbaugh (2000) also explored the relationship of male and female numbers, using the thallose liverwort *Marchantia inflexa* (Figure 8) in Trinidad. They found that among 209 individual patches of this liverwort along a stream, 83% were not expressing sexual characters at all, 9% had both male and female thalli, and the remainder were 4% all male and 4% all female. In bisexual patches, the proportion of males ranged 22-80%. This is hardly an image of sexual dominance by either sex and is one of the examples of infraspecific variation mentioned by Bisang & Hedenäs (2005). Furthermore, when gemmae from non-sex-expressing field collections were planted, the resulting ratio of plants was 10 females to 8 males.

But in those 209 patches of *Marchantia inflexa* (Figure 8), the role of environment in affecting sex expression began to emerge (McLetchie & Puterbaugh

2000). Those patches with the lowest canopy openness, *i.e.* less light, exhibited less sex expression. And, growth patterns of male and female plants differed. The female plants grew faster and produced more meristematic tips, but they had lower levels of asexual reproduction (gemmae) than did the male plants. In fact, asexual reproduction was negatively correlated with the number of meristematic tips (see also 3.4, Reproductive Trade-off). This suggests that the female plants might be more competitive through more rapid growth and soil coverage, but male plants might have greater ability to disperse and occupy new ground. And, this behavior could lead to large numbers of single-sex patches and biased sex ratios among mature, sex-expressing plants.

In the Mojave Desert of southern Nevada, USA, the female-biased desert moss *Syntrichia caninervis* (Figure 15) is a dominant moss in the blackbrush (*Coleogyne ramosissima*) community. Bowker *et al.* (2000) demonstrated that microhabitat can play a major role in sex expression in this xerophytic dioicous moss. On one 10-ha site, sex-expressing female ramets dominated males 14:1 (890 samples). In this harsh environment, it is not surprising that 85% of the ramets did not show sexual expression during their entire life span. Demonstrating responses similar to those of *Sphaerocarpos texanus* (Figure 10), *Syntrichia caninervis* showed more sexual expression in shaded sites, where there was more moisture and plants were taller. Predictably, ramet height was positively correlated with soil surface moisture in more exposed sites. Male ramets were restricted to shaded sites, whereas female ramets and populations occurred in both shaded and exposed locations. There were no mature sporophytes in the ramets sampled, and only 3% of the populations overall had mature sporophytes. Among the reasons for the success of females are their greater ability to produce biomass and to produce new protonemata and shoots from detached leaves that have experienced desiccation (Figure 16-Figure 17), an inevitable event in this habitat (Stark *et al.* 2005). In this case it appears that there is a strong selection against males in some environments and that females are more tolerant.
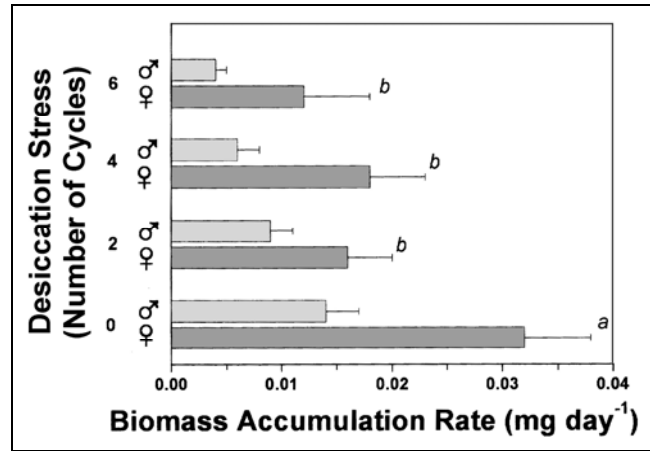

Figure 16. Effect of varying numbers of desiccation cycles on biomass accumulation rates in males and females of *Syntrichia caninervis* leaves. Values are means (n=20) ± 1 SE. Cycles with different letters are significantly different (*P*<0.05, Tukey's multiple comparison). Graph modified from Stark *et al.* (2005).
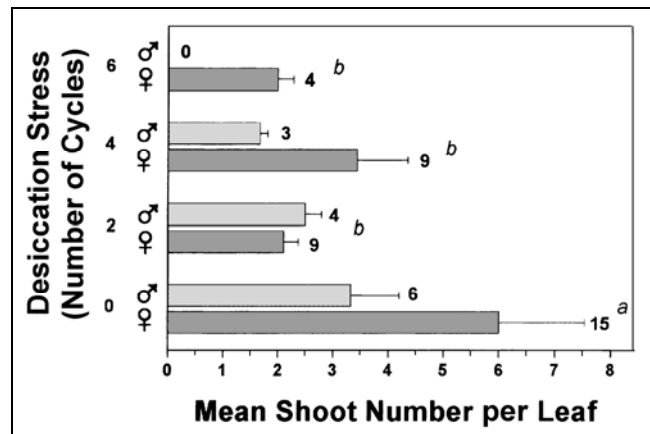
Figure 17. Shoot production from regenerating leaves of males and females of *Syntrichia caninervis* subjected to varying numbers of desiccation cycles. Values are means (n=20) ± 1 SE. Cycles with different letters are significantly different (*P*<0.05, Tukey's multiple comparison); data were log-transformed before analysis. Graph modified from Stark *et al.* (2005).

Sex-expressing males of *Syntrichia caninervis* (Figure 15) are restricted to higher elevations in the Mojave Desert where they are tucked under shrubs (Stark *et al.* 2005). Female plants, on the other hand, have no such habitat restriction and are relatively widespread along the elevational gradient. Whenever only one sex is expressed, it is always the female. Stark *et al.* (2005) found that the greatest stress results from rapid drying cycles (Figure 16). The plants need 72 hours to deharden after a gradual drying event. In their desert habitat, they experience 40-70°C temperatures in a dry condition, but may experience 30-40°C while still hydrated. Differential abilities to handle such stress can have severe effects on sex ratios.

Blackstock (2015) investigated sex expression rate, sporophyte frequency, and sex ratios of the dioicous liverwort *Frullania tamarisci* (Figure 18) in western Britain, comparing woodland populations with exposed coastal colonies. Whereas the former were highly fertile, the coastal population exhibited a distinct female sex ratio bias, spatial segregation of the sexes, and male scarcity, which appear to limit sporophyte formation.

Figure 15. *Syntrichia caninervis*. Photo by John Game, through Creative Commons.

Figure 18. *Frullania tamarisci*, a species in which habitat affects the sex ratio. Photo by Hermann Schachner, through Creative Commons.

As seen in the moss genus *Macromitrium* (Figure 19), both geographic and ecological differences are present (Une 1985). Dwarf males (see Chapter 3-3, Dwarf Males, in this volume) of the isosporous species *M. gymnostomum* and *M. japonicum* are widely distributed in Japan, whereas normal males are rare and occur only in low altitudes and latitudes on the Pacific Sea side of Japan. Experimental results suggest that this difference is due to suppression of growth of males at low temperatures, whereas females and dwarf males are less affected by the cold.

Figure 19. Neotropical *Macromitrium* sp. with capsules. Photo by Michael Lüth, with permission.

Hassel *et al*. (2005a) compared mountain and lowland populations of *Pogonatum dentatum* (Figure 20) in Fennoscandia. They found differences in age of maturation, with females in the lowlands producing spores in their second year, whereas those in the mountains required three years. Nevertheless, both populations produced sex organs in the second year.

Fisher (2011) examined differences between sex ratios at the edges vs the centers of distributions in *Syrrhopodon involutus* (Figure 21). In this species, he found that female sex expression was significantly lower at the margins than in central areas of the species complex. Furthermore, the margins had a higher proportion of non-sex-expressing individuals. On the other hand, the proportion of male-expressing plants did not differ significantly between marginal and central areas. Nor did the percentage of female-expressing successfully producing sporophytes

plants differ between margins and the centers of distribution. Fisher concluded that this indicates the availability of males constrains sporophyte production for this species in both the margins and the centers of distribution. Could it also mean that the two sexes are more likely to differ genetically on the margins?

Figure 20. *Pogonatum dentatum* in Norway. Photo by Michael Lüth, with permission.

Figure 21. *Syrrhopodon involutus*. Photo by Jan-Peter Frahm, with permission.

The only study so far that explores the association between genetic adult sex ratio and environmental factors was conducted with the wetland moss *Drepanocladus trifarius* (Figure 6), a species that rarely produces sexual structures or sporophytes (Bisang *et al.* 2015). In a total of 277 shoots representing 214 locations, Bisang and coworkers determined sex using a female-targetting molecular marker. They found that the sexes did not differ in shoot biomass. The sexes were randomly distributed and environmental factors associated with the localities of the two sexes did not differ. Nevertheless, the sex ratio had a strong female bias of 28:1! In this case, the environment does not appear to be the cause of the biased genetic sex ratio.

When Are Some Males More Stress Tolerant?

Loss of males due to stressful environments is not true for all species. Cameron and Wyatt (1990) found that males of *Splachnum* are able to survive in more stressful habitats than are females. Using experimental cultures, they found that for *S. ampullaceum* (Figure 22), *S. rubrum* (Figure 23-Figure 24), and *S. sphaericum* (Figure 25), low light and low *p*H favored production of males over females,

whereas good nutrition seemed only to affect *S. ampullaceum*. Nevertheless, the sexes are highly clumped and the sex ratio is typically 2:1 female to male.

Figure 22. *Splachnum ampullaceum* with sporophytes in southern Europe. Photo by Michael Lüth, with permission.

Figure 23. *Splachnum rubrum* males, which are more abundant than females in this species when provided with low light, low *p*H, and good nutrition. Photo by Dick Haaksma, with permission.

Figure 24. *Splachnum rubrum* with capsules on Isle Royale, Michigan, USA. This is a species where males are favored over females by low light, low *p*H, and good nutrition. Photo by Janice Glime.

Figure 25. *Splachnum sphaericum* with capsules, a species where males are favored over females by low light, low *p*H, and good nutrition. Photo through Creative Commons.

Other Differences between Populations

Even within a species complex (based on isozyme analysis), the ratios can vary in size and bias. For example, in the liverwort *Aneura pinguis* (Figure 26) complex, in one cryptic species there were equal frequencies of males and females, in one male plants numbered more, and in a third female plants were more numerous (Buczkowska *et al.* 2006). In *Ceratodon purpureus* (Figure 3) cultivated from spores to maturity, the sex ratio is also heterogeneous (Shaw & Gaughan 1993), but a female bias occurred in more than half of the eleven studied populations.

Figure 26. *Aneura pinguis* with perianths and one black capsule. Photo by Dick Haaksma, with permission.

Another possibility might be that dispersal and germination success differ between male and female propagules, causing more females to colonize. Such a difference would not present itself in experiments on germination of spores from individual capsules or other propagules because these would not have been subjected to the stresses of long-distance dispersal. Males and females

would then not arrive and / or establish together and long periods of time may elapse before both sexes are present. And these sexes may represent different cryptic species. Using allozyme electrophoresis, Cronberg (2002) showed that **Hylocomium splendens** (Figure 27) presented 103 haplotypes in a sample of 694 shoots on 10 Baltic islands. The number of clones, sex expression, and sporophyte frequency increased, and sex ratios became more balanced with the age of the islands.

Figure 27. *Hylocomium splendens*, a moss with many haplotypes in Europe. Photo through Wikimedia Commons.

Frequency and Timing of Sex Expression

Functional sex ratio is dependent on the frequency with which it is expressed and whether sequential sex changes ever occur. Do males express sex only once, or do they continue to do it year after year? Likewise, are females able to repeat their high-cost sexual endeavors?

Zoologists have named two strategies of sexual frequency as **iteroparity** and **semelparity.** The story behind the term **semelparity** helps one to remember its meaning. *Semel* comes from the Latin *semel*, meaning once, a single time. *Parous* is derived from *pario*, meaning to beget. The origin seems to be in Greek mythology, where Semele, daughter of Cadmus and Harmonia, was the mortal mother of Dionysus by Zeus. In the myth, Semele asked Zeus to reveal himself as his true entity. Because he had promised to grant her a boon, he could not break his promise, revealing himself as the lightning bolts he represented, and that cause any human that views them to incinerate. Hence, Semele could bear a child only that once, then died.

The terms semelparity and iteroparity have been applied to plants, as for example the century plant that blooms only once, then dies, certainly an example of semelparity. But the terms are rarely used for bryophytes. Hassel *et al*. (2005a) used it in relation to the populations of **Pogonatum dentatum** (Figure 20) in the mountains vs lowlands of Fennoscandia to describe their differences in sexual parity. Among mountain females, 41% of the shoots branched following reproduction the first time, a condition known as **iteroparous** (having sexual crossing in iterations, *i.e.*, successive years). On the other hand, the lowland female populations did not produce branches, thus being unable to produce sexual organs the next year, a behavior one could call **semelparous** (having sexual crossing only once), assuming it never produces such innovations. On the other hand, new plants might arise from rhizomes.

More likely representatives of semelparity are the **Splachnaceae** (Figure 28-Figure 29). As they mature, their habitat changes. They produce capsules and their substrate is no longer able to support the early stages of the life cycle, nor do they produce additional sporophytes in subsequent years.
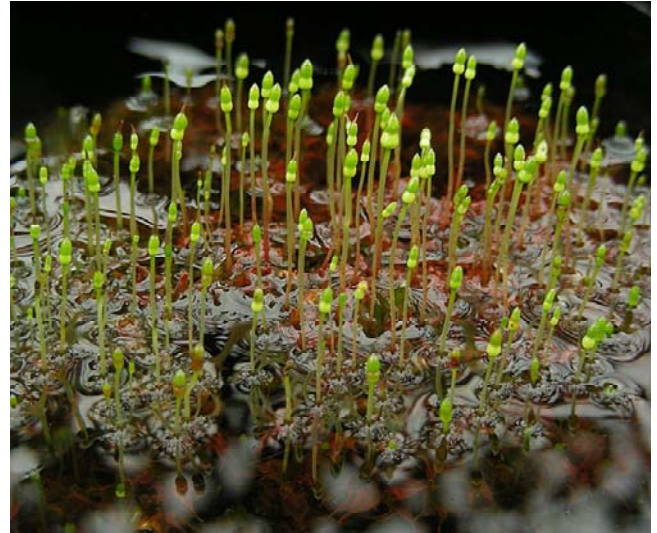
Figure 28. *Splachnum rubrum* females with young sporophytes. Photo by Michael Lüth, with permission.

Figure 29. *Splachnum rubrum* females with mature capsules. Photo by Michael Lüth, with permission.

Among **pleurocarpous** plants, both males and females continue to produce ramets or side branches where new gametangia can form, making them **iteroparous**. But what is the general case in **acrocarpous** mosses? Observations of clumps suggest that individual sporophytes are produced annually, but do these come from the same branches or from new plants formed within the clump? For example, in *Weissia* spp. (Figure 30) most shoots are **unisexual** (only male or female) during a given reproductive cycle (Anderson & Lemmon 1973, 1974). To answer these questions we must understand the differences in growth habits among the bryophytes.

Figure 30. *Weissia controversa* with capsules. Photo by J. C. Schou, with permission.

Branching Patterns and Gametangial Location

Among mosses there are two overall branching patterns: **sympodial** for **acrocarpous** mosses and **monopodial** for **pleurocarpous** mosses (with some exceptions). **Sympodial** growth is growth in the absence of apical dominance, *i.e.*, apical growth is terminated (in acrocarpous mosses it is terminated by the gametangia) and the main axis produces branches by innovations or produces ramets at the base. **Monopodial** growth is growth with apical dominance wherein new apical stem and leaf tissue continues to be added. In pleurocarpous mosses, the primary axis produces side branches where the gametangia develop, while the primary axis continues growth. In acrocarpous mosses, growth appears at first to be monopodial, but once gametangia occupy the apex, new growth of that axis ceases. (See Mishler & De Luna 1991 for a discussion of branching in mosses.)

The family **Polytrichaceae** exhibits both of these branching patterns, often in the same species. In *Polytrichum* (Figure 31), we know that new growth originates in the antheridial splash cup and that new splash cups are produced in successive years on the primary axis. In his studies on *Pogonatum dentatum* (Figure 20), Kristian Hassel (pers. comm. 24 January 2014) found that both male and females were able to produce innovations just below their gametangia, but this behavior seemed to be affected by the environment. Furthermore, in Scandinavia he found that production of innovations varied among species in **Polytrichaceae** as well as between males and females. For example, Hassel never observed innovations on shoots of *Polytrichum commune* (Figure 31) that had sporophytes, but in *Polytrichastrum alpinum* (Figure 32) and *Pogonatum urnigerum* (Figure 33) such innovations are common. Males of these species usually produce new antheridial splash cups on the primary axis year after year, reliably enough that these have been used as growth markers. In the genus *Atrichum* (Figure 59-Figure 61), sex expression occurs via branching (Linley Jesson, unpubl., pers. comm. 25 January 2014).

Figure 31. *Polytrichum commune* with capsules, a species where innovations apparently do not occur. Photo by David T. Holyoak, with permission.

Figure 32. *Polytrichastrum alpinum*. Photo from Botany Department, University of British Columbia, Canada, with permission.

Figure 33. *Pogonatum urnigerum* males with splash cups. Photo by David T. Holyoak, with permission.

But it appears that among **acrocarpous mosses** in the **Bryopsida**, the formation of archegonia at least terminates the apical growth, requiring subapical innovations for further extension of that gametophore. For example, in *Philonotis* (Figure 34), new male inflorescences can appear on innovations in successive years. Mishler and Oliver (1991) reported that female gametangia terminated growth of annual innovations in the dioicous acrocarpous moss *Syntrichia ruralis* (Figure 35). Bisang and Ehrlén (2002)

have observed perichaetia terminating each annual innovation of female stems of *Dicranum polysetum* (a species with dwarf males; Figure 36-Figure 38). *Tortella rigens* (Figure 39) females have similar innovations, although the perigonia could not be located (Lars Hedenäs, pers. comm. 23 January 2014). The multiyear behavior in males seems to be less obvious, although the **Polytrichaceae** demonstrate the possibility for growth to continue apically, even when a splash cup is present (Figure 40).

Figure 34. *Philonotis fontana* with antheridia and innovations. Photo by Michael Becker, through Creative Commons.

Figure 36. *Dicranum polysetum* showing multiple sporophytes in one apex. The apical production of archegonia stops growth of that apex (see Figure 37). Picture by Janice Glime.

Figure 35. *Syntrichia ruralis* with sporophytes arising from archegonia that terminate its apical growth. Photo by Peggy Edwards, with permission.

Figure 37. *Dicranum polysetum* tomentum and innovations. Photo by Walter Obermayer, with permission.
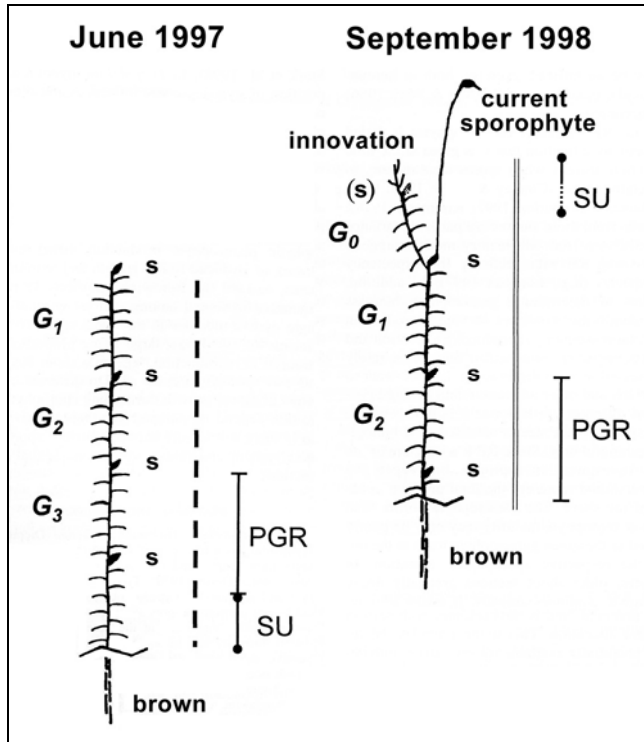
Figure 38. Representation of unbranched plant of *Dicranum polysetum* indicating locations of shoot sections and reproductive structures. G_x indicates annual growth interval, with G_0 indicating current year's growth as innovation. Note that the innovation is just below the apex where the sporophyte emerges from the sexual structure. **s** indicates location of sexual organs, in this case perichaetia. **Brown** portions are at the base and move progressively upward as the stem grows. **SU** indicates summer growth; **PGR** indicates proximal green portion. **Broken line** on **1997 drawing** indicates green gametophyte; **thin double line** on **1998 drawing** indicates the green gametophyte at the time of the G_1 sporophyte maturation. Modified from Bisang & Ehrlén 2002.

Figure 39. *Tortella rigens*, a species with female innovations, growing or exposed rock. Photo by Michael Lüth, with permission.

Figure 40. *Polytrichum juniperinum* with new growth arising from the splash cups. Photo by Janice Glime.

Also those acrocarpous mosses that have horizontal growth forms much like those of pleurocarpous taxa do produce antheridia and archegonia in multiple years, but usually not at the original apex. In fact, it is the innovations, growing horizontally, that make them look pleurocarpous. This group includes such taxa as *Racomitrium* (Figure 41), *Hedwigia* (Figure 42) (Sean Edwards, pers. comm. 23 January 2014), and some members of the **Orthotrichaceae** (Figure 43). For example, Arno van der Pluijm (pers. comm. 23 January 2014) tells me that his search for males of the dioicous acrocarpous *Zygodon* (*Zygodon viridissimus*, Figure 43) in **Orthotrichaceae**) in old herbarium collections revealed male plants with multiple male buds on the same stem. He found that one or two innovations can develop directly below the perigonium, make a new perigonium, then branch again. He was able to observe up to five generations of male buds in 19th century collections. This family has members that often appear to be pleurocarpous, with predominantly horizontal growth like that of *Macromitrium* (Figure 19).

Figure 41. *Racomitrium heterostichum* with capsules. Photo by Jan-Peter Frahm, with permission.

Figure 42. *Hedwigia ciliata* with capsules. Photo by Robert Klips, with permission.

Figure 44. *Riccia glauca* showing apices where growth occurs (at end of rib). Photo by Jan-Peter Frahm, with permission.

Figure 43. *Zygodon viridissimus* var *viridissimus* with capsules. Photo by David T. Holyoak, with permission.

Figure 45. *Riccia glauca* spores showing their location behind the apex. Photo by Rick Haaksma, with permission.

In **thallose liverworts**, the apex typically continues to grow or divides to produce two branches for the succeeding year. For example, in perennial *Riccia* (Figure 44-Figure 45) species, the apex continues growing, and if fertilized, leaving successive sporangia to mature – and decay – behind (Rod Seppelt, pers. comm. 23 January 2014). Similarly, in Australia populations of fertilized *Lunularia cruciata* (Figure 46) produce white scalelike conical structures on the upper surface of the thallus. These enclose fully developed sporangia with spores and elaters as well as the **carpocephalum** (sporangial receptacle in most thallose liverworts). When autumn rains arrive, the stalks suddenly elongate to elevate the mature sporangia. It appears that in thallose liverworts, growth continues at the apex following gametangial formation and new gametangia later arise near the new apex.

Leafy liverworts have a growth pattern in which most species have terminal perianths surrounding the archegonia and sporophytes, but with antheridia in leaf axils along the branches. This pattern permits the male branches to continue growing at the apex, but alas, the female has a terminator in the presence of the perianth and archegonia, whether it is terminal on the stem or terminal on a branch. Hence, only new branches can form subsequent archegonia.

Figure 46. *Lunularia cruciata* with archegoniophores and young sporophytes. Note the scales at the base of the archegoniophores. Photo by Ken-Ichi Ueda, with permission.

It is likely that we should find examples where bryophytes expend so much energy on capsule development that they must wait a year or more to provide enough energy for another sexual endeavor. A negative relationship between sporophyte production and future perichaetia initiation was actually demonstrated in *Dicranum polysetum* (Figure 36-Figure 38) (Bisang & Ehrlén 2002; see also Chapter 3.4, Reproductive Trade-

off).　If we are to use growth increment markers such as splash cups, we need to understand this relationship lest we underestimate the age of the plants.

Protogyny and Protandry

Protogyny, the maturation of female reproductive structures before those of the male, and **Protandry**, the maturation of male reproductive structures before those of the female, are not commonly reported in the bryophytes [but see for example Lackner 1939; Crum 1972 for *Atrichum undulatum* (Figure 59-Figure 58) and *Funaria hygrometrica* (Figure 47); Longton & Schuster 1983; Stark 2002].　That does not mean they are effectively absent or even rare.　One of the problems in identifying maturation of the male and female sexual organs at different times is that this may occur even in different years and give the appearance of having the two sexes on separate plants. Deguchi (1978) sums this up well in his study of *Grimmia* (Figure 48):　"When successive branchings, including subfloral innovations, continue, and lower, older branches are decomposed in time, the upper newer branches, with different sexual organs, appear to be of different individuals.　This circumstance often leads bryologists to a misunderstanding of the sexuality."

Ken Kellman (Bryonet 17 April 2014) excitedly reported the discovery of a plant that had been thought to be dioicous, but that in reality was synoicous and protogynous, an undescribed species of *Bryum* (*Gemmabryum*).　The antheridia are produced after the archegonia have senesced.　As he aptly pointed out, this is an effective mechanism to prevent selfing in monoicous species, while retaining the advantage of a clone that contains both sexes and achieves adequate spore dispersal for later mixing of genes.

This discovery by Kellman brought other Bryonetters to report their observations.　Brent Mishler (Bryonet 18 April 2014) reported that in *Syntrichia princeps* (Figure 49) mature archegonia are present while antheridia in the same inflorescence are just beginning their development. We can't be certain whether this is maturing of archegonia first, or if the antheridia of that year have already matured and disintegrated, but one would assume that since they are in the same inflorescence this is protogyny.　Stark (1985) likewise found evidence of brief protogyny in both species of *Forsstroemia* (Figure 50) in Virginia, USA.　The monoicous *Phaeoceros carolinianus* (Figure 51) is an example of a typically protandrous hornwort.

Figure 47.　*Funaria hygrometrica*, a monoicous annual shuttle species that produces prolific capsules with long-lived spores, shown here growing on fresh charcoal.　Photo by Janice Glime.

Figure 49.　*Syntrichia princeps*, a species that exhibits protogyny.　Photo by Jan-Peter Frahm, with permission.

Figure 48.　*Grimmia affinis*, a species that produces mature antheridia and archegonia at different times.　Photo by Michael Lüth, with permission.

Figure 50.　*Forsstroemia trichomitria*, a protogynous moss. Photo by Misha Ignatov, with permission.

Figure 51. *Phaeoceros carolinianus* with sporophytes, a protandrous hornwort. Photo by Hermann Schachner, through Creative Commons.

Age-related Differences

Could differences be due to fewer males expressing sex in their lifetimes, or do they take longer to reach sexual maturity? Since antheridia frequently require a longer time to develop than do archegonia (Clapham & Oldroyd 1936; Miles *et al.* 1989; Stark 1997, 2002; Milne 2001), it seems logical that males might require more maturity before they produce their first antheridia.

In *Anastrophyllum hellerianum* (Figure 52), Pohjamo and Laaka-Lindberg (2004) found that a **threshold size** exists not only for sexual reproduction, but also for asexual reproduction. This threshold could account for a large number of non-expressing plants in some populations and some species might even exhibit a different threshold for male and female expression.

Figure 52. *Anastrophyllum hellerianum* with gemmae. Photo by Michael Lüth, with permission.

Sexual Plasticity

It seems that bryophytes may have their own version of the alligator and crocodile story. In these reptiles, and some other animals, the temperature during development of the embryo determines the sex. At high temperatures ca 34°C all the hatchlings are males and when it is ca 30°C, all are female (Woodward & Murray 1993). In this case, there are no sex chromosomes, so temperature during incubation is a crucial factor in sex determination. The planktonic microcrustacean *Daphnia* is dependent on environmental triggers for sex determination of its progeny (Innes & Dunbrack 1993; Tessier & Cáceres 2004).

Bisexual through Ramets and Rhizautoicy

Dioicous plants may not always be what they seem. Stark and Delgadillo (2001) became curious when the Mojave Desert moss *Aloina bifrons* (Figure 53), reputedly dioicous, appeared frequently with sporophytes. This was most unusual for a xerophytic, dioicous moss. Upon further investigation, they found that **ramets** (individual members of a clone) (Figure 54-Figure 55) of the same clone could on some individual ramets bear **perichaetia** (modified leaves enclosing archegonia) (Figure 56) and on others bear **perigonia** (modified leaves enclosing antheridia) (Figure 57), but that underground these ramets were connected by single rhizoids, rhizoid strands, or masses of rhizoids (Figure 54-Figure 55). In an experimental approach, Stark & Brinda (2013) recently confirmed **rhizautoicy** in this species, *i.e.* the sexual condition of separate male and female shoots connected by protonemata (Crandall-Stotler & Bartholomew-Began 2007) (or rhizoids), often beneath the substrate surface. Such a strategy, apparently from a single spore, would increase the probability of fertilization while permitting a somewhat greater chance for somatic variation between the sexes.

Figure 53. *Aloina bifrons*, a dioicous species with frequent sporophytes. Some individuals can bear both archegonia and antheridia. Photo by Martin Hutten, with permission.

Figure 54. Seven **ramets** from one individual of *Syntrichia caninervis*. Photo courtesy of Lloyd Stark.

Figure 55. *Bryoerythrophyllum rubrum* **ramets**. Examples of branching is indicated by arrows. Photo by Jan-Peter Frahm, with permission.

Figure 56. *Diphyscium foliosum* females with capsules and **perichaetial leaves**. This species is dioicous (the male and female sexual organs occur on separate individuals), with photosynthetic males with leaves and females that consist of only a protonema and perichaetial leaves that surround the archegonia and subsequent sporophyte. Photo by David Holyoak, with permission.

One example of possibly environmentally determined sex in bryophytes is that of *Splachnum ampullaceum* (Figure 22). In this species a protonema from a single spore can produce both male and female shoots (Cameron & Wyatt 1990), fitting the definition of rhizautoicy. The spores are bisexual, but the individual gametophores are unisexual. Therefore, it appears that selection against female-expressing plants is determined later in the developmental stage, although field conditions might cause quite different responses from those in the lab. What is it that determines the sex in these gametophores? Could density of the population in the dung habitat influence sexual differentiation or survival in this functionally dioicous moss? Or could presence of external hormones in the dung habitat influence sexual differentiation or survival? Such factors as ethylene concentrations,

regulated by population density or other environmental factors, could alter the sex ratio. For example, in the flowering plant *Cucurbita texana*, an injection of ethylene into the stem resulted in a greater proportion of female-expressing flowers (Krupnick *et al.* 2000). It is possible that bryophytes, like flowering plants (Lebel-Hardenack & Grant 1997), have environmental means of sex determination. But, alas, it seems we know little about the ability of a single protonema to produce gametophores of different sexes and what might control those differences.

Figure 57. **Perigonial leaves** and antheridia of *Diphyscium foliosum*. Photo from Botany 321 website at the University of British Columbia, with permission.

How common is rhizautoicy in bryophytes? Is this a facultative trait that responds to absence of the opposite sex? Does it involve genetic mutations on the branches, or suppression of genes? And what environmental stimuli are involved in triggering the formation of each sex? Does the environmental trigger cause a physiological response that changes the sex of a newly developing ramet? What is the role of hormone concentration in determining sex expression? Do these rhizautoicous plants retain their sex, or can they switch from year to year based on their stored energy or growing conditions or even age? Is rhizautoicy involving rhizoid connections really the same phenomenon as the production of separate male and female gametophores produced from a single protonema in *Splachnum ampullaceum*?

Sex Reversal

This brings us to attempting to answer the question of sex change in bryophytes. Do bryophytes behave like the Jack-in-the-pulpit (*Arisaema triphyllum*) and remain non-expressing until they have sufficient energy, then change sex in a pattern determined by their sizes? This species does not flower when it is small, produces males flowers when somewhat larger, and produces female flowers in its largest size range (Bierzychudek 1982). Hence, as these perennial plants increase or decrease in size from year to year, they also may change sex.

This model would seem only to work for perennials with underground overwintering structures like the Jack-in-

the-pulpit, but consider another scenario. Annual growth of an innovation after sporophyte production can decrease due to energy transfer to the sporophyte, as seen in *Dicranum polysetum* (Figure 36-Figure 38) (Bisang & Ehrlén 2002). Then the new branch would represent the shorter "plant." In *D. polysetum* sporophyte development reduced the probability of development of future perichaetia and/or reduced the mass of new perichaetia. In short, it exhibited an energy tradeoff much like the Jack-in-the-pulpit, but there is no sex change involved.

Is there evidence that any bryophytes can change sex in response to stored nutrients or nutrient availability? Crum (1976) reports that *Atrichum undulatum* (Figure 59-Figure 58) behaves this way in Michigan, USA. He observed that this species does not produce male and female gametangia on the same plant at the same time, but that at least some populations produce antheridia the first year and archegonia the next (Braithwaite 1887-1905; Dixon 1924; Nyholm 1954-1969; Smith 1978). Thank you to Bryonetters, we can cite further personal observations to shed light on this matter. Linley Jesson, in response to my question on Bryonet in January 2014, shared her observations that in *Atrichum* (**Polytrichaceae**; Figure 59-Figure 61), because new innovations arise after sex expression, sex indicators remain over 2 or sometimes 3+ years. In triploid *Atrichum undulatum* (Figure 59-Figure 58) and diploid *Atrichum altecristatum* (Figure 60-Figure 61; or possibly *A. undulatum*) it appears that sequential sex expression occurs. Often the first gametangia produced are male and in the next year either female or both gametangia appear. The age of reproduction in both sexes certainly needs further investigation.

Figure 58. *Atrichum undulatum* with capsules. Photo by Michael Lüth, with permission.

This leaves us with two pieces that we have not been able to connect in bryophytes. *Dicranum polysetum* (Figure 36-Figure 38) demonstrates the tradeoff due to energy cost, with innovations behaving like the subsequent year of growth from the Jack-in-the-pulpit rhizome. *Atrichum undulatum* (Figure 59-Figure 58) demonstrates the ability to change sex in subsequent years. But we lack

the link to demonstrate that energy/nutrient availability cause a change to the less costly sex.

Figure 59. *Atrichum undulatum* males with splash cups. Photo by Janice Glime.

Figure 60. *Atrichum altecristatum* showing male splash cups. Photo by Robert Klips, with permission.

Figure 61. *Atrichum altecristatum* in its first year of invasion. There was no evidence of sexual structures. Photo by Eric Schneider, with permission.

Dan Norris, in his discussion on Bryonet (2 May 2003), helps to answer this question. He expressed his observations on the variability of sexual type within species: "I find myself very skeptical about published data

on dioicy and monoicy. As I write my own manual of California mosses with all descriptions based upon observation of actual specimens, I have found too many contradictions to published reports... I have found the **Polytrichaceae** is so often male in early few years of its life and female later. Too many presumed **cladoicous** (having archegonia and antheridia on different stems of the same plant) specimens can only be guessed as such because actual connections of the stems cannot clearly be demonstrated...The frequency of sporophytes is hardly a reliable indication of sexuality; *Orthotrichum lyellii* (Figure 87) in my California region seems to be dioicous, as universally reported, but nearly all bunches of the plant – bunches I first thought to be clones – contain both sexes and are almost always with sporophytes."

Even in the well-known dioicous *Polytrichum* (Figure 40) and *Atrichum* (Figure 59-Figure 58), both archegonia and antheridia can occur on the same plant, either mixed together or in separate locations, a condition known as **polyoicous** or **heteroicous** (Vitt 1968). We have much to learn about sex determination in bryophytes!

Mechanisms of Labile Sex Expression

Korpelainen (1998) compared the **lability** (flexibility) of sex expression among the plant phyla and found that while it exists in all the major plant phyla, it is the rule only among homosporous ferns. Furthermore, most of the plants that have labile sex expression are perennials with long life cycles. She found that environmental stresses such as low light, nutrition, unfavorable weather, and too much or too little moisture often favor male expression. Unfortunately, we know little of these mechanisms in bryophytes.

In the monoicous *Tetraphis pellucida* (Figure 63-Figure 62), density affects sex expression, with male shoots dominating when densities are greater (Kimmerer 1991). Selkirk (1979) found that nitrate levels affected sexual expression in *Riccia duplex* (Figure 64), but she did not show differences between male and female expression. In *Riccia rhenana* (Figure 65), some clones produced archegonia in both soil and nutrient solutions, whereas others did not produce any sexual structures during the same six-month cultivation period, suggesting that either they differed genetically or that their past history (*e.g.* age, environmental conditions, time since last production of sporophytes) affected their ability to respond.

Figure 63. *Tetraphis pellucida* antheridia. Photo from Botany Department UBC, with permission.

Figure 64. *Riccia duplex*, a species in which nitrate affects sexual expression. Photo by Michael Lüth, with permission.

Figure 65. *Riccia rhenana*, a species for which sexual expression is not affected by nitrates. Photo by Jan-Peter Frahm, with permission.

Figure 62. *Tetraphis pellucida* with capsules. Photo by Bob Klips, with permission.

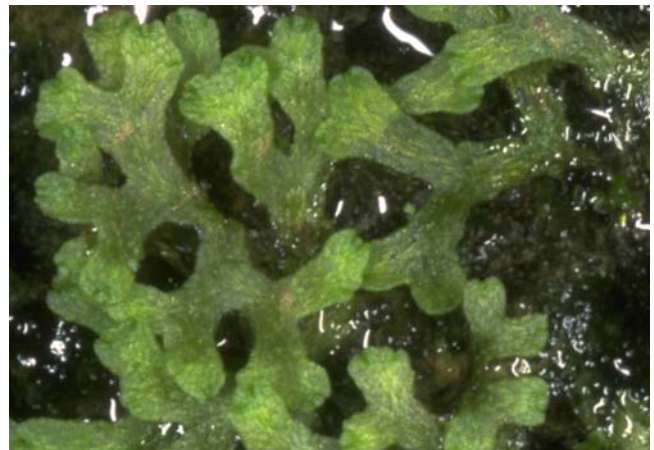Hormones undoubtedly contribute to sex expression and we might expect their concentrations to be subject to environmental conditions. When experimenting with the mostly vegetative liverwort *Riccia crystallina* (Figure 66), Chopra and Sood (1973) found that **gibberellin** and **ethrel** enhanced antheridial formation, whereas **glycocel** enhanced archegonial formation. In the dioicous *Bryum argenteum* (Figure 7), Bhatla and Chopra (1981) stimulated expression of male gametangia with **auxin** and **gibberellin**, whereas these same hormones inhibited development of female gametangia. Instead, **cytokinins** stimulated the development of female gametangia, slightly inhibiting development of gametangia in male clones. Studies such as these suggest that hormones could control sex expression either by genetic control or environmental control on gene expression. Furthermore, gaseous hormones such as **ethylene** or fungal exudates such as **gibberellin**, present in the environment, could influence sexual expression, differing between years and environments and causing the differences and changes in sexual expressions that have been observed in some species.

Figure 66. *Riccia cf crystallina*, a species in which **gibberellin** and **ethrel** enhance antheridial formation, whereas **glycocel** enhances archegonial formation. Photo by Michael Lüth, with permission.

We cannot overlook the importance of hormonal interactions on development (see Chapter 5-1 of this volume, Ecophysiology of Development: Hormones). In their experiments with *Bryum argenteum* (Figure 7), Bhatla and Chopra (1981) showed that **IAA** and **cytokinin** could counteract each other's individual hormonal inhibitory effects on the female and male clones, respectively. **ABA**, known as a stress hormone, inhibited both sexual expression and vegetative growth in this species, with sexual induction in the female being more sensitive. In addition to interactions, concentrations are important in developmental control.

Plasticity vs Genetic Differentiation

Transplant experiments can be used to help us understand plasticity that permits environmentally induced changes vs genetic characters that may prevent living in some environments. Hassel *et al.* (2005b) used *Pogonatum dentatum* (Figure 67) transplants to demonstrate such plasticity. They found that vegetative growth was greater in the mountain areas than in lowland areas. Furthermore, reproductive investment was greater in

the lowland areas. But lowland plants tended to produce larger sporophytes than those from the mountain when grown in the same environment, suggesting a genetic difference between the two populations. What is interesting is that the transplanted shoots often outperformed the native ones by growing larger and producing larger sporophytes. They suggested that plasticity may have permitted the range expansion of *P. dentatum*.

Figure 67. *Pogonatum dentatum*. Photo by Michael Lüth, with permission.

Using reciprocal transplants, Hedderson and Longton (2008) likewise found both genetic variation and plasticity in life history traits in upland and lowland sites of several other **Polytrichaceae**: *Pogonatum aloides* (Figure 68-Figure 69), *Polytrichum commune* (Figure 31), and *P. juniperinum* (Figure 40, Figure 70). These differences were apparent in male reproductive effort and investment in vegetative shoots by females. Variation included tradeoffs between number and size of spores and between vegetative reproduction and spore production.

Is There an Asexual Role for Males?

Is it possible that male bryophytes may have more vegetative reproductive success while females have the primary sexual reproductive role? A sexually reproducing female bryophyte needs to nurture the developing sporophyte (see Chapter 3-4, Reproductive Trade-off). Reproductive output may be increased if the female individual is large, increasing fitness by permitting that female to occupy more space and obtain more light, and possibly more water and nutrients. But a male may be able to maintain the population, and enlarge it, through asexual means.

Figure 68. *Pogonatum aloides* males. Photo by David Holyoak, with permission.

Figure 69. *Pogonatum aloides* females with capsules. Photo by Michael Lüth, with permission.

Figure 70. *Polytrichum juniperinum* males showing old antheridial splash cups (**arrows**) with new growth and splash cups above that previous apex. Photo by Jan-Peter Frahm, with permission.

As discussed above (Genetic vs Expressed Sex Ratio), in *Marchantia inflexa* (Figure 8) the growth patterns of males and females differ, with the females in some habitats producing more meristematic tips, but the males producing more gemmae (Brzyski *et al*. 2013), giving the females more coverage in the immediate area and more chance for long-distance dispersal through spores, but giving males more opportunity to spread locally away from the immediate clump.

Among mosses in Great Britain about 18% (Longton 1992) to 29% (Hill *et al*. 1991, 1992, 1994) produce specialized vegetative propagules, and there are significantly more of these in dioicous mosses than in monoicous taxa (Longton 1992; During 2007). Among Belgian and Dutch liverworts, 69% of the dioicous species produce vegetative propagules, compared to 54% for monoicous taxa (During 2007). Such a strategy of asexual reproduction in males could be cost effective in dioicous taxa, permitting the females to put energy into producing spores while males could maintain the local population through asexual means (see *e.g.* Laaka-Lindberg *et al*.

2000). Even if both sexes produce vegetative propagules, this may be suppressed while sexual reproductive processes occur. In *Marchantia polymorpha* gemma cup (Figure 71) production ceases while it is producing sexual reproductive structures (Terui 1981).

Recently, Pereira *et al*. (2016) noted in Amazonian **Calymperaceae** that gemmae-bearing shoots produced fewer gametangia than shoots without gemmae, although both sexual and asexual reproduction were positively related to monthly precipitation amounts. Likewise, in his assessment of life cycle strategies, During (2007) concluded that there is a negative correlation between processes and structures (such as propagules and sexual structures) that serve the same functions in the life of the bryophyte.

Figure 71. *Marchantia polymorpha* with gemmae cups. Photo by Ralf Wagner <www.dr-ralf-wagner.de>, with permission.

Stieha *et al*. (2014) confirmed that in *Marchantia inflexa* (Figure 8), male plants produce gemmae more quickly and prolifically than do females. Nevertheless, this is not necessarily an indication of a greater role for asexual reproduction in males. Once gemma cups are produced, male plants of this species increase production of gemmae to week 4 and stop at about week 9. Female plants, on the other hand, have stable production of gemmae during the first three weeks of cup existence, increasing sharply in week 4, then declining in subsequent weeks. On the other hand, male gemmae suffer greater desiccation effects, resulting in greater gemmae mortality than that of female plants. But once gemmae are dispersed (about 20 cm per minute in light rain), they have a high survival rate if they remain moist and are critical for maintaining both sexes.

Differential survival may account for the observed sex imbalance (see above in Germination Patterns and Spore Mortality; Environmental and Geographic Differences). And it appears this could diminish the role of males in asexual reproduction. Newton (1972) demonstrated the loss of young males from leaf regeneration in *Mnium hornum* (Figure 72) and *Plagiomnium undulatum* (Figure 73-Figure 74) where none of these survived desiccation, but 77% of the leaf regenerates from females did survive.

Figure 72. *Mnium hornum* males at Bretagne, France. Photo by Michael Lüth, with permission.

Figure 73. *Plagiomnium undulatum* habitus, a species in which male regenerants are more likely to die than those of females. Photo by Ralf Wagner <www.dr-ralf-wagner.de>, with permission.

Figure 74. *Plagiomnium undulatum* with antheridial splash cups. Photo by Jan-Peter Frahm, with permission.

When Males Are Dominant

But we must remember that females are not always the dominant sex. In her 1972 study Newton showed that isolated spores of *Plagiomnium undulatum* (Figure 73-Figure 74) had a sex ratio of 4.1♀:1♂, changing little to 3.5♀:1♂ in the first protonemal buds, but in the same family *Mnium hornum* (Figure 72) had a ratio of 0.89♀:1♂, becoming more skewed in favor of males (0.45♀:1♂) in the first protonemal buds. Other examples exist of expressed male dominance in some populations within a species. This could be an advantage in species where differences in stress tolerance favor males. And

having more males increases the chances for some of the sperm reaching eggs.

Laaka-Lindberg (2005) found that only 8% of the females were sex-expressing whereas 17% of the males were sex expressing in the leafy liverwort *Lophozia ventricosa* var. *silvicola* (Figure 75), with a female to male sexual ratio of 0.61:1. Furthermore, the timing of gametangia production and conditions needed for development differed between the males and females. This timing in females varied among years, suggesting that the environmental signals differed between the sexes. Such timing differences could cause a mismatch between male and female maturation that could reduce fertilization.

Figure 75. *Lophozia ventricosa* from Europe. Photo by Michael Lüth, with permission.

Alvarenga Pereira *et al*. (2013) found a highly male-biased condition (0.43 ♀:1 ♂ at ramet level, n=604) in the epiphyllous moss *Crossomitrium patrisiae* (Figure 76) in the Brazilian Atlantic rainforest. In this study of 797 ramets, a high rate of 76% were expressing sexual structures. This species had an extremely high rate of sporophyte production, with 40% of all female ramets, and 74% of female ramets occurring in mixed colonies bearing sporophytes. For this species, arriving and establishing on a new leaf, a short-lived habitat, is a necessity for the species to continue, and this is best achieved by spores that can more easily become airborne than many larger vegetative propagules. Low levels of abortion and high investment in sporophyte maturation provide this species with the dispersal units to survive in this ephemeral habitat.

Figure 76. *Crossomitrium patrisiae* habit in Costa Rica. Photo by Michaela Sonnleitner, with permission.

Also the aquatic liverwort *Scapania undulata* (Figure 77) exhibits a clearly male-skewed expressed sex ratio (Holá *et al.* 2014). The authors suggest that the high production of males is a strategy to overcome sperm dilution and ensure fertilization over longer distances in water.

Figure 77. *Scapania undulata* with capsules, a species with more males than females. Photo by Michael Lüth, with permission.

Maintaining Sexual Dimorphism in a Population

What factors might maintain the balance of males to females to retain the dioicous character in a bryophyte species? We have seen many cases of male suppression, some so strong that they could lead to male extinction in some populations, at least when we look at sex-expressing plants. Maintenance of both sexes is important for fitness and evolution. We find that the same factors that separate the environments of males and females might contribute to the continuation of both sexes. That is, some years and conditions may favor one sex, whereas other years and modified conditions may favor the other. For the slow-growing bryophytes, this slows competition between the sexes and prevents rapid extinctions.

Marchantia inflexa (Figure 8) demonstrates the complex way in which sexual expression might occur. In this as in many other bryophyte species, it is common for males to be rare. Single-sex populations, especially of females, are common (Garcia-Ramos *et al.* 2002). In the USA, only single-sex populations are known, but in tropical sites, populations with both sexes occur. Spread of both sexes by clonal growth and vegetative propagules is common. Garcia-Ramos and coworkers found that in *Marchantia inflexa* seasonal disturbances (desiccation) delay the elimination of males within the patch, whereas large scale disturbances permit re-establishment by spores. It is these large-scale disturbances that permit both sexes to coexist at a **metapopulation** level (*i.e.* group of partially isolated local populations of same species, but connected by migration). In this species, isolated clonal populations seem independent of sexual reproduction, but at the landscape scale, sexual reproduction is crucial for re-establishment by spores.

Fuselier and McLetchie (2002) explored the question of what maintains sexual dimorphism, using *Marchantia inflexa* (Figure 8) as a model system. They suggested that there is sex-specific selection, as already seen for *Syntrichia caninervis* (Figure 15) (Stark *et al.* 2005) and discussed above for this species (Environmental and Geographic Differences), causing one sex to be favored over the other under certain stressful conditions. When the habitats of the sexes do not overlap, the sex with the higher cost of sexual reproduction should experience higher mortality in the more stressful habitats (Lloyd & Webb 1977; Charnov 1982; Bierzychudek & Eckhart 1988; Fuselier & McLetchie 2002). Whereas habitat specialization can lead to difficulty in obtaining mating success, it leads to a wider habitat range for the species, albeit by separating males and females. In this case, the species must be maintained by asexual reproduction. Fuselier and McLetchie (2002) reasoned that such a strategy would favor males with a high degree of asexual reproduction, but females with a low asexual reproduction.

In *Marchantia chenopoda* (Figure 78), Moyá (1992) found that there was a large female bias, even when the population seemed to be relying on its abundant sporophytes. The **selective forces** acting on asexual vs sexual fitness can act in opposition and may help to explain the persistence of sexual dimorphism and the smaller number of males.

Figure 78. *Marchantia chenopoda* in Puerto Rico, a dioicous species. **Upper:** male population; **Lower:** female population. Photos by Janice Glime.

Sexual dimorphism may occur at the clump level while seemingly absent at the shoot level. Moore *et al.* (2014) found that when 25 male and 25 female shoots of *Bryum argenteum* were cultured, no differences in water-holding

capacity could be ascertained between the sexes. However, when 1 cm^2 samples were saturated with water and then centrifuged to remove external water, the female clumps retained more water per unit of clump area. The researchers suggested that this water retention ability could favor greater growth of females and contribute to a female bias in expressed sex ratio.

Season and Sex Expression

Those of us in the temperate and arctic climates expect bryophytes to be dormant in the winter and that many species will take advantage of rainy or melting periods in spring for fertilization. But not all species conform to those expectations (Arnell 1878, 1905). In the majority of species in temperate regions, phenology of fertilization and sporophyte formation are clearly seasonal, and differ among families and habitats.

Capsules take varying periods to mature, some taking more than a year, so those can be found almost year-round, albeit on different species (Milne 2001). In the tropics, a seasonal cold period is absent, but precipitation may cause seasonality. Maciel-Silva and Marques Válio (2011) examined the effects of season on bryophyte sexual expression in Brazilian tropical rainforests. They found that many of the species exhibited sexual expression continuously over the 15-month study in both the sea level and montane sites.

Seasons did, however, affect the length of time required for gametangia to mature in the tropics (Maciel-Silva & Marques Válio 2011). Male gametangia typically matured by the end of the dry season, providing sperm when the rains were present, presumably facilitating their dispersal during the following rainy season. Female gametangia, on the other hand, were receptive over the entire period, even having many mature before the start of the rainy season. This strategy would assure that females were ready at any time the rains came, allowing for year-to-year differences. It is interesting that the male gametangia took longer to develop and that many aborted. This scheme also maximizes the dispersal of spores, permitting them to mature near the end of the dry season when conditions are best for dispersal; rains will soon follow to induce germination.

If seasons are indeed important, then there should be differences between sea level and montane reproductive cycles at the same latitude, in this case the Brazilian Atlantic rainforest. Maciel-Silva *et al.* (2012) found that species at sea level produced more sexual branches and had a more strongly female-biased sex ratio than did the montane populations. There were more frequent fertilizations among the montane populations, but ultimately, the number of successful sporophytes was about the same at the two elevations. Fertilization occurred mostly during the rainy season of October to December. Moreover, monoicous species exhibited a higher reproductive performance in terms of number of sexual branches, fertilization, and sporophyte formation. The authors concluded that both the breeding system and the environment influenced the sexual expression and mating strategies.

Role of Asexual Reproduction in Dioicy

By now it should be clear that dioicous bryophytes suffer from lack of sexual reproduction in many populations. On the other hand, asexual reproduction can maintain the population and help it spread. But is specialized asexual reproduction more common among dioicous taxa?

It appears that among British mosses, asexual propagules are common among dioicous colonists (Longton 1992), but this relationship does not exist among the liverworts (Longton 1997). Rather, among the British liverworts the production of asexual propagules is not related to sexuality (monoicous vs dioicous).

In examining the Japanese flora, Une (1986) found support for the concept of vegetative success in the relationships of specialized vegetative reproduction. Of the 111 moss taxa that produced **asexual diaspores** (any structures that become detached and are dispersed) (Figure 79), 86 were dioicous (77.5%), whereas only 11 (9.9%) were monoicous. A further phenomenon in this story is the presence of more asexual propagules in the erect-growing dioicous mosses than in the prostrate (creeping) taxa. Could it be that these rarely sporulating but upright taxa take advantage of vegetative propagules to facilitate movement "in search" of the opposite sex?
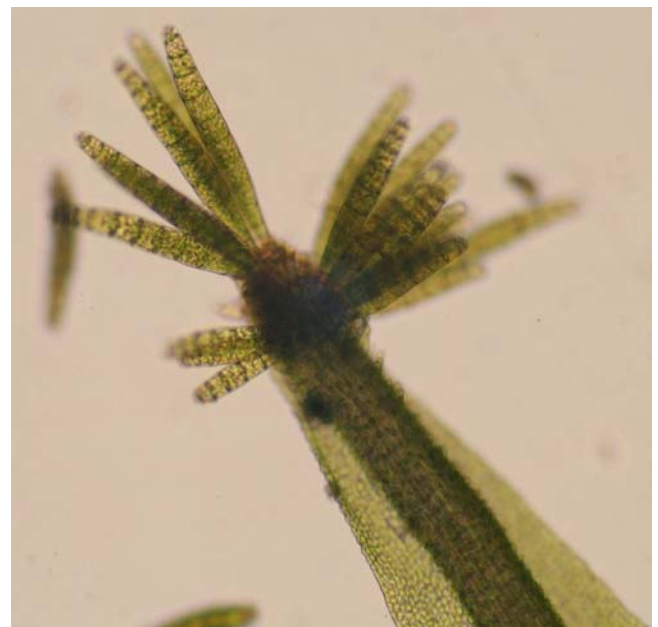

Figure 79. *Calymperes erosum* with gemmae on the leaf tip. Photo by Li Zhang, with permission.

Similarly, During (2007) was able to show that among bryophytes in Belgium and The Netherlands, dioicous taxa more commonly had vegetative propagules than did monoicous ones. But among the mosses, this relationship only held true for acrocarpous species; the pleurocarpous taxa were able to achieve significant expansion by clonal growth, thus negating much of the advantage of vegetative propagules. During suggested that the tradeoff between

propagules and vegetative growth seen in the liverwort ***Marchantia inflexa*** (Figure 80) might be a common phenomenon among bryophytes. He found that negative correlations generally occur between processes and structures that serve the same functions in the life of the bryophyte, suggesting that vegetative diaspores and sexual organs compete for the same energy reserves. A more detailed discussion of asexual reproduction follows.

Gemma-bearing Dioicous Taxa

We have previously mentioned (Chapter 3-1, Or the Dioicous Advantage?) the importance of asexual propagules in dioicous taxa. To the examples cited above, we can add that of 715 species of mosses examined in eastern North America, 13% have some obvious means of specialized asexual reproduction (Crum 2001). Of these, 76% are dioicous, 19% monoicous, 5% of unknown sexuality. Old data from Germany (Correns 1899) indicated that of 915 species, 12% had true gemmae, with 86% of these dioicous and 14% monoicous.

Longton (1992) indicated that producing asexual propagules in many dioicous moss taxa provided them with a safety net, permitting reproduction under conditions when sexual reproduction was not possible. Such a strategy permitted them to survive in marginal habitats and in years when the weather was unfavorable to fertilization due to drought or frost (Longton 1990). Furthermore, it appeared that a greater number of rare taxa relied on asexual reproduction – not surprising due to the greater ease of dispersal of spores (Schuster 1988; Miles & Longton 1990; Söderström & Herben 1997; Bolker & Pacala 1999).

Figure 80. ***Marchantia inflexa***. Photo by Scott Zona, through Wikimedia Commons.

The moss genus ***Aulacomnium*** is known for special brood bodies (Figure 81-Figure 82). In most species, these are comprised of reduced and thickened leaves in a cluster on stalks at the tips of plants (Figure 81-Figure 82). However, in ***Aulacomnium heterostichum*** (Figure 83), sporophytes are common and these brood bodies were overlooked until 1991 when Imura *et al.* reported them from Japan. In this species, brood bodies are on a terminal stalk, but the individual propagules are not thickened as in other ***Aulacomnium*** species and only slightly modified

from the leaves (Figure 84). It is likely that brood bodies have been overlooked in other bryophyte taxa as well, particularly rhizoidal tubers and protonemal gemmae.

Figure 81. Brood body production in dioicous ***Aulacomnium androgynum***. Photo by Michael Lüth, with permission.

Figure 82. ***Aulacomnium palustre*** with brood bodies. Photo by Zen Iwatsuki, with permission.

Figure 83. ***Aulacomnium heterostichum***, a monoicous moss with abundant sporophytes. Photo by Janice Glime.

Figure 84. ***Aulacomnium heterostichum*** in Japan, with brood bodies (arrows). Photo by Janice Glime.

Spores, Asexual Propagula, and Rarity

There seems to be a distinct correlation between spore size and asexual propagules, with plants having small spores being more likely to have propagula (Longton & Schuster 1983; During 2007). If having small spores means having more of them, such a species would seem to have the best of all worlds, with a good chance for long-distance dispersal through spores, and colony expansion through readily sprouting propagula. Its price would be in lower viability of small spores compared to large ones.

The possibility to self-fertilize would suggest that sexual reproduction should be more frequent in the monoicous condition, with the possibility of cross fertilization with sister plants in the same clone, if not on the same plant. Longton (1997, 1998) agrees. He predicts that at least among the colonists, fugitives, and annual shuttle species (all inhabiting newly available substrata; Figure 47), the trend toward monoicy will be accompanied by an increased reproductive effort, decrease in life span, and decrease in the age of first reproduction. To facilitate such a strategy, he predicts that the life cycle will have substantial **phenological** (timing of events) flexibility and that the success of establishment from spores will increase. He suggests that the specialized asexual propagules that are common among dioicous colonists compensate for their more limited sexual reproduction.

To sum up what we know now, it appears that species that rarely produce capsules are more likely themselves to be rare (Miles & Longton 1990; Söderström 1992; Laaka-Lindberg 2000). Monoicous species produce capsules much more frequently than do dioicous species, with the distance between archegonia and antheridia being a limiting factor (Longton & Schuster 1983; Wyatt & Anderson 1984; Longton 1990; Laaka-Lindberg 2000; Bisang *et al.* 2004). Even monoicous species may become rarer in severe habitats where weather conditions may prevent even short-range dispersal of sperm to egg (Laaka-Lindberg 2000). Asexual propagules are more common among dioicous moss species. (See Chapter 4-7, Adaptive Strategies: Vegetative vs Sexual Diaspores, for more information on asexual vs sexual reproduction.)

Why Are Liverworts Different?

Laaka-Lindberg (2000) found that the relationship between rarity and presence of asexual vs sexual strategy differs markedly between British mosses (Longton 1992) and liverworts. Whereas only 18% of the mosses produce asexual propagules, 46% of the liverworts do (Longton 1992), a group that is 68% dioicous (Villarreal & Renner 2013). And, unlike the mosses, production of asexual propagules in liverworts is not linked to the dioicous condition, but is nearly equal to that in the monoicous condition. The researchers warn us, however, that the ephemeral nature of liverwort sporophytes could create a bias in herbarium data since liverworts are more likely to be collected in sterile condition than are non-sporophytic mosses with persistent capsules elsewhere in the population. This could also increase the collected representation of propaguliferous plants among liverworts compared to mosses. There also seems to be less evidence of fragmentation success in leafy liverworts (see, for example, Miller & Howe Ambrose 1976).

Nevertheless, the long-identified association between dioicy and the ability to produce vegetative propagules in mosses in different regions and at different scales has recently also been challenged by Laenen *et al.* (2015). The authors applied comparative phylogenetic methods with 303 out of 382 liverwort genera currently recognized globally. They were unable to find a correlation between dioicy and the formation of vegetative propagules. They did not compare 'rarity' with reproductive system, but used size of geographic ranges. Interestingly, the production of vegetative propagules was positively correlated with range size, but sexual system and spore size were not. This suggests that asexual reproduction may play a more important role than hitherto thought in long-range dispersal of liverworts, and calls for further investigation of the spatial genetic structure of bryophyte populations in relation to their mating systems.

Laaka-Lindberg *et al.* (2000) concluded that those British liverwort taxa that produce neither spores nor vegetative propagules tend to be rare (Figure 85). Rarity of capsule production does correlate with rarity of the species, with those failing to produce spores being three times as likely to be rare. Monoicous taxa have a higher proportion with sporophytes than do dioicous taxa, but among those species of both mating systems that do produce capsules, there is greater rarity among the monoicous taxa. This suggests that there is a fitness price for selfing or sibling crosses due to suppression of genetic variation that would be available through outcrossing. Data are needed to support this hypothesis.

The production of asexual propagules is not related to rarity in British liverworts, with propagules occurring as often in common species as in rare ones (Laaka-Lindberg *et al.* 2000). It is interesting that whereas there are few liverwort taxa in which sporophytes are unknown anywhere (Figure 85), there are many taxa in which vegetative propagules are unknown (Figure 86), and the frequency of those lacking such propagules is twice as great among dioicous liverworts as among monoicous liverworts, although the proportion is about the same in both (Figure 86) (Laaka-Lindberg *et al.* 2000). Spores are more likely to provide long-range dispersal, but among seeds Thompson *et al.* (1999) concluded that the best predictor of range among British plants was diversity of habitats used. It is likely that this is true for bryophytes as well.

Could it be that liverworts, rather than using specialized asexual means as a safety net, more frequently are opportunistic, having occasional sexual reproduction, but gaining the advantages of both means of reproduction (Green & Noakes 1995; McLellan *et al.* 1997)? Their horizontal growth habit, producing ramets, permits them to expand on their substrate without having to reproduce. Asexual reproduction, including ramification, is suggested to require less energy, particularly on the part of females, and therefore may be useful under stressful conditions (Longton & Schuster 1983; Newton & Mishler 1994). This concept is supported by greater occurrence of species with asexual propagation in arctic and alpine areas than in the tropics (Schuster 1988). In stable environments, maintenance will permit survival of the population, but in habitats subject to frequent disturbance, dispersal of progeny is essential (Schuster 1988; Söderström 1994) and

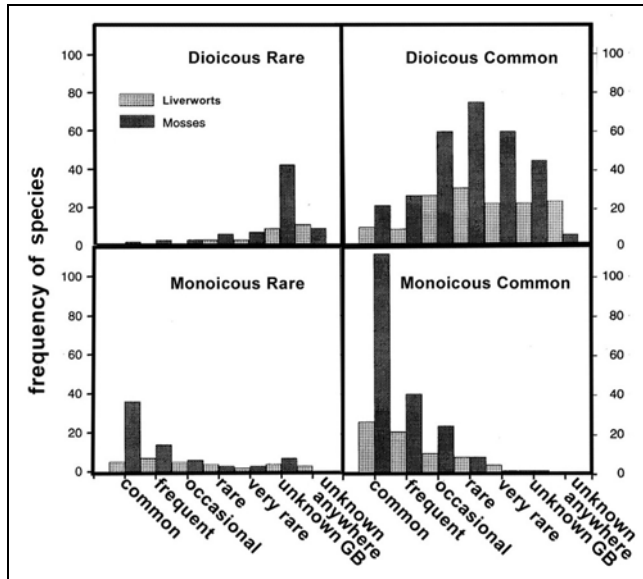may even depend on delay through dormancy (McPeek & Kalisz 1998).

Figure 85.   Comparison of frequencies (seven classes) of sporophyte production for mosses and liverworts in four sexuality groups within Britain.   Modified from Laaka-Lindberg *et al.* 2000.
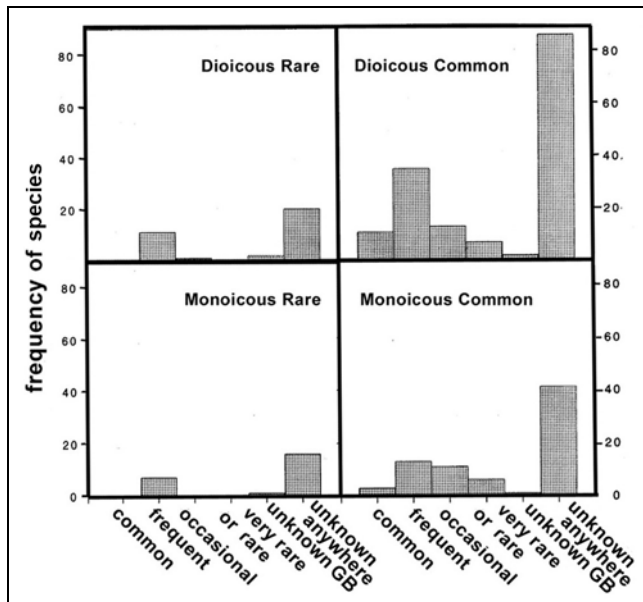

Figure 86.   Comparison of frequencies (six classes, rare and very rare combined) of asexual reproductive structures for liverworts in four sexuality groups within Britain.   Modified from Laaka-Lindberg *et al.* 2000.

Are Epiphytes a Special Case?

For epiphytic species such as the presumed dioicous *Orthotrichum lyellii* (Figure 87), the same tree needs to be colonized by both sexes to facilitate sexual reproduction. Norris (see Sex Reversal above) finds that colonies frequently have both sexes.   Fortunately, sperm can be washed downward considerable distances by rainfall, facilitating fertilization.   The presence of numerous gemmae permits this species to spread vegetatively and the

gemmae may help it to become established on its vertical substrate, increasing chances for both sexes to survive. But this begs the point Norris tried to make about sexual expression (see Sex Reversal above).   We need to be cautious about generalizations and look closely for variability due to age relationships, habitat expressions, or hidden connections.

Figure 87.   *Orthotrichum lyellii*, an epiphytic dioicous species. Note brown gemmae on leaves. Photo by Michael Lüth, with permission.

Smith (1982) reported that the proportion of monoicous taxa among those restricted to bark greatly exceeds that among mosses in general. Devos and coworkers (2011) found that the mostly epiphytic liverwort genus *Radula* (Figure 88) exhibits evidence of shifts from dioicy to monoicy multiple times as new species arose, with some epiphytes having facultative shifts.   It is interesting that they found no correlation between asexual gemmae and either dioicy or strict epiphytism in *Radula*. Rather, the obligate epiphytes tend to disperse by whole gametophyte fragments, avoiding the protonemal stage that is more susceptible to the ravages of rapid changes in moisture.   The former is in line with findings of Laaka-Lindberg (2000) for British liverworts and by Laenen *et al*. (2015) for liverworts at the global scale (see above, "Why Are Liverworts Different?").

Figure 88.   *Radula complanata* growing epiphytically and exhibiting gemmae. Photo by J. C. Schou, with permission.

As a result of their dispersal by fragments and often the absence of successful sexual reproduction, many epiphytes may have a special problem in maintaining the species due to lack of genetic variability. Because of the limited success of establishment on the vertical substrate of tree trunks and vertical rocks, these substrates often have only one clone and therefore only one sex in dioicous taxa. Hence, in the frequent absence of sexual reproduction, reproduction is accomplished by clonality or possibly selfing or among siblings. This may result in a lack of genetic diversity, as exemplified by *Leucodon sciuroides* (Figure 89) in Europe (Cronberg 2000). Glaciated areas had lower genetic diversity, as might be predicted for an area of lower age. Furthermore, the unglaciated populations from the Mediterranean region reproduce sexually, whereas the younger and more isolated populations from glaciated areas reproduce asexually, leading further to lack of genetic variability. This lack of variability may contribute to the disappearance of epiphytic populations under stress of air pollution and climate change.

Figure 89. *Leucodon sciuroides* on tree bole in Europe. Photo by Dick Haaksma, with permission.

Summary

Many species exhibit a strongly female-biased phenotypically expressed sex ratio that likewise is in some cases genetic and in others possibly due to varying responses of sex expression to environmental conditions. The "**shy male**" hypothesis lacks support in explaining most of this female bias. Examples of distinct male bias in expressed sex ratios also exist. Sex ratios based on genetic information on non-expressing plants is known for a very limited number of species.

Some species, perhaps more than we realize, have sexual plasticity. That is, they have different sex expressions in different years, possibly dependent on age or available energy resources. This can be due to hormonal expressions of the same or neighboring plants.

When sexual reproduction fails, asexual reproduction by specialized propagules can compensate, and this is especially true for dioicous mosses at the same scales. In addition, clonal growth and fragmentation can help the species spread. Because of the energy cost of producing sporophytes, males may exhibit higher vegetative performance. A modelling study suggests that disturbance level (weather, pollution, fire, etc) affects sexes differentially, hence maintaining both sexes in the long term. Epiphytes are frequently isolated on a tree with only one sex present. Although there seems to be no correlation between epiphytism and asexual propagules, there is a greater proportion of monoicous taxa among epiphytes than in general.

Literature Cited

Alvarenga Pereira, L. D., Pôrto, K. C., and Zartman, C. E. 2013. Sex ratio, spatial segregation, and fertilization rates of the epiphyllous moss *Crossomitrium patrisiae* (Brid.) Müll. Hal. in the Brazilian Atlantic rainforest. J. Bryol. 35: 88-95.

Anderson, L. E. and Lemmon, B. E. 1973. *Weissia sharpii*, a new species from North America, with cytological and ecological correlations. Bryologist 76: 131-143.

Anderson, L. E. and Lemmon, B. E. 1974. Gene flow distances in the moss *Weissia controversa* Hedw. J. Hattori Bot. Lab. 38: 76-90.

Arnell, H. W. 1878. A proposal on phaenological observations on mosses. Rev. Bryol. 5,2: 1-6.

Arnell, H. W. 1905. Phaenological observations on mosses. Bryologist 8: 41-44.

Bell, G. 1980. The costs of reproduction and their consequences. Amer. Nat. 116: 45-76.

Bhatla, S. C. and Chopra, R. N. 1981. Hormonal regulation of gametangial formation in the moss *Bryum argenteum* Hedw. J. Exper. Bot. 32: 1243-1256.

Bierzychudek, P. 1982. The demography of Jack-in-the-pulpit, a forest perennial that changes sex. Ecol. Monogr. 52: 335-351.

Bierzychudek, P. and Eckhart, V. 1988. Spatial segregation of the sexes of dioecious plants. Amer. Nat. 132: 34-43.

Bisang, I. and Ehrlén, J. 2002. Reproductive effort and cost of sexual reproduction in female *Dicranum polysetum*. Bryologist 105: 384-397.

Bisang, I. and Hedenäs, L. 2005. Sex ratio patterns in dioicous bryophytes re-visited. J. Bryol. 27: 205-219.

Bisang, I. and Hedenäs, L. 2013. Males are not shy in the wetland moss *Drepanocladus lycopodioides*. Internat. J. Plant Sci. 174: 733-739.

Bisang, I., Ehrlén, J., and Hedenäs, L. 2004. Mate limited reproductive success in two dioicous mosses. Oikos 104: 291-298.

Bisang, I., Ehrlén, J., and Hedenäs, L. 2006. Reproductive effort and costs of reproduction do not explain female-biased sex ratios in the moss *Drepanocladus trifarius* (Amblystegiaceae). Amer. J. Bot. 93: 1313-1319.

Bisang, I., Korpelainen, H., and Hedenäs, L. 2010. Can the sex-specific molecular marker of *Drepanocladus trifarius* uncover gender in related species? J. Bryol. 32: 305-308.

Bisang I., Ehrlén, J., Persson, C., and Hedenäs, L. 2014. Family affiliation, sex ratio and sporophyte frequency in unisexual mosses. J. Linn. Soc. Bot. 174: 163-172.

Bisang, I., Ehrlén, J., Korpelainen, H., and Hedenäs, L. 2015. No evidence of sexual niche partitioning in a dioecious moss with rare sexual reproduction. Ann. Bot. mcv133: 9 pp.

Bisang, I., Hedenäs, L., and Cronberg, N. 2017. Can the meiotic sex ratio explain the sex ratio bias in adult populations in the dioecious moss *Drepanocladus lycopodioides*? J. Bryol. in press.

Blackstock, T. H. 2015. Sex expression and sporophyte frequency in *Frullania tamarisci* (L.) Dumort. J. Bryol. 37: 202-208.

Bolker, B. M. and Pacala, S. W. 1999. Spatial moment equations for plant competition: Understanding spatial strategies and the advantages of short dispersal. Amer. Nat. 153: 575-602.

Bowker, M. A., Stark, L. R., McLetchie, D. N., and Mishler, B. D. 2000. Sex expression, skewed sex ratios and microhabitat distribution in the dioecious desert moss *Syntrichia caninervis* (Pottiaceae). Amer. J. Bot. 87: 517-526.

Braithwaite, R. 1870-1905. The British Moss Flora. London.

Brzyski, J. R., Wade, T., and McLetchie, D. N. 2013. Reproductive allocation between the sexes, across natural and novel habitats, and its impact on genetic diversity. Evol. Ecol. 28: 247-261.

Buczkowska, K., Adamczak, M., and Bączkiewicz, A. 2006. Morphological and anatomical differentiation within the *Aneura pinguis* complex (Metzgeriales, Hepaticae). Biol. Lett. 43: 51-68.

Cameron, R. G. and Wyatt, R. 1990. Spatial patterns and sex ratios in dioecious and monoecious mosses of the genus *Splachnum*. Bryologist 93: 161-166.

Charnov, E. L. 1982. The theory of sex allocation. Princeton University Press, Princeton, NJ.

Chopra, R. N. and Sood, S. 1973. In vitro studies in Marchantiales. I. Effects of some carbohydrates, agar, *p*H, light and growth regulators on the growth and sexuality in *Riccia crystallina*. Phytomorphology 23: 230-244.

Clapham, P. M. and Oldroyd, M. 1936. A time-table for the life cycles of some of the liverworts in Yorkshire. Naturalist 1936: 253-259.

Correns, C. 1899. Untersuchungen über die Vermehrung der Laubmoose durch Brutorgane und Stecklinge. Gustav Fischer, Jena, 472 pp.

Crandall-Stotler, B. J. and Bartholomew-Began, S. E. 2007. Morphology. In: Flora of North America North of Mexico, Vol. 27. Bryophyta, part 1. Oxford University Press, New York & Oxford, pp. 3-13.

Cronberg, N. 2000. Genetic diversity of the epiphytic bryophyte *Leucodon sciuroides* in formerly glaciated versus nonglaciated parts of Europe. Heredity 84: 710-720.

Cronberg, N. 2002. Colonization dynamics of the clonal moss *Hylocomium splendens* on islands in a Baltic land uplift area: Reproduction, genet distribution and genetic variation. J. Ecol. 90: 925-935.

Cronberg, N., Andersson, K., Wyatt, R., and Odrzykoski, I. J. 2003. Clonal distribution, fertility and sex ratios of the moss *Plagiomnium affine* (Bland.) T. Kop. in forests of contrasting age. J. Bryol. 25: 155-162.

Cronberg, N., Rydgren, K., and Økland, R. H. 2006. Clonal structure and genet-level sex ratios suggest different roles of vegetative and sexual reproduction in the clonal moss *Hylocomium splendens*. Ecography 29: 95-103.

Cronberg, N., Wyatt, R., Odrzykoski, I. J., and Andersson, K. 2005. Genetic diversity of the moss *Plagiomnium affine* in forests of contrasting age. Lindbergia 30: 49-58.

Crowley, P. H., Stieha, C. R., and McLetchie, D. N. 2005. Overgrowth competition, fragmentation and sex-ratio dynamics: A spatially explicit, sub-individual-based model. J. Theoret. Biol. 233: 25-42.

Crum, H. 1972. The geographic origins of the mosses of North America's eastern deciduous forest. J. Hattori Bot. Lab. 35: 269-298.

Crum, H. 1976. Mosses of the Great Lakes forest, revised edition. Univ. Mich. 10: 1-404.

Crum, H. 2001. Structural Diversity of Bryophytes. University of Michigan Herbarium, Ann Arbor, 379 pp.

Deguchi, H. 1978. A revision of the genera *Grimmia*, *Schistidium* and *Coscinodon* (Musci) of Japan. J. Sci. Hiroshima Univ. Ser. B, Div. 2 (Botany) 16: 121-256.

Devos, N., Renner, M. A. M., Gradstein, R., Shaw, A. J., Laenen, B., and Vanderpoorten, A. 2011. Evolution of sexual systems, dispersal strategies and habitat selection in the liverwort genus *Radula*. New Phytol. 192: 225-236.

During, H. J. 2007. Relations between clonal growth, reproduction and breeding system in the bryophytes of Belgium and The Netherlands. Beih. Nova Hedw. 131: 133-145.

Fisher, K. M. 2011. Sex on the edge: Reproductive patterns across the geographic range of the *Syrrhopodon involutus* (Calymperaceae) complex. Bryologist 114: 674-685.

Fuselier, L. C. 2004. Maintenance of Sexually Dimorphic Patterns of Growth and Reproduction in *Marchantia inflexa*. Ph. D. Dissertation, University of Kentucky, Lexington, KY, 203 pp.

Fuselier, L. and McLetchie, N. 2002. Maintenance of sexually dimorphic pre-adult traits in *Marchantia inflexa* (Marchantiaceae). Amer. J. Bot. 89: 592-601.

Fuselier, L. and McLetchie, N. 2004. Microhabitat and sex distribution in *Marchantia inflexa*, a dioicous liverwort. Bryologist 107: 345-356.

Garcia-Ramos, G., Stieha, C., McLetchie, N., and Crowley, P. 2002. Maintenance of sexes under metapopulation dynamics: Modeling a liverwort case. Abstracts of the 87th Annual Meeting of the Ecological Society of America and the 14th Annual International Conference of the Society for Ecological Restoration, August 4-9, 2002, Tucson, AZ.

Green, R. F. and Noakes, D. L. G. 1995. Is a little bit of sex as good as a lot? J. Theoret. Biol. 174: 87-96.

Groen, K. E., Stieha, C. R., Crowley, P. H., and McLetchie, D. N. 2010a. Sex-specific plant responses to two light levels in the liverwort *Marchantia inflexa* (Marchantiaceae). Bryologist 113: 81-89.

Groen, K. E., Stieha, C. R., Crowley, P. H., and McLetchie, D. N. 2010b. Sex-specific plant responses to light intensity and

canopy openness: Implications for spatial segregation of the sexes. Oecologia 162: 561-570.

Hassel, K., Pedersen, B., and Söderström, L. 2005a. Age and size at maturity in mountain and lowland populations of the expanding moss *Pogonatum dentatum*. Plant Ecol. 179: 207-216.

Hassel, K., Pedersen, B., and Söderström, L. 2005b. Changes in life-history traits in an expanding moss species: Phenotypic plasticity or genetic differentiation? A reciprocal transplantation experiment with *Pogonatum dentatum*. Ecography 28: 71-80.

Hedderson, T. A. and Longton, R. E. 2008. Local adaptation in moss life histories: Population-level variation and a reciprocal transplant experiment. J. Bryol. 30: 1-11.

Hedenäs, L., Bisang, I., Korpelainen, H., and Cronholm, B. 2010. The true sex ratio in European *Drepanocladus trifarius* (Bryophyta: Amblystegiaceae) revealed by a novel molecular approach. Biol. J. Linn. Soc. 100: 132-140.

Hill, M. O., Preston, C. D., and Smith, A. J. E. 1991. Atlas of the Bryophytes of Britain and Ireland Vol. 1. Liverworts (Hepaticae and Anthocerotae). Colchester.

Hill, M. O., Preston, C. D., and Smith, A. J. E. 1992. Atlas of the Bryophytes of Britain and Ireland. Vol. 2. Mosses (except Diplolepidae). Colchester.

Hill, M. O., Preston, C. D., and Smith, A. J. E. 1994. Atlas of the Bryophytes of Britain and Ireland Vol. 3. Mosses (Diplolepidae). Colchester.

Holá, E., Vesalainen, T., Těšitel, J., and Laaka-Lindberg, S. 2014. Sex ratio, sex - specific pattern in vegetative growth and gemma production in an aquatic liverwort, Scapania undulata (Marchantiophyta: Scapaniaceae). Bot. J. Linn. Soc. 175: 229-241.

Horsley, K., Stark, L. R., and McLetchie, D. N. 2011. Does the silver moss *Bryum argenteum* exhibit sex-specific patterns in vegetative growth rate, asexual fitness or prezygotic reproductive investment? Ann. Bot. 107: 897-907.

Imura, S., Glime, J. M., and Iwatsuki, Z. 1991. Propagula of *Aulacomnium heterostichum* in Japan. Bryologist 94: 67-69.

Innes, D. J. and Dunbrack, R. L. 1993. Sex allocation variation in *Daphnia pulex*. J. Evol. Biol. 6: 559-575.

Kimmerer, R. W. 1991. Reproductive ecology of *Tetraphis pellucida* I. Population density and reproductive mode. Bryologist 94: 255-260.

Korpelainen, H. 1998. Labile sex expression in plants. Biol. Rev. 73: 157-180.

Krupnick, G. A., Avila, G., Brown, K. M. and Stephenson, A. G. 2000. Effects of herbivory on internal ethylene production and sex expression in *Cucurbita texana*. Funct. Ecol. 14: 215-225.

Laaka-Lindberg, S. 2000. Ecology of Asexual Reproduction in Hepatics. E-thesis. University of Helsinki, Finland, 28 pp.

Laaka-Lindberg, S. 2005. Reproductive phenology in the leafy hepatic *Lophozia silvicola* Buch in southern Finland. J. Bryol. 27: 253-259.

Laaka-Lindberg, S., Hedderson, T. A., and Longton, R. E. 2000. Rarity and reproductive characters in the British hepatic flora. Lindbergia 25: 78-84.

Lackner, L. 1939. Über die Jahresperiodizität in der Entwickung der Laubmoose. Planta 29: 534-616.

Lebel-Hardenack, S. and Grant, S. R. 1997. Genetics of sex determination in flowering plants. Trends Plant Sci. 2: 130-136.

Laenen, B., Machac, A., Gradstein, S. R., Shaw, B., Patiño, J., Désamoré, A., Goffinet, B., Cox, C. J., Shaw, J., and Vanderpoorten, A. 2015. Geographical range in liverworts: Does sex really matter? J. Biogeogr. 43: 627-635.

Lidforss, B. 1904. Über die Reizbewegungen der *Marchantia*-Spermatozoiden. Jahrb. Wiss. Bot. 41: 65-87.

Longton, R. E. 1990. Sexual reproduction in bryophytes in relation to physical factors of the environment. In: Chopra, R. N. and Bhatla, S. C. (eds.). Bryophyte Development: Physiology and Biochemistry. CRC Press, Boca Raton, pp. 139-166.

Longton, R. E. 1992. Reproduction and rarity in British mosses. Biol. Conserv. 59: 89-98.

Longton, R. E. 1997. Reproductive biology and life-history strategies. Adv. Bryol. 6: 65-101.

Longton, R. E. 1998. Reproductive biology and life-history strategies. In: Bates, J. W., Ashton, N. W., and Duckett, J. G. (eds.). Bryology for the Twenty-first Century. Maney Publishing and the British Bryological Society, UK, p. 369.

Longton, R. E. and Schuster, R. M. 1983. Reproductive biology. In: Schuster, R. M. (ed.). New Manual of Bryology, Vol. 1. Hattori Botanical Laboratory, Nichinan, Japan, pp. 386-462.

Maciel-Silva, A. S. and Marques Válio, I. F. 2011. Reproductive phenology of bryophytes in tropical rain forests: The sexes never sleep. Bryologist 114: 708-719.

Maciel-Silva, A. S., Marques Valio, I. F., and Rydin, H. 2012. Altitude affects the reproductive performance in monoicous and dioicous bryophytes: Examples from a Brazilian Atlantic rainforest. AoB PLANTS <www.aobplants.oxfordjournals.org>, 14 pp.

McDaniel, S. F., Willis, J. H., and Shaw, A. J. 2007. A linkage map reveals a complex basis for segregation distortion in an interpopulation cross in the moss *Ceratodon purpureus*. Genetics 176: 2489-2500.

McLellan, A. J., Prati, D., Kaltz, O., and Schmid, B. 1997. Structure and analysis of phenotypic and genetic variation in clonal plants. In: Kroon, H. de and Groenendael, J. van. (eds.). The Ecology and Evolution of Clonal Plants. Backhuys Publishers, Leiden, pp. 185-210.

McLetchie, D. N. 1992. Sex ratio from germination through maturity and its reproductive consequences in the liverwort *Sphaerocarpos texanus*. Oecologia 92: 273-278.

McLetchie, D. N. 2001. Sex-specific germination in *Sphaerocarpos texanus*. Bryologist 104: 69-71.

McLetchie, D. N. and Puterbaugh, M. N. 2000. Population sex ratios, sex-specific clonal traits and tradeoffs among these traits in the liverwort, *Marchantia inflexa*. Oikos 90: 227-237.

McPeek, M. A. and Kalisz, S. 1998. On the joint evolution of dispersal and dormancy in metapopulations. Ergebn. Limnol. 52: 33-51.

Miles, C. J. and Longton, R. E. 1990. The role of spores in reproduction in mosses. J. Linn. Soc. Bot. 104: 149-173.

Miles, C. J., Odu, E. A., and Longton, R. E. 1989. Phenological studies on British mosses. J. Bryol. 15: 607-621.

Miller, N. G. and Howe Ambrose, L. J. 1976. Growth in culture of wind-blown bryophyte gametophyte fragments from arctic Canada. Bryologist 79: 55-63.

Milne, J. 2001. Reproductive biology of three Australian species of *Dicranoloma* (Bryopsida, Dicranaceae): Sexual reproduction and phenology. Bryologist 104: 440-452.

Mishler, B. D. and Luna, E. De. 1991. The use of ontogenetic data in phylogenetic analyses of mosses. Adv. Bryol. 4: 121-167.

Mishler, B. D. and Oliver, M. J. 1991. Gametophytic phenology of *Tortula ruralis*, a desiccation-tolerant moss, in the Organ

Mountains of southern New Mexico. Bryologist 94: 143-153.

Moore, J. D., Kollar, L., and McLetchie, D. N. 2014. Sex-specific clump morphology leads to differences in water retention ability in *Bryum argenteum*. Botany 2014 Abstract Book, 26-30 July 2014, Boise, Idaho.

Moyá, M. T. 1992. Phenological observations and sex ratios in *Marchantia chenopoda* L. (Hepaticae: Marchantiaceae). Trop. Bryol. 6: 161-170.

Newton, M. E. 1971. A cytological distinction between male and female *Mnium undulatum* Hedw. Trans. Brit. Bryol. Soc. 6: 230-243.

Newton, M. E. 1972. Sex-ratio differences in *Mnium hornum* Hedw. and *M. undulatum* Sw. in relation to spore germination and vegetative regeneration. Ann. Bot. 36: 163-178.

Newton, A. E. and Mishler, B. D. 1994. The evolutionary significance of asexual reproduction in mosses. J. Hattori Bot. Lab. 76: 127-145.

Norrell, T. E., Jones, K. S., Payton, A. C., and McDaniel, S. F. 2014. Meiotic sex ratio variation in natural populations of *Ceratodon purpureus* (Ditrichaceae). Amer. J. Bot. 101: 1572-1576.

Pedersen, N., Russell, S. J., Newton, A. E., and Ansell, S. W. 2006. A novel molecular protocol for the rapid extraction of DNA from bryophytes and the utility of direct amplification of DNA from a single dwarf male. Bryologist 109: 257-264.

Pereira, M. R., Dambros, C. S., and Zartman, C. E. 2016. Prezygotic resource-allocation dynamics and reproductive trade-offs in Calymperaceae (Bryophyta). Amer. J. Bot. 103: 1838-1846.

Pettet, A. 1967. Sex ratios of *Riccia frostii* Aust. in Khartoum Province, Sudan. Trans. Brit. Bryol. Soc. 5: 332-337.

Pohjamo, M. and Laaka-Lindberg, S. 2004. Demographic population structure of a leafy epixylic hepatic *Anastrophyllum hellerianum* (Nees ex Lindenb.) R. M. Schust. Plant Ecol. 173: 73-81.

Reese, W. D. 1984. Reproductivity, fertility and range of *Syrrhopodon texanus* Sull. (Musci: Calymperaceae), a North American endemic. Bryologist 87: 217-222.

Rydgren, K., Halvorsen, R., and Cronberg, N. 2010. Infrequent sporophyte production maintains a female-biased sex ratio in the unisexual clonal moss *Hylocomium splendens*. J. Ecol. 98: 1224-1231.

Schuster, R. M. 1983. Reproductive biology, dispersal mechanisms, and distribution patterns in Hepaticae and Anthocerotae. Sonderb. Naturwiss. Vereins Hamburg 7: 119-162.

Schuster, R. M. 1988. Ecology, reproductive biology and dispersal of Hepaticae in the tropics. J. Hattori Bot. Lab. 64: 237-269.

Selkirk, P. M. 1979. Effect of nutritional conditions on sexual reproduction in *Riccia*. Bryologist 82: 37-46.

Shaw, A. J. 2000. Population ecology, population genetics, and microevolution. In: Shaw, A. J. and Goffinet, B. (eds.). Bryophyte Biology. Cambridge University Press, Cambridge, pp. 369-402.

Shaw, A. J. and Gaughan, J. F. 1993. Control of sex-ratios in haploid populations of the moss, *Ceratodon purpureus*. Amer. J. Bot. 80: 584-591.

Shaw, J. and Beer, S. C. 1999. Life history variation in gametophyte populations of the moss *Ceratodon purpureus* (Ditrichaceae). Amer. J. Bot. 86: 512-521.

Smith, A. J. E. 1982. Epiphytes and epiliths. In: Smith, A. J. E. (ed.). Bryophyte Ecology. Chapman & Hall, New York, pp. 191-228.

Söderström, L. 1992. Invasions and range expansions and contractions of bryophytes. In: Bates, J. W. and Farmer, A. M. (eds.). Bryophytes and Lichens in a Changing Environment. Oxford University Press, Oxford, pp. 131-158.

Söderström, L. 1994. Scope and significance of studies on reproductive biology of bryophytes. J. Hattori Bot. Lab. 76: 97-103.

Söderström, L. and Herben, T. 1997. Dynamics of bryophyte metapopulations. Adv. Bryol. 6: 205-240.

Stark, L. R. 1985. Phenology and species concepts. Bryologist 88: 190-198.

Stark, L. R. 1997. Phenology and reproductive biology of *Syntrichia inermis* (Bryopsida, Pottiaceae) in the Mojave Desert. Bryologist 100: 13-27.

Stark, L. R. 2002. Phenology and its repercussions on the reproductive ecology of mosses. Bryologist 105: 204-218.

Stark L. R. and Brinda, J. C. 2013. An experimental demonstration of rhizautoicy, self-incompatibility, and reproductive investment in *Aloina bifrons* (Pottiaceae). Bryologist 116: 43-52.

Stark, L. R. and Delgadillo, M. C. 2001. Rhizoautoicous *Aloina bifrons* in the Mojave Desert, a possible adaptation to increase spore production. Bryologist 104: 104-108.

Stark, L. R., Nichols, L. II, McLetchie, D. N., and Bonine, M. L. 2005. Do the sexes of the desert moss *Syntrichia caninervis* differ in desiccation tolerance? A leaf regeneration assay. Internat. J. Plant Sci. 166: 21-29.

Stark, L. R., McLetchie, D. N., and Eppley, S. M. 2010. Sex ratios and the shy male hypothesis in the moss *Bryum argenteum* (Bryaceae). Bryologist 113: 788-797.

Stieha, C. R., Middleton, A. R., Stieha, J. K., Trott, S. H., and McLetchie, D. N. 2014. The dispersal process of asexual propagules and the contribution to population persistence in *Marchantia* (Marchantiaceae). Amer. J. Bot. 101: 1-9

Taylor, D. R. 1990. Evolutionary consequences of cytoplasmic sex ratio distorters. Evol. Ecol. 4: 235-248.

Terui, K. 1981. Growth and gemma-cup formation in relation to archegoniophore protrusion in *Marchantia polymorpha* L. Ann. Rept. Fac. Ed. Iwate Univ. 40: 19-28.

Tessier, A. J. and Cáceres, C. E. 2004. Differentiation in sex investment by clones and populations of *Daphnia*. Ecol. Lett. 7: 695-703.

Thompson, K., Gaston, K. J., and Band, S. R. 1999. Range size, dispersal and niche breadth in the herbaceous flora of central England. J. Ecol. 87: 150-155.

Une, K. 1985. Geographical distribution of male and female plants in species of *Macromitrium* Brid. (Musci: Orthotrichaceae) in Japan. J. Hattori Bot. Lab. 59: 515-521.

Une, K. 1986. Sexuality of the Japanese mosses. Hikobia 9: 339-344.

Villarreal, J. C. and Renner, S. S. 2013. Correlates of monoicy and dioicy in hornworts, the apparent sister group to vascular plants. Evol. Biol. 13: 1-8.

Vitt, D. H. 1968. Sex determination in mosses. Mich. Bot. 7: 195-203.

Woodward, D. E. and Murray, J. D. 1993. On the effect of temperature-dependent sex determination on sex ratio and survivorship in crocodilians. Proc. Royal Soc. Lond. Ser. B Biol. Sci. 252: 149-155.

Wyatt, R. and Anderson, L. E. 1984. Breeding systems in bryophytes. In: Dyer, A. F. and Duckett, J. G. (eds.). The Experimental Biology of Bryophytes. Academic Press, London, pp. 39-64.

CHAPTER 3-3
SEXUALITY: SIZE AND SEX DIFFERENCES

JANICE M. GLIME AND IRENE BISANG

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CHAPTER 3-3
SEXUALITY: SIZE AND SEX DIFFERENCES

Figure 1. *Plagiomnium* producing male splash cups as it grows amid *Thuidium delicatulum*. Photo by Janice Glime.

Sex-related Differences in Gametophores

For most bryophytes, secondary sexual characteristics are subtle and are noticed only by the most observant. Fuselier and Stark (2004) consider size, morphology, physiology, reproductive investment, and stress response all to be expressed among sexual differences in bryophytes. Une (1985 a, b) with the moss *Macromitrium* and Fuselier and McLetchie (2004) with the thallose liverwort *Marchantia inflexa* (Figure 3) have shown that males and females of the sex-expressing individuals of these species can respond differently to stress. Even at the spore stage, size and morphology are traditional characteristics used to determine **anisospory** (two spore sizes) and **anisogamy** (size, shape, or behavioral differences in gametes) in bryophytes as well as in algae.   For bryophyte gametophytes, reproductive investment has been shown to

differ between antheridia and archegonia in some species (*e.g.* Stark *et al*. 2000; Horsley *et al*. 2011), but not in others (Bisang *et al*. 2006).

Shaw and Gaughan (1993) noted non-reproductive differences between the sexes in the moss *Ceratodon purpureus* (Figure 2). Among 160 single-spore isolates representing 40 sporophytes from one population, female gametophytes outnumbered males by a ratio of 3:2 at the time of germination. The resulting female gametophytic clones formed significantly more biomass, and individual female shoots were more robust than in male clones. On the other hand, male clones produced more numerous stems. Shaw and Gaughan suggest that this strategy may permit the females to provide more nutritional support for the sporophytic generation.
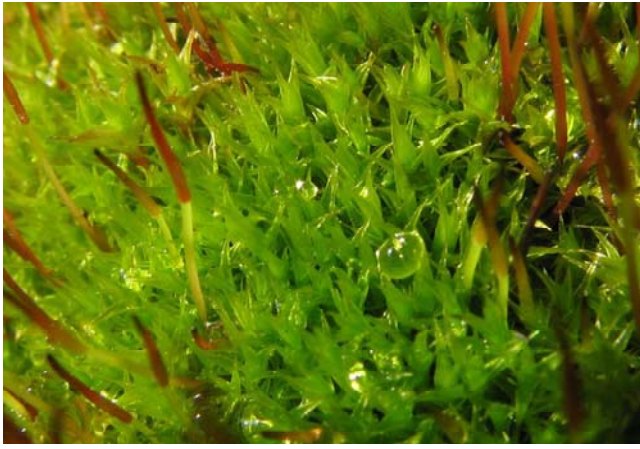
Figure 2. *Ceratodon purpureus* with young sporophytes. Photo by Jiří Kameníček, with permission.

Even in *Ceratodon purpureus* (Figure 2) that lacks **dwarf males** (see below under Dwarf Males) and where sex is chromosomally determined, sexes differ in size and in maturation rate, a character that Shaw and Beer (1999) suggest may prove to be widespread among bryophytes.

Even factors related to photosynthesis can differ between sexes. In their study Groen *et al.* (2010) found that females of *Marchantia inflexa* (Figure 3) had higher chlorophyll *a:b* ratios. And in the same study they found that females had a negative relationship between thallus thickness and gross photosynthesis whereas males did not, but they were unable to explain that negative relationship. Finally, differences between sexes in physiological traits may also occur at the clump level, as recently demonstrated in *Bryum argenteum* (Moore *et al.* 2016). Female clumps held more water and included more robust shoots than male clumps.

Figure 3. *Marchantia inflexa*, a species in which photosynthetic factors differ between males and females. Photo by Scott Zona, with permission.

Size and Sex Differences

"Why is the world full of large females?" (Lewin 1988). Particularly among insects, fish, amphibians, and reptiles, females are larger than males (Lewin 1988). Darwin explained this as the need of the species to produce a large number of eggs, a concept known as the fecundity-advantage model. Shine (1988) feels the concept is flawed in that evolution should maximize lifetime reproductive success, not instantaneous reproductive success. He

suggests that the fecundity-advantage model implies one large reproductive effort late in life, thus subjecting the female to great energy costs, and would only be of benefit when energy resources are non-limiting. With that in mind, it is interesting that mammals that must carry their young within do not generally have larger females than males. It is also the case in seed plants that are dioecious; only the female must bear the fruits. Yet it is not typical among seed plants for the female plant to be larger.

Bryophytes present an interesting contrast here. No other group of plants or algae is characterized by the need for the gametophyte to persist through the entire development of the sporophyte (there are individual exceptions, such as the fern *Botrychium*). In bryophytes, the female must supply the energy to support the developing sporophyte. Indeed, some bryophytes do have larger females than males [*e.g.* the liverworts *Cryptothallus* (Figure 4), *Pallavicinia* (Figure 5), *Pellia* (Figure 6-Figure 8), *Riccia* (Figure 9), and *Sphaerocarpos* (Figure 10)]. There are also a number of mosses with **dwarf males** [male plants that are considerably reduced in size relative to female plants, usually occurring on leaves (Figure 14) or in the tomentum of female plants, *e.g. Micromitrium* (Figure 11)] – about 60 genera already identified by Fleischer (1900-23, 1920). Females smaller than males are rare, with the non-sexual part of *Diphyscium foliosum* (Figure 12) being a notable exception.

Figure 4. *Cryptothallus mirabilis* with young capsules. This is a genus with females larger than males. Photo by David Holyoak, with permission.

Figure 5. *Pallavicinia levieri*, in a genus with females larger than males. Photo by Li Zhang, with permission.

Figure 6. *Pellia endiviifolia* males with reddish antheridial cavities and females in center; females are the larger sex. Photo by David Holyoak, with permission.

Figure 7. *Pellia endiviifolia* with antheridia. Photo by Ralf Wagner <www.dr-ralf-wagner.de>, with permission.
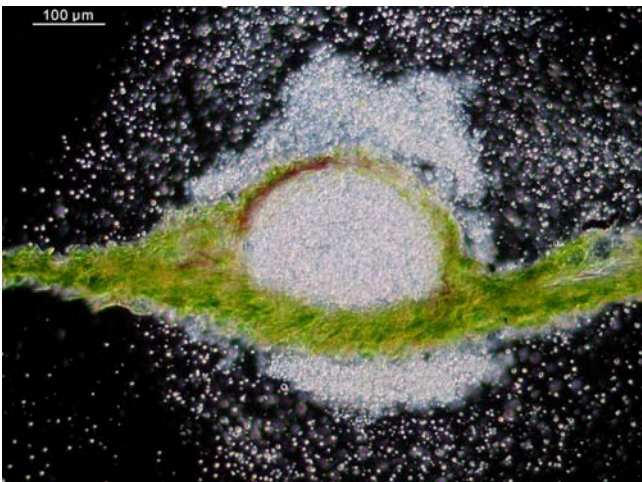

Figure 8. *Pellia endiviifolia* with antheridium cross section and spermatocytes. Photo by Ralf Wagner <www.dr-ralf-wagner.de>, with permission.

Figure 9. *Riccia sorocarpa*, a genus with females that are larger than males. Photo by Michael Lüth, with permission.

Figure 10. *Sphaerocarpos* sp., a species in which females are larger than males. Photo by David T. Holyoak, with permission.
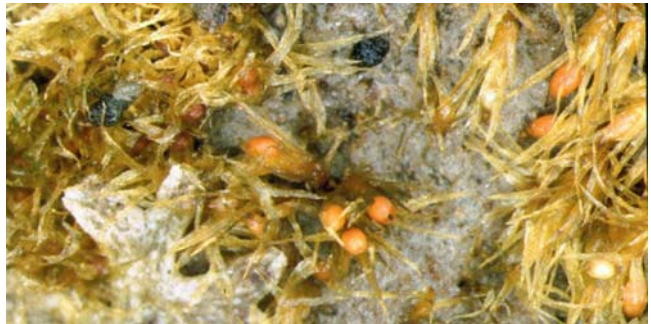

Figure 11. *Micromitrium tenerum* with capsules, a genus with females that are larger than males. Photo by Jan-Peter Frahm, with permission.
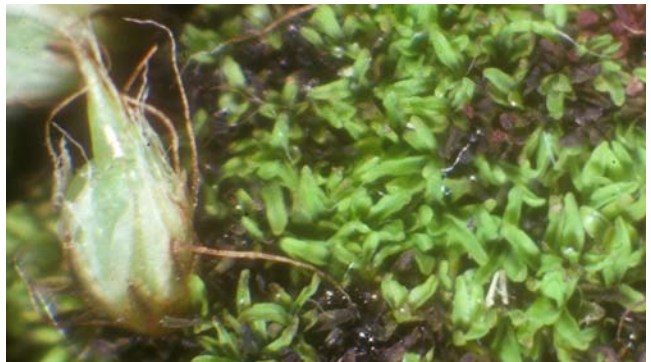

Figure 12. *Diphyscium foliosum* female (**left**) with only perichaetial leaves visible and reduced vegetative gametophyte; male plants are to its **right**, showing conspicuous leafy plants. Photo by Janice Glime.

Dwarf Males

Dwarf males are a notable exception to the observation that there is little, if any, size difference between males and females among most bryophytes. Nevertheless, early publications on bryophytes recognized examples of sharp size distinctions (Bruch *et al.* 1851-1855; Limpricht 1895-1904; Fleischer 1920). Where spores germinate on the leaves (**phyllodioicy**; Figure 13-Figure 18) or other parts of the female, some species produce **dwarf males** (**nannandrous males**) whose primary function is to produce sperm (Crum 1976). This production of dwarf males is unique to bryophytes among land plants [but is present in some species of the green alga *Oedogonium* (Figure 19) in Oedogoniaceae (Maier & Müller 1986)].
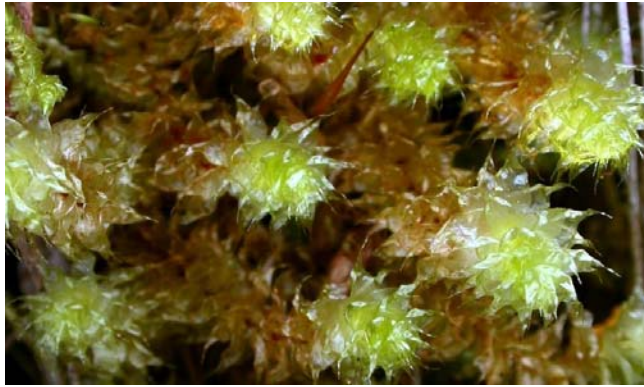
Figure 13. ***Ptychomnion aciculare***. Photo by David Tng, with permission.
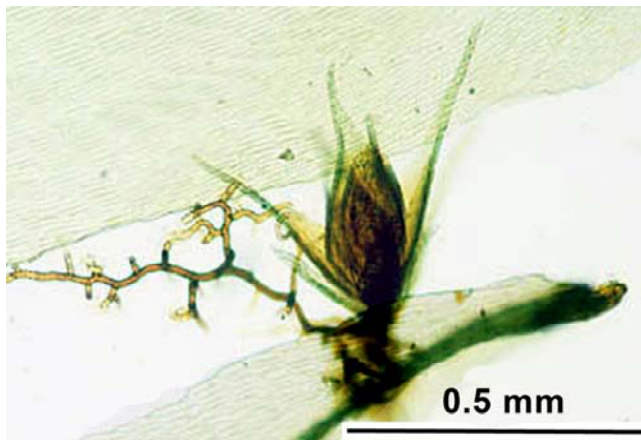
Figure 14. ***Ptychomnion aciculare*** with dwarf male on leaf. Photo modified from one by John Braggins, with permission.
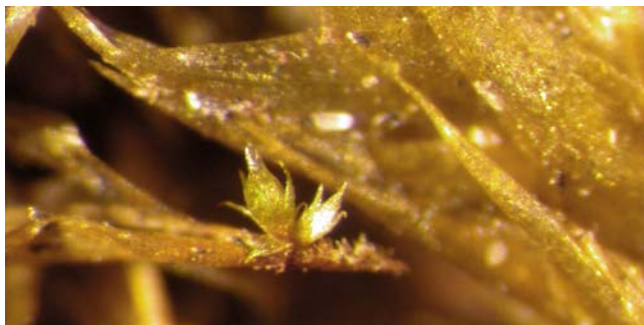
Figure 15. ***Acroporium stramineum*** dwarf male on leaf of mature female. Photo courtesy of Lars Hedenäs.

Figure 16. ***Isothecium alopecuroides*** dwarf male on leaves. Photo courtesy of Lars Hedenäs.

Figure 17. ***Isothecium alopecuroides*** dwarf male. Photo courtesy of Lars Hedenäs.

Figure 18. ***Eurhynchium angustirete*** dwarf males on female plant. Photo courtesy of Lars Hedenäs.

Figure 19. *Oedogonium* sp. with enlarged oogonium (female gametangium) and two dwarf males curved toward the oogonium. Photo by Janice Glime.

Although dwarf males have been known for many decades in some genera, their widespread occurrence among many more genera has been overlooked (Hedenäs & Bisang 2011). This is especially true for facultative dwarf males. Hedenäs and Bisang (2011, 2012) estimate that 25-44% of the dioicous pleurocarpous moss species exhibit dwarf males, with about 75% of these producing them **facultatively**, *i.e.*, the species has the ability to form both normal-sized and dwarf males. The underlying mechanisms (discussed below under How Do Facultative Males Develop) are currently unresolved in most cases, although at least some seem to produce normal males on a non-moss substrate and dwarf males on a moss substrate. Nearly 60% of the 1737 species in the total data set investigated by Hedenäs and Bisang are dioicous. Of the 178 species reported to produce dwarf males, 113 are considered to form obligate dwarf males. When they examined in detail a subset of 162 species, 72 produced observable dwarf males, but only 18 of these had obligate dwarf males. Hedenäs and Bisang (2011) reason that these dwarf males are likely to be overlooked when counting male presence.

This **phyllodioicous** strategy has been repeated in at least 27 separate families of mosses (Fuselier & Stark 2004), including both acrocarpous (Schellenberg 1920; Ramsay 1979; Yamaguchi 1993; Une & Yamaguchi 2001; Hedenäs & Bisang 2004) and pleurocarpous species (Une 1985a; Goffinet 1993; Hedenäs & Bisang 2011). Hedenäs and Bisang (2011) found dwarf males in 22 pleurocarpous families.

Even when we find dwarf males, we can't be certain of the sex unless they have gametangia. For example, Fleischer (1900-23) suggested a strategy for *Trismegistia brauniana*, wherein spores that germinate on leaves of normal females all develop into dwarfs – both male and female. But these were non-expressing dwarfs, so there was no way for Fleischer to determine if there were really females (Lars Hedenäs, pers. comm. 4 April 2013).

The dwarf male strategy may increase fitness for the species by saving space and conserving resources. A sexually reproducing female bryophyte needs to nurture the developing sporophyte. Fitness of the reproductive output may be increased if the female individual is large, permitting that female to occupy more space and obtain more light, and possibly more water and nutrients. Males,

on the other hand, need only produce sperm and do not sacrifice nutrients and energy to a developing embryo.

Vollrath (1998) referred to the condition of dwarf males associated with females as being short of true parasitism. Although the females provide a kind of room and board for the males, the males provide sperm to the females. But we are unaware of any evidence that the females provide nutrition. Rather, they provide a safe habitat that offers protection from desiccation and a short route to the egg.

Revisiting the Sex Ratio

Realization that 10-20% of the pleurocarpous moss species worldwide produce functional dwarf males requires re-examination of our data on sex ratios (Hedenäs & Bisang 2011) (discussed in Chapter 3-2). Using herbarium specimens of five Macaronesian species as models, Hedenäs and Bisang (2012) examined the effect of adding these newly recognized dwarf males to the calculation of sex ratio. If dwarf males were not counted, male availability was reduced by 51-61%, with that reduction increasing to 74-76% for sporophyte-producing plants. As one might expect, presence of sporophytes was positively correlated with presence of dwarf males. Hence, in those species with dwarf males, the sex ratio at the specimen level was balanced if dwarf males were counted, but strongly female biased if they were not.

Dwarf males in *Homalothecium lutescens*

Rosengren and co-workers examined the nanandrous sexual system in the pleurocarpous moss ***Homalothecium lutescens*** (Figure 20) in grassland habitats in southern Sweden and on the Baltic island Öland. These detailed studies, covering both ecological and genetic aspects, greatly advanced our knowledge on the conditions for and consequences of male dwarfism in mosses. ***Homalothecium lutescens*** has facultative dwarf males, but large males are extremely rare in this species (Wallace 1970; Rosengren *et al.* 2014 and references therein). In one of the study sites, dwarf males were almost exclusively found on sporophytic shoots (Rosengren *et al.* 2014). Investigating 90 colonies from three localities, Rosengren and Cronberg (2014) found that dwarf male density was positively related to colony moisture (two localities).

Figure 20. ***Homalothecium lutescens***, a species with facultative dwarf males. Photo by Michael Lüth, with permission.

In addition, fertilization frequency was positively affected by dwarf male density, but also by canopy cover in one locality (Rosengren & Cronberg 2014). Their findings suggest that nannandry reduces the problem of short fertilization distances in bryophytes, but that the presence of water is still critical. In terms of genetic affinity, dwarf males are most closely related to their host shoot, then to neighbors within their colony of 0.5m^2, and finally, to plants in the remaining population (Figure 21) (Rosengren *et al.* 2015). This means that spores giving rise to the dwarf males are at most commonly produced by the mother shoot or by a shoot in the close vicinity. Occasionally, however, dwarf males seemed even to originate from outside the host population, *i.e.* from another of the four study populations within a radius of 60 m^2. The researchers conclude that although dwarf males have in general local origin, sporadic dispersal to greater distances happens. These events contribute to the gene flow across populations and to the accumulation of genetic diversity within a population. Overall, the levels of genetic diversity were comparable between dwarf males and females within each population (Rosengren *et al.* 2015).
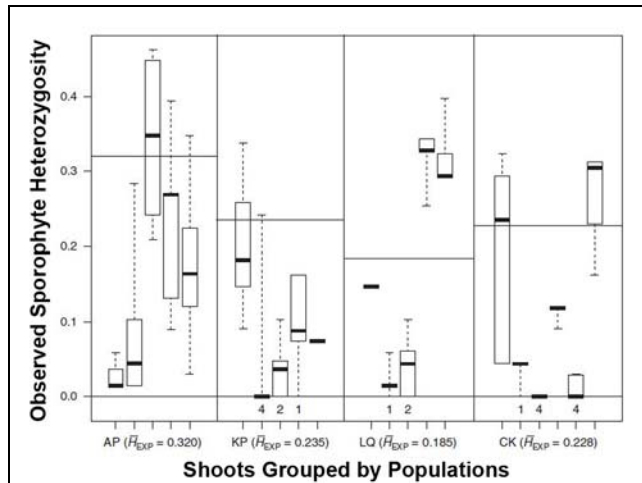

Figure 21. Inbreeding vs outcrossing in *Homalothecium lutescens* from four populations in Sweden. Each box represents the lower and upper quartile of 4-6 sporophytes on a single female shoot. The thick horizontal lines within boxes represent the median and whiskers denote the total range of data (minimum and maximum values outside the quartiles). Horizontal lines across each population section represent the mean H_{exp} (mean expected sporophyte heterozygosity over all loci, based on male and female allele frequencies). Sporophytes falling below that line could be considered inbred, with a few exceptions. Numbers below the y=0 line represent the number of sporophytes on the shoot that are homozygous in all loci, *i.e.* probably self-fertilized or inbred. Modified from Rosengren *et al.* 2016.

Rosengren *et al.* (2016) also genotyped sporophytes, female host shoots, and dwarf male plants in these populations. The high proportion of entirely homozygous sporophytes confirms frequent mother-son mating. Nevertheless, 23% of sporophytes exhibited a higher heterozygosity level than the expected population mean, which gives evidence of occasional fertilizations by non-host males (Figure 21). Further, almost 60% of the sporophytes were sired by distinct fathers (Rosengren *et al.* 2016). The extent of **polyandry** (multiple male parents) in bryophytes is poorly understood, but Szövény *et al.* (2009)

also reported prevalent multiple paternity (polyandry) among sporophytes in *Sphagnum lescurii* (Figure 22).

Figure 22. *Sphagnum lescurii*, a species that has multiple paternity of its sporophytes. Photo by Bob Klips, with permission.

In an *in vitro* experimental approach by sowing spores from three species [*Homalothecium lutescens* (Figure 20), *H. sericeum* (Figure 23), *Isothecium alopecuroides* (Figure 16-Figure 17) on shoots of *H. lutescens*, Rosengren and Cronberg (2015) noted distinct differences in germinability of the sown spores among the three species (Figure 24). While no dwarf males were formed from spores of the distantly related *I. alopecuroides*, both *H. lutescens* and *H. sericeum* spores developed into dwarf males (Figure 25). The latter points to a possible pathway for hybridization between the two species (Rosengren & Cronberg 2015).
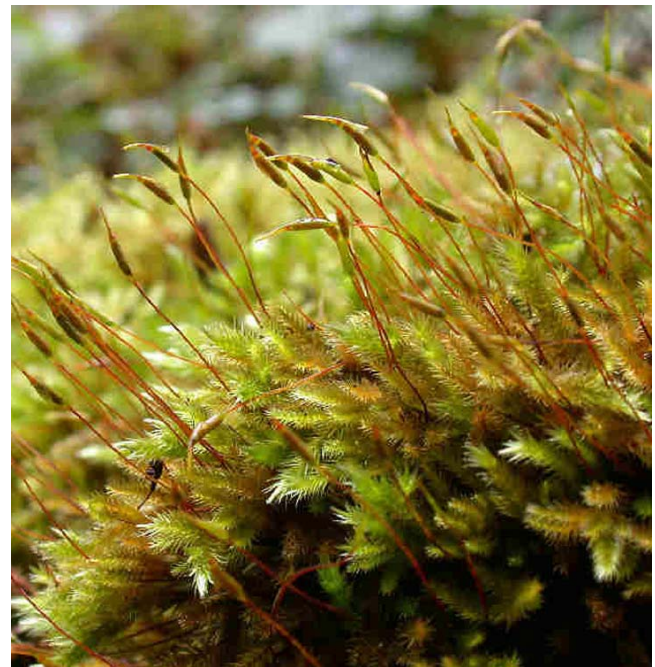

Figure 23. *Homalothecium sericeum* with capsules, indicating successful fertilization. Photo by David Holyoak, with permission.
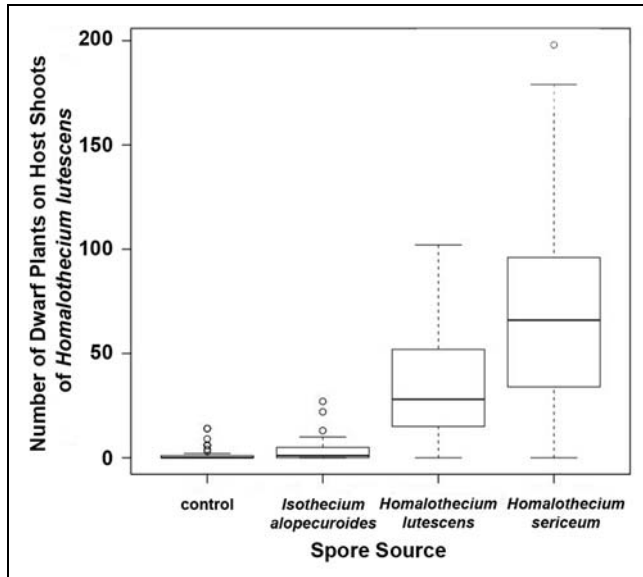
Figure 24.   Total number of dwarf plants of each source species on *Homalothecium lutescens* 10 months after sowing spores of three species on *H. lutescens* (n-46).   Redrawn from Rosengren & Cronberg 2015.
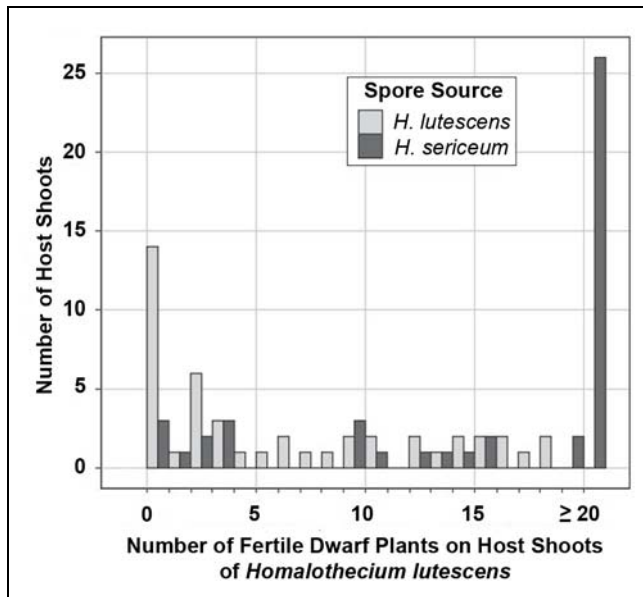

Figure 25.   Number of fertile dwarf male-expressing plants of *Homalothecium lutescens* and *H. sericeum* that germinated from spores placed on *Homalothecium lutescens*.   Redrawn from Rosengren & Cronberg 2015.

What Is the Role of Vegetative Propagules?

As noted above, some species of the alga *Oedogonium* (Figure 19) (Chlorophyta) have a similar dimorphism in the size of the filaments, whereupon a male spore produces a dwarf male when it germinates upon a female (Rawitscher-Kunkel & Machlis 1962).   However, if a male spore develops away from a female, it will grow into a larger filament and produce asexual spores that again have the opportunity to locate a female and form a dwarf male, a possible strategy that has apparently received no consideration among bryophytes.

I (Glime) became curious as to a similar relationship between vegetative propagules (since asexual spores do not

exist in bryophytes) and facultative dwarf males in bryophytes.   That is, do vegetative propagules develop into normal-sized male plants when establishing on "ordinary" substrate away from a female, but form minute males on a female individual, as has been observed for spores in some species (see below).   Would the non-dwarfed males then produce vegetative propagules that might develop dwarf males if they were to land on a female?   Bryonetters brought me several examples, predominantly in the genus *Dicranoloma* (Figure 26-Figure 27).   But species bearing both dwarf males and gemmae in Asia and Australia [*D. bartramianum*, *D. dicarpum* (Figure 26), *D. platycaulon*, *D. leichhardtii* (Figure 27)] do not produce gemmae in New Zealand (Milne 2000; Pina Milne and Allan Fife, pers. comm. 9 January 2014).   In southeastern Asia, Malesia, and Oceania, *D. braunii* has the most frequent and conspicuous gemmae and produces dwarf males (Niels Klazenga, pers. comm. 8 January 2014).   But this still begs the question, do gemmae that land on females produce dwarf males, and do those that land on soil continue to produce gemmae-producing non-sex-expressing plants?

Figure 26. *Dicranoloma dicarpum*, a moss with both dwarf males and gemmae. Photo by Tom Thekathyil, with permission.

Figure 27.   *Dicranoloma leichhardtii*, a moss with both dwarf males and gemmae.   Photo by Niels Klazenga, with permission.

Several other examples exist.   *Platygyrium repens* (Figure 28) produces brood branches and sometimes produces facultative dwarf males (Lars Hedenäs, pers. comm. 8 January 2014).   Many species of *Garovaglia* (**Ptychomniales**) have both dwarf males and produce filamentous gemmae, with *G. elegans* (Figure 29)

producing gemmae rather frequently (Neil Bell, Bryonet 8 January 2014). But despite these examples, Pedersen and Newton (2007) found no correlation between the evolution of dwarf males and the filamentous gemmae in the order **Ptychomniales**.

Figure 28. *Platygyrium repens* with bulbils clustered at the branch tips. Photo by Dick Haaksma, with permission.

The problem with trying to interpret these observations is that if a non-expressing individual has propagules, we have been unable to tell if it is a male or a female. Hence, it is difficult to assess the importance of vegetative propagation in males that developed away from a female. Do bryophyte male propagules in any species behave as do nannandrous species of *Oedogonium*, reproducing asexually until they land on a female? Do the gemmae of asexual (sterile) male plants of some species develop into dwarf males if they land on a female substrate? Fortunately, we now have genetic means to identify sex of non-sex-expressing plants using DNA markers. As markers become available in more species, we may be able to answer these questions more easily.

Figure 29. *Garovaglia elegans* with capsules. Photo by Li Zhang, with permission.

For my *Oedogonium* comparison to work for bryophytes, we need evidence that asexual propagules, *e.g.* gemmae or bulbils, produced by male plants, are able to germinate on females and produce dwarf males. Tamás

Pócs (pers. comm. 14 January 2014) kindly pointed me to his publication (Pócs 1980) on the liverwort *Cololejeunea borhidiana* (Figure 30) as a new species. He illustrates a dwarf male, complete with antheridia, developing from a gemma from this species (Figure 30), a much smaller version than a male that develops into a normal-sized plant (Figure 31). This the only evidence that dwarf males exist among liverworts, and is the only evidence we know of a dwarf male developing from an asexual propagule. The complete story for this species is not known and we have no evidence that the spores ever form dwarf males. However, it suggests the possibility that an asexual strategy for males that fail to land on a female might exist among some bryophytes. *Cololejeunea borhidiana* is **epiphyllous**, and the ability to produce vegetative plants until a gemma reaches a female to induce formation of a dwarf male could be very advantageous for a species that occupies a somewhat short-lived substrate that is difficult to reach and colonize. But was it a female that stimulated this gemma to become a dwarf male, or was it the current environmental conditions? And is this an isolated occurrence, with dwarf males otherwise unknown in liverworts? Knowledge of gemmae of any bryophyte developing into dwarf males seems to be otherwise lacking, so we cannot measure its importance among the bryophytes. In the case of *Cololejeunea borhidiana*, we don't know if the gemma came from a male or a female plant. If the nannandrous *Oedogonium* strategy does exist among some bryophytes, it presents an interesting adaptation that could be quite beneficial in difficult habitats.
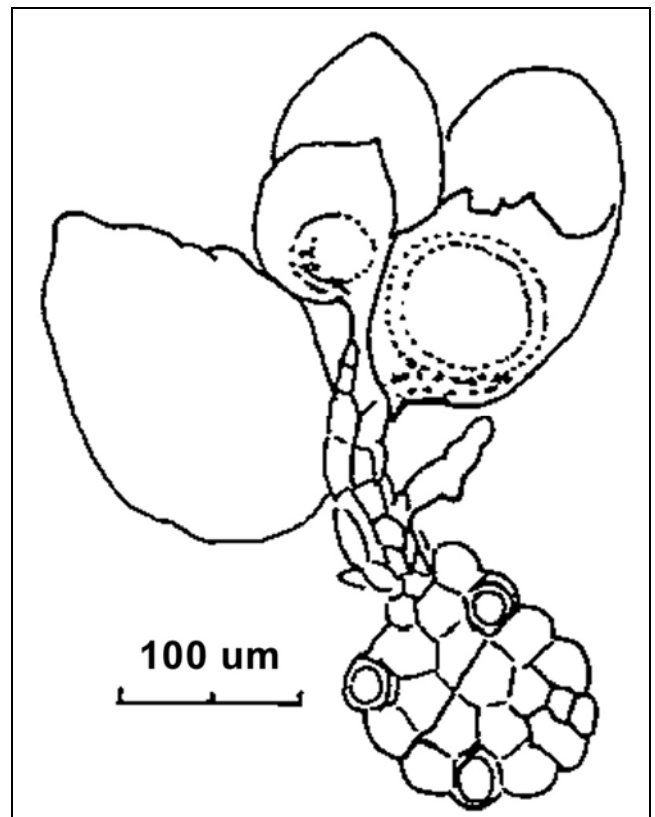

Figure 30. *Cololejeunea borhidiana* dwarf male developing from a gemma. Drawing by Tamás Pócs, with permission.
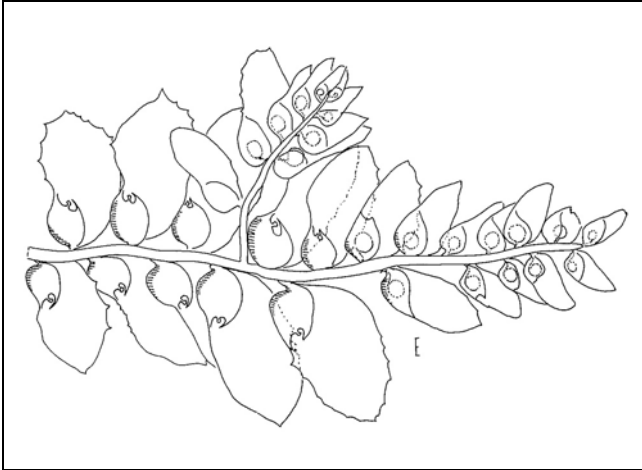
Figure 31. *Cololejeunea borhidiana* normal male developing from a gemma. Drawing by Tamás Pócs, with permission.

How Do Facultative Males Develop?

Dicranum has a well-established record of dwarf males. Based on a literature review, Pichonet and Gradstein (2012) estimate that such dwarf males occur in about 20% of the *Dicranum* species, with most species being obligately nannandrous. However, in at least two species, *D. bonjeanii* (Figure 32) and *D. scoparium* (Figure 38), both normal-sized and dwarfed males occur. In this genus, the environment seems important to control male plant size.

Figure 32. *Dicranum bonjeanii* showing the dense tomentum that provides a habitat for dwarf males. Photo from Frullania Data Portal, through Creative Commons.

One must ask how a spore can become a full-sized male on soil or other substrate, but when it lands on a female of its own species, it develops into a dwarf. This facultative behavior may support the suggestion of Loveland (1956) that the dwarfism on leaves of the same species was the result of some chemical interaction with the substrate leaf. For example, in *Trachybryum megaptilum* (Figure 33) normal-sized males never have dwarf males on them (Wallace 1970), suggesting that the female has some sort of chemical, most likely hormonal, control over expression of the dwarf male – or could it be that the male plant prohibits germination of the male spore.

Figure 33. *Trachybryum megaptilum*, a moss that may have several hundred dwarf males growing on the female. Photo by Martin Hutten, with permission.

Hormones – Hormonal suppression seems to account for the development of males in a number of taxa (Loveland 1956; Wallace 1969, 1970). In fact, some species prevent growth of males among females, but those spores fortunate enough to germinate away from a female become males (Crum 2001). This would seem to be maladaptive for purposes of fertilization but reduces competition for resources between the sexes.

In the moss genus *Dicranum* (Loveland 1956), *D. drummondii* (Figure 34), *D. sabuletorum* (Figure 35), *D. polysetum* (Figure 36-Figure 37), and *D. scoparium* (Figure 38) (Preston & Mishler 1997) and in other dimorphic bryophyte species, spores cultured on agar produce normal-sized males, suggesting hormonal control of plant size that is determined by the female. Briggs (1965) provides further evidence in this genus, with those species that have a variety of sizes of males only producing dwarf males in culture when they are grown near females.

Figure 34. *Dicranum drummondii* from Europe, a species that produces normal-sized males on agar, but produces dwarf males on female plants. Photo by Michael Lüth, with permission.
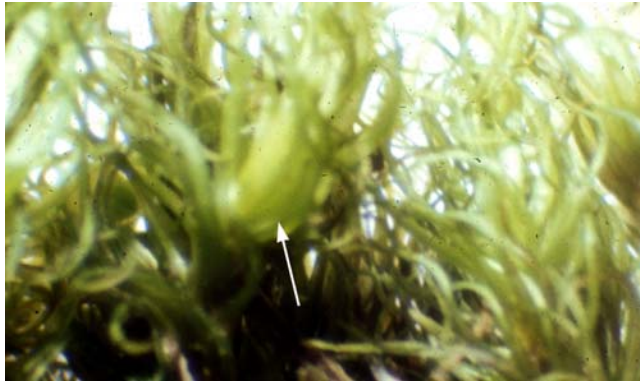
Figure 35. *Dicranum sabuletorum* dwarf male (**arrow**) growing on a female plant. Photo by Janice Glime.

Figure 36. *Dicranum polysetum*, showing tomentum where dwarf males often develop. Photo by Robert Klips, with permission.

Figure 37. *Dicranum polysetum* dwarf males on the tomentum of a female. Photo courtesy of Lars Hedenäs.

In *Leucobryum*, *L. glaucum* (Figure 39) and *L. juniperoideum* (Figure 40) males can be dwarf to full size (Blackstock 1987). Dwarf males form on the tomentum of *L. bowringii* and *L. juniperoideum* (Figure 40), but normal males also form on non-*Leucobryum* substrates (Une & Yamaguchi 2001).   Furthermore, Une and Yamaguchi

found that dwarf *Leucobryum* males removed from the female and grown on a different substrate grew into tall male plants. Suggesting physiological differences between the sexes, males of these *Leucobryum* species, particularly normal males, are restricted to lower altitudes and latitudes in Japan, but females are not. This is also the case in some *Macromitrium* species (Figure 41) (Ramsay 1979; Une 1985c).

Figure 38. *Dicranum scoparium* with dwarf male in Norway. Photo by Michael Lüth, with permission.

Figure 39. *Leucobryum glaucum* with tomentum (at arrow) and what appears to be a dwarf male. Photo by Aimon Niklasson, with permission.

Figure 40. *Leucobryum juniperoideum*, a moss that gets dwarf males on its tomentum. Photo by Jan-Peter Frahm, with permission.

Figure 41. *Macromitrium* from the Neotropics. Photo by Michael Lüth, with permission.

There seem to be a number of possible hypotheses to explain ways that hormones from the female could influence the sizes of males.

1. The spore must land and probably germinate before the female produces the "hormone" that determines the size, with the spore or germling serving as a stimulant. Hence, the "hormone" would act on the protonema. This would be like a response to a fungus or herbivory that stimulates production of a secondary compound in seed plants and similar to the response of the alga *Oedogonium* that produces its oogonium after the spore lands on the filament (Rawitscher-Kunkel & Machlis 1962).
2. The "hormone" from the female is highly volatile and thus only works when the spore/protonema/young plant is in direct contact with the plant that provides it. Ethylene could do this.
3. The "hormone" is rendered inactive by contact with soil (binding by soil). That, however, would not explain the epiphytic *Macromitrium*, assuming bark does not have the binding properties known for soils.
4. The level of "hormone" is too weak anywhere but on the female plant.
5. Similar to 4, but the "hormone" is water soluble and is soon washed away elsewhere, but is continually produced on the female.
6. Similar to 1; there is some sort of complementation between male and female plant – both must be present for the female to produce the "hormone."

Heinjo During (Bryonet 27 February 2009) suggested that the variation in sizes of males may in some cases relate to the **distance from females** (possibly related to hypotheses 2, 4, & 5). He has observed this size variation in *Leucobryum* (Figure 39-Figure 40). A possible explanation for this observation is that a hormone gradient exists, but it is also possible there is a male size gradient due to an environmental gradient away from the female colony in this cushion-former. The colony could create this gradient through such factors as moisture retention, nutrient usage, or *p*H alteration.

During (Bryonet 27 February 2009) reports that *Garovaglia* (Figure 29) seems to lack those intermediates, with males being either full size (similar to the size of females) on a non-leaf substrate, or dwarf when sitting on a female leaf. During suggests that a lack of intermediates, as in *Garovaglia*, indicates that dwarfing is genetically fixed and not dependent on effects of female neighbors. One possible explanation is that the large *Garovaglia* males are mutants in which the dwarfing is inactivated. We can also consider that if a certain level of hormones is required for dwarf males to develop in a species, a hormonal gradient away from the female could reach a threshold at a certain distance from the female, with those farther away and beyond the threshold becoming full-sized males.

Inhibitors – Absence of dwarf males on older parts of mosses suggests that emission of some inhibitor, perhaps the gaseous hormone **ethylene**, may suppress germination, thus being adaptive by avoiding the waste of energy and resources on a part of the plant too far from apical female reproductive organs for fertilization success. Alternative explanations might be that the stimulant hormone has been leached out of older parts and is not being replaced, or that growth conditions, especially with respect to light, are unfavorable.

Nutrient Considerations – Rod Seppelt (Bryonet 1 March 2009) suggested another possibility – a nutritional limitation. He suggested that when the spore germinates on a moss leaf, it could be at first rain after a dry period. At this time, the moss would leak nutrients due to membrane damage during desiccation. This would provide the nutrients needed for the male plant to start growing, but once the membranes were repaired in the substrate leaf, the nutritional source would be gone, hence limiting the further growth of the male, causing it to be a dwarf. Those spores on soil would obtain nutrients from the soil and the male gametophyte plant could grow to a full size. I have observed this in flowering plants. In one of my early attempts at gardening I grew poppies in very poor soil. Instead of growing to 60 cm tall, they were only 3-4 cm tall, but nevertheless produced miniature flowers. However, Hedenäs and Bisang (2012) could find no support for this nutrient limitation hypothesis in the pleurocarpous mosses they examined. Rather, they observed that dwarf males are most common shortly after spore release, the dwarfs being dead and more difficult to detect during the period before spore maturation.

Genetically Obligatory Dwarfs – In Japanese *Macromitrium* (Figure 41), eight species are dimorphic, producing dwarf males (Une 1985a; 2009). In these **anisosporous** (**anisospory** – having 2 spore sizes in the same tetrad following meiosis, see also the section on Anisospory below) species the dwarf males are genetically determined, whereas in **isosporous** (one spore size) species the dwarfness is apparently regulated by hormones from the female plants, with the potential to develop into normal plants in absence of the hormones. In his experiments, Une found that the hormone **2,4-d** (an auxin – growth hormone) caused dwarf males to develop in the **isosporous** species, supporting the hypothesis that hormones produced by the substrate leaf are the factor determining the development into a dwarf male.

Andréa Pereira Luizi-Ponzo (Bryonet 2 March 2009) and her students examined dwarf male biology in *Orthotrichum* (Figure 42-Figure 45). They found that in all species that have dwarf males, there are two spore sizes (**anisospory**). In those that exhibit full-sized males, the spores are **isomorphic** (all the same in form and size; Figure 45). So far they have found no species with both dwarf males and full-size males that also exhibit anisospory.

Hedenäs and Bisang (2011) present evidence that the presence of male dwarfism is related to family membership, and that it does not correlate with geographic area. Such examples of dwarf male relatedness occur in the currently configured family **Miyabeaceae**: *Homaliadelphus*, *Miyabea*, *Bissetia* (Olsson *et al.* 2009). Olsson *et al.* have placed these three genera in the same family, **Miyabeaceae**, based on a molecular phylogenetic analysis. *Homaliadelphus* (formerly in **Neckeraceae**; Figure 46) produces normal-sized males or facultatively produces dwarf males, whereas *Miyabea* (formerly in **Thuidiaceae**) and *Bissetia* (formerly in **Neckeraceae**; Figure 47) produce obligatory dwarf males. *Homaliadelphus* has all the spores the same size, but those of the obligatory dwarf male genera *Miyabea* and *Bissetia* are of two distinct sizes.

Figure 44. *Orthotrichum alpestre*, an isosporous species. Photo by Michael Lüth, with permission.
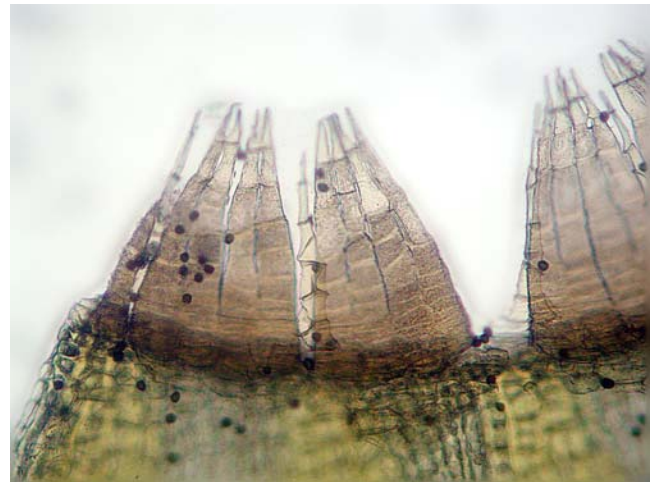
Figure 45. Peristome and spores of *Orthotrichum alpestre*, an isosporous species. Photo by Michael Lüth, with permission.

Figure 42. *Orthotrichum lyellii* habit. This species exhibits **anisospory** and **dwarf males**. Photo by Malcolm Storey at Discover Life, through Creative Commons.

Figure 43. *Orthotrichum lyellii* with gemmae (brown structures on leaf margins). Photo by Michael Lüth, with permission.

Figure 46. *Homaliadelphus sharpii*. Photo by Paul Redfearn, Ozarks Regional Herbarium, with permission.

Figure 47. *Bissetia ligulata*, a species of obligatory dwarf males. Photo by Digital Museum Hiroshima University, with permission.

The Dwarf Male Advantage

So, we repeat the question here, what is the advantage to having a larger female? Lewin (1988) suggests that bigger females may produce more fit offspring; smaller males may have increased mobility (an animal bias but could be applied to small spores); females may survive longer after reaching sexual maturity and continue growing. Do these explanations apply to bryophytes?

For those species with small male spores and large female spores, the greater dispersal distance that correlates with small spore size could be an advantage, especially in species where asexual diaspores are produced by the males. This could eventually increase reproductive success by providing males with greater possibilities to reach females.

Among *Dicranum majus* (Figure 48) female plants with dwarf males, there was an 84% success rate in fertilization compared to 75% when including those identifiable females without dwarf males (Sagmo Solli *et al.* 1998). In the northern part of Lower Michigan, monoicous species of mosses achieve the same rate (75%; Rohrer 1982). Also in *Homalothecium lutescens* (Figure 20), fertilization rate was positively associated with dwarf male density (Rosengren *et al.* 2014, see above). The dwarf male mechanism seems to ensure fertilization success while wasting little on production of male plant tissue, thus avoiding competition with female plants for resources. Nevertheless, it appears that in some cases males must be reborn each year, as Sagmo Solli *et al.* (1998) were unable to find any males on female *Dicranum majus* parts more than one year old.

Figure 48. *Dicranum majus*, a species with dwarf males from Bretagne. Photo by Michael Lüth, with permission.

So it appears that one strategy of energy conservation and assurance of having males nearby females that works for a number of genera is to dwarf any male that develops on a female plant (Wallace 1970) (see also below, evolutionary drivers). Wallace found that in *Trachybryum* (=*Homalothecium*) *megaptilum* (Figure 33) only one plant in 200 is a normal-sized male, whereas a single female may have several hundred dwarf male plants growing on her.

Some bryophytes make certain that sperm dispersal distance is absolutely minimal. *Leucobryum martianum* (Figure 49) produces rhizoidal heads (Salazar Allen 1989). Yamaguchi (1993) later reported that the characteristic rhizoid formation in *Leucobryum* occurs at the lower abaxial side of the inner perichaetial leaves. Young plants develop on this rhizoidal tomentum and this was originally considered a means of asexual reproduction. Further examination revealed that these young plants were actually dwarf males developed from spores, located conveniently close to the archegonia.

Figure 49. *Leucobryum martianum*, a species with rhizoidal tufts on the inner perichaetial leaves where dwarf males grow. Photo by Jan-Peter Frahm, with permission.

Hedenäs and Bisang (2011) suggest that **evolutionary drivers** toward dwarf males in bryophytes may have included (1) competing selective pressures on cytoplasmic and nuclear genomes, (2) selection for reduced mate competition, in particular when resources are limited, and (3) selection for reduced fertilization distances. In many cases it is likely that combinations of these drivers existed. Furthermore, the associated niche shift of the males may provide them with a habitat that is both humid and nutrient-rich (but see above - nutrient considerations under How Do Facultative Males Develop? by Seppelt).

Species Interactions

If females can inhibit the development of males of their own species through nutrition or hormonal control, can they likewise do this to other species?

Mishler and Newton (1988; Newton & Mishler 1994) experimented with interaction effects of moss leaves and leaf extracts on spore germination. They determined the effect of *Dicranum scoparium* (Figure 38) and four species of *Syntrichia* (previously in *Tortula*) on *Syntrichia* spore germination. Spores planted on agar or sand had normal germination and growth, but spores (either sex) of *S. ruralis* (Figure 50) and *S. laevipila* (Figure 51) that were planted on *Dicranum scoparium* or *Syntrichia* leaves either didn't germinate or germinated very slowly. *Syntrichia princeps* (Figure 52) germination was inhibited by extracts from leaves of its own species. Even a water extract of *D. scoparium* caused a significantly slower spore germination or resulted in significantly smaller plants than those grown with no extracts. At least in this case, it appears that when the inhibition of other species exists, it is to a degree that sexual maturity is not reached. What is puzzling is that in three of the species germination was inhibited by leaf extracts of their own species.

In contrast, spores of *Homalothecium lutescens* (Figure 22) and *H. sericeum* (Figure 23) both germinated on shoots of the former, but spores of the more distantly related *Isothecium alopecurioides* (Figure 16-Figure 17) did not (Rosengren & Cronberg 2015; see above). This suggests that the regulation of spore germination on host shoots is associated with the degree of relatedness between species. In contrast, spores of *Homalothecium lutescens* and *H. sericeum* both germinated on shoots of the former, but spores of the more distantly related *Isothecium alopecurioides* did not (Rosengren & Cronberg 2015; see above). This suggests that the regulation of spore germination on host shoots is associated with the degree of relatedness between species.

Figure 50. *Syntrichia ruralis* in Europe. Spore germination in this species is inhibited by extracts of both other members of its own genus and of *Dicranum scoparium*. Photo by Michael Lüth, with permission.

Figure 51. *Syntrichia laevipila* with capsules in Europe. Spore germination in this species is inhibited by extracts of both other members of its own genus and of *Dicranum scoparium*. Photo by Michael Lüth, with permission.

Figure 52. *Syntrichia princeps*, a species for which spore germination is inhibited by both *S. princeps* and *Dicranum scoparium*. Photo by Jan-Peter Frahm, with permission.

Spore Differences

Spore differences can account for male-female differences. Mogensen (1981) elaborated on the types of spores in bryophytes; note that these definitions refer to the species, not to individuals, and are based on spore size frequencies (SSF) and mean spore size frequencies (MSSF) across populations:

isospory – one SSF and MSSF; spore mortality none or only a few percent
ex.: *Fissidens limbatus* (Figure 53), *Funaria hygrometrica* (Figure 54-Figure 55), *Mnium hornum* (Figure 56); probably the most common type in bryophytes

[**heterospory** – large female and small male spores present [microspores produced in microsporangia and mega(macro)spores produced in megasporangia] – bryophytes have only one type of sporangium
ex.: not known in bryophytes]

pleurispory – 2 or more SSF grouped around 1-2 MSSF
ex.: *Ditrichum difficile*

anisospory – SSF and MSSF grouped around 2 mean sizes in 1:1 ratio; probably in 2-3% of mosses
ex.: some *Orthotrichum* (Figure 42-Figure 43) & *Macromitrium* spp (Figure 41)

pseudoanisospory (= false anisospory) – SSF & MSSF grouped around 2 mean sizes, usually in 1:1 ratio; small spore fraction is aborted
ex.: *Cinclidium* spp. (Figure 64), *Ceratodon purpureus* (Figure 69), *Rhizomnium magnifolium* (Figure 66), *Fissidens* spp. (Figure 67), *Macromitrium* spp. (Figure 41)

amphispory – SSF & MSSF grouped around 2 mean sizes in varying ratios; small spore fraction is aborted
ex.: *Pleurozium schreberi* (Figure 57)

combispory – SSF & MSSF grouped around 3 or more mean sizes; may have aborted spores but also living spores in at least 2 sizes
ex.: *Macromitrium* spp. (Figure 41)

Figure 53. *Fissidens limbatus* from Europe. Photo by Michael Lüth, with permission.

Figure 54. *Funaria hygrometrica* with capsules. Photo by Li Zhang, with permission.
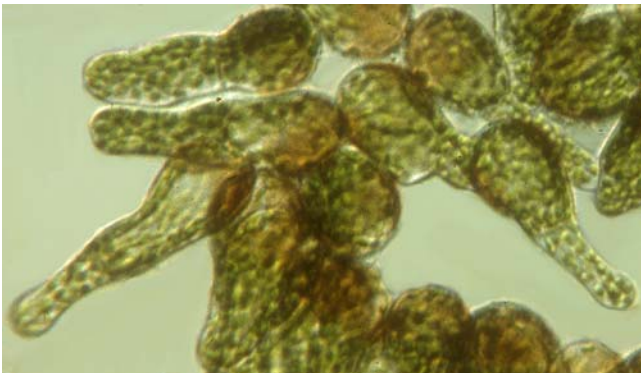

Figure 55. *Funaria hygrometrica* spore germination from isosporous spores. Photo by Yenhung Li, with permission.

Figure 56. *Mnium hornum*. Photo by Andrew Spink, with permission.

Figure 57. *Pleurozium schreberi* growing on sand. Photo by Janice Glime.

Anisospory

The "big female" concept has been based on animals, but like so many other evolutionary concepts, the broader concept is applicable throughout living organisms. Haig and Westoby (1988) have applied this concept to the origin of heterospory in plants. But bryophytes are not quite there yet. Instead, they have evolved (in relatively few species) only to **anisospory** with some related variants.

Spores in bryophytes are always **homosporous** and generally **isosporous** (all the same size). **Heterospory** can be defined as bearing spores of distinctly different types; it is the condition when **microspores** are produced in microsporangia and **mega(macro)spores** in megasporangia. Micro- and megaspores differ in size and sex. Heterospory has evolved independently several times in vascular plants, but does not occur in bryophytes. Early diverging ferns are homosporous; several families of aquatic ferns are heterosporous. All bryophytes are homosporous in this sense, all seed plants are heterosporous, and in ferns both conditions exist.

Anisospory in bryophytes refers to a bimodal size difference between spores produced in the same sporangium (Magill 1990). In this case, meiosis results in a tetrad of two small spores that generally produce male gametophytes and two larger spores that produce female gametophytes (Ramsay 1979; Magill 1990).

Anisospory has been reported in a variety of mosses, not just in connection with male dwarfism as described above, with several explanations for their occurrence. But the usage of the term may not always be precise. Pant and Singh (1989) reported several possible cases of anisospory in liverworts: *Targionia indica*, *Targionia hypophylla* (Figure 58), *Cyathodium aureonitens*, and *Cyathodium barodae*. They based this conclusion on the wide variations in size of spores, similar to those in the moss family **Orthotrichaceae**. They did not determine sex or viability, hence we cannot eliminate the possibility of **false anisospory**. Multiple spore sizes can occur in bryophytes as a result of unequal growth of the spores, or in some cases abortion of spores (Ramsay 1979). These cases do not have any known relationship to sex.

Figure 58. *Targionia hypophylla* with marsupium (black), a structure that houses the archegonia and sporophyte. Photo by Des Callaghan, with permission.

Support for the anisospory concept comes from some species with dwarf males (see paragraphs above). In several dioicous taxa [*Lorentziella*, some *Macromitrium* (Figure 41), including the former *Schlotheimia* (Figure 59)], small, yellow spores produce males and larger, green spores produce females (Ernst-Schwarzenbach 1938, 1939, 1944). But this differentiation in spore size seems to be rare among the bryophytes.

Alfayate *et al*. (2013) have recently provided irrefutable evidence of anisospory in two more genera – irrefutable because both sizes of spores germinated. In *Leucodon canariensis* (Figure 60) viable spores were of two classes - uni- or multicellular, medium-sized (26-48 µm) spores and multicellular, large (50-94 µm) spores. In *Cryptoleptodon longisetus*, viable spores are likewise of two kinds in the same capsule, unicellular, small spores (11-24 µm) and medium-sized (26-35 µm) spores. Furthermore, in both species, germination was present within the capsules. Somewhat similar anisospory occurs

in *Brachythecium velutinum*, with both sizes germinating (Herguido & Ron 1990).

Figure 59. *Macromitrium trichomitrium* (=*Schlotheimia trichomitria*) with capsules. This dioicous genus has small and large spores and produces dwarf males. Photo by George J. Shepherd, through Creative Commons.

Figure 60. *Leucodon canariense* in habitat. Photo by Jonathan Sleath, with permission.

False Anisospory – Spore Abortion

Mogensen (1978a) described **false anisospory** (appearing to have two sizes, one chlorophyllous and one not), later (1981) referring to it as **pseudoanisospory**; false anisospory seems to be the terminology most used. Mogensen does not include any sex relationship for this condition.

In several species that exhibit **dimorphic** (having two forms) spores, one can find on closer examination that the small ones are dead (thus not implying a difference in sex) and satisfying the condition Mogensen (1978a) termed false anisospory. (Dimorphic does not imply that the size difference is genetically based.) He first reported aborted

spores in *Cinclidium* (Figure 61-Figure 64) (Mogensen 1978a) and later in *Macromitrium japonicum* (=*M. incurvum*; Figure 65), *Rhizomnium magnifolium* (Figure 66), and *Fissidens cristatus* (Figure 67) (Mogensen 1978b). In *Cinclidium arcticum* (Figure 61-Figure 62) and *C. stygium* (Figure 63) 50% of the spores abort, whereas in *C. subrotundum* (Figure 64) only 11% abort. It is also known in *Lorentziella imbricata* (Figure 68) (Crum 2001). The result is that large, green, live spores cohabit the capsule with small, brown, dead ones.

Figure 63. *Cinclidium stygium*, a species in which a ~50% of the spores abort as the spores mature. Photo by Michael Lüth, with permission.

Figure 61. *Cinclidium arcticum* with capsules. Photo by Michael Lüth, with permission.

Figure 64. *Cinclidium subrotundum*, a species in which only 11% of the spores abort. Photo by Michael Lüth, with permission.

Figure 62. *Cinclidium arcticum*, a species in which 50% of the spores abort as the spores mature. Photo by Michael Lüth, with permission.

Figure 65. *Macromitrium japonicum*. Photo from Digital Museum of Hiroshima University, with permission.
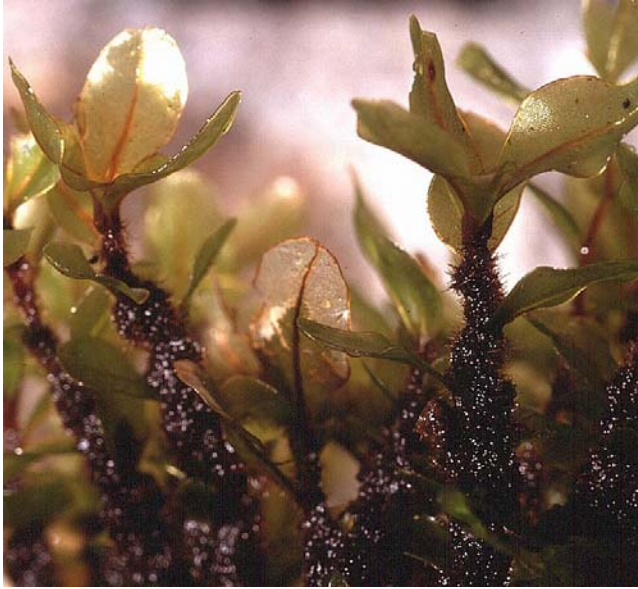
Figure 66. *Rhizomnium magnifolium* from Europe, a species with false anisospory. Photo by Michael Lüth, with permission.
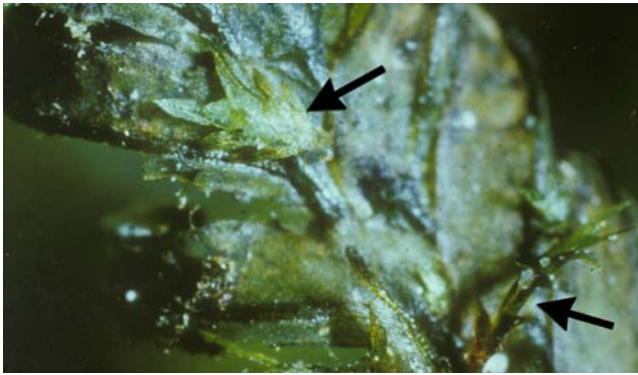

Figure 67. Dwarf males (arrows) on *Fissidens cristatus*. Photo by Janice Glime.

Figure 68. *Lorentziella imbricata*. Photo by Claudio Delgadillo Moya, with permission.

Mogensen (1978a, 1981) tracked the spore sizes of *Cinclidium arcticum* (Figure 61) as the capsule dried. He concluded that the columella serves as a reservoir of water (Mogensen 1978a). He demonstrated a range of spore sizes in a single capsule and that as the columella dries and shrinks, the smaller spores die first. A similar loss of smaller spores during maturation was present in *Ceratodon purpureus* (Figure 69) (Mogensen 1981). Premature drying can cause the operculum to be released before the spores reach their potential size, stopping their development (Mogensen 1981).

Figure 69. *Ceratodon purpureus* capsules. Photo by Hermann Schachner, through Creative Commons.

Glime and Knoop (1986) observed a similar phenomenon in the dioicous aquatic moss *Fontinalis squamosa* (Figure 70-Figure 75). Because its capsules are constantly wet in nature (Figure 70), it was possible to simulate their maturation conditions in the laboratory and examine the spores at various times during development (Figure 71). In that species, death did not occur to all spores simultaneously. At any point in time during development, large and small spores were present (Figure 72-Figure 75). However, small spores at later points in time were larger than small spores at earlier points in time. It was not clear whether the first degenerate spores disintegrated before larger ones appeared, or if different spores accomplished abortion at different developmental stages. Some already were abortive in their tetrads following meiosis (Figure 75). Glime and Knoop suggest that at least in *Fontinalis squamosa*, spore abortion is a gradual and continual process as the capsule matures, and that it is determined either randomly or by location of developing spores in the capsule, rather than by genetic predetermination. This species is not known to have dwarf males. The smaller spores had a much lower germination rate.

Figure 70. **Fontinalis squamosa** var. **curnowii** with capsules, showing their tough structure. Note the perichaetial leaves that cover about half the capsule. Photo by David T. Holyoak, with permission.

Figure 73. Normal and aborting spores of **Fontinalis squamosa** in white light (**left**) and the same spores fluorescing under ultraviolet light (**right**), showing red healthy spores and yellow or green dying spores. Note the lack of fluorescence in the small, deflated spores and the yellow edges of some that are beginning to abort. Smaller spores with no remaining chlorophyll are not visible in this image through fluorescence. Photo by Janice Glime.

Figure 71. Longitudinal section through capsule of **Fontinalis squamosa** showing the tightly packed spores. Photo by Janice Glime.
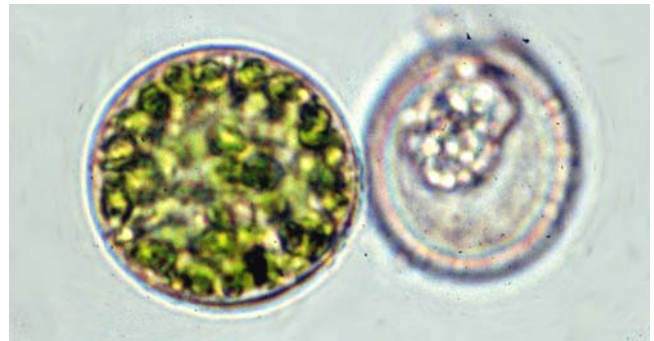
Figure 74. Normal (**left**) and smaller aborted (**right**) spores of **Fontinalis squamosa**. Photo by Janice Glime.

Figure 72. Spores of **Fontinalis squamosa** showing large, healthy green spores, smaller white dying or dead spores, and small brown spores that may be dead. These are not anisosporous because they are not of two sizes at the end of meiosis. Photo by Janice Glime.
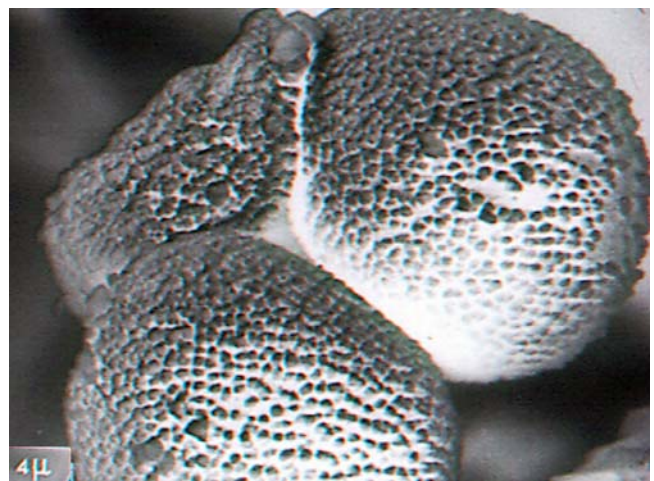
Figure 75. Tetrad of spores from **Fontinalis squamosa**. Note one abortive spore. Photo by Janice Glime.

Zander (1972) reported a similar situation for **Leptodontium viticulosoides** var. **viticulosoides** (Figure 76). In this case, the seeming anisospory was actually a large, chlorophyllous spore and a small, non-chlorophyllous spore, *i.e.* false anisospory. The small

spores were, as in most for *Fontinalis squamosa* (Figure 74), not viable. So I would add another possibility, although with absolutely no proof for *Fontinalis* or any other species. If the smaller spores in some species are indeed viable, they could produce a smaller gametophyte due to reduced starting nutrition. In this case, a leaf producing inhibitory substances would not be needed. However, such a function for small spores is not known for *Fontinalis* or any other bryophyte.

Figure 76. *Leptodontium viticulosoides*. Photo by Li Zhang, with permission.

Rhizomnium punctatum (Figure 77), a species closely related to *Rhizomnium magnifolium* (Figure 66), provides further support for the hypothesis that false anisospory can result from the progressive abortion of spores during the stages leading up to spore maturity. This species exhibits false anisospory during early capsule development but in the mature capsule the spores are isosporous (Mogensen 1978b). Mogensen further points out that there is no correlation of spore size with the monoicous or dioicous condition, at least in his small sample of taxa.

Figure 77. *Rhizomnium punctatum* with capsules in Europe, a species in which mature spores are isosporous. Photo by Michael Lüth, with permission.

But not all capsules have the progressive abortion we have been describing. In *Bryowijkia ambigua*, abortion occurs in the tetrad stage, with two spores aborting and two

presumably remaining viable (De Luna 1990). This brings to mind the image a spore tetrad from *Fontinalis squamosa* above (Figure 75) where one visible spore is likewise aborted in the tetrad stage. In the case of *F. squamosa*, spore abortion may begin as early as the tetrad and continue throughout development, or it might be that I have misinterpreted the continual abortion throughout development. Nevertheless, as seen in Figure 78, three spores can abort in one tetrad, suggesting that the number of abortions is not a programmed event in the tetrad stage.
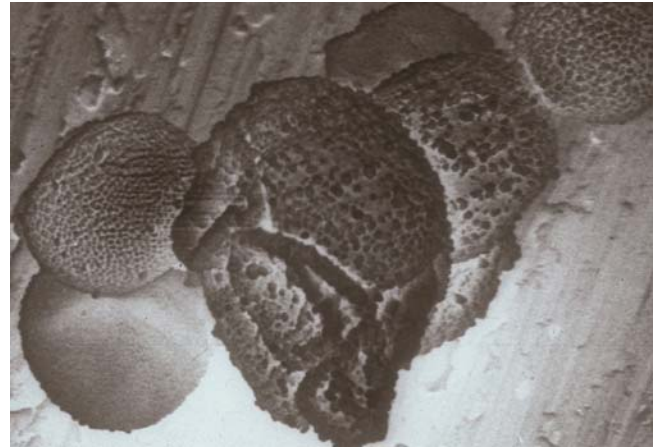
Figure 78. *Fontinalis squamosa* showing what to be three aborted spores in one tetrad. Photo by Janice Glime.

Figure 79. *Bryowijkia ambigua*, a species in which spore abortion occurs in the tetrad stage. Photo by Li Zhang, with permission.

It is likely that abortion of some spores is the rule among bryophytes, and it would be interesting to investigate how widespread the process is. It might be that in seasons of low water or nutrient availability the abortion is more common. This would be an interesting topic to explore for both its control and its adaptive value. The number of studies of changes in spore size during sporogenesis are insufficient to make accurate generalizations. Mogensen (1981) suggests that the abortion is a selection against certain genotypes, and he (1978a) interpreted this phenomenon to be a genetic factor that is lethal to a fraction of the spores prior to vegetative growth of the spore. Without further detailed study we cannot rule out random abortion between the sexes or

resource-related abortion, perhaps based on crowding, water availability, or nutrient availability.

Evolution of Spore Differences

In studying the evolution of heterospory in ferns, Haig and Westoby (1988) predicted that sporophytes would produce spores of a size that would maximize return in gametophyte fitness per unit investment. He postulated that the evolution of heterospory would occur in three steps:

1. a gradual increase of spore size in a homosporous population
2. the sudden introduction of smaller microspores
3. subsequent divergence in size and specialization of the two spore types.

This implies that larger spores would occur in those taxa that depend on stored reserves of the spore for successful reproduction. No surprises there. Their model predicts that because there are only minimal costs for male reproduction compared to that of female reproduction, larger food reserves would therefore evolve for female reproduction. Following this model, above some critical spore size, the population can be invaded by smaller spores that are predominately males (assuming that small spores travel farther?).

If one continues with this logic, it would then imply that the population would have few large females and more small males. A larger number of small males would increase chances of some of these males being near a female and strategically placed so that sperm can reach and fertilize the egg. Whenever male reproductive cells must travel by themselves to the female, many will be lost, literally unable to find the female, or perishing before the distance is accomplished. Hence, such a system will necessarily require many male gametes. In bryophytes, by having many small gametophytes, it would be possible for more gametophytes to occupy available small spaces near the female and offer more opportunities for successful fertilization.

The theory presented by Haig and Westoby (1988) would seem to make sense for the heterosporous ferns where the gametophyte is contained within the spore wall. And it makes sense for the seed plants where male gametophytes can travel reasonably long distances. But does this concept really work for evolution of anisosporous bryophytes where the sporophyte and sporangia have no sex distinction and the gametophyte is **exosporic** (develops outside the spore wall)? The number of male and female spores produced in the bryophyte case should be equal, dividing in a 1:1 ratio at meiosis, at least in the absence of sex ratio distorters. The model would only seem to be applied in bryophytes if size differentiation occurred after meiosis, during spore development. Then, it would require that being a small spore caused differentiation into a male while larger spores containing more stored nutrients became female. But unlike heterosporous ferns such as *Marsilea*, the bryophytes do not have gametophyte development and fertilization within the spore wall and the spore is not used to nourish the developing embryo. And to satisfy the Haig and Westoby model, the distinction in spore size would have to favor few large spores and many

small spores. This possibility cannot be ruled out, and there may be some support for it in **Fontinalis** (Figure 70-Figure 75), where a distinction between small and large spores occurs throughout spore development (Glime & Knoop 1986), but linkage of size, number, and sex has not been established.

Advantages of Anisospory and False Anisospory in Bryophytes

One must wonder if the progressive death of spores is a waste of energy, or a way of saving or even providing resources. Dead spores may serve a useful function by reducing the rate and extent of desiccation, and by reducing the drain of nutrients, until the remaining spores are larger and crowded, thus protecting each other. Finally, they could be a reservoir of nutrients readily available as they abort. It would be interesting to explore whether seasons of low water or nutrient availability increase the percent abortion.

Whereas the anisosporous condition seems to be favorable for dioicous taxa, the false anisosporous condition can occur in monoicous taxa (Mogensen 1981), but is not restricted to them. This leads us to consider the space-nutrient need as a possible selection factor for false anisospory.

New Methods

Our understanding of bryophyte sexuality should become increasingly easier with the development of molecular techniques. Pedersen *et al.* (2006) amplified DNA from nine mosses and one liverwort. This technique permitted them to obtain sufficient DNA from a single dwarf male of **Dicranum scoparium** (Figure 38). This will permit us to study genetic variation even in such small plants as dwarf males.

Summary

Males and females can differ in non-sexual ways, including size, biomass, branching, maturation rate, chlorophyll content, and photosynthetic rate and other physiological traits. Large female and small male plants (**dwarf males**) are known among bryophytes, but not the converse, except in non-sporophytic *Diphyscium*. Most dwarf males develop on the leaves or tomentum of females of the species. Dwarf males are often missed in surveys and this omission can cause misleading results in sex ratio determination. Spores of some species develop dwarf males on females of the species but normal males on other substrates. Dwarfism can increase the success of fertilization while decreasing the competition for resources with the females.

Bryophytes are **isosporous**, but some species exhibit **anisospory**; some exhibit **false anisospory** due to abortion of spores. The anisosporous condition seems to present a potential advantage for fertilization when it is correlated with the presence of dwarf males. On the other hand, this strategy reduces the dispersal of the larger female spores compared to that of the smaller

male spores. This is less of a problem if nearly all females get fertilized. Many **anisosporous** and **false anisosporous** conditions occur in species with no dwarf males (Mogensen 1981). This causes us to seek other explanations for their presence, including abortion related to water, space, and nutrient limitations within the capsule. The abortions can provide room for remaining developing spores while maintaining protection and resources for them.

Acknowledgments

Literature Cited

Alfayate, C., Ron, E., Estébanez, B., and Pérez-Batista, M. A. 2013. Mature spores of four pleurocarpous mosses in the Canary Islands: Ultrastructure and early germination stages. Bryologist 116: 97-112.

Bisang, I., Ehrlén, J., and Hedenäs, L. 2006. Reproductive effort and costs of reproduction do not explain female-biased sex ratios in the moss *Drepanocladus trifarius* (Amblystegiaceae). Amer. J. Bot. 93: 1313-1319.

Blackstock, T. H. 1987. The male gametophores of *Leucobryum glaucum* (Hedw.). Ångstr. and *L. juniperoideum* (Brid.) C. Muell. in two Welsh woodlands. J. Bryol. 14: 535-541.

Briggs, D. A. 1965. Experimental taxonomy of some British species of genus *Dicraum*. New Phytol. 64: 366-386.

Bruch, P., Schimper, W. P., and Gümbel, T. 1851-1855. Bryologia Europaea seu genera muscorum Europaeorum monographice illustrata. Vol. 5. Sumptibus Librariae E. Schweizerbart. Stuttgartiae.

Crum, H. 1976. Mosses of the Great Lakes Forest, revised edition. Univ. Mich. 10: 1-404.

Crum, H. 2001. Structural Diversity of Bryophytes. University of Michigan Herbarium, Ann Arbor, 379 pp.

Ernst-Schwarzenbach, M. 1938. Dimorphismus der Sporen und Zwergmännchen-Problem in der Laubmoos-Gattung *Macromitrium*. Ann. Bryol. 11: 46-55.

Ernst-Schwarzenbach, M. 1939. Zur Kenntnis des sexuellen Dimorphismus der Laubmoose. Arch. Julius Klaus-Stiftung Verebungsf. 14: 361-474.

Ernst-Schwarzenbach, M. 1944. La sexualité et le dimorphìsme des spores des Mousses. Rev. Bryol. Lichénol. N.S. 14: 105-113.

Fleischer, M. 1900-23. Die Musci der Flora von Buitenzorg, zugleich Laubmoosflora von Java mit Berücksichtigung aller Familien und Gattungen der gesamten Laubmooswelt. 4 Vols. Brill, Leiden.

Fleischer, M. 1920. Über die Entwicklung der Zwergmännchen aus sexuell differenzierten Sporen bei den Laubmoosen. Ber. Deutsch. Bot. Ges. 38: 84-92.

Fuselier, L. and McLetchie, N. 2004. Microhabitat and sex distribution in *Marchantia inflexa*, a dioicous liverwort. Bryologist 107: 345-356.

Fuselier, L. and Stark, L. 2004. Sexual dimorphism in bryophytes: Patterns and consequences. Abstracts, Scientific Meeting, American Bryological and Lichenological Society, 31 July – 5 Aug. 2004, Snowbird, Utah.

Glime, J. M., and Knoop, B. C. 1986. Spore germination and protonemal development of *Fontinalis squamosa*. J. Hattori Bot. Lab. 61: 487-497.

Goffinet, B. 1993. Taxonomic and floristic notes on neotropical Macromitrioideae (Orthotrichaceae). Trop. Bryol. 7: 149-154.

Groen, K. E., Stieha, C. R., Crowley, P. H., and McLetchie, D. N. 2010. Sex-specific plant responses to two light levels in the liverwort *Marchantia inflexa* (Marchantiaceae). Bryologist 113: 81-89.

Haig, D. and Westoby, M. 1988. A model for the origin of heterospory. J. Theor. Biol. 134(2): 257-272.

Hedenäs, L. and Bisang, I. 2004. Key to European *Dicranum* species. Herzogia 17: 179-197.

Hedenäs, L. and Bisang, I. 2010. The overlooked dwarf males in mosses – Unique among green land plants. Perspect. Plant Ecol. Evol. Syst. 13: 121-135.

Hedenäs, L. and Bisang, I. 2011. The overlooked dwarf males in mosses – Unique among green land plants. Perspect. Plant Ecol. Evol. Syst. 13: 121-135.

Hedenäs, L. and Bisang, I. 2012. Sex expression and sex ratios in dwarf male-producing pleurocarpous mosses – have we missed something? Plant Ecol. Div. 5: 387-393.

Herguido, P. and Ron, M. E. 1990. Contribución al estudio de la espora de *Brachythecium velutinum* (Hedw.) B., S. & G. Anales Jará. Bot. Madrid 46: 413-420.

Horsley, K., Stark, L. R., and McLetchie, D. N. 2011. Does the silver moss *Bryum argenteum* exhibit sex-specific patterns in vegetative growth rate, asexual fitness or prezygotic reproductive investment? Ann. Bot. 107: 897-907.

Lewin, R. 1988. Why is the world full of large females? Science 240: 884.

Limpricht, K. G. 1895-1904. Die Laubmoose Deutschlands, Oesterreichs und der Schweiz. III. Abtheilung. Verlag von Eduard Kummer, Leipzig, Germany.

Loveland, H. F. 1956. Sexual Dimorphism in the Moss Genus *Dicranum* Hedw. Ph.D. Dissertation. University of Michigan, Ann Arbor.

Luna, E. De. 1990. Multicellular spores and false anisospory in *Bryowijkia ambigua* (Musci: Trachypodaceae). Lindbergia 16: 73-79.

Magill, R. E. (ed.). 1990. Glossarium Polyglottum Bryologiae. A Multilingual Glossary for Bryology. Missouri Botanical Garden, St. Louis, MO, 297 pp.

Maier, I. and Müller, D. G. 1986. Sexual pheromones in algae. Biol. Bull. 170: 145-175.

Milne, P. 2000. Gemmae in *Dicranoloma serratum* (Broth.) Paris. J. Bryol. 22: 70-72.

Mishler, B. D. and Newton, A. E. 1988. Influences of mature plants and desiccation on germination of spores and gametophytic fragments of *Tortula*. J. Bryol. 15: 327-342.

Mogensen, G. S. 1978a. Spore development and germination in *Cinclidium* (Mniaceae, Bryophyta), with special reference to spore mortality and false anisospory. Can. J. Bot. 56: 1032-1060.

Mogensen, G. S. 1978b. False anisospory in *Macromitrium incurvum*, *Rhizomnium magnifolium* and *Fissidens cristatus* (Bryophyta). Lindbergia 4: 191-195.

Mogensen, G. S. 1981. The biological significance of morphological characters in bryophytes: The spore. Bryologist 84: 182-207.

Moore, J. D., Kollar, L. M., and McLetchie, D. N. 2016. Does selection for gamete dispersal and capture lead to a sex difference in clump water-holding capacity? Amer. J. Bot. 103: 1449-1457.

Newton, A. E. and Mishler, B. D. 1994. The evolutionary significance of asexual reproduction in mosses. J. Hattori Bot. Lab. 76: 127-145.

Norrell, T. E., Jones, K. S., Payton, A. C., and McDaniel, S. F. 2014. Meiotic sex ratio variation in natural populations of Ceratodon purpureus (Ditrichaceae). Amer. J. Bot. 101: 1572-1576.

Olsson, S., Buchbender, V., Enroth, J., Hedenäs, L., Huttunen, S., and Quandt, D. 2009. Phylogenetic analyses reveal high levels of polyphyly among pleurocarpous lineages as well as novel clades. Bryologist 112: 447-466.

Pant, D. D. and Singh, R. 1989. On the possible occurrence of anisospory in some Hepaticae. Bot. J. Linn. Soc. 100: 183-196.

Parihar, N. S. 1970. An Introduction to Embryophyta. Volume I. Bryophyta, 4th edn. Central Book Depot, Allahabad.

Pedersen, N. and Newton, A. E. 2007. Phylogenetic and morphological studies within the Ptychomniales, with emphasis on the evolution of dwarf males. In: Newton, A. E. and Tangney, R. (eds.). Pleurocarpous Mosses: Systematics and Evolution. Systematics Association Special Volume Series 71: 367-392.

Pedersen, N., Russell, S. J., A.E. Newton, A. E., and Ansell, S. W. 2006. A novel molecular protocol for the rapid extraction of DNA from bryophytes and the utility of direct amplification of DNA from a single dwarf male. Bryologist 109: 257-264.

Pichonet, A. and Gradstein, S. R. 2012. Male dwarfism in the genus Dicranum (Dicranaceae) – a review. Cryptog. Bryol. 33: 299-311.

Pócs, T. 1980. New or little known epiphyllous liverworts, II. Three new Cololejeunea from East Africa. J. Hattori Bot. Lab. 48: 305-320.

Preston, K. A. and Mishler, B. D. 1997. Ecological and developmental studies on the dwarf male breeding system of the moss Dicranum scoparium in North Carolina Piedmont. Amer. J. Bot. (Suppl.) 84(6): 20.

Ramsay, H. 1979. Anisospory and sexual dimorphism in the Musci. In: Clarke, G. C. S. and Duckett, J. G. (eds.). Bryophyte Systematics. Academic Press, London, UK, pp. 281-316.

Rawitscher-Kunkel, E. and Machlis, L. 1962. The hormonal integration of sexual reproduction in Oedogonium. Amer. J. Bot. 49: 177-183.

Rohrer, J. R. 1982. Sporophyte production and sexuality of mosses in two northern Michigan habitats. Bryologist 85: 394-400.

Rosengren, F. and Cronberg, N. 2014. The adaptive background of nannandry: Dwarf male distribution and fertilization in the moss Homalothecium lutescens. Biol. J. Linn. Soc. 113: 74-84. <http://dx.doi.org/10.1111/bij.12332>.

Rosengren, F. and Cronberg, N. 2015. Selective spore germination on shoots of Homalothecium lutescens, a moss with dwarf males. Biol. Lett. 11: <http://dx.doi.org/10.1098/rsbl.2015.0427>.

Rosengren, F., Cronberg, N., Reitalu, T., and Prentice, H. C. 2013. Genetic variation in the moss Homalothecium lutescens in relation to habitat age and structure. Botany 91: 431-441. <http://dx.doi.org/10.1139/cjb-2012-0258>.

Rosengren, F., Cronberg, N., Reitalu, T., and Prentice, H. C. 2014. Sexual reproduction in the phyllodioicous bryophyte Homalothecium lutescens (Hedw.) H. Rob. in relation to habitat age, growth conditions and genetic variation. J. Bryol. 36: 200-208. <http://dx.doi.org/doi:10.1179/1743282014Y.0000000114>.

Rosengren, F., Hansson, B., and Cronberg, N. 2015. Population structure and genetic diversity in the nannandrous moss Homalothecium lutescens: Does the dwarf male system facilitate gene flow? BMC Evol. Biol. 15: 1-12. <http://dx.doi.org/10.1186/s12862-015-0545-4>.

Rosengren, F., Cronberg, N., and Hansson, B. 2016. Balance between inbreeding and outcrossing in a nannandrous species, the moss Homalothecium lutescens. Heredity 116: 107-113.

Sagmo Solli, I. M., Söderström, L., Bakken, S., Flatberg, K. I., and Pedersen, V. 1998. Reproductive phenology of Dicranum majus in central Norway. J. Bryol. 20: 311-321.

Salazar Allen, N. 1989. A preliminary report of rhizoidal heads in Leucobryum martianum. Bryologist 92: 493-494.

Schellenberg, G. 1920. Über die Verteilung der Geschlechtsorgane bei den Bryophyten. Beih. Bot. Centralb. 1919/20: 1-39.

Shaw, A. J. and Gaughan, J. F. 1993. Control of sex-ratios in haploid populations of the moss, Ceratodon purpureus. Amer. J. Bot. 80: 584-591.

Shaw, J. and Beer, S. C. 1999. Life history variation in gametophyte populations of the moss Ceratodon purpureus (Ditrichaceae). Amer. J. Bot. 86: 512-521.

Shine, R. 1988. The evolution of large body size in females: A critique of Darwin's fecundity advantage model. Amer. Nat. 131: 124-131.

Stark, L. R., Mishler, B. D., and McLetchie, D. N. 2000. The cost of realized sexual reproduction: Assessing patterns of reproductive allocation and sporophyte abortion in a desert moss. Amer. J. Bot. 87: 1599-1608.

Szövényi, P., Ricca, M., and Shaw, A. J. 2009. Multiple paternity and sporophytic inbreeding depression in a dioicous moss species. Heredity 103: 394-403.

Une, K. 1985a. Sexual dimorphism in the Japanese species of Macromitrium Brid. (Musci: Orthotrichaceae). J. Hattori Bot. Lab. 59: 487-513.

Une, K. 1985b. Factors restricting the formation of normal male plants in the isosporous species of Macromitrium Brid. (Musci: Orthotrichaceae) in Japan. J. Hattori Bot. Lab. 59: 523-529.

Une, K. 1985c. Geographical distribution of male and female plants in species of Macromitrium Brid. (Musci: Orthotrichaceae) in Japan. J. Hattori Bot. Lab. 59: 515-521.

Une, K. 2009. Sexual dimorphism in the Japanese species of Macromitrium Brid. (Musci: Orthotrichaceae). J. Hattori Bot. Lab. 59: 487-513.

Une, K. and Yamaguchi, T. 2001. Male plants of the Japanese species of Leucobryum Hampe (Leucobryaceae, Musci). Hikobia 13: 579-590.

Vollrath, F. 1998. Dwarf males. Trends Ecol. Evol. 13: 159-163.

Wallace, M. H. 1969. Sexual dimorphism and developmental morphology of Camptothecium megaptilum Sull. Abstracts XI Internat. Bot. Congr. 232.

Wallace, M. H. 1970. Developmental Morphology and Sexual Dimorphism in Homalothecium megaptilum (Sull.) Robins. Ph. D. dissertation. Washington State University, Pullman.

Yamaguchi, T. 1993. A revision of the genus Leucobryum (Musci) in Asia. p. 15. J. Hattori Bot. Lab. 73: 1-123.

Zander, R. H. 1972. Revision of the genus *Leptodontium* (Musci) in the New World. Bryologist 75: 213-180.

Glime, J. M. and Bisang, I. 2017. Sexuality: Reproductive Barriers and Tradeoffs. Chapt. 3-4. In: Glime, J. M. Bryophyte Ecology.   3-4-1
Volume 1. Physiological Ecology. Ebook sponsored by Michigan Technological University and the International Association of
Bryologists. Last updated 6 March 2017 and available at <http://digitalcommons.mtu.edu/bryophyte-ecology/>.

CHAPTER 3-4
SEXUALITY: REPRODUCTIVE BARRIERS AND TRADEOFFS

JANICE M. GLIME AND IRENE BISANG

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CHAPTER 3-4
SEXUALITY: REPRODUCTIVE BARRIERS
AND TRADEOFFS

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 monoicous and gametophyte (haploid) 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 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). 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*. Photo from Proyecto Musgo, through Creative Commons.

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 Thekathyil, 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 Thekathyil, 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.
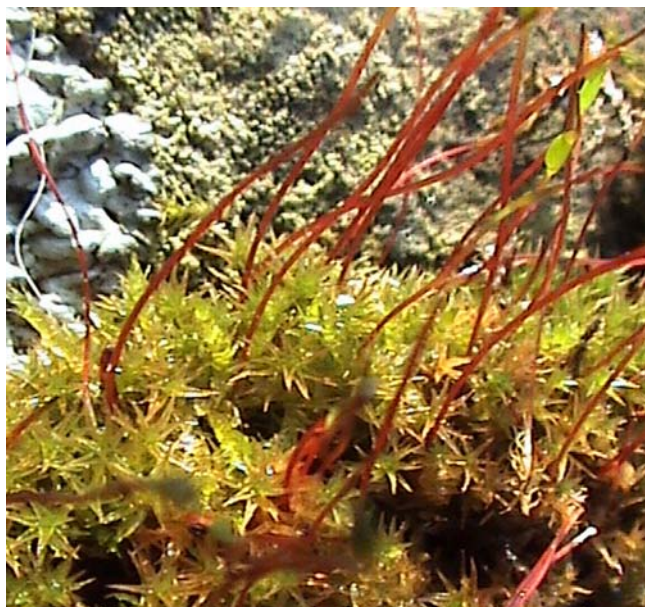

Figure 10. ***Funaria hygrometrica*** in southern Europe. Photo by Michael Lüth, with permission.

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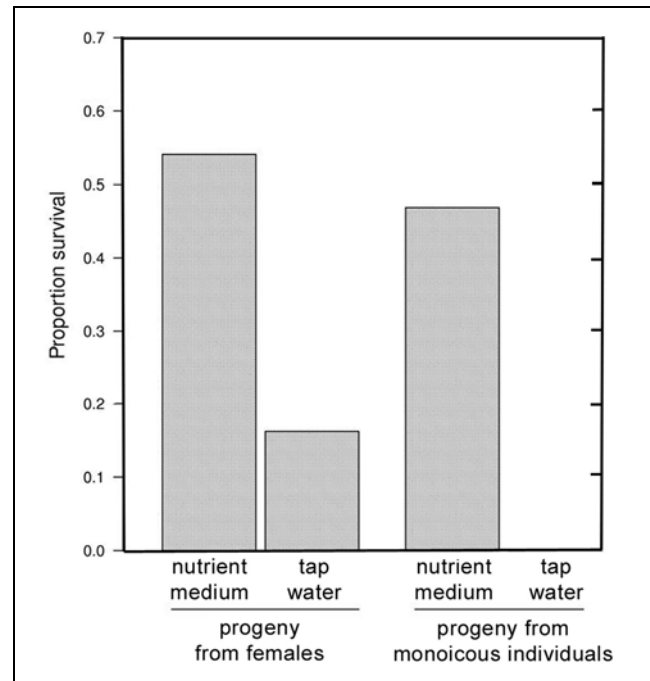
Figure 12. Proportion of culture plates with spores from females (*n*=39) compared to progeny of monoicous individuals (*n*=30) of **Atrichum undulatum** s.l. (Figure 2-Figure 3) germinating after 6 months on nutrient medium (Bold's basic media) vs tap water (stressful condition). Modified from Jesson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

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

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

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

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

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 dehisced. 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.
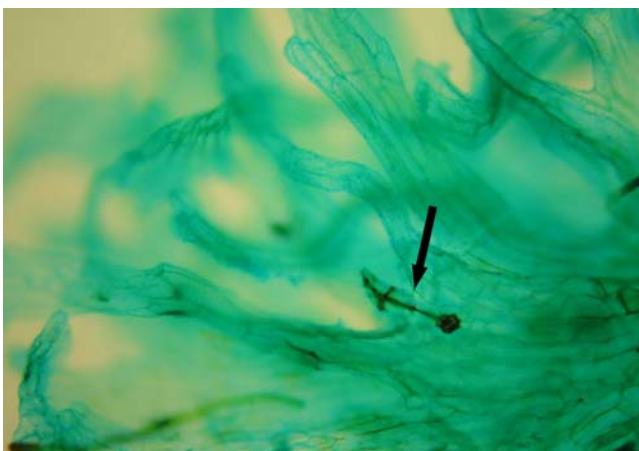

Figure 33. *Equisetum* prothallus with archegonium (**arrow**). Note the neck projecting from the gametophyte. Eggs of this species can be penetrated by sperm of other phyla, including the moss **Mnium hornum**. Photo by Ross Koning, with permission.

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.
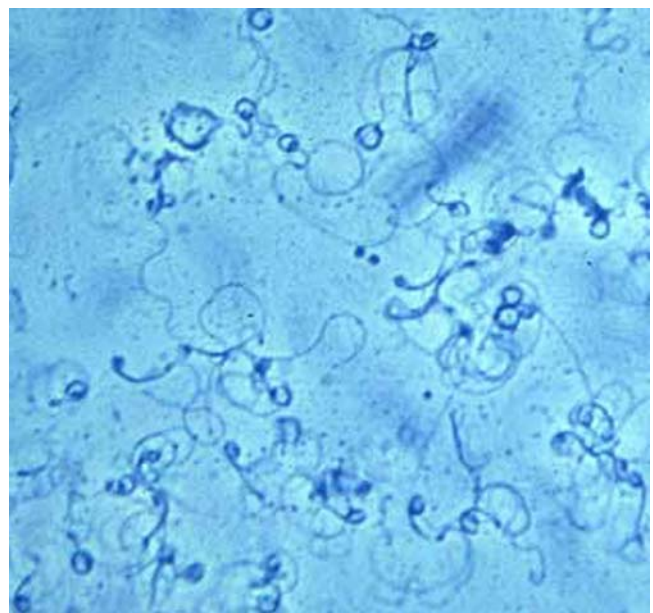
Figure 34. **Marchantia polymorpha** sperm swarming. Photo from Botany 321 website at the University of British Columbia, with permission.
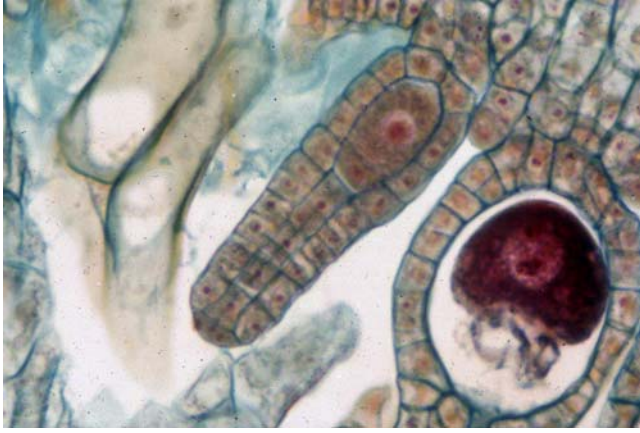
Figure 35. *Marchantia polymorpha* with immature archegonium with neck canal cells intact (**left**) and archegonium venter with large purplish egg and sperm attached, penetrating the egg. When the neck canal cells break down, they exude a mucilage that attracts the sperm. Photo by Janice Glime.

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

Figure 36. *Radula complanata* with dehisced 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.

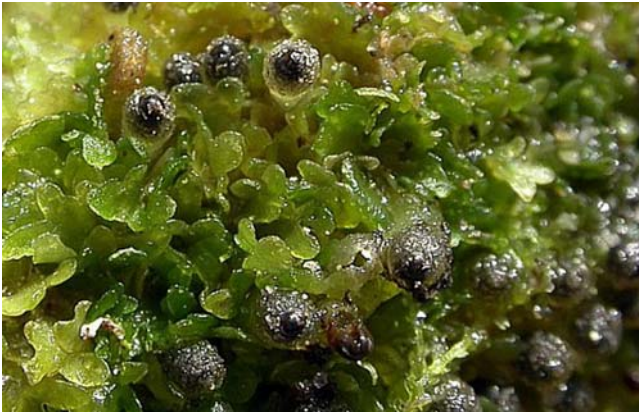More recent compendia ignore the topic completely (Chopra & Bhatla 1990; Crum 2001; Vanderpoorten & Goffinet 2009). We find it hard to believe that the archegonia of bryophytes lack such attractants. But are they able to attract only specific sperm? Perhaps it is the clonal nature of bryophytes that 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 Såstad (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 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 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 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.

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.

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).
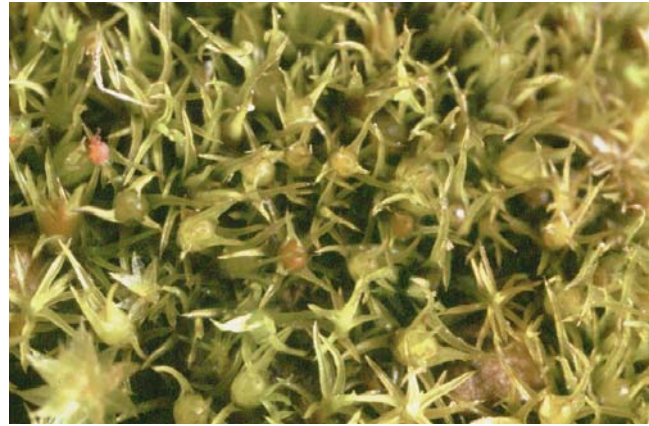
But if one tracks chromosome numbers in bryophytes, it becomes clear that some of these hybrids have succeeded in making new species (see 3.1, Genome Doubling in Mosses). Hence, from the basic chromosome number of 10 in bryophytes, we find that *Weissia* (=*Astomum*) *muhlenbergianum* (Figure 51) has 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 autoploidy, 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, measureable 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*. 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.

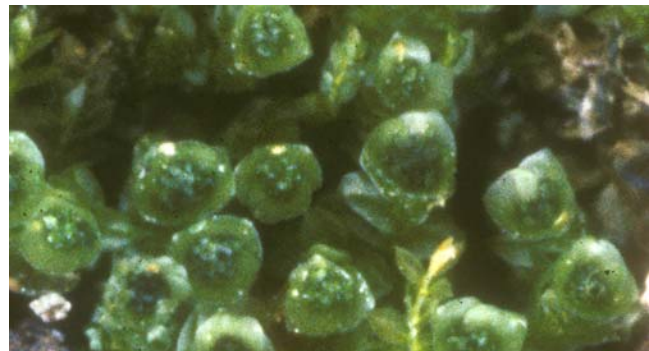Figure 63. Top view of ***Tetraphis pellucida*** showing terminal gemma cups that prevent simultaneous development of reproductive structures. Photo by Janice Glime.

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 pre-zygotic 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 Lloyd 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).
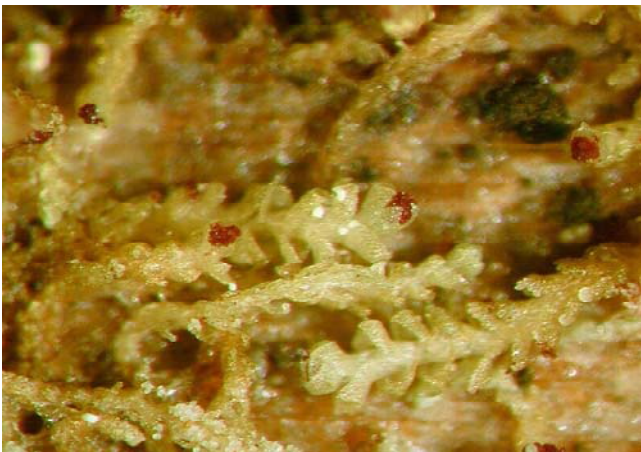

Figure 71. ***Anastrophyllum hellerianum*** with gemmae in Europe. Photo by Michael Lüth, with permission.

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.
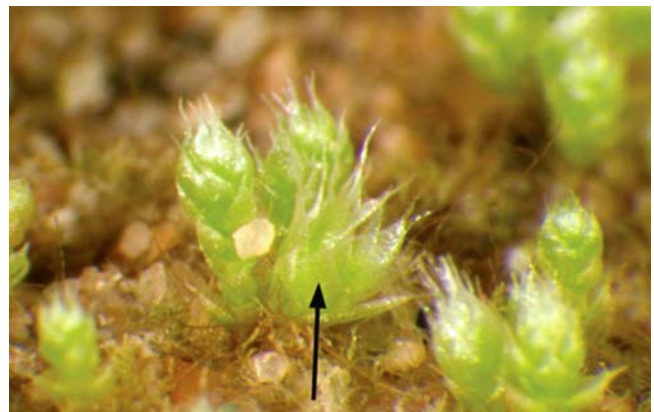

Figure 73. ***Bryum argenteum*** female plants. Photo from Horsley *et al.* 2011.

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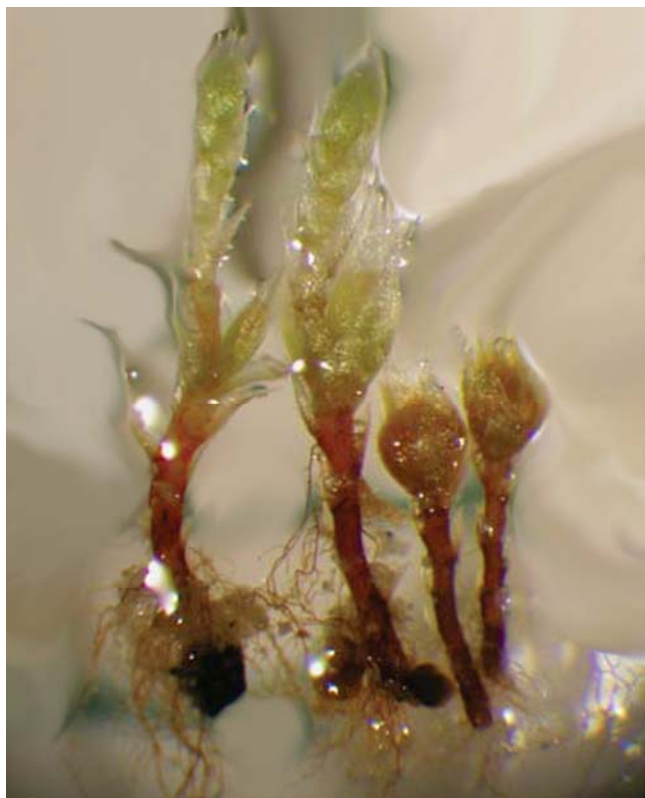

Figure 75. *Bryum argenteum* female (left) and male (right) plants, illustrating sexual dimorphism. Photo modified from Horsley *et al.* 2011.
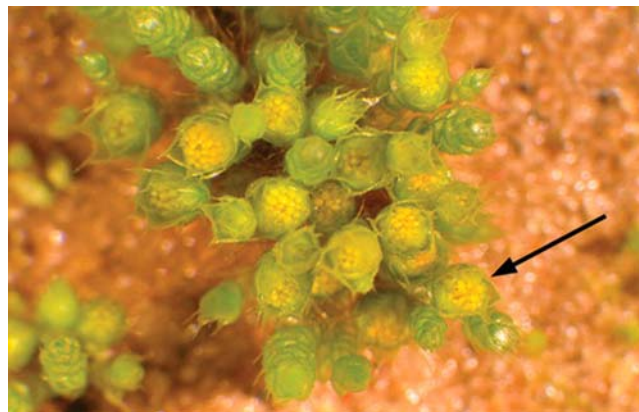
Figure 76. *Bryum argenteum* male plants, illustrating the numerous perigonia and antheridia present. Photo from Horsley *et al.* 2011.

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

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

2*n* 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 (1*n*) plant has the ability to express its alleles in the generation where they first occur, whereas the diploid (2*n*) plants have the ability to mask deleterious recessive alleles. The haploid (1*n*) 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 2*n* 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.
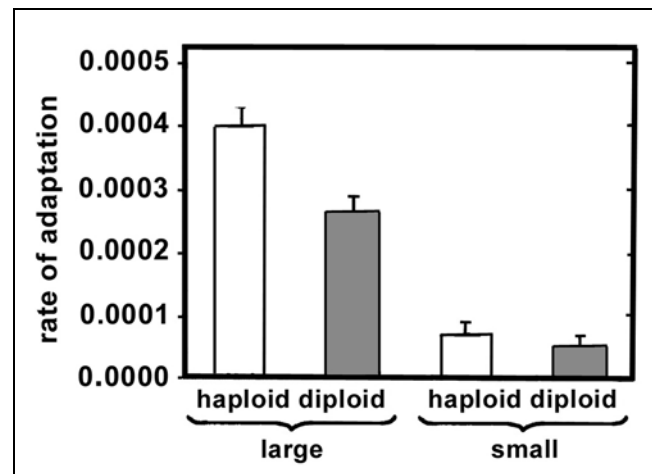

Figure 78. Rates of adaptation in large and small haploid and diploid populations of yeast. Bar length and 95% confidence interval was determined by slopes using linear regression of fitness on the generation number (n=5 pooled for 4 regressions). Ploidy was highly significant for large populations ($p<0.001$), 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 monoicous 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? Bryonetters 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 has 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 Såstad (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 (2*n*), 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 *Potamopygrus 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, *Fossombronia 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 dioicy 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

Monoicy (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 monoicy 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.

Literature Cited

Allen, C. H. 1937. Fertility and compatibility in *Sphaerocarpos*. Cytol. Fujii Jubilee Volume 1937: 494-501.

Anderson, L. E. 1980. Cytology and reproductive biology of mosses. In: Taylor, R. J. and Levontin, L. E. (eds.). The Mosses of North America. California Academy of Sciences, San Francisco, Calif. pp. 37-76.

Anderson, L. E. and Lemmon, B. E. 1972. Cytological studies of natural intergeneric hybrids and their parental species in the moss genera, *Astomum* and *Weissia*. Ann. Missouri Bot. Gard. 59: 382-416.

Anderson, L. E. and Lemmon, B. E. 1973. *Weissia sharpii*, a new species from North America, with cytological and ecological correlations. Bryologist 76: 131-143.

Anderson, L. E. and Lemmon, B. E. 1974. Gene flow distances in the moss, *Weissia controversa*. J. Hattori Bot. Lab. 38: 67-90.

Anderson, L. E. and Snider, J. A. 1982. Cytological and genetic barriers in mosses. J. Hattori Bot. Lab. 52: 241-254.

Andrews, A. L. 1920. *Hymenostomum* in North America. I. Delimitation of the genus. Bryologist 23: 23-31.

Andrews, A. L. 1922. *Hymenostomum* in North America. II. The case of *Astomum sullivantii*. Bryologist 25: 66-71.

Andrews, A. L. 1942. Taxonomic notes. II. Another natural hybrid in the Funariaceae. Bryologist 45: 176-178.

Andrews, A. L. and Hermann, F. J. 1959. A natural hybrid in the Ditrichaceae. Bryologist 62: 119-122.

Asakawa, Y. 1982. Terpenoids and aromatic compounds as chemosystematic indicators in Hepaticae and Anthocerotae. J. Hattori Bot. Lab. 53: 283-293.

Asakawa, Y. 1988. Chemical evolution of mono- and sesqui-terpenoids of liverworts. J. Hattori Bot. Lab. 64: 97-108.

Asakawa, Y. 2004. Chemosystematics of the Hepaticae. Phytochemistry 65: 623-669.

Asakawa, Y., Hattori, S., Mizutani, M., Tokunaga, N., and Takemoto, T. 1979a. Chemosystematics of bryophytes. III. Terpenoids of the primitive Hepaticae, *Takakia* and *Haplomitrium*. J. Hattori Bot. Lab. 46: 77-90.

Asakawa, Y., Takunaga, N., Toyota, M., Takemoto, T., and Suire, C. 1979b. Chemosystematics of bryophytes 1. The distribution of terpenoid of bryophytes. J. Hattori Bot. Lab. 45: 395-407.

Asakawa, Y., Tokunaga, N., Toyota, M., Takemoto, T., Hattori, S., Mizutani, M., and Suire, C. 1979c. Chemosystematics of bryophytes II. The distribution of terpenoids in Hepaticae and Anthocerotae. J. Hattori Bot. Lab. 46: 67-76.

Asakawa, Y., Inoue, H., Toyota, M., and Takemoto, T. 1980a. Sesquiterpenoids of fourteen *Plagiochila* species. Phytochemistry 19: 2623-2626.

Asakawa, Y., Tokunaga, N., Takemoto, T., Hattori, S., Mizutani, M., and Suire, C. 1980b. Chemosystematics of bryophytes. IV. The distribution of terpenoids and aromatic compounds in Hepaticae and Anthocerotae. J. Hattori Bot. Lab. 47: 153-164.

Asakawa, Y., Matsuda, R., Toyota, M., Hattori, S., and Ourisson, G. 1981. Terpenoids and bibenzyls of 25 liverwort *Frullania* species. Phytochemistry 20: 2187-2194.

Asakawa, Y., Tori, M., Masuya, T., and Frahm, J.-P. 1990. Chemosystematics of bryophytes. 34. Ent-sesquiterpenoids and cyclic bis (bibenzyls) from the German liverwort *Marchantia polymorpha*. Phytochemistry 29: 1577-1584.

Asakawa, Y., Sono, M., Wakamatsu, M., Kondo, K., Hattori, S., and Mizutani, M. 1991. Geographical distribution of tamariscol, a mossy odorous sesquiterpene alcohol, in the liverwort *Frullania tamarisci* and related species. Phytochemistry 30: 2295-2300.

Asakawa, Y., Ludwiczuk, A., and Nagashima, F. 2012. Chemical constituents of bryophytes: Bio- and chemical diversity, biological activity and chemosystematics. In: Progress in the Chemistry of Organic Natural Products Vol. 93, pp. 1-796.

Ashton, N. W. and Cove, D. J. 1976. Auxotrophic and developmental mutants of *Physcomitrella patens*. Bull. Brit. Bryol. Soc. 27: 10.

Bell, G. 1980. The costs of reproduction and their consequences. Amer. Nat. 116: 45-76.

Bengtsson, B. O. and Ceplitis, A. 2000. The balance between sexual and asexual reproduction in plants living in variable environments. J. Evol. Biol. 13: 415-422.

Bisang, I. and Ehrlén, J. 2002. Reproductive effort and cost of sexual reproduction in female *Dicranum polysetum*. Bryologist 105: 384-397.

Bisang, I. and Hedenäs, L. 2008. Mate limitation does not explain the lack of capsules in a *Drepanocladus trifarius* population. J. Bryol. 30: 97-99.

Bisang I. and Hedenäs, L. 2016. Mass-occurrence of springtails on *Tortula cernua*: A field-observation of possible animal-mediated fertilization. J. Bryol. 37: 339-341.

Bisang, I., Ehrlén, J., and Hedenäs, L. 2004. Mate limited reproductive success in two dioicous mosses. Oikos 104: 291-298.

Bisang, I., Ehrlén, J., and Hedenäs, L. 2006. Reproductive effort and costs of reproduction do not explain female-biased sex ratios in the moss *Drepanocladus trifarius* (Amblystegiaceae). Amer. J. Bot. 93: 1313-1319.

Boisselier-Dubayle, M. C. and Bischler, H. 1999. Genetic relationships between haploid and triploid *Targionia* (Targioniaceae, Hepaticae). Internat. J. Plant Sci. 160: 1163-1169.

Boisselier-Dubayle, M. C., Lambourdière, J., and Bischler, H. 1996. Progeny analysis by isozyme markers in the polyploid liverwort *Plagiochasma rupestre*. Can. J. Bot. 74: 521-527.

Bowker, M. A., Stark, L. R., McLetchie, D. N., and Mishler, B. D. 2000. Sex expression, skewed sex ratios and microhabitat distribution in the dioecious desert moss *Syntrichia caninervis* (Pottiaceae). Amer. J. Bot. 87: 517-526.

Brzyski, J. R., Wade, T., and McLetchie, D. N. 2014. Reproductive allocation between the sexes, across natural and novel habitats, and its impact on genetic diversity. Evol. Ecol. 28: 247-261.

Chopra, R. N. and Bhatla, S. C. 1990. Bryophyte Development: Physiology and Biochemistry. CRC Press, Ann Arbor, 300 pp.

Clare, D. and Terry, T. B. 1960. Dispersal of *Bryum argenteum*. Trans. Brit. Bryol. Soc. 3: 748.

Clay, K. and Kover, P. X. 1996. The Red Queen Hypothesis and plant/pathogen interactions. Ann. Rev. Phytopath. 34: 29-50.

Cleavitt, N. L. 2000. Relating rarity and phylogeny to the autecology of mosses: A comparative study of three rare-common species pairs in the Front Ranges of Alberta, Canada. Ph.D. thesis, University of Alberta, Edmonton, AB, Canada, 293 pp.

Cleavitt, N. L. 2002a. A test of physical limitation to specific substrata during establishment for *Didymodon johansenii*, a rare moss. J. Bryol. 24: 197-206.

Cleavitt, N. L. 2002b. Stress tolerance of rare and common moss species in relation to their occupied environments and asexual dispersal potential. J. Ecol. 90: 785-795.

Cleavitt, N. L. 2005. Patterns, hypotheses and processes in the biology of rare bryophytes. Bryologist 108: 554-566.

Cove, D. J. 1983. Genetics of bryophytes. In: Schuster, R. M. (ed.). 1983. New Manual of Bryology. Vol. 1. Hattori Botanical Laboratory, Nichinan, Japan, pp. 222-231.

Cronberg, N., Natcheva, R., and Hedlund, K. 2006. Microarthropods mediate sperm transfer in mosses. Science 313: 1255.

Crum, H. 2001. Structural Diversity of Bryophytes. University of Michigan Herbarium, Ann Arbor, 379 pp.

Crundwell, A. C. and Nyholm, E. 1964. The European species of the *Bryum erythrocarpum* complex. Trans. Brit. Bryol. Soc. 4: 597-637.

Delgadillo, M. C. 1989. *Astomiopsis* x *altivallis* (Musci: Ditrichaceae), a putative interspecific hybrid in Mexico. Bryologist 92: 225-227.

Derda, G. S. and Wyatt, R. 2000. Isozyme evidence regarding the origins of three allopolyploid species of *Polytrichastrum* (Polytrichaceae, Bryophyta). Plant Syst. Evol. 220: 37-53.

Duckett, J. G. 1979. An experimental study of the reproductive biology and hybridization in the European and North American species of *Equisetum*. Bot. J. Linn. Soc. 79: 205-229.

Duckett, J. G., Carothers, Z. B., and Miller, C. C. J. 1983. Gametogenesis. In: Schuster, R. M. (ed.). New Manual of Bryology. The Hattori Botanical Laboratory, Nichinan, Japan, pp. 232-258.

During, H. J. 1992. Ecological classifications of bryophytes and lichens. In: Bates, J. W. and Farmer, A. M. (eds.). Bryophytes and Lichens in a Changing Environment, Clarendon Press, Oxford, pp. 1-31.

Ehrlén, J., Bisang, I., and Hedenäs, L. 2000. Costs of sporophyte production in the moss, *Dicranum polysetum*. Plant Ecol. 149: 207-217.

Elder, J. 2013. Secret life of moss could end world hunger. The Sydney Morning Herald 16 March 2013. Accessed 17 February 2014 at <http://www.smh.com.au/national/secret-life-of-moss-could-end-world-hunger-20130316-2g7rp.html#ixzz2tbX3yxnn>.

Eppley, S. M., Taylor, P. J., and Jesson, L. K. 2007. Self-fertilization in mosses: A comparison of heterozygote deficiency between species with combined versus separate sexes. Heredity 98: 38-44.

Flatberg, K. I., Thingsgaard, K., and Såstad, S. M. 2006. Interploidal gene flow and introgression in bryophytes: *Sphagnum girgensohnii* x *S. russowii*, a case of spontaneous neotriploidy. J. Bryol. 28: 27-37.

Fuselier, L. and McLetchie, D. N. 2002. Maintenance of sexually dimorphic pre-adult traits in *Marchantia inflexa* (Marchantiaceae). Amer. J. Bot. 89: 592-601.

Gayet, L. A. 1897. Recherches sur le développement de l'archegone chez les muscinées. Ann. Sci. Nat. Bot. sér. 8, 3: 161-258.

Glime, J. M. 2011. Why do bryophytes seem to lack extensive diversity? Field Bryol. 104: 84.

Glime, J. M., Nissila, P. D., Trynoski, S. E., and Fornwall, M. D. 1979. A model for attachment of aquatic mosses. J. Bryol. 10: 313-320.

Goffinet, B. and Shaw, A. J. 2009. Bryophyte Biology. 2nd ed. Cambridge University Press, Cambridge.

Greig, D. and Travisano, M. 2003. Haploid superiority. Science 299: 524-525.

Gustafsson, A. 1946. Apomixis in the higher plants. I. The mechanism of apomixis. Lunds Univ. Arsskr. 43: 166. pp.

Haig, D. and Wilczek, A. 2006. Sexual conflict and the alternation of haploid and diploid generations. Philosoph. Trans. Royal Soc. B Biol. Sci. 361: 335-343.

Haupt, A. W. 1929. Studies in California Hepaticae. I. *Asterella californica*. Bot. Gaz. 87: 302-318.

Hedrick, P. W. 1987. Population genetics of intragametophytic selfing. Evolution 41: 137-144.

Holá, E., Vesalainen, T., Těšitel, J., and Laaka-Lindberg, S. 2014. Sex ratio, sex - specific pattern in vegetative growth and gemma production in an aquatic liverwort, *Scapania undulata* (Marchantiophyta: Scapaniaceae). Bot. J. Linn. Soc. 175: 229-241.

Horsley, K., Stark, L. R., and McLetchie, D. N. 2011. Does the silver moss *Bryum argenteum* exhibit sex-specific patterns in vegetative growth rate, asexual fitness or prezygotic reproductive investment? Ann. Bot. 107: 897-907.

Hutsemekers, V., Hardy, O. J., and Vanderpoorten, A. 2013. Does water facilitate gene flow in spore-producing plants? Insights from the fine-scale genetic structure of the aquatic moss *Rhynchostegium riparioides* (Brachytheciaceae). Aquat. Bot. 108: 1-6.

Jesson, L. K., Cavanagh, A. P., and Perley, D. S. 2011. Polyploidy influences sexual system and mating patterns in the moss *Atrichum undulatum* sensu lato. Ann. Bot. 107: 135-143.

Jesson, L. K., Perley, D. S., Cavanagh, A. P., Cameron, J. A. C., and Kubien, D. S. 2012. Mating and fitness consequences of sexual system in the moss *Atrichum undulatum* s.l. (Polytrichaceae). Internat. J. Plant Sci. 173: 16-25.

Johnson, M. G. and Shaw, A. J. 2015. Genetic diversity, sexual condition, and microhabitat preference determine mating patterns in *Sphagnum* (Sphagnaceae) peat-mosses. Biol. J. Linn. Soc. 115: 96-113.

Jokela, J., Dybdahl, M., and Lively, C. 2009. The maintenance of sex, clonal dynamics, and host-parasite coevolution in a mixed population of sexual and asexual snails. Amer. Nat. 174: 43-53.

Kerfoot, W. C. and Weider, L. J. 2004. Experimental paleoecology (resurrection ecology): Chasing Van Valen's Red Queen hypothesis. Limnol. Oceanogr. 49: 1300-1316. .

Kew. 2014. The Plant List, ver. 1. Accessed 28 January 2014 at <http://www.theplantlist.org/>.

Khanna, K. R. 1960. Studies in natural hybridization in the genus *Weisia*. Bryologist 63: 1-16.

Klekowski, E. J. 1969. Reproductive biology of the Pteridophyta. II. Theoretical considerations. Bot. J. Linn. Soc. 62: 347-359.

Klips, R. A. 2015. DNA microsatellite analysis of sporophytes of the short-lived moss *Physcomitrium pyriforme* reveals a predominantly self-fertilizing mating pattern. Bryologist 118: 200-211.

Krueger-Hadfield, S. A., Roze, D., Mauger, S., and Valero, M. 2013. Intergametophytic selfing and microgeographic genetic structure shape populations of the intertidal red seaweed *Chondrus crispus*. Molec. Ecol. 22: 3242-3260.

Laaka-Lindberg, S. 2000. Ecology of asexual reproduction in hepatics. Academic Dissertation, Publications in Botany from the University of Helsinki No. 29, Helsinki, Finland, 28 pp.

Laaka-Lindberg, S. 2001. Biomass allocation to sexual and asexual reproduction in a leafy hepatic *Lophozia silvicola* Buch. J. Bryol. 23: 3-8.

La Farge, C., Williams, K. H., and England, J. H. 2013. Regeneration of Little Ice Age bryophytes emerging from a polar glacier with implications of totipotency in extreme environments. Proc. Nat. Acad. Sci. 110: 9839-9844.

Longton, R. E. 1997. Reproductive biology and life-history strategies. Adv. Bryol. 6: 65-101.

Longton, R. E. 1998. Reproductive biology and life-history strategies. In: Bates, J. W., Ashton, N. W., and Duckett, J. G. (eds.). Bryology for the Twenty-first Century. Maney Publishing and the British Bryological Society, UK, pp. 369-370.

Longton, R. E. 2006. Reproductive ecology of bryophytes: What does it tell us about the significance of sexual reproduction? Lindbergia 31: 16-23.

Longton, R. E. and Miles, C. J. 1982. Studies on the reproductive biology of mosses. J. Hattori Bot. Lab. 52: 219-239.

Longton, R. E. and Schuster, R. M. 1983. Reproductive biology. In: Schuster, R. M. (ed.). New Manual of Bryology. Vol. 1. Hattori Botanical Laboratory, Nichinan, Japan, pp. 386-462.

Machlis, L. and Rawitscher-Kunkel, E. 1967. 4. Mechanisms of gametic approach in plants. In: Metz, C. B. and Monroy, A. Fertilization. I. Academic Press, N. Y., 489 pp.

Maciel-Silva, A. S. and Válio, I. F. M. 2011. Reproductive phenology of bryophytes in tropical rain forests: The sexes never sleep. Bryologist 114: 708-719.

McLetchie, D. N. 1996. Sperm limitation and genetic effects on fecundity in the dioecious liverwort *Sphaerocarpos texanus*. Sex. Plant Repro. 9: 87-92.

McLetchie, D. N. and Puterbaugh, M. N. 2000. Population sex ratios, sex-specific clonal traits and tradeoffs among these traits in the liverwort, *Marchantia inflexa*. Oikos 90: 227-237.

McLetchie, D. N. and Stark, L. R. 2006. Sporophyte and gametophyte generations differ in responses to thermotolerance in the moss *Microbryum*. Ann. Bot. 97: 505-511.

Mishler, B. D. 1988. Reproductive ecology of bryophytes. In: Lovett Doust, J. and Lovett Doust, L. (eds.). Plant Reproductive Ecology, Patterns and Strategies, Oxford University Press, New York, pp. 285-306.

Morran, L. T., Schmidt, O. G., Gelarden, I. A., Parrish, R. C. II, and Lively, C. M. 2011. Running with the Red Queen: Host-parasite coevolution selects for biparental sex. Science 333: 216-218.

Natcheva, R. and Cronberg, N. 2004. What do we know about hybridization among bryophytes in nature? Can. J. Bot. 82: 1687-1704.

Newton, A. E. and Mishler, B. D. 1994. The evolutionary significance of asexual reproduction in mosses. J. Hattori Bot. Lab. 76: 127-145.

Newton, M. E. 1984. The cytogenetics of bryophytes. In: Dyer, A. F. and Duckett, J. G. (eds.). The Experimental Biology of Bryophytes. Academic Press, New York, pp. 65-96.

Nicholson, W. E. 1905. Notes on two forms of hybrid *Weisia*. Rev. Bryol. 32: 19-25.

Noguchi, A. and Miyata, I. 1957. Sporelings and regenerants in some mosses. Kumamoto J. Sci. Ser. B Sec. 22, 3(1): 1-19.

Nyholm, E. 1958. Illustrated Moss Flora of Fennoscandia. II. Musci. Fasc. 3. Swedish Natural Science Research Council, Lund.

Ochi, H. 1971. Preliminary notes on the speciation in the subfamily Bryoideae, Musci. Misc. Bryol. Lichenol. 5: 159-161.

Parihar, N. S. 1970. An Introduction to Embryophyta. Volume I. Bryophyta, 4th edn. Central Book Depot, Allahabad.

Patiño, J., Bisang, I., Hedenäs, L., Dirkse, G., Bjarnason, Á. H., Ah-Peng, C., and Vanderpoorten, A. 2013. Baker's law and the island syndromes in bryophytes. J. Ecol. 101: 1245-1255.

Pépin, F., Hugonnot, V., and Celle, J. 2013. Sex ratio patterns and fertility of *Hamatocaulis vernicosus* (Mitt.) Hedenäs at different spatial scales. J. Bryol. 35: 20-26.

Pereira, M. R., Dambros, C. S., and Zartman, C. E. 2016. Prezygotic resource-allocation dynamics and reproductive trade-offs in Calymperaceae (Bryophyta). Amer. J. Bot. 103: 1838-1846.

Pfeffer, W. F. P. 1884. Locomotorische richtungsbewegungen durch chemische Reize. Unter. Bot. Tübingen 1: 364-482.

Pohjamo, M. and Laaka-Lindberg, S. 2004. Demographic population structure of a leafy epixylic hepatic *Anastrophyllum hellerianum* (Nees ex Lindenb.) R. M. Schust. Plant Ecol. 173: 73-81.

Preston, K. A. and Mishler, B. D. 1997. Ecological and developmental studies on the dwarf male breeding system of the moss *Dicranum scoparium* in North Carolina Piedmont. Amer. J. Bot. (Suppl.) 84(6): 20.

Proskauer, J. 1967. The variable ploidy life history of a monoecious self-fertilizing hornwort (Anthocerotales). Amer. J. Bot. 54: 641-642.

Proskauer, J. 1969. Studies on Anthocerotales. VIII. Phytomorphology 19: 52-66.

Red Queen Hypothesis. 2014. Wikipedia. Last updated 10 March 2014. Accessed 13 March 2014 at <http://en.wikipedia.org/wiki/Red_Queen_hypothesis>.

Reese, W. D. and Lemmon, B. E. 1965. A natural hybrid between *Weissia* and *Astomum* and notes on the nomenclature of the North American species of *Astomum*. Bryologist 68: 277-283.

Ricca, M., Szovenyi, P., Temsch, E. M., Johnson, M. G., and Shaw, A. J. 2011. Interploidal hybridization and mating patterns in the *Sphagnum subsecundum* complex. Molec. Ecol. 20: 3202-3218.

Richards, A. J. 1997. Agamospermy. In: Plant Breeding Systems. Springer, US, pp. 396-450.

Ricklefs, R. E. 1990. Ecology, 3rd ed. W. H. Freeman and Co., New York, 896 pp.

Roads, E., Longton, R. E., and Convey, P. 2014. Millennial timescale regeneration in a moss from Antarctica. Curr. Biol. 24: 222-223.

Ros, R. M., Guerra, J., and Cano, M. J. 1994. *Pottia* x *andalusica* (Musci: Pottiaceae), un híbrido interespecífico en Pottiaceae. Cryptog. Bryol. Lichénol. 15: 199-204.

Rosengren, F. and Cronberg, N. 2014. The adaptive background of nannandry: Dwarf male distribution and fertilization in the moss *Homalothecium lutescens*. Biol. J. Linn. Soc. 113: 74-84.

Rosengren, F., Cronberg, N., Reitalu, T., and Prentice, H. C. 2014. Sexual reproduction in the phyllodioicous bryophyte *Homalothecium lutescens* (Hedw.) H. Rob. in relation to habitat age, growth conditions and genetic variation. J. Bryol. 36: 200-208.

Rosenstiel, T. N., Shortlidge, E. E., Melnychenko, A. N., Pankow, J. F., and Eppley, S. M. 2012. Sex-specific volatile compounds influence microarthropod-mediated fertilization of moss. Nature 489: 431-433.

Ruthe, R. 1891. Die Laubmoose Deutschlands, Oesterreichs und der Schweiz 2: 200.

Rydgren, K. and Økland, R. H. 2002. Ultimate costs of sporophyte production in the clonal moss *Hylocomium splendens*. Ecology 83: 1573-1579.

Rydgren, K. and Økland, R. H. 2003. Short-term costs of sexual reproduction in the clonal moss *Hylocomium splendens*. Bryologist 106: 212-220.

Sakakibara, K., Ando, S., Yip, H. K., Tamada, Y., Hiwatashi, Y., Murata, T., Deguchi, H., Hasebe, M., and Bowman, J. L. 2013. KNOX2 genes regulate the haploid-to-diploid morphological transition in land plants. Science 339: 1067-1070.

Schofield, W. B. 1985. Introduction to Bryology. Macmillan Publishing Co., New York, 431 pp.

Schuster, R. 1991. Diagnosis of new taxa of Hepaticae, I. Jungermanniidae. J. Hattori Bot. Lab. 70: 143-150.

Shaw, A. J. 1991. The genetic structure of sporophytic and gametophytic populations of the moss, *Funaria hygrometrica* Hedw. Evolution 45: 1260-1274.

Shaw, A. J. 2000. Population ecology, population genetics, and microevolution. In: Shaw, A. J. and Goffinet, B. (eds.). Bryophyte Biology. Cambridge University Press, Cambridge, UK, p. 377.

Shaw, A. J., Shaw, B., Ricca, M., and Flatberg, K. I. 2012. A phylogenetic monograph of the *Sphagnum subsecundum* complex (Sphagnaceae) in eastern North America. Bryologist 115: 128-152.

Shaw, J. 1986. A new approach to the experimental propagation of bryophytes. Taxon 35: 671-675.

Shortlidge, E. E., Rosenstiel, T. N., and Eppley, S. M. 2012. Tolerance to environmental desiccation in moss sperm. New Phytol. 194: 741-750.

Showalter, A. M. 1926. Studies in the cytology of the Anacrogynae. I. Antherozoids. Ann. Bot. 40: 691-707.

Showalter, A. M. 1928. Studies in the cytology of the Anacrogynae. V. Hybrid fertilization in *Riccardia pinguis*. Cellule 38: 295-348.

Smith, A. J. E. 1978. Cytogenetics, biosystematics and evolution in the Bryophyta. Adv. Bot. Res. 6: 165-276.

Smith, A. J. E. 1979. Towards an experimental approach to bryophyte taxonomy. In: Clarke, G. C. S. and Duckett, J. G. (eds.). Bryophyte Systematics. Academic Press, New York, pp. 195-206.

Söderström, L. and During, H. J. 2005. Bryophyte rarity viewed from the perspectives of life history strategy and metapopulation dynamics. J. Bryol. 27: 261-268.

Stark L. R. and Brinda, J. C. 2013. An experimental demonstration of rhizautoicy, self-incompatibility, and reproductive investment in *Aloina bifrons* (Pottiaceae). Bryologist 116: 43-52.

Stark, L. R. and Stephenson, A. G. 1983. Reproductive biology of *Entodon cladorrhizans* (Bryopsida, Entodontaceae). II. Resource-limited reproduction and sporophyte abortion. Syst. Bot. 8: 389-394.

Stark, L. C., Brinda, J. C., and McLetchie, D. N. 2009. An experimental demonstration of the cost of sex and a potential resource limitation on reproduction in the moss *Pterygoneurum* (Pottiaceae). Amer. J. Bot. 96: 1712-1721.

Stark, L. R., Mishler, B. D., and McLetchie, D. N. 2000. The cost of realized sexual reproduction: Assessing patterns of reproductive allocation and sporophyte abortion in a desert moss. Amer. J. Bot. 87: 1599-1608.

Stark, L. R., Oliver, M. J., Mishler, B. D., and McLetchie, D. N. 2007. Generational differences in response to desiccation stress in the desert moss *Tortula inermis*. Ann. Bot. 99: 53-60.

Stenøien, H. K. and Såstad, S. M. 2001. Genetic variability in bryophytes: Does mating system really matter? J. Bryol. 23: 313-318.

Stoneburner, A. 1990. Are bryophytes primitive or advanced? Bryol. Times 57/58: 11.

Strasburger, E. 1869-1870. Die Geschlechtsorgane und die Befruchtung bei *Marchantia polymorpha*. Jahrb. Wiss. Bot. 7: 409-422.

Szövényi, P., Ricca, M., and Shaw, A. J. 2009. Multiple paternity and sporophytic inbreeding depression in a dioicous moss species. Heredity 103: 394-403.

Täckholm, G. 1922. Zytologische Studien über die Gattung *Rosa*. Acta Hort. Berg. 7.

Taylor, P. J., Eppley, S. M., and Jesson, L. K. 2007. Sporophytic inbreeding depression in mosses occurs in a species with separate sexes but not in a species with combined sexes. Amer. J. Bot. 94: 1853-1859.

Terui, K. 1981. Growth and gemma-cup formation in relation to archegoniophore protrusion in *Marchantia polymorpha* L. Ann. Rep. Fac. Ed. Iwate Univ. 40: 19-28.

Une, K. 1984. A field observation on the reproductive mode in *Marchantia polymorpha* L. Hikobia 9: 15-18.

Une, K. 1985a. Sexual dimorphism in the Japanese species of *Macromitrium* Brid. (Musci: Orthotrichaceae). J. Hattori Bot. Lab. 59: 487-513.

Une, K. 1985b. Factors restricting the formation of normal male plants in the isosporous species of *Macromitrium* Brid. (Musci: Orthotrichaceae) in Japan. J. Hattori Bot. Lab. 59: 523-529.

Une, K. 1985c. Geographical distribution of male and female plants in species of *Macromitrium* Brid. (Musci: Orthotrichaceae) in Japan. J. Hattori Bot. Lab. 59: 515-521.

Une, K. 2009. Sexual dimorphism in the Japanese species of *Macromitrium* Brid. (Musci: Orthotrichaceae). J. Hattori Bot. Lab. 59: 487-513.

Une, K. and Yamaguchi, T. 2001. Male plants of the Japanese species of *Leucobryum* Hampe (Leucobryaceae, Musci). Hikobia 13: 579-590.

Valen, L. Van. 1973. A new evolutionary law. Evol. Theory 1: 1-30.

Vanderpoorten, A. and Goffinet, B. 2009. Introduction to Bryophytes. Cambridge University Press, Cambridge.

Velde, M. Van Der and Bijlsma, R. 2004. Hybridisation and asymmetric reproductive isolation between the closely related taxa *Polytrichum commune* and *Polytrichum uliginosum*. Molec. Ecol. 13: 1447-1454.

Vitt, D. H. 1971. Infrageneric evolution, phylogeny, and taxonomy of the genus *Orthotrichum* (Musci) in North America. Nova Hedw. 21: 683-711.

Watson, E. V. 1964. The Structure and Life of Bryophytes. Hutchinson University Library, London.

Wettstein, F. V. 1923. Kreuzungsversuche mit multiploiden Moosrassen. Biol. Zentralbl. 43: 71-82.

Wiese, L. and Wiese, W. 1977. On speciation by evolution of gametic incompatibility: A model case in *Chlamydomonas*. Amer. Nat. 111: 733-742.

Wiklund, K. and Rydin, H. 2004. Ecophysiological constraints on spore establishment in bryophytes. Funct. Ecol. 18: 907-913.

Williams, C. 1966. A natural hybrid in the genus *Weissia*. Bryologist 69: 361-365.

Williams, G. C. 1996. Adaptation and Natural Selection. Princeton University Press, Princeton, N.J., 320 pp.

Winkler, H. 1908. Über Parthenogenesis und Apogamie im Pflanzenreich. Progressus Rei Botanicae 2: 293-454.

Xu, S. 2000. Phylogenetic analysis under reticulate evolution. Molec. Biol. Evol. 17: 897-907.

Zeyl, C., Vandeford, T., and Carter, M. 2003. An evolutionary advantage of haploidy in large yeast populations. Science 299: 555-558.