Glime, J. M. and Bisang, I. 2017. Sexuality: Size and Sex Differences. Chapt. 3-3. In: Glime, J. M. Bryophyte Ecology.
Volume 1. Physiological Ecology. Ebook sponsored by Michigan Technological University and the International Association of
Bryologists. Last updated 3 April 2022 and available at <http://digitalcommons.mtu.edu/bryophyte-ecology/>.

3-3-1

CHAPTER 3-3
SEXUALITY: SIZE AND SEX DIFFERENCES

JANICE M. GLIME AND IRENE BISANG

TABLE OF CONTENTS

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.
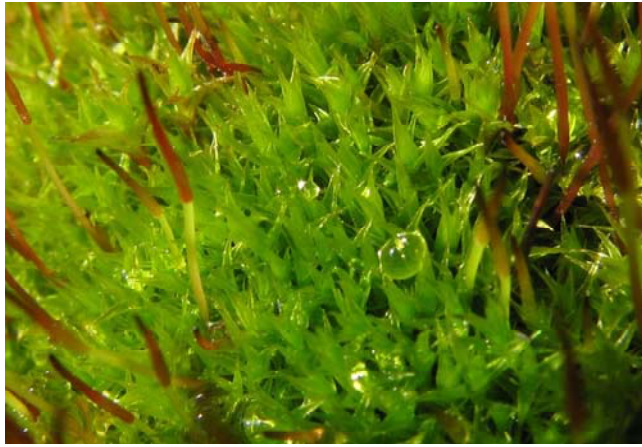
Figure 2. *Ceratodon purpureus* with young sporophytes. Photo by Jiří Kameníček, with permission.

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.
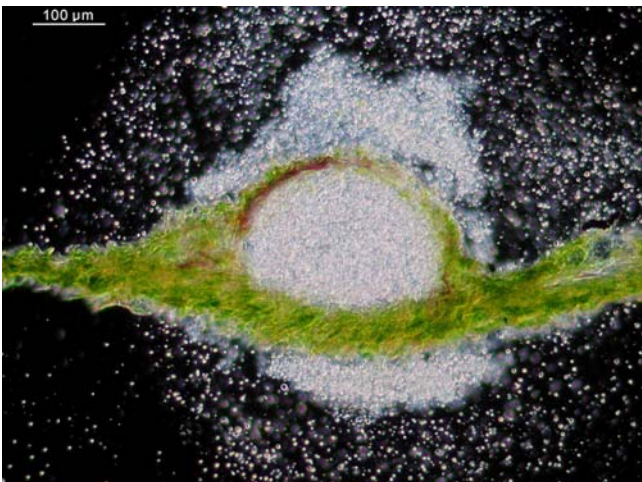

Figure 8. *Pellia endiviifolia* with antheridium cross section and spermatocytes. Photo by Ralf Wagner <www.dr-ralf-wagner.de>, with permission.

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.
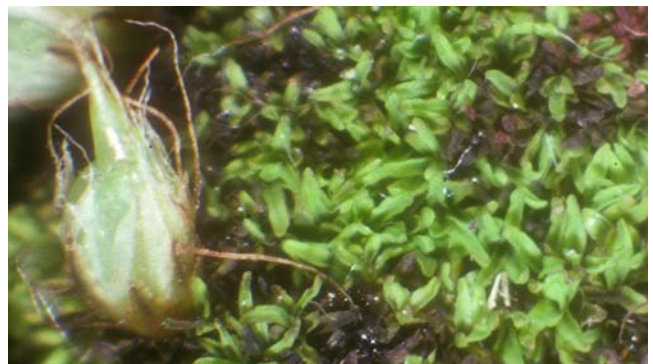

Figure 12. *Diphyscium foliosum* female (**left**) with only perichaetial leaves visible and reduced vegetative gametophyte; male plants are to its **right**, showing conspicuous leafy plants. Photo by Janice Glime.

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. Even early publications on bryophytes recognized examples of sharp size distinctions (Bruch *et al.* 1851-1855; Limpricht 1895-1904; Fleischer 1920). Dwarf males occur in approximately 60 genera and 22 families (Rosengren & Cronberg 2015); estimations suggest that they occur in 10-20% of all moss species (Hedenäs *et al*. 2011). 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)].
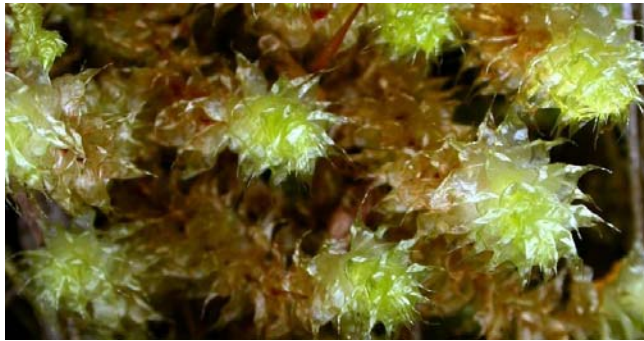

Figure 13. *Ptychomnion aciculare*, a species that produces dwarf males. Photo by David Tng, with permission.
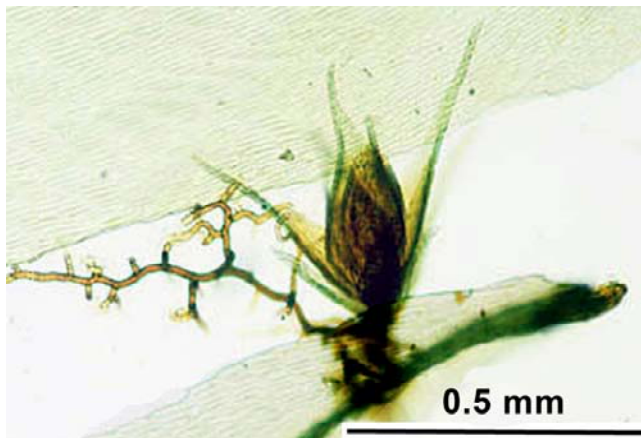

Figure 14. *Ptychomnion aciculare* with dwarf male on leaf. Photo modified from one by John Braggins, with permission.
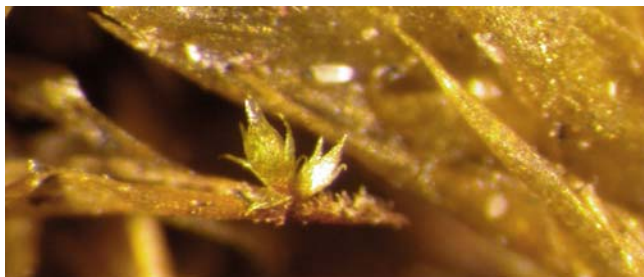

Figure 15. *Acroporium stramineum* dwarf male on leaf of mature female. Photo courtesy of Lars Hedenäs.

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 (2014) 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).
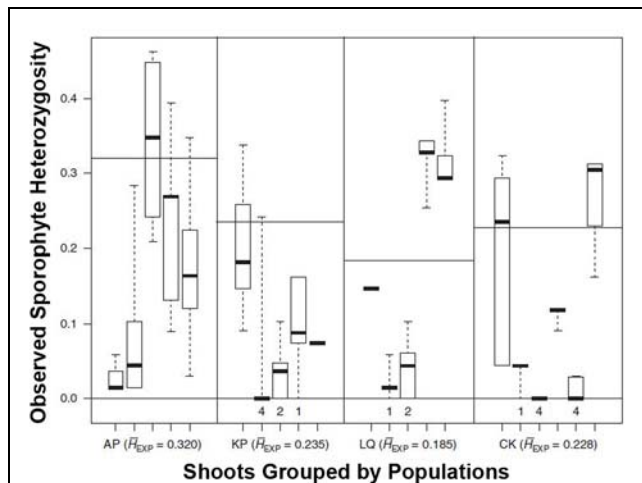

Figure 21. Inbreeding vs outcrossing in *Homalothecium lutescens* from four populations in Sweden. Each box represents the lower and upper quartile of 4-6 sporophytes on a single female shoot. The thick horizontal lines within boxes represent the median and whiskers denote the total range of data (minimum 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.
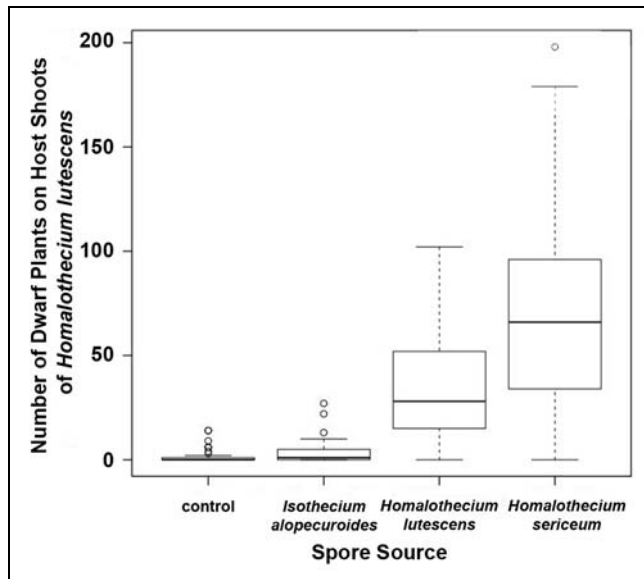
Figure 24.   Total number of dwarf plants of each source species on *Homalothecium lutescens* 10 months after sowing spores of three species on *H. lutescens* (n-46).   Redrawn from Rosengren & Cronberg 2015.
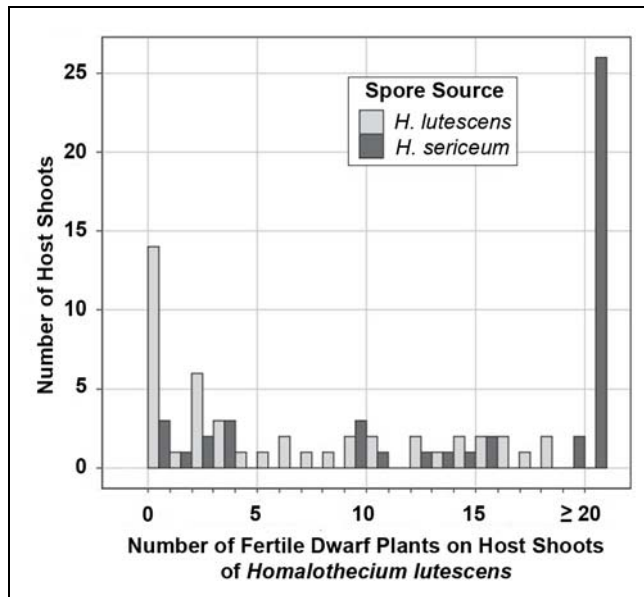

Figure 25.   Number of fertile dwarf male-expressing plants of *Homalothecium lutescens* and *H. sericeum* that germinated from spores placed on *Homalothecium lutescens*.   Redrawn from Rosengren & Cronberg 2015.

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 is 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** (growing on leaves of other plants), 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.
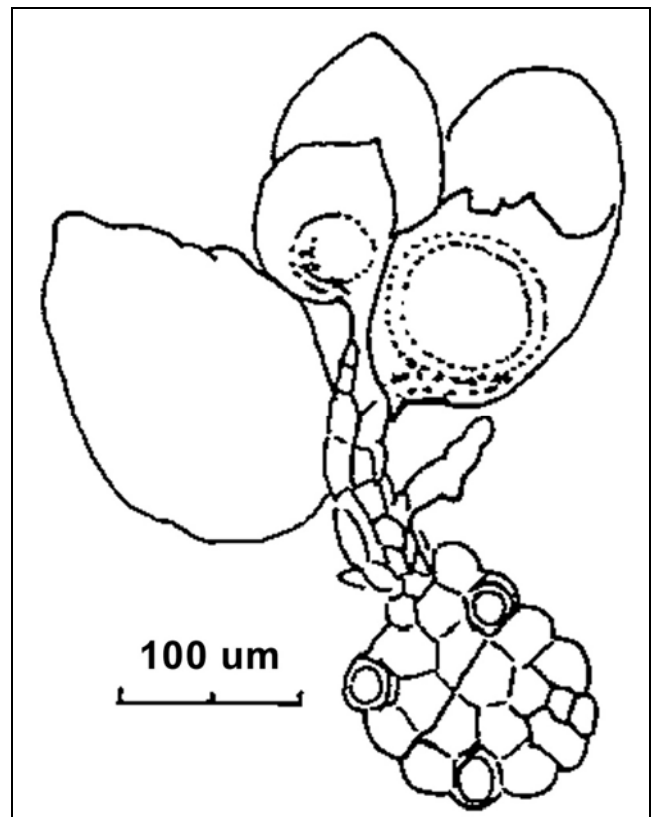

Figure 30. *Cololejeunea borhidiana* dwarf male developing from a gemma. Drawing by Tamás Pócs, with permission.
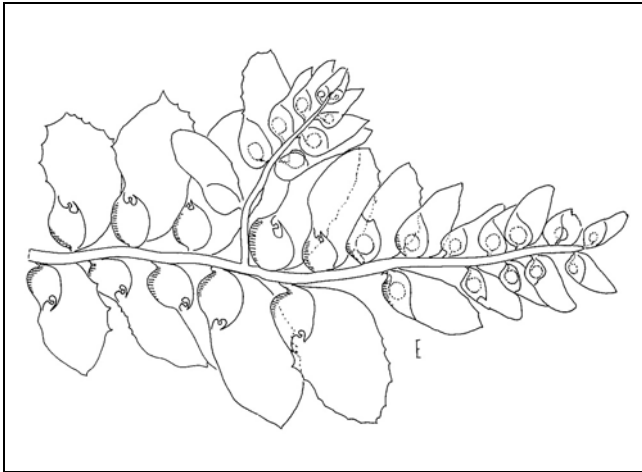
Figure 31.   *Cololejeunea borhidiana* normal male developing from a gemma.   Drawing by Tamás Pócs, with permission.

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.
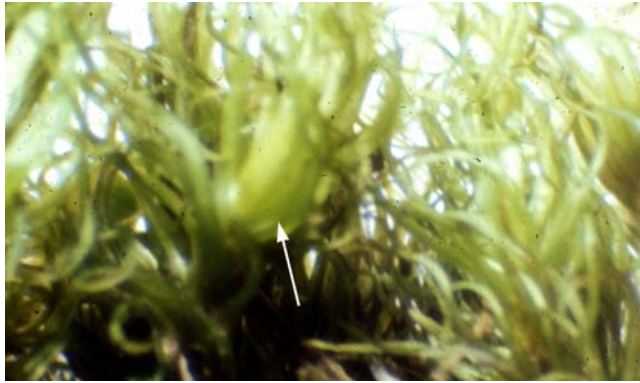
Figure 35. *Dicranum sabuletorum* dwarf male (**arrow**) growing on a female plant. Photo by Janice Glime.

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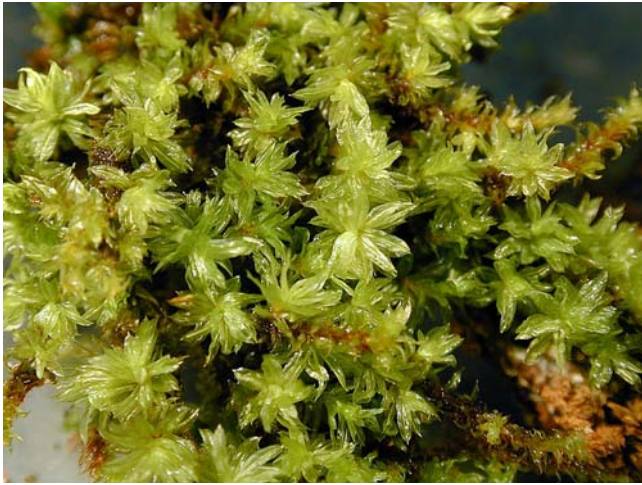
Figure 41. *Macromitrium* from the Neotropics. Photo by Michael Lüth, with permission.

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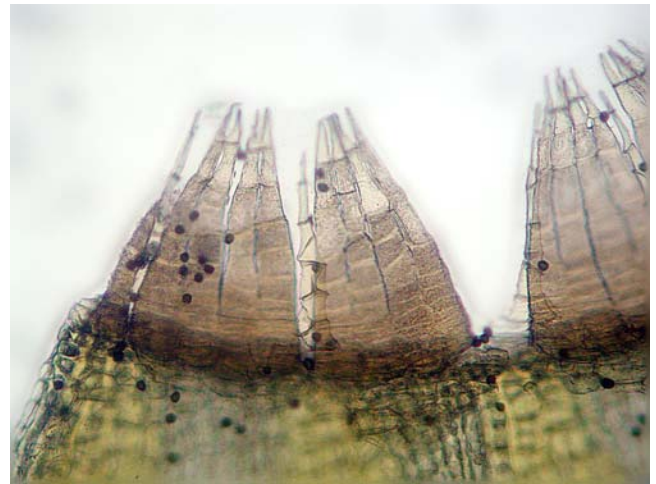
Figure 45. Peristome and spores of *Orthotrichum alpestre*, an isosporous species. Photo by Michael Lüth, with permission.

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.

Neoteny vs Progenesis

These two conditions are easy to confuse because they have similar results. **Neoteny** is the condition of slowing down developmental growth (**somatic** or body cell), resulting in sexual reproduction that happens in what was ancestrally a juvenile morphologic stage. **Progenesis** is the condition of developmental growth at a normal rate, but the production of gametangia occurs at a faster rate, resulting in **paedomorphosis** (retention by organism of juvenile traits into later life), *i.e.*, reproduction also happens in what was ancestrally a juvenille morphologic stage. Mishler and DeLuna (1991) consider the development of dwarf males to be **progenesis**.

Dwarf males typically attach to the female by rhizoids (Johannes Enroth, pers. comm 29 December 2020) and at least in *Garovaglia*#and#*Euptychium* also protonemata (Heinjo During, Bryonet, 29 December 2020).

Neoteny and progenesis should not be confused with **sexual dimorphism** such as that found in *Sphaerocarpus* (Irene Bisang, pers. comm., 28 December 2020).

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 perichetial 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).

Dwarf males seem to be sensitive to microclimate, living shorter lives than the females of the species. This resultes in variation of male frequencies among years (Sagmo Solli *et al*. 2000; Rosengren & Cronberg 2014; Lang *et al*. 2021).

Lang *et al*. (2021) investigated the facultative dwarf males in *Dicranum scoparium* (Figure 38). They found that numbers of dwarf males differed greatly between sites. When present, the dwarf males usually outnumbered both females and normal males. Genetic variation was low within a locality. It was strongest between cushions for both females and normal males and within cushions of dwarf males. They found indications that inbreeding occurs some of the time.

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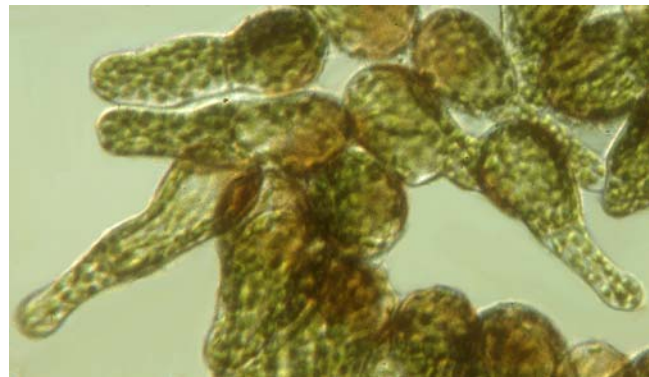

Figure 55. *Funaria hygrometrica* spore germination from isosporous spores. Photo by Yenhung Li, with permission.

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

Figure 53. *Fissidens limbatus* from Europe. Photo by Michael Lüth, 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 61.   *Cinclidium arcticum* with capsules. 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 63. *Cinclidium stygium*, a species in which a ~50% of the spores abort as the spores mature. 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 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.
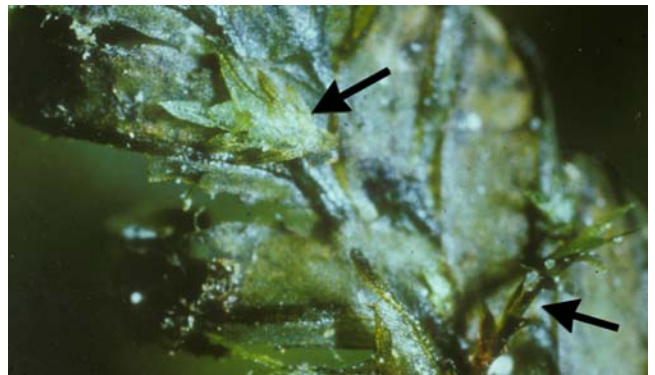

Figure 67. Dwarf males (arrows) on *Fissidens cristatus*. Photo by Janice Glime.

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 71.   Longitudinal section through capsule of **Fontinalis squamosa** showing the tightly packed spores. Photo by Janice Glime.
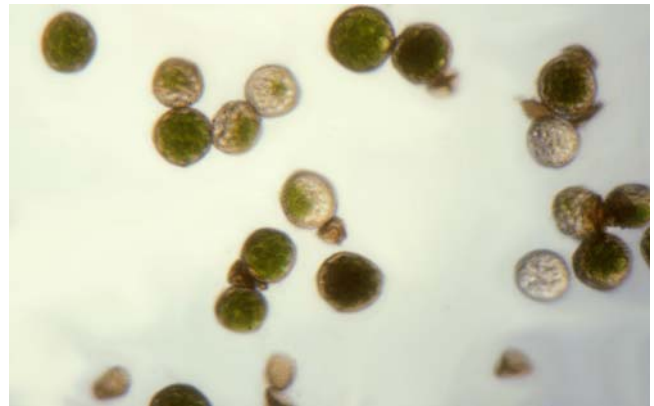

Figure 72. Spores of **Fontinalis squamosa** showing large, healthy green spores, smaller white dying or dead spores, and small brown spores that may be dead. These are not anisosporous because they are not of two sizes at the end of meiosis. Photo by Janice Glime.
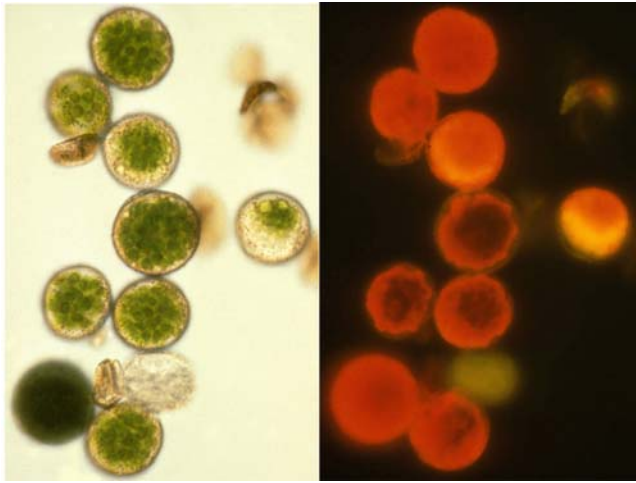
Figure 73.   Normal and aborting spores of *Fontinalis squamosa* in white light (**left**) and the same spores fluorescing under ultraviolet light (**right**), showing red healthy spores and yellow or green dying spores. Note the lack of fluorescence in the small, deflated spores and the yellow edges 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.
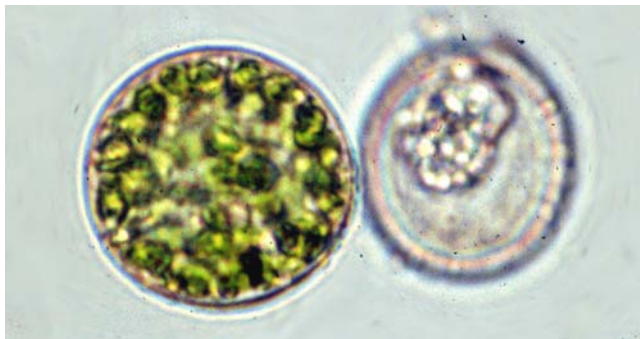

Figure 74.   Normal (**left**) and smaller aborted (**right**) spores of *Fontinalis squamosa*. Photo by Janice Glime.
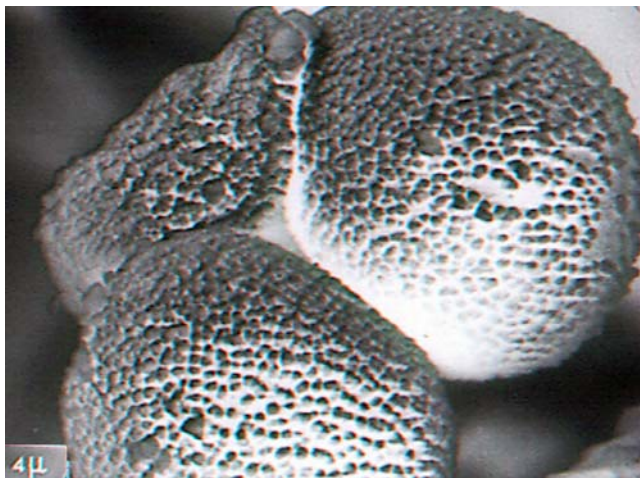

Figure 75.   Tetrad of spores from *Fontinalis squamosa*. Note one abortive spore. Photo by Janice Glime.

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.
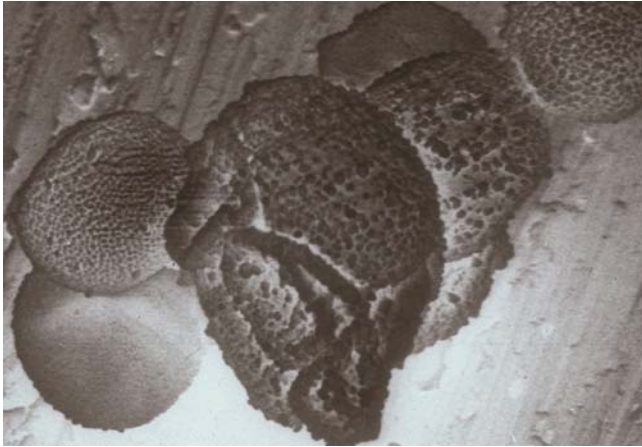

Figure 78. *Fontinalis squamosa* showing what to be three aborted spores in one tetrad. Photo by Janice Glime.

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.

Lang, A. S., Gerhrmann, T., and Cronberg, N. C. 2021. Genetic diversity in bryophyte populations with facultative nannandry. Front. Plant Sci. 12: 525, 14 pp. 10.3389/fpls.2021.517547.

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. and DeLuna, E. 1991. The use of ontogenetic data in phylogenetic analyses in mosses. Adv Bryol. 4: 121-167.

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: 1-4. <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.

Sagmo Solli, I. M. S, Söderström, L., Bakken, S., Flatberg, K. I., and Pedersen, B. 2000. Studies of fertility of *Dicranum majus* in two populations with contrasted sporophyte production. J. Bryol. 22: 3‑8.

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.