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CHAPTER 3-2
SEXUALITY: SEX RATIO AND SEX EXPRESSION

JANICE M. GLIME AND IRENE BISANG

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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 ***Pseudocalliergon lycopodioides*** (Figure 8). 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 8) 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 vegetative 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 ***Pseudocalliergon trifarium*** (=***Drepanocladus trifarius***) (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. ***Pseudocalliergon trifarium***. 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. Field collections revealed a greater than 80% female bias among 154 collections in the USA, with male expressions being even more rare (as little as 2%) 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 sexually expressing field populations. Hence, the "shy male hypothesis" lacks support in ***Bryum argenteum***. Populations grown in the lab from spores, on the other hand, had a 1:1 sex ratio. This leads us to the conclusion that in this species there are factors between sporeling and mature gametophyte that differentially affect the two sexes.

Using herbarium samples from a wide geographic range, Bisang and Hedenäs (2013) assessed the sex ratio in expressing and non-expressing ***Pseudocalliergon lycopodioides*** (Figure 8), 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). As in ***Bryum argenteum*** (Figure 7), biased population sex ratios in ***Pseudocalliergon lycopodioides*** 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 montane and disturbed habitats in western USA and up to 98% females in native lowland desert and chaparral, but with a 1:1 ratio of plants grown in the lab from spores. Photo from India Biodiversity Portal, through Creative Commons.

Figure 8. ***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.

Eppley *et al.* (2018) set out to understand the origin of biased sex ratios in ***Ceratodon purpureus*** (Figure 3). They started with equal populations of male biased and female biased sex ratios to test the hypothesis that male-biased populations would become female-biased and that stress could increase the transition. After 18 months there was no significant change in the originally female-biased populations. However, the male-biased populations became more female. This provides concrete evidence that sex ratio change can occur during the gametophyte generation. Furthermore, sporophytes were produced only in female-biased populations. Eppley and coworkers suggested that females in male-biased populations may have invested energy in producing ramets, thus increasing the number of females. They noted that these results, combined with previous work, indicate that both the gametophyte and the sporophyte operate in determining the sex ratio in ***Ceratodon purpureus***. Yes, for than one mechanism exists in a single species!

Brzyski *et al.* (2013) cultivated ***Marchantia inflexa*** (Figure 9) 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.

Figure 9. ***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?

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

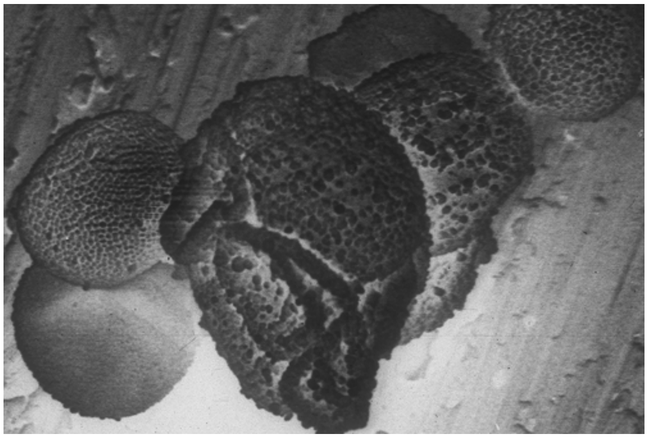

Figure 11. SEM image of spores of ***Fontinalis squamosa*** showing abortion of two spores in the tetrad. Photo by Janice Glime.

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 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).

It is likely that both phylogenetic history and extreme environments play a role in determining sex ratio, depending on the species and location. Stark *et al.* (2001) provide supporting data that the greatest departure from a 1:1 sex ratio in mosses is seen in the extreme desert environment (high temperatures and extreme drought) and always leads to male rarity. In fact, there are four species in the Mojave desert that are known there only from females (Llo Stark, pers. comm. 28 March 2023).

In ***Marchantia inflexa*** (Figure 9), 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 9) 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 9) 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 9) 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 9), 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 any 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 pressure, based on available moisture, against males in some environments and that females are more tolerant of exposed, desiccating conditions.

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

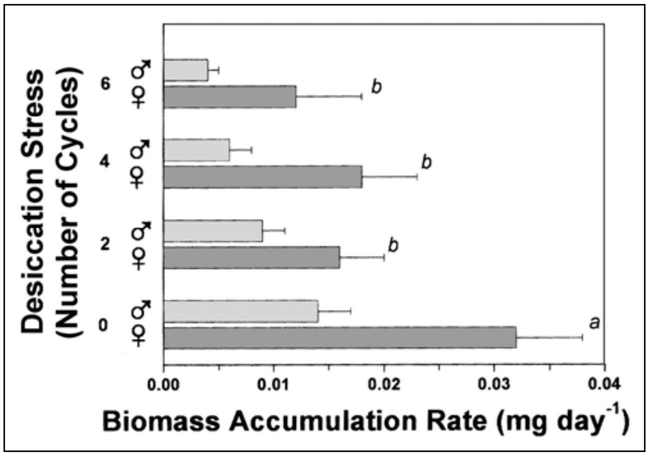

Figure 16. Effect of varying numbers of desiccation cycles on biomass accumulation rates in males and females of ***Syntrichia caninervis*** leaves. Values are means (n=20) ± 1 SE. Cycles with different letters are significantly different ($P<0.05$, Tukey's multiple comparison). Graph modified from Stark *et al.* (2005).

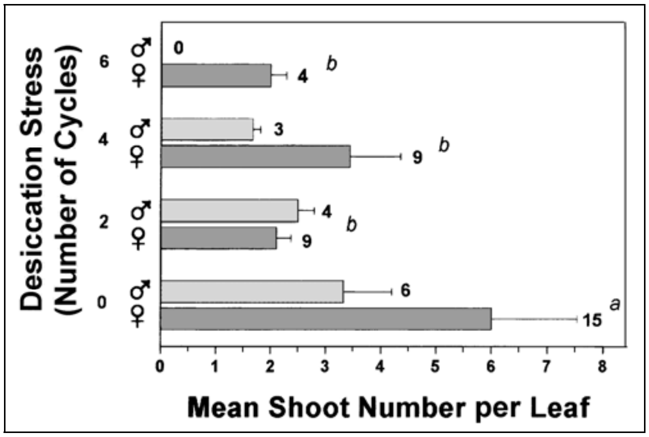

Figure 17. Shoot production from regenerating leaves of males and females of ***Syntrichia caninervis*** subjected to varying numbers of desiccation cycles. Values are means (n=20) ± 1 SE. Cycles with different letters are significantly different ($P<0.05$, Tukey's multiple comparison); data were log-transformed 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 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.

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.

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

Fisher (2011) examined differences between sex ratios at the edges vs the centers of distributions in ***Syrrhopodon involutus*** (Figure 21). In this species, she 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 ***Pseudocalliergon trifarium*** (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.

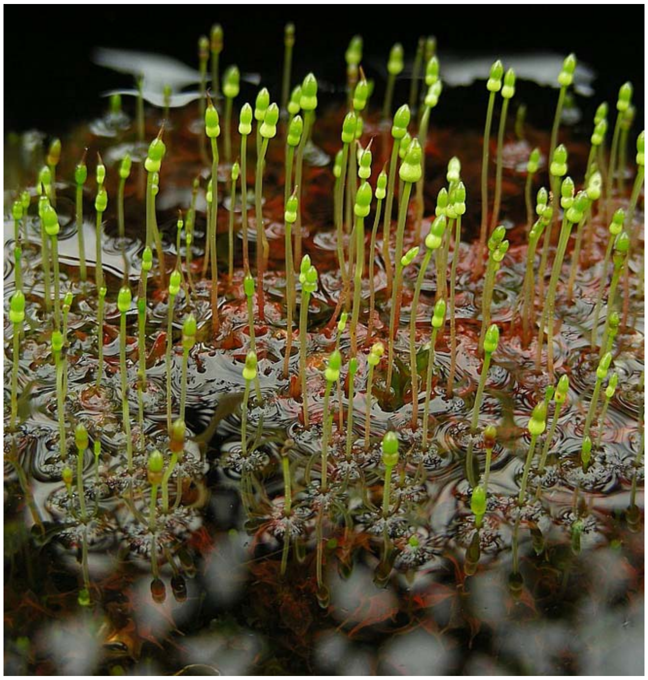

Figure 28. ***Splachnum rubrum*** females with young sporophytes. Photo by Michael Lüth, with permission.

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

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 37. ***Dicranum polysetum*** tomentum and innovations. Photo by Walter Obermayer, with permission.

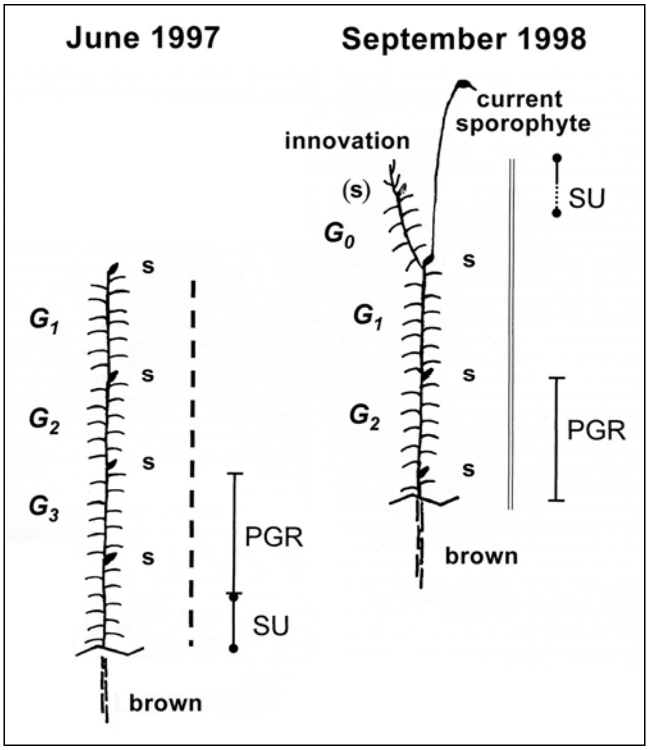

Figure 38. Representation of unbranched plant of ***Dicranum polysetum*** indicating locations of shoot sections and reproductive structures. G_x indicates annual growth interval, with G_0 indicating current year's growth as innovation. Note that the innovation is just below the apex where the sporophyte 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 43. ***Zygodon viridissimus*** var ***viridissimus*** with capsules. Photo by David T. Holyoak, 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 scale-like 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.

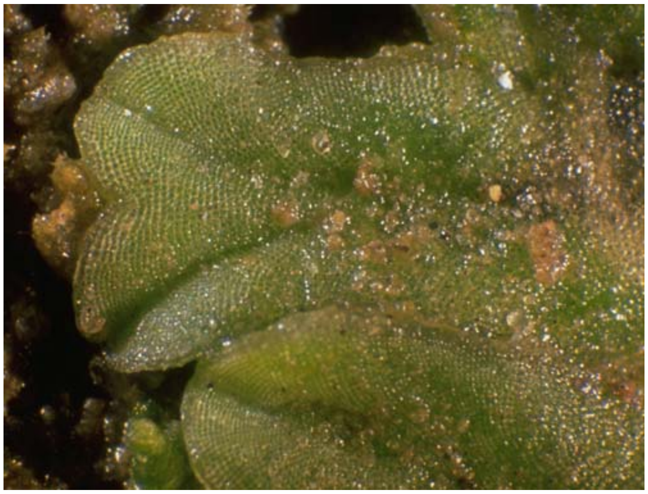

Figure 44. ***Riccia glauca*** showing apices where growth occurs (at end of rib). Photo by Jan-Peter Frahm, with permission.

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

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."

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

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 49. ***Syntrichia princeps***, a species that exhibits protogyny. Photo by Jan-Peter Frahm, 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. Stark *et al.* (1998) also report a threshold size for sex expression in ***Syntrichia caninervis*** at 2.1 mm height; this usually is reached at about 6 years. 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 Llo Stark.

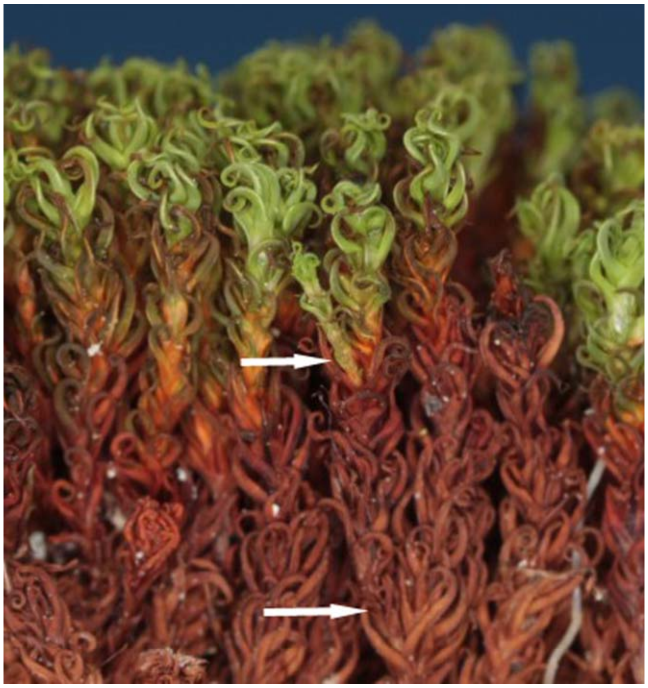

Figure 55. ***Bryoerythrophyllum rubrum* ramets**. Examples of branching is indicated by arrows. Photo by Jan-Peter Frahm, with permission.

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.

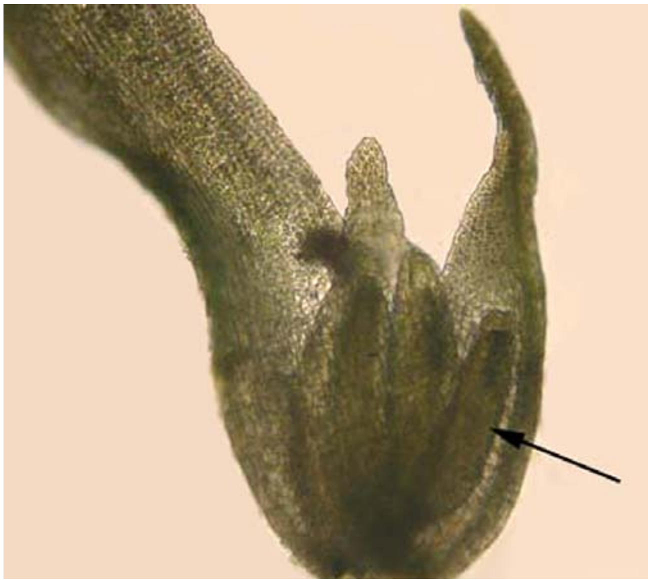

Figure 57. **Perigonial leaves** and antheridia of ***Diphyscium foliosum***. Photo from Botany 321 website at the University of British Columbia, with permission.

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

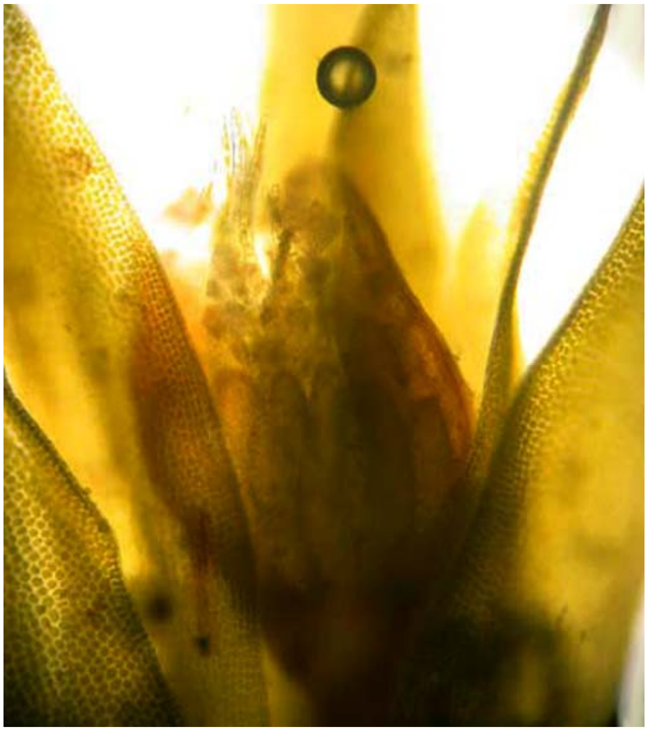

Figure 63. ***Tetraphis pellucida*** antheridia. Photo from Botany Department UBC, with permission.

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

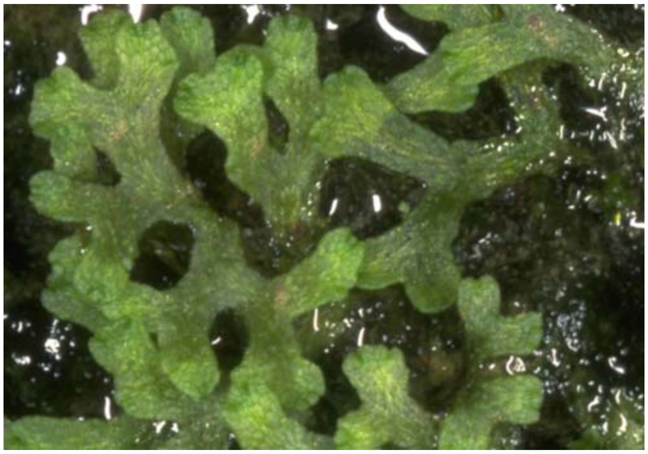

Figure 65. ***Riccia rhenana***, a species for which sexual expression is not affected by nitrates. Photo by Jan-Peter Frahm, with permission.

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 9) 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 9), 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.

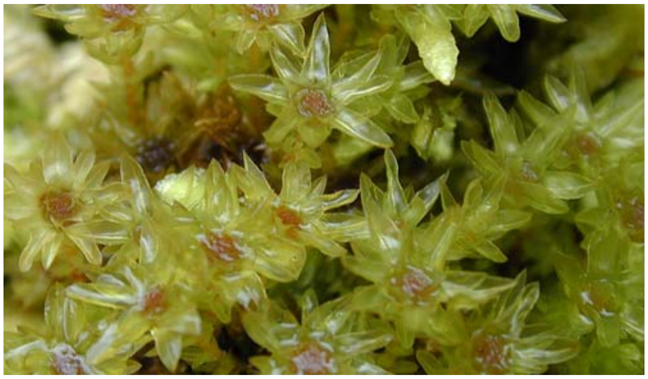

Figure 72. ***Mnium hornum*** males at Bretagne, France. Photo by Michael Lüth, with permission.

Figure 73. ***Plagiomnium undulatum***, 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.

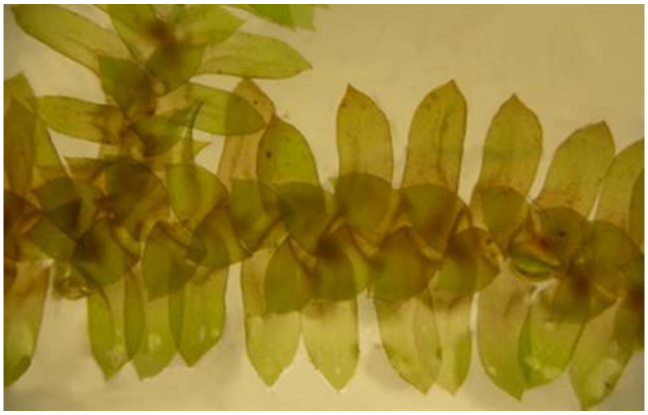

Figure 76. ***Crossomitrium patrisiae*** habit in Costa Rica. Photo by Michaela Sonnleitner, with permission.

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 9) 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 9) 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.

But even when sex is expressed in plants within proximal distance suitable for fertilization, that sporophyte production might not occur (Bisang & Hedenäs 2008). This is the case in ***Pseudocalliergon trifarium***, based on transplantation experiments. Even when the archegonium was present, it was never swollen, and no sporophytes became evident. Instead, the archegonia withered. Although ***Rhytidiadelphus triquetrus*** had sporophytes in 100% of the transplant plots that made both sexes available, ***Abietinella abietina*** produced them in only 41% of the plots, and ***Pseudocalliergon trifarium*** in none! Although we can postulate potential causes for the lack of sporophyte development, we lack the kind of evidence needed to support such hypotheses.

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?

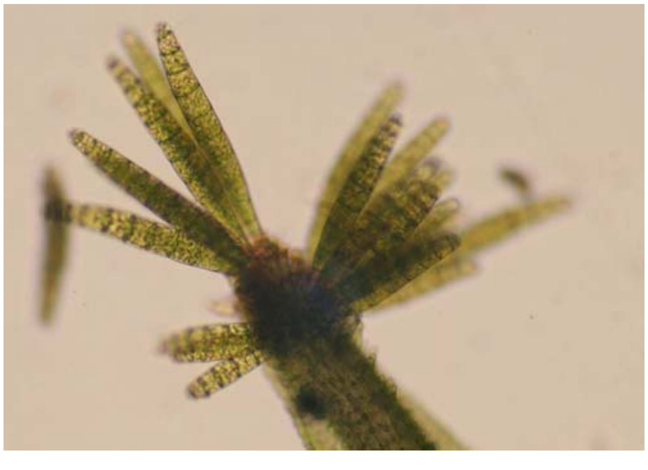

Figure 79. ***Calymperes erosum*** with gemmae on the leaf tip. Photo by Li Zhang, with permission.

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.

Protonemal gemmae predominate among the **Pottiaceae**, even when the plants are expressing gametangia. ***Dicranella heteromalla*** produces chloronemal gemmae with well defined tmema cells (Duckett & Matcham 1995). The tiny ***Ephemeropsis*** produces protonemal gemmae on its leaf substrate (Pressel & Duckett 2009). Duckett *et al.* (2004) found propagules on protonemata in species where they were previously unknown, including ***Ditrichum cornubicum***, ***Saelania glaucescens***, ***Seligeria carniolica***, and ***Zygodon gracilis***. And the aquatic moss ***Fontinalis antipyretica*** produces protonemal gemmae (Ares *et al.* 2014).

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

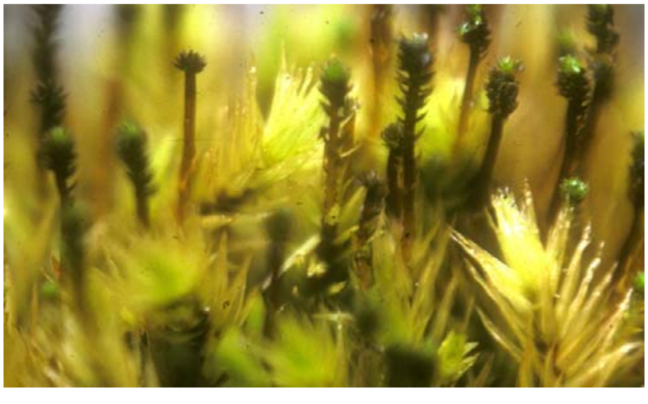

Figure 82. ***Aulacomnium palustre*** with brood bodies. Photo by Zen Iwatsuki, with permission.

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).

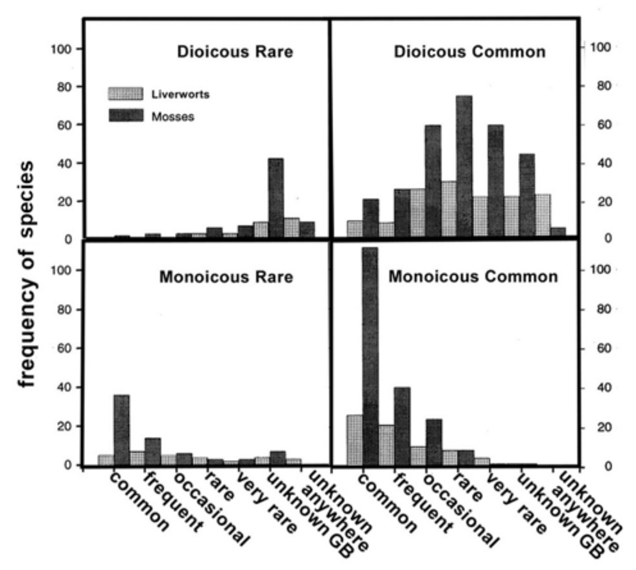

Figure 85. Comparison of frequencies (seven classes) of sporophyte production for mosses and liverworts in four sexuality groups within Britain. Modified from Laaka-Lindberg *et al.* 2000.

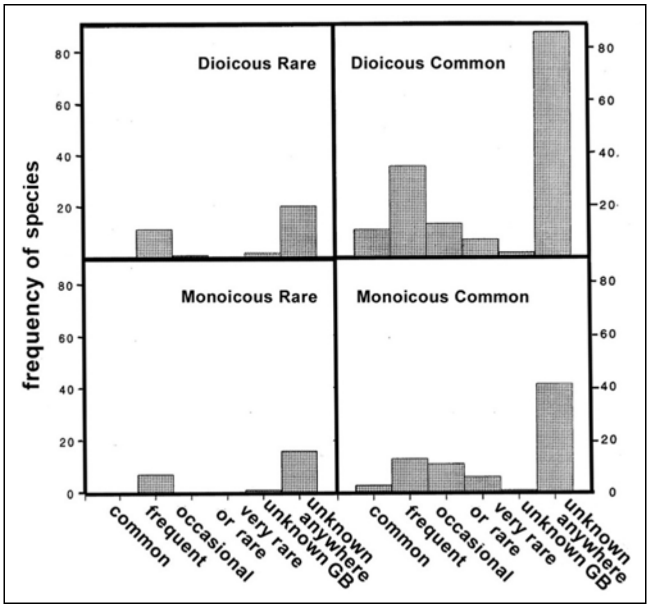

Figure 86. Comparison of frequencies (six classes, rare and very rare combined) of asexual reproductive structures for liverworts in four sexuality groups within Britain. Modified from Laaka-Lindberg *et al.* 2000.

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.

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