ORIGINAL ARTICLE

Self-fertilization in mosses: a comparison of heterozygote deficiency between species with combined versus separate sexes

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Self-fertilization is a key difference of adaptive significance between species with combined versus separate sexes. In haploid-dominant species such as mosses and ferns, species with either combined or separate sexes (monoicous and dioicous, respectively) have the potential to self-fertilize (intergametophytic selfing), but being monoicous allows an additional mode of selfing (intragametophytic selfing). We used allozyme electrophoresis to estimate deviations from expected levels of heterozygosity under Hardy–Weinberg equilibrium to infer selfing rates in 10 moss species from 36 New Zealand populations. We found that while there were deficiencies of heterozygotes compared to expectation in both monoicous and dioicous mosses, monoicous species had significantly higher levels of heterozygote deficiency than dioicous species ($F_{IS} = 0.89 \pm 0.12$ and 0.41 ± 0.11, respectively). Estimated selfing rates suggest that selfing occurs frequently in monoicous populations, and rarely in dioicous populations. However, in two dioicous species (*Polytrichadelphus magellanicus* and *Breutelia pendula*), we found significant indications of mixed mating or biparental inbreeding in a handful of populations. These data provide the first analysis of heterozygote deficiency and selfing among haploid-dominant species with breeding system variation, and we discuss our results with respect to the consequences of inbreeding depression and the evolution of breeding systems.
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Introduction

Breeding systems influence patterns of genetic diversity because they govern the transmission of genes between generations. The breeding systems that offer the starkest contrasts are those with organisms expressing both sex functions in an individual (hermaphroditism) versus those with only a single sex function, as hermaphrodites have the potential to self-fertilize. Population genetic theory predicts that selfing may be selected against because it increases levels of homozygosity in offspring, which in turn, allows the expression of recessive, deleterious alleles (Fisher, 1949; Wright, 1965; Nei *et al.*, 1975; Charlesworth and Charlesworth, 1998). On the other hand, once deleterious alleles are purged from the genome, selfing can be adaptive because a selfer can fertilize both its own eggs and the eggs of others in the population (Fisher, 1949). Theoretical models predict that both selfing and outcrossing are stable states, depending on levels of inbreeding depression (Lande and Schemske, 1985), and general surveys in angiosperms reveal that both complete selfing and complete outcrossing commonly occur (Barrett and Eckert, 1990). However,

the distribution of selfing rates in other taxa with breeding system variation is less known (e.g. Jarne and Charlesworth, 1993; Carlon, 1999).

The consequences of selfing in populations will depend not only on the breeding system but also on whether the dominant life stage of the organism is haploid and/or diploid (Hedrick, 1987a, b; Otto and Marks, 1996). In animals and seed plants the dominant life stage is diploid (referred to as a sporophyte in plants) and can have either combined or separate sexes while in many nonseed plants, such as some ferns and bryophytes, variation in sexual form occurs at the haploid stage (gametophyte). Whereas selfing in diploid organisms results in a 50% reduction in heterozygosity, in haploid organisms, selfing can occur in more than one fashion and can result in more than a 50% reduction in heterozygosity (Klekowski, 1979). Mating between gametes from different haploid individuals produced from the same diploid parent (intergametophytic selfing) results in a 50% reduction in homozygosity, which is equivalent to selfing in animals and seed plants. In contrast, mating between gametes produced from the same haploid individual (intragametophytic selfing) results in complete homozygosity in a single generation (Hedrick, 1987a).

Several methods exist to estimate levels of selfing in homosporous ferns and mosses. Hedrick (1987b) showed that if inbreeding equilibrium is assumed (e.g. selfing is constant between generations), then the two different

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types of selfing can be estimated from:

$$F_e = \frac{S + 2S_I}{2 - S} \quad (1)$$

where F_e is the equilibrium inbreeding coefficient, S is the proportion of the progeny in a population produced by intergametophytic selfing and S_I is the proportion produced by intragametophytic selfing. Thus, if the level of selfing within gametophytes (S_I) is assumed to be zero (e.g. as in dioicous mosses), the above equation can be rearranged to estimate selfing between gametophytes (S):

$$S = \frac{2F_e}{1 + F_e} \quad (2)$$

which is analogous to selfing in seed plants (Wright, 1968). Similarly, if there is no selfing between gametophytes then the level of selfing within gametophytes is:

$$S_I = F_e \quad (3)$$

(see McCauley *et al.*, 1985; Holsinger, 1987). As intragametophytic selfing results in equilibrium proportions in only one generation (McCauley *et al.*, 1985), the assumption of equilibrium conditions for Eq. (3) will always apply. In this way, assuming the absence of other factors (e.g. no selection, genetic drift or microspatial population structuring), Wright's fixation index (F_{IS}) is equal to the inbreeding coefficient (F_e) and thus can be used to estimate an amalgam of both intergametophytic and intragametophytic selfing in haploid-dominant species with combined sexes and intergametophytic selfing in haploid-dominant species with separate sexes. While many studies have used F_{IS} as an estimate of selfing in homosporous ferns (by assuming that intergametophytic selfing is not present; for example McCauley *et al.*, 1985; Holsinger, 1987), in populations with significant levels of heterozygosity in two unlinked loci, Ritland's (1990) maximum likelihood method can be used to estimate both types of selfing. Low levels of heterozygosity are characteristic of many populations of mosses (Shaw, 2000), and therefore this method has only limited application.

While some data exist on levels of average genetic variation in ferns and mosses (McCauley *et al.*, 1985; Shaw, 1991; Shaw, 2000) and on levels of intragametophytic selfing in ferns (McCauley *et al.*, 1985; Holsinger, 1987; Ritland *et al.*, 1990; Soltis and Soltis, 1992), there is a paucity of data examining how variation in breeding system influences deviations from expected levels of heterozygosity or other measures of self-fertilization rates at the diploid stage of haploid-dominant species (see Wyatt and Anderson, 1984; Wyatt, 1994; Shaw, 2000). In particular, it is important to compare selfing rates between populations with combined and separate sexes in haploid-dominant species, as individuals with combined sexes can experience both inter- and intragametophytic selfing, whereas individuals with only one sex function can only engage in intergametophytic selfing (for review see Wyatt and Anderson, 1984). We are unaware of any studies that have made this comparison, despite the important ecological and genetic consequences caused by differential levels of inbreeding depression and genetic diversity in natural populations. In this study we compared deviations from expected levels of heterozygosity in five moss species with combined sexes (monoicous) and five with separate sexes (dioicous). Specifically, we used allozyme markers to estimate Wright's coefficient of inbreeding (F_{IS}) in order to (1) test the hypothesis that populations of monoicous and dioicous mosses differ significantly in the amount they deviate from expected levels of heterozygosity, with dioicous populations predicted to have an excess of heterozygotes compared with monoicous populations due to lower levels of selfing and (2) to generate estimates of self-fertilization rates in species of mosses with monoicous and dioicous breeding systems.

Materials and methods

Population sampling
We sampled 35 moss species from New Zealand between December 2003 and July 2005. Population coordinates are available from the authors on request. From these species we selected 10 study species (five monoicous and five dioicous) that had: (1) three or more known populations containing sporophytes, and (2) sporophytes showing polymorphism at *PGM* or *PGI* allozyme loci either within or between populations. We collected at least 25 sporophytes from each population, unless a population had fewer than 25 individuals (which was true for three populations; Table 1), in which case we collected the entire population. If a population was not discrete, and therefore did not have distinct edges, for instance, a species that occurred continuously along a roadside, we sampled sporophytes for a subsection stretching approximately 100 m. To increase the number of sampled genets, we maximized the distance between sampled sporophytes within each population or subsection of nondiscrete populations. Although genetic variation can be low in many moss gametophytic populations due to gametophytic clonal growth (Shaw, 2000), this did not constrain our ability to sample large numbers of distinct sporophytic genets in such populations because by definition each sporophyte is a separate genet created by a single fertilization event and cannot spread vegetatively. Sporophyte samples were stored at 4°C and used for allozyme electrophoresis within 3 weeks.

Allozyme electrophoresis
We used starch gel electrophoresis to screen sporophyte tissue of all species for variation at 16 allozyme markers. Sporophyte capsules were ground in a drop of extraction buffer (100 ml 0.1 M Tris-HCl (pH 7.5), 2 drops (0.2%) 2-mercaptoethanol, 0.04 g ethylenediamine-N,N,N',N'-tetraacetic acid (EDTA)-3Na salt, 0.23 g 10 mM MgCl$_2$, 0.08 g 10 mM KCl, 0.1 ml (0.1%) Triton X-100 and 5 mg polyvinylpolypyrrolidone per 0.2 ml). A TEB electrode buffer system (0.5 M Tris, 0.016 M EDTA, 0.57 M Boric acid, pH 8.0 and diluted 1:10 for the gel buffer) was used to separate enzymes. Wicks containing extract were run in electrophoresis tanks on 2.5% starch gels for 20 min after which time the wicks were removed, and gels were run for a further 3 h at 180 V. Staining protocols followed Soltis and Soltis (1989).

We focused on *PGM* and *PGI* because all 10 species were variable and resolvable at one or two loci of *PGM*, and a subset of species were also variable for *PGI*. Variability at the other 14 allozyme markers was

Table 1 Summary of Wright's F_{IS} values for monoicous and dioicous mosses

Species (location)	Breeding system	Habitat	N	Loci (N)	F_{IS}	S	P (S=0)	P (S=1)
Acrocladium chlamydophyllum	Monoicous		87		**0.62**			
Harwood's Hole		Forest understorey	32	2	1	—	0.844	0.000*
Glenorchy		Forest understorey	41	1	−0.14	—	0.182	1.000
Papatowai		Forest understorey	14	1	1	—	0.359	1
Funaria hygrometrica	Monoicous		228		**0.98**			
Akatarawa Road		Roadside	35	3	0.95	—	0.000*	0.779
Khandallah		Garden	25	2	1	—	0.123	1
Otari Wilton's Bush		Pathway	25	2	0.93	—	0.004*	0.682
Queenstown		Garden	64	2	1	—	0.134	1
Victoria University		Greenhouse	51	2	1	—	0.365	1
Wairau Falls		Riverbank	28	2	1	—	0.001*	1
Rhynchostegium tenuifolium	Monoicous		81		**0.85**			
Belmont Regional Park		Forest understorey	27	1	1	—	0.000*	1
Orongorongo Valley		Forest understorey	25	1	1	—	0.357	1
Otari Wilton's Bush		Forest understorey	29	2	0.55	—	0.352	0.500
Syntrichia antartica	Monoicous		117		**0.83**			
Otari Wilton's Bush		Wall	36	3	1	—	0.365	1
Wellington Botanic Gardens		Wall	51	2	0.5	—	0.725	0.358
Orongorongo Valley		Coastal rocks	30	1	1	—	NA	NA
Tortula muralis	Monoicous		99		**0.92**			
Okato		Wall	27	2	0.77	—	0.003*	0.502
Khandallah		Wall	40	1	1	—	0.000*	1
Victoria University		Wall	32	2	1	—	0.679	1
Breutelia pendula	Dioicous		187		**0.29**			
Akatarawa Road		Roadside	29	3	0.69	0.82	0.695	0.000*
Bryophyte Gully		Roadside	41	2	1	1	0.721	0.497
Glenorchy		Forest gap	56	3	−1	−1	0.342	0.658
Kaitoke		Forest gap	61	2	0.45	0.62	0.000*	0.000*
Hypnodendron arcuatum	Dioicous		85		**0.17**			
Butterfly Creek		Forest understorey	30	3	0.93	0.96	0.217	0.684
Haast		Forest understorey	30	2	−0.05	−0.11	0.544	0.177
Kaitoke		Forest understorey	25	1	−0.37	−1.17	1	0.000*
Hypopterygium filiculaeforme	Dioicous		115		**0.62**			
Belmont Regional Park		River bank	88	2	0.49	0.66	0.319	0.507
Orongorongo Valley		Forest understorey	12	3	1	NA	NA	NA
Otari Wilton's Bush		River bank	15	2	0.38	0.55	0.906	0.000*
Macromitrium longipes	Dioicous		112		**0.16**			
Glenorchy		Forest understorey	27	2	0.36	0.53	0.994	0.000*
Kaitoke		Forest understorey	27	3	0.45	0.62	0.502	0.019*
Milford Sound		Forest understorey	33	2	0.06	0.11	0.493	0.000*
Washbourne Reserve		Forest understorey	25	2	−0.22	−0.56	0.841	0.064
Polytrichadelphus magellanicus	Dioicous		177		**0.97**			
Akatarawa Road		Roadside	44	3	1	1	0.001*	1
Bryophyte Gully		Roadside	27	3	0.98	0.98	0.079	0.798
Glenorchy		Forest gap	81	3	1	1	0.000*	0.186
Papatowai		Garden	25	3	0.91	0.95	0.359	1

Values of F_{IS} in bold are species means (total sample size is given italics). F_{IS} in monoicous mosses is an amalgam of intergametophytic and intragametophytic selfing. S is intergametophytic selfing in dioicous mosses; P-values followed by * are significant at the $\alpha = 0.05$ level.

generally low, and because of the small size of the sporophytes, resolution was often insufficient for scoring. To eliminate the confounding effects of fixed heterozygosity through polyploidy, for a subset of individuals for each species, we separately electrophoretically assayed the gametophyte (haploid) as well as the sporophyte (diploid) tissue. Using these results, we only scored a locus if a band was consistently absent from some gametophytic individuals. With this information, we were able to unam biguously score every individual as either a heterozygote or a homozygote.

Population statistics and estimation of selfing rates
For each population, we calculated Wright's inbreeding coefficient (F_{IS}), as a measure of divergence of the

proportion of heterozygotes from those expected under the assumptions of Hardy–Weinberg equilibrium, following Hartl and Clark (1997). Additionally, we estimated (F_{ST}) as a measure of population differentiation, using POPGENE (Yeh *et al.*, 1997).

There was insufficient heterozygosity in either the monoicous or dioicous species in this study to use existing algorithms that estimate levels of intergametophytic and intragametophytic selfing (Ritland *et al.*, 1990). Instead we used the indirect approach for measuring intragametophytic selfing rates described above (McCauley *et al.*, 1985; Hedrick, 1987b; Holsinger, 1987). For the monoicous mosses we examined F_{IS} as a measure of both intergametophytic and intragametophytic selfing, and for the dioicous species, selfing rates were estimated using Eq. (2) above. One monoicous and one dioicous population were monomorphic for all loci examined, and we were therefore unable to estimate selfing rates for these two populations. While these estimates of selfing are insightful for each breeding system, we did not test for differences between the two estimates because each mode of selfing has different genetic consequences.

Data analyses

To assess how breeding system (a fixed effect) and species (a random effect, nested in breeding system) affected F_{IS} we used a mixed model, nested analysis of co-variance ANCOVA (using EMS variance calculations because REML calculations violated model assumptions) using JMP (SAS Institute, 2004). As not all loci were resolvable and variable for all species, the number of loci that was scored was included as a covariate. The residuals were normally distributed, and variances were homogeneous. To analyse the effects of breeding system on F_{ST}, and to determine if either F_{IS} or F_{ST} differed from zero in either dioicous or monoicous mosses, we used a Student's *t*-test of species means, with no assumption of equal variances.

To assess whether estimated selfing rates differed from zero or one, we used a nonparametric bootstrapping technique described by Johnston *et al.* (1998) that examines whether levels of heterozygosity are significantly different than expectation. We chose to bootstrap levels of heterozygosity rather than selfing rates as resampling the observed genotypes could result in samples with no expected heterozygosity, and in these samples *S* could not be calculated. For each of 10 000 iterations we randomly sampled with replacement the observed genotypes of individuals in a population *N* times (where *N* is the number of individuals in a population). If a population was polymorphic for more than one locus, each locus was chosen with equal probability. From this resampled data, we used the allele frequency to calculate the expected heterozygosity. Hedrick (1987a) showed that the expected heterozygosity (assuming mating types are constant over time) for intergametophytic selfing (*S*) or intragametophytic selfing (S_I) should equal:

$$H_e = \frac{4pq(1 - S - S_I)}{2 - S}$$

If outcrossing equals one (i.e. there is no selfing of any type) under the expectation of random mating the observed heterozygosity should be equal to the expected heterozygosity. One-tailed *P*-values for the null hypothesis (selfing = 0) versus the alternative (selfing > 0) were calculated as the proportion of the iterations in which the observed heterozygosity of the original data were greater than or equal to the expected heterozygosity of the resampled genotypes.

In dioicous mosses, which have no intragametophytic selfing, if selfing rates were equal to one then H_e would equal zero. In monoicous mosses, if intragametophytic selfing was 1, intergametophytic selfing must be zero, and H_e would therefore also equal zero. For both breeding systems, to test whether *S* or S_I was significantly different from 1, we counted the proportion of the 10 000 iterations in which the heterozygosity of the resampled genotypes was zero.

Results

Population statistics
For the five monoicous species, the mean species inbreeding coefficients were extremely high: $F_{IS} = 0.89 \pm 0.12$ (s.e.), and ranged from 0.62 to 0.98 (Table 1). For the five dioicous species, mean inbreeding coefficients were much lower: $F_{IS} = 0.41 \pm 0.12$ (s.e.), ranging from 0.16 to 0.97. For both the monoicous and dioicous species, F_{IS} was significantly different from zero ($t_{1,18} = 13.75$; $P = 0.0002$ and $t_{1,18} = 2.83$; $P = 0.05$, for monoicous and dioicous species, respectively), indicating that the average estimated frequency of heterozygotes diverged significantly from Hardy–Weinberg expectations. In each case, significant, positive values indicate a deficiency of heterozygotes, or significant substructure within populations. Additionally, F_{IS} was significantly higher in monoicous species compared with dioicous species ($F_{1,24} = 6.53$, $P = 0.02$). There were no significant effects of species or the covariate, number of allozyme loci ($F_{8,24} = 0.89$, $P = 0.54$ and $F_{2,24} = 0.28$, $P = 0.76$, respectively).

On average, populations of the same species showed significant levels of genetic differentiation (Table 2). For both breeding systems, F_{ST} was significantly different from zero ($t_{1,4} = 3.20$, $P = 0.03$ and $t_{1,4} = 3.50$, $P = 0.03$, for monoicous and dioicous species, respectively). For monoicous mosses, mean $F_{ST} = 0.56 \pm 0.07$ (s.e.) with a range from 0.37 to 0.78, and for dioicous mosses, mean $F_{ST} = 0.55 \pm 0.02$ (s.e.) with a range from 0.09 to 0.61. F_{ST} did not vary significantly between monoicous and dioicous moss species ($t_{1,8} = -0.25$, $P = 0.81$).

Table 2 Wright's measurement of population divergence (F_{ST})

Breeding system	Species	F_{ST}
Monoicous	*Acrocladium chlamydophyllum*	0.4860
	Funaria hygrometrica	0.6015
	Rhynchostegium tenuifolium	0.3672
	Syntrichia antarctica	0.7840
	Tortula muralis	0.5829
Dioicous	*Breutelia pendula*	0.5584
	Hypnodendron arcuatum	0.6089
	Macromitrium longipes	0.5010
	Polytrichadelphus magellanicus	0.5498
	Hypopterygium filiculaeforme	0.0873

Selfing rates

For the monoicous mosses, the amalgam of intergametophytic selfing and intragametophytic selfing is equivalent to the F_{IS} already given (mean ± s.e. = 0.88 ± 0.12; Table 1). In 35% of monoicous populations (six of 17), selfing rates were significantly different from zero, and in only 6% of monoicous populations (one of 17) were selfing rates significantly different from one. Among populations of dioicous mosses, the intergametophytic selfing rate ranged from −1.17 to 1.00 (mean ± s.e. = 0.41 ± 0.17). In only 18% of dioicous populations (three of 17), intergametophytic selfing rates were significantly different from zero, while in 41% of dioicous populations (seven of the 17), selfing rates were significantly different from one (Table 1).

Discussion

Here, we show that moss species with combined sexes have levels of heterozygosity that diverge more from expectation than species with separate sexes. Only a handful of studies have used molecular markers to examine diploid moss tissue (Shaw, 2000); however, our result is consistent with two allozyme studies in mosses from which we calculated F_{IS}. Shaw (1999), using 14 allozyme loci, found that in the monoicous moss *Funaria hygrometrica*, sporophytes were never heterozygous, suggesting an F_{IS} value of one and high heterozygote deficiency. In contrast, Innes (1990) found exceedingly high levels of heterozygote excess in the dioicous moss *Polytrichum juniperinum*, using six allozyme loci ($F_{IS} = -0.10 \pm 0.16$; according to our calculations from the reported data). We infer from our results that the difference found by these researchers in the divergence of heterozygosity levels from expectations may be due to differences in breeding system.

Our results also suggest that levels of selfing may be high in monoicous mosses, and less so in many dioicous mosses. This finding is consistent with suggestions that monoicous mosses experience high levels of selfing (for reviews see Wyatt and Anderson, 1984; Wyatt, 1994). In dioicous populations, widespread spore dispersal may result in little spatial population structure and consequently low intergametophytic selfing (see Holsinger, 1987). If spore dispersal is widespread in mosses, negative F_{IS} values in outcrossing species (such as those measured by Innes (1990) in *Polytrichum* and occur in several of the dioicous populations we sampled; Table 1) are not unexpected because gametophytic variability can be exceedingly low – one to two clones per population being the norm in many species (Shaw, 2000). As a result, one haploid genet may be the maternal parent and the other the paternal parent of all sporophytic individuals in an outcrossing population, and if the parental pair shares few alleles because of long-distance spore dispersal, a negative population-wide F_{IS} will result. If widespread spore dispersal occurs in dioicous populations, then negative F_{IS} values will be common, intergametophytic selfing will be low, and mean values of F_{IS} for these dioicous species as a whole may be much lower than would be expected for monoicous species where even low levels of intragametophytic selfing occur.

Interestingly, in three populations of dioicous mosses from two species (*Polytrichadelphus magellanicus*; Polytrichaceae and *Breutelia pendula*; Bartramiaceae) selfing rates were significantly greater than zero. Thus, significant levels of intergametophytic selfing and/or biparental inbreeding do occur, and the breeding system of some populations of dioicous mosses may be more mixed mating than expected. This finding also suggests that it cannot be assumed that no intergametophytic selfing occurs in monoicous species: intergametophytic selfing depends on similar processes in both monoicous and dioicous mosses (spore dispersal and sperm movement), and it could be expected that levels may be similar in monoicous and dioicous mosses with similar life histories.

Differences in selfing rates are not the only explanation for significant differences in F_{IS} between breeding systems (Hartl and Clark, 1997). For example, small-scale spatial structure within the sampled populations may lead to an overall deficiency of heterozygotes, for example, through a Wahlund effect (Wahlund, 1928). Under this scenario, mating system differences may result in changes to the frequency of matings between close neighbours. This could occur, for example, if splash cups found in males of some dioicous species result in increased sperm dispersal distances. While we did not test for spatial structuring of genotypes within populations, F_{IS} did not differ between the dioicous species with splashcups (*P. magellanicus* and *B. pendula*) and those without (*Hypnodendron arcuatum*, *Macromitrium longipes*, and *Hypopterygium filiculaeforme*), suggesting that the morphological differences that are often associated with a dioicous mating system do not always influence small-scale population structuring.

Another explanation for the differences in F_{IS} between breeding systems may be a more frequent history of population bottlenecks in monoicous versus dioicous mosses. Indeed, the high variability in F_{IS} measured within species suggests that demographic differences between populations may contribute to our estimates of F_{IS}, as histories of population size fluctuations and extinction/recolonization events have theoretically and empirically been found to contribute to heterozygote deficiency in other species (e.g. Viard *et al.*, 1997; for a review see Hanski and Gaggiotti, 2004). Moreover, differences in selfing rates and population dynamics between breeding systems can be confounded as both high levels of selfing and a history of bottlenecks are likely to be characteristics of species with an ephemeral life-history (Baker, 1965; Hamrick and Godt, 1989; Schoen and Brown, 1991). The advantage to an individual in being able to self-fertilize during repeated colonization or recolonization events – and thus being able to establish a population on its own – is the most compelling current explanation for the evolution of self-compatibility in a wide-range of unrelated plant taxa (see Pannell and Barrett, 1998 for a review).

Determining the relative importance of selfing and population bottlenecks for reducing heterozygosity excess is difficult; however, some of our data may be informative. Measurements of F_{ST} in monoicous and dioicous species did not differ significantly despite a significant difference in F_{IS}. While the lack of differences found between the breeding systems could be an artefact of restricted sampling of allozyme loci (and indeed some of our estimates are higher than estimates between moss populations reported elsewhere; Shaw *et al.*, 1990), it may also be due to the similar, disturbed habitat occupied by

many of the monoicous and dioicous mosses we collected (e.g. monoicous *F. hygrometrica*, *Tortula muralis*, *Syntrichia antarctica*, and dioicous *P. magellanicus* and *B. pendula*). In more stable habitats, many mosses had few sporophytes, and thus we were unable to include these species in our collections. If monoicous species were more likely to undergo bottlenecks because of frequent colonization and/or recolonization events (see Freckleton and Watkinson, 2002; Hanski and Gaggiotti, 2004), and thus have higher levels of homozygosity, F_{ST} values would also be higher in monoicous species due to greater population differentiation (assuming that the number of individuals founding colonies is small relative to the number of migrants moving into extant populations; see Wade and McCauley, 1988). While values of F_{ST} will also increase in selfing populations (Wright, 1968; Hamrick and Godt, 1989), intragametophytic selfing will result in a complete loss of heterozygosity in one generation, and thus F_{IS} should increase at a much faster rate. Our finding of no significant difference in F_{ST} between breeding systems may indicate that the significant difference we measured in heterozygote deficiency between monoicous and dioicous mosses is not due primarily to histories of bottlenecks, but either to a combination of bottlenecking and selfing, or to selfing alone.

Differences in selfing rates between breeding systems should influence levels of inbreeding depression in the sporophyte. In monoicous bryophytes, complete homozygosity after one generation of intragametophytic selfing suggests that levels of inbreeding depression will be extremely high at this stage (Hedrick, 1987a), but purging of deleterious alleles will result in no or little inbreeding depression in future generations, and selfing would likely be favoured. Under such conditions, the presence of separate sexes (dioicy) may seem unintuitive. One possibility is that the maintenance of separate sexes in haploid-dominant species may depend on the levels of intergametophytic selfing. If intergametophytic selfing in dioicous mosses is low or nonexistent (which in this study may be as many as 41% of the sampled dioicous populations), the accumulation of deleterious alleles at the diploid stage would lead to a high cost of selfing (e.g. through sporophyte abortion) and may maintain separate sexes. If intergametophytic selfing is universally high, then purging of deleterious alleles would occur (as in selfing in seed plants) and monoicy should be selected over dioicy in these species. In this situation, inbreeding depression is unlikely to be the explanation for the selection and maintenance of separate sexes in mosses, and other possibilities may include genetic constraints (particularly if dioicy is the ancestral condition), sexual specialization (Charlesworth, 1999) or fitness advantages to heterozygosity (Wright, 1968; Roff, 2002).

The evolution of biphasic lifecycles may also be influenced by selfing rates and the differential expression of inbreeding depression at the haploid and diploid stage. Theoretical work has suggested that selection for haploid-dominant life stages should be correlated with selfing, as deleterious alleles are revealed and more quickly eliminated by selection. In contrast, diploidy masks mutations and diploid dominance should be selected in outcrossing species (Otto and Marks, 1996). If having separate sexes is primarily an outcrossing mechanism, it is predicted that this breeding system would correlate with haploid dominance. Interestingly, separate sexes occur in 58% of moss species (Wyatt and Anderson, 1984), compared to approximately 10% of angiosperm species (Geber et al., 1999), which is contrary to these predictions.

Breeding systems in haploid-dominant organisms are almost unknown, and yet may provide information on the evolution of diploidy from haploidy and may also provide a context to test theoretical models of breeding system evolution based on angiosperms (Lloyd, 1975; Charlesworth and Charlesworth, 1978). Measurements of selfing rates within and between gametophytes are needed, and algorithms exist to distinguish the two (Ritland et al., 1990). At present, estimates have been restricted by a lack of heterozygosity at allozyme loci, especially in monoicous populations. Microsatellite data may overcome these problems.

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