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SPOROPHYTIC INBREEDING DEPRESSION IN MOSSES OCCURS IN A SPECIES WITH SEPARATE SEXES BUT NOT IN A SPECIES WITH COMBINED SEXES[1]

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Inbreeding depression is a critical factor countering the evolution of inbreeding and thus potentially shaping the evolution of plant sexual systems. Current theory predicts that inbreeding depression could have important evolutionary consequences, even in haploid-dominant organisms. To date, no data have been reported on inbreeding depression in moss species. Here, we present data on the magnitude of inbreeding depression in sporophytic traits of moss species with contrasting breeding systems. In *Ceratodon purpureus* (Ditrichaceae), a moss species with separate sexes, self-fertilizations between sibling gametophytes (intergametophytic selfing) significantly reduced fitness in two of four traits quantified, with seta length and capsule length having inbreeding coefficients significantly different from zero, resulting in a cumulative inbreeding depression that was also significantly greater than zero ($\delta = 0.619 \pm 0.076$). In hermaphroditic *Funaria hygrometrica* (Funariaceae), there was no evidence of inbreeding depression in seta length, spore number, capsule mass, or capsule length resulting from sporophytes generated by self-fertilization within an individual (intragametophytic selfing), and cumulative inbreeding depression was also not different from zero ($\delta = 0.038 \pm 0.022$). These results provide evidence that, despite haploid dominance, inbreeding depression can be expressed at the diploid stage in mosses and may have implications for the evolution and maintenance of combined versus separate sexes in mosses.

Key words: *Ceratodon purpureus*; *Funaria hygrometrica*; gender dimorphism; inbreeding depression; moss; sexual system; sporophytes.

Inbreeding depression, the reduced fitness of inbred progeny relative to outcrossed progeny, has been considered one of the most important topics of research in evolutionary biology because of its implications for mating system evolution, conservation biology, animal and plant breeding, and levels of genetic variation (Crnokrak and Barrett, 2002). For example, inbreeding depression is thought to play a key role in opposing the evolution of self-fertilization in plants. Current theory predicts that selfing will result in initially high levels of inbreeding depression in a population, but once recessive deleterious alleles have been purged from the population by selection, there will be no cost to selfing, and selfing can become adaptive (Fisher, 1941; Lande and Schemske, 1985). Because of purging of recessive, deleterious alleles by selfing, there should be a negative relationship between the frequency of self-fertilization and the magnitude of inbreeding depression (Lande and Schemske, 1985). Indeed, empirical studies have demonstrated that in highly selfed populations, inbreeding depression is lower than in populations that are predominantly outcrossed (e.g., Johnston and Schoen, 1996; Crnokrak and Barrett, 2002).

Because of the potential impact of inbreeding depression on the evolution of self-fertilization, theoretical and empirical investigations suggest that inbreeding depression can be highly influential in the evolution of plant sexual systems. For example, separate sexes in seed plants (dioecy) has been considered to function primarily as an outcrossing mechanism to avoid strong inbreeding depression (Mather, 1940; Thomson and Barrett, 1981), while combined sexes, such as hermaphroditism, allows higher frequencies of self-fertilization, and thus hermaphrodites generally have lower levels of inbreeding depression (Stebbins, 1957; Husband and Schemske, 1996). Theoretical models of animals and diploid-dominant seed plants suggest that selection should favor separate sexes when outcrossing produces progeny that are twice as fit as progeny produced from self-fertilization (i.e., $\delta > 0.5$; Lande and Schemske, 1985). This hypothesis is supported by several empirical investigations (e.g., Kohn, 1988; Dorken et al., 2002; Ramsey et al., 2006).

Sexual systems of mosses vary widely, with over 50% of moss species having separate sexes (Wyatt, 1982; Une, 1986). Despite the potential importance of inbreeding depression for the evolution of moss sexual systems, to the best of our knowledge inbreeding depression has never been measured in mosses. Mosses have a haploid-dominant life cycle, and thus selection can act directly on the genotypes of haploid gametophytes (as opposed to seed plants where recessive deleterious alleles can be sheltered in heterozygote diploid sporophytes), thereby eliminating deleterious alleles before they can accumulate (Wyatt, 1982; Hedrick, 1987a; Charlesworth, 1991). If there is extensive overlap between haploid and diploid gene expression, accumulation of deleterious alleles in the diploid stage may also be reduced (Mulcahy, 1970; Shaw and Beer, 1997; Joseph and Kirkpatrick, 2004). Despite these arguments, however, inbreeding depression could occur in the diploid stage of the life cycle if recessive deleterious mutations are only mildly deleterious (Lande and Schemske, 1985; Charlesworth et al., 1990; Dudash and Carr, 1998), if gene

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duplication arising from polyploidy initially shelters deleterious alleles (Klekowski, 1970), or if certain genes are only rarely expressed or only expressed at the sporophyte stage (Klekowski, 1973a; Lloyd, 1974; Bull, 1978; Charlesworth and Charlesworth, 1992; Shaw and Beer, 1999). Indeed, inbreeding depression has been observed in haploid male wasps (Henter, 2003), and in several fern species, mutations in the gametophyte can persist and can cause severe inbreeding depression in subsequent sporophyte generations (e.g., Ganders, 1972; Klekowski, 1973a, b; Lloyd, 1974; Hedrick, 1987a).

Here, we used experimental fertilizations to measure inbreeding depression in two moss species with contrasting sexual systems. Intragametophytic selfing (mating between gametes produced from the same haploid individual) is unique to haploid-dominant species with combined sexes and produces 100% homozygous progeny after one generation (Klekowski, 1973b; Lloyd, 1974; Hedrick, 1987b). In species with high rates of intragametophytic selfing, selection should rapidly purge any mutations, and inbreeding depression should therefore be negligible (Ewing, 1977; Wyatt, 1982; Hedrick, 1987a; Stenøien and Såstad, 2001). In contrast, mosses with separate sexes (gametophyte-dioecy) can only undergo outcrossing or intergametophytic selfing (mating between gametes from different haploid individuals produced from the same diploid parent). Intergametophytic selfing results in a 50% reduction in heterozygosity (analogous to selfing in seed plants), and previously we demonstrated that mosses with separate sexes have lower levels of inbreeding than mosses with combined sexes (Eppley et al., 2007). Therefore, mutations could potentially accumulate in a moss population with separate sexes, resulting in the expression of significant inbreeding depression at the diploid stage. In this study, we examined the fitness of sporophytes resulting from intergametophytic selfing in gender-dimorphic *Ceratodon purpureus* and from intragametophytic selfing in cosexual *Funaria hygrometrica*. Specifically, we predicted that inbreeding depression at the diploid (sporophyte) stage should be higher in the gametophyte-dioecious mosses than in cosexual mosses because of masking of recessive mutations.

MATERIALS AND METHODS

Study species—Two ecologically similar species, *Funaria hygrometrica* Hedw. (Funariaceae) and *Ceratodon purpureus* (Hedw.) Brid. (Ditrichaceae), were used for this experiment. Both species are common cosmopolitan mosses that are often found in recently disturbed habitats. *Funaria hygrometrica* is widespread on bare soil, especially on the sites of old fires (Southorn, 1976). The gametophytes of *F. hygrometrica* are cosexual: the antheridia and archegonia are borne within the same gametophyte but are spatially separated from one another. Sporophytes are commonly observed, and the capsules are usually 2–4 mm long and produce between 30 000–600 000 wind-dispersed spores (Shaw, 1991). *Ceratodon purpureus* can be found in a broad range of habitats, including clay banks and various concrete surfaces. The gametophytes of *C. purpureus* are unisexual, and gametophytes either carry antheridia or archegonia. Unlike many gametophyte-dioecious mosses, *C. purpureus* commonly produces abundant sporophytes. The sporophyte capsules are approximately 3 mm long and produce between 40 000–180 000 spores (Beever et al., 1992; Shaw and Beer, 1999).

Plant culture—To establish greenhouse populations consisting of individuals derived from a single spore, we collected a minimum of 10 mature sporophyte capsules from five populations of *F. hygrometrica* and four populations of *C. purpureus* between November 2003 and December 2004. For both species, sporophyte capsules were collected far apart from one another to minimize genetic relatedness, thereby avoiding biparental inbreeding occurring in the experimental crosses. Voucher specimens are deposited in the herbarium at the Museum of New Zealand Te Papa Tongarewa, Wellington, New Zealand [*Ceratodon purpureus* (Hedwig) Bridel, M037877; *Funaria hygrometrica* Hedwig, M037879].

In the laboratory, each sporophyte was surface sterilized for 20 s in undiluted household bleach (6% sodium hypochlorite) under a dissecting microscope. Spores were dissected and immediately placed in a 3 : 1 solution of sterilized double distilled water (ddH_2O) and glycerol, then pipetted onto sterile plastic petri dishes filled with 1.2% agar. When the spores began to germinate (after 7 d), individual sporelings were transferred to a new petri dish containing non-nutrient based agar medium and grown in a temperature controlled room on a (8 : 16) night/day rotation at 18°C under fluorescent lights. When gametophyte development was obvious, they were placed onto individual plastic containers containing a 50 : 50 mixture of potting mix and propagation sand.

Genetic screening—Genetic markers were required to determine selfed and outcrossed sporophytes in cosexual *F. hygrometrica*. Gametophytes were scored for the allozyme marker phosphoglucomutase (*PGM*) because prior screening revealed allelic variation in all *F. hygrometrica* populations. Three alleles were detected in the Akatarawa Road population, while all remaining populations had two. Gametophyte tissue was ground in one drop of extraction buffer (100 mL 0.1 M Tris-HCL [pH 7.5], two drops 2-mercaptoethanol, 0.04 g EDTA-3Na salt, 0.23 g $MgCl_2$, 0.08 g KCl, 0.1 mL Triton X-100, and 5 mg polyvinylpolypyrrolidone per 0.2 ml). A TBE 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 12.5% starch gels; after 20 min, the wicks were removed from the gels, and the gels were run for a further 3 h at 180 V. The starch gels were stained for enzyme activity using recipes in Soltis and Soltis (1989).

Experimental fertilizations—For *F. hygrometrica*, once the cultivated gametophytes had developed sufficient leafy tissue, three or four leaves were removed from each gametophyte and genotyped using *PGM*. In total, 1420 crosses were established by growing two gametophytes with separate segregating *PGM* alleles that originated from separate sporophytes in the same container. In this way, any sporophyte with a heterozygous phenotype at the *PGM* locus must have resulted from an outcrossing event, while sporophytes homozygous at that marker must be self-fertilized (with an inbreeding coefficient of $F = 1$). Because none of the original sporophytes was heterozygous (i.e., the haploid gametophytes produced from one sporophyte always had the same allozyme genotype), it was not possible to use this method to produce sporophytes resulting from intergametophytic self-fertilizations (i.e., $F = 0.5$), and so the results of this type of cross are not presented for this species. Genetic markers were required to identify selfing and outcrossing in *F. hygrometrica,* and the majority of crosses were made between populations because there was insufficient within-population allelic diversity. From the 1420 *F. hygrometrica* capsules screened, 20 outcrossed sporophytes (approx. 0.01%) were found. Of these, 16 of the outcrossed sporophytes were the result of between-population crosses, while four were from separate within-population crosses. Student's *t* tests did not reveal a difference in any of the fitness traits measured for outcrossed sporophytes resulting from between- or within-population crosses (results not shown), and so all crosses were retained in the analysis.

For *C. purpureus,* outcrossed sporophytes were produced by placing gametophytes from different sporophytes together, using the same method as for *F. hygrometrica* (see previous paragraph). In total 155 crosses were established, with the expectation that approximately 50% of these would be pairs of male and female gametophytes. Crosses were preferentially conducted using sporophytes from within a population, although one cross that was harvested came from a cross between populations. The fitness values of the sporophyte resulting from this cross was not an outlier in any of the analyses and was retained in the sample. In *C. purpureus*, only intergametophytic selfing (inbreeding coefficient $F = 0.5$) is possible, and self-fertilizations were induced by growing two gametophytes derived from the same parent sporophyte in a single pot. To ensure that no intragametophytic selfing or contamination occurred during the experiment, we also established 35 control treatments by growing a single gametophyte in a container; none of these gametophytes produced sporophytes. Allozyme screening was not necessary for *C. purpureus*

because any resulting sporophytes must have come from either an outcrossing event or intergametophytic selfing.

The gametophyte pairs were grown on a medium consisting of a 50 : 50 mixture of potting mix and propagation sand inside six-celled pots (cell dimensions: $4 \times 5 \times 5$ cm). An inverted clear plastic container was placed over the top of the pairs to prevent contamination by neighboring gametophytes or airborne spores. Because of the importance of water for male gamete dispersal for both moss species, each cross was misted daily with a watering gun to maximize the chance of fertilization.

Data collection—To control for differences in fitness estimations that could be biased by sampling sporophytes at different developmental stages, we harvested each sporophyte and seta from the experimental population when the calyptra had naturally fallen from the sporophyte capsule (progress of the capsules was monitored daily). Once collected, the seta and the sporophyte capsule length were measured using digital calipers, capsule weight was recorded using an analytical balance (Mettler-Toledo, Columbus, Ohio, USA brand series PJ-300), and the reproductive output was estimated as the number of spores produced by each sporophyte capsule. Spores were dissected from each capsule under a stereomicroscope. Care was taken to remove all the spores from the capsules, which were stored for counting in a solution of 0.4 mL water and 0.1 mL Tween 20. In *F. hygrometrica*, the capsule tissue was retained for allozyme electrophoresis to identify outcrossed sporophytes. Following the identification of selfed and outcrossed samples (see next paragraph), samples were randomized and coded to ensure no knowledge of the sporophytic origin (i.e., whether selfed or outcrossed). Spores were counted using a hemacytometer, and a mean of six replicates from each tube was taken.

For identifying selfed and outcrossed progeny of *F. hygrometrica,* empty sporophyte capsules were placed on ice and scored using allozyme electrophoresis following the previously described protocol, then staining for *PGM*. Double-banded (heterozygous) allozyme phenotypes were considered to result from outcrossing, whereas single-banded (homozygous) phenotypes were considered to result from selfing.

Statistical analyses—For all analyses, fitness values were log-transformed to assess whether the ratio of fitness responses at different levels of F differed between species. All data were log-transformed because log-transformation of fitness data converts a multiplicative model to an additive one, and this transformation allows the examination of whether levels of inbreeding depression differ among species (Johnston and Schoen, 1994). We used the coefficient of inbreeding (F) as a covariate because self-fertilizations were conducted between gametophytes for *C. purpureus* but within a gametophyte for *F. hygrometrica*. Because response variables were not independent (i.e., all four traits were recorded from the same individual), a multivariate analysis of variance (MANOVA) was initially used to conservatively test for the effects of F, species, and their interaction on seta length, capsule mass, capsule length, and spore number using the Wilks' lambda (λ) ratio (Tabachnick, 2001). The MANOVA was followed by an independent analysis of covariance (ANCOVA) for each fitness trait to identify the contribution of each response variable to the overall test of significance and to test the hypothesis for both species that the slope of F on each trait was significantly different from zero. All statistical analyses were performed in the statistical package R, version 2.3.1 (R Development Core Team, 2007).

The level of inbreeding depression (δ) in *C. purpureus* and *F. hygrometrica* was estimated as $\delta = 1 - w_s/w_x$; where w_s and w_x are the mean values of fitness of self-fertilized and outcrossed progeny, respectively, for each species. Cumulative inbreeding depression for these traits was estimated by multiplying seta and capsule length, capsule mass, and spore number for each cross type. Means and SE were obtained from the standard deviation of 1000 bootstrap replicates. This method of resampling repeatedly generates a 95% confidence interval using: $\delta \pm 1.96$ SE with the population mean of selfed and outcrossed sporophytes as the unit of resampling. The proportion of bootstrapped values from 1000 replicates that include zero was generated, which is approximately equivalent to a P value (Seber, 1973).

RESULTS

Multiple analyses of variance (MANOVA) revealed a significant interaction between F and species for the four log-transformed fitness values (df = 1, 92; Wilks' $\lambda = 0.864$; $P = 0.01$). Analysis of covariance revealed a significant interaction between F and species for log-transformed values of seta length and capsule length, but not capsule mass or spore number (Table 1; Fig. 1). In *F. hygrometrica*, the slope of the regression of the log of fitness on F was not significantly different from zero for any trait (seta length: $\beta = -0.02$, SE = 0.074, $t = -0.03$, $P = 0.975$; capsule length: $\beta = -0.061$, SE = 0.055, $t = -1.12$, $P = 0.26$; capsule mass: $\beta = 0.00$, SE = 0.0003, $t = 0.13$, $P = 0.89$; spore number: $\beta = 0.06$, SE = 0.49, $t = 0.128$, $P = 0.89$). In *C. purpureus*, in contrast, the slope was significantly different from zero for two of the four measured traits (seta length $\beta = -0.42$, SE = 0.126, $t = -3.30$, $P = 0.0014$; capsule length: $\beta = -0.369$, SE = 0.093, $t = -3.96$, $P < 0.001$; capsule mass: $\beta = -0.0001$, SE = 0.0005, $t = -0.251$, $P = 0.803$; spore number: $\beta = -0.317$, SE = 0.833, $t = -0.381$, $P = 0.898$).

TABLE 1. Summary of analyses of covariance (ANCOVA) for the effect of treatment (selfed vs. outcrossed diploid progeny) on the relative performance of four life history traits between cosexual (*Funaria hygrometrica; N* = 20 in each cross) and dioecious (*Ceratodon purpureus*; $N = 28$ in each cross) mosses. Fitness values were log-transformed. P values in bold are significant at the $\alpha = 0.05$ level.

Fitness component	Source of variation	df	F	P
Seta length	Species	1	11 416.78	**<0.001**
	F	1	2.73	0.101
	$F \times$ Species	1	8.15	**0.005**
	Error	92		
Capsule length	Species	1	2230.48	**<0.001**
	F	1	8.87	**0.003**
	$F \times$ Species	1	8.06	**0.006**
	Error	92		
Capsule mass	Species	1	161.31	**<0.001**
	F	1	0.0001	0.990
	$F \times$ Species	1	0.08	0.777
	Error	92		
Spore number	Species	1	2301.48	**<0.001**
	F	1	0.007	0.934
	$F \times$ Species	1	0.154	0.695
	Error	92		

There was evidence of significant inbreeding depression in gametophyte-dioecious *C. purpureus* but not in cosexual *F. hygrometrica*. In *F. hygrometrica*, the inbreeding coefficient (δ) was not significantly different from zero for all fitness traits (Table 2). *Ceratodon purpureus* showed significant evidence of inbreeding depression in two fitness traits, capsule length and seta length (Table 2). The total cumulative amount of inbreeding depression expressed in these traits was significantly different from zero for *C. purpureus* ($\delta = 0.619 \pm 0.076$) but not for *F. hygrometrica* ($\delta = 0.038 \pm 0.022$).

DISCUSSION

Inbreeding depression is a primary factor influencing levels of genetic variability and patterns of selection in populations, and in mosses, despite haploid dominance, the evolutionary consequences of inbreeding depression could be significant. Here, we provide evidence of significant inbreeding depression in the sporophytic generation of gametophyte-dominant dioecious *C. purpureus,* but not in gametophyte-cosexual *F. hygrometrica*. These data are the first to estimate inbreeding

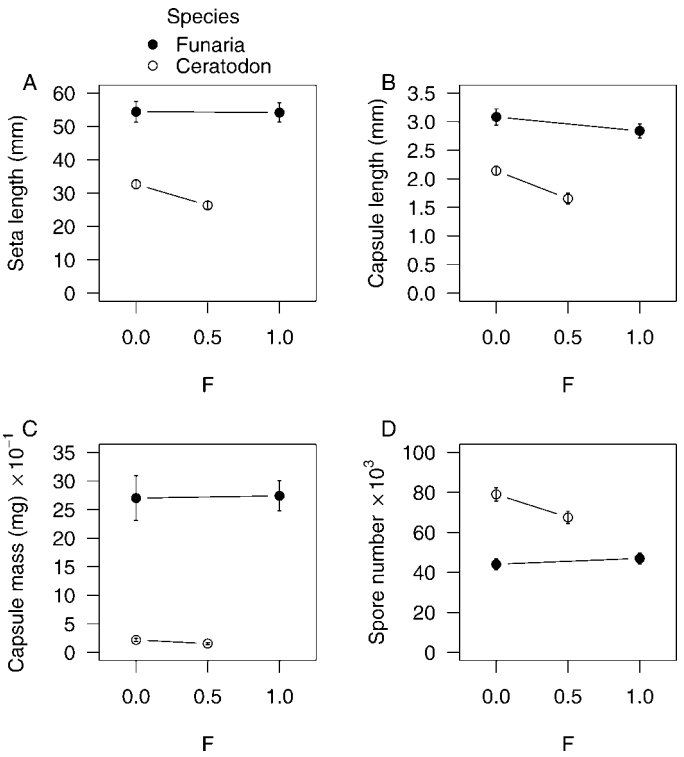

Fig. 1. Relations between coefficient of inbreeding F and relative performance of sporophytes in the cosexual moss *Funaria hygrometrica* and the dioecious moss *Ceratodon purpureus* based on four fitness traits: (A) seta length; (B) capsule length; (C) capsule mass; and (D) spore number. All values are means ± 1 SE.

depression in mosses with contrasting sexual systems, and although there are no other studies for comparison in mosses, these data are consistent with several empirical and theoretical studies that have estimated genetic load and inbreeding depression in other organisms with free-living haploid life stages (Lloyd, 1974; Hedrick, 1987a; Henter, 2003). Klekowski (1973b, 1982) reported significant genetic load in two species of ferns as a result of both intragametophytic and intergametophytic selfing. Similarly, a general survey of 18 fern species supported the hypothesis that predominantly outcrossed species have greater genetic loads than those that self-fertilize, either intra- or intergametophytically (Lloyd, 1974). While this difference is consistent with predictions that intragametophytic selfing in cosexual mosses should purge deleterious alleles, inbreeding depression can be influenced by many life history parameters. Next, we discuss potential differences that might contribute to differential expression of inbreeding depression and speculate on the role that inbreeding depression may play in the maintenance of combined or separate sexes.

Correlations with sexual system and inbreeding depression—Polyploidy and sexual system are correlated in many plant species (Lloyd, 1974; Miller and Venable, 2000; Charlesworth, 2001; Pannell et al., 2004), and in many species of mosses, allopolyploidy has been implicated in the evolution of combined sexes from separate sexes (Wyatt et al., 1988, 1992). The effect of polyploidy on inbreeding depression in mosses is likely to be intertwined with changes in the sexual system. Polyploidy is a mechanism by which genes for both male and female sex organs might be combined into one gametophytic individual, and depending on the pairing of chromosomes, gametophytes resulting from polyploidy should either segregate into hermaphroditic and unisexual progeny or completely hermaphroditic progeny (Smith, 1978). Self-fertilization of the resulting hermaphroditic gametophytes would provide a mechanism for the establishment of cosexual populations and as a consequence would lead to an almost total absence of genetic load (see Hedrick, 1987a). In contrast, when intragametophytic selfing is not involved, chromosome doubling may either raise or lower inbreeding depression. Theoretical work by Lande and Schemske (1985) showed that inbreeding depression in tetraploids should be between one half or equivalent to that of diploids, depending on the degree of recessivity of deleterious alleles, while Ronfort (1999) showed that inbreeding depression in diploids and autotetraploids depended on scenarios of dominance and selection coefficients. Experimental studies of diploid–polyploid species have revealed lower inbreeding depression in polyploidy lineages (Husband and Schemske, 1997; Rosquist, 2001), though more recent work suggests that polyploids may have inbreeding depression levels quite similar to diploids (see Galloway and Etterson, 2007). However, it is possible that the levels of inbreeding depression measured here may reflect differential changes in ploidy in the evolutionary history of the two species. *Funaria hygrometrica* has reported chromosome numbers of $n = 14$, 21, 28, 42, and 58 (Anderson, 1980), and recent polyploidy has played a role in its evolutionary history. In contrast, the most frequently reported haploid chromosome numbers in *C. purpureus* ($n = 13$) are consistent with other members of the genus (Fritsch, 1991), but chromosome numbers of $n = 6$ were reported in one population (Shaw and Beer, 1999), and so the history of polyploidy in this species is unknown.

In flowering plants, inbreeding depression is also correlated with plant stature, with smaller plants having lower inbreeding depression in early fitness measures than larger plants (Husband and Schemske, 1996). Recent models suggest that the biological reasons for this may be attributed to the number of mitotic cell divisions because plants do not separate germ line from soma and thus any mutations during the mitotic phase may become fixed into gametes (Morgan, 2001; Scofield and Schultz, 2006). This effect may be mitigated somewhat by selection against deleterious mutations at the cellular level (Klekowski, 1988; Otto and Orive, 1995). In mosses that are highly clonal, there may be an association between inbreeding depression and age, size, or propensity for asexual reproduction. Both species used in this study are weedy and were grown from single spores, so much of these differences have been reduced. However, further research into interactions of life history, mating system, and inbreeding depression is clearly warranted.

Because the moss species chosen for this study were common colonizers of disturbed environments, periodic bottlenecks experienced by these populations will also remove deleterious mutations (Lynch et al., 1995; Kirkpatrick and Jarne, 2000). Therefore, it is reasonable to assume that deleterious mutations in gender-dimorphic mosses that occupy similar habitats could frequently be eliminated. In gender-dimorphic mosses that are frequent colonizers, high levels of intergametophytic selfing should result in lower levels of deleterious mutations and low levels of inbreeding depression

TABLE 2. Mean inbreeding depression (δ), standard errors (SE), and *P* values (H_o: values are not significantly different from zero) for *Funaria hygrometrica* and *Ceratodon purpureus*. Means and standard errors were generated from 1000 bootstrap replications. *P* values in bold are significant at the $\alpha = 0.05$ level.

	F. hygrometrica			*C. purpureus*		
Fitness component	δ	SE	*P*	δ	SE	*P*
Seta length	−0.0004	0.076	0.947	0.193	0.047	**0.041**
Capsule length	0.078	0.057	0.689	0.227	0.0514	**0.006**
Capsule mass	−0.034	0.171	0.948	0.289	0.107	0.266
Spore number	−0.067	0.088	0.892	0.142	0.057	0.235
Cumulative inbreeding depression	0.038	0.222	0.897	0.619	0.076	<**0.001**

because of purging of deleterious alleles (Lande and Schemske, 1985; Hedrick, 1987a, b). Intergametophytic selfing is determined by the degree of population structuring and the proximity of related gametophytes as a result of the short distances that spores and sperm are dispersed (Solbrig, 1976; Clegg, 1980; Bisang et al., 2004; Taylor et al., 2005), and so may be higher in smaller populations. However, if inbreeding depression is caused by only mildly deleterious mutations, and if such mutations remain in the population because they are not easily purged (Charlesworth and Charlesworth, 1999), then partial purging of mutations could still cause populations to undergo low but significant levels of inbreeding (see Dudash and Carr, 1998).

Implications for the evolution of sexual systems—While other explanations for the differences in inbreeding depression exist, sexual system differences between the two species will likely change the frequency and history of inbreeding and hence the expression of inbreeding depression. In a study examining heterozygote deficiency in 36 populations from 10 moss species, Eppley et al. (2007) found evidence for frequent self-fertilization in approximately 35% of cosexual populations (six of 17) but in only 18% of gender-dimorphic populations (three of 17). Selfing in the cosexual species *F. hygrometrica* was shown to be approximately 98% ± 0.02 (SE; averaged across five populations). No estimates of selfing are currently available for *C. purpureus*, which further highlights the importance of quantifying outcrossing rates for a wide range of mosses. Providing such estimates will allow for a more thorough interpretation of the importance of selfing for influencing the genetic load of moss sporophytes.

The data from this study, however, provide us with enough information to suggest that the magnitudes of inbreeding depression in these species are at least consistent with stable evolutionary strategies for sexual system. According to classical theory of sexual systems evolution (Lloyd, 1975, 1976; Charlesworth and Charlesworth, 1978), if sex is controlled nuclearly and if there are no other factors influencing the evolution of dioecy, establishment of females in hermaphroditic populations requires that the product of the selfing rate and inbreeding depression (sδ) be greater than 0.5. For *F. hygrometrica*, selfing is estimated at 98% and thus $s\delta = 0.98 \times 0.038 = 0.037$. This result suggests that the low levels of inbreeding depression documented in *F. hygrometrica* are sufficient to explain why females cannot invade these populations and why these populations are evolutionarily stable as hermaphroditic entities.

On the other hand, theory predicts that outcrossing mechanisms, such as separate sexes, should be maintained if the inbreeding depression is greater than 0.5 (Lande and Schemske, 1985). This theoretical result was devised for the diploid stage (such as occurs in seed plants); however, in gametophyte-dioecious mosses, inbreeding between sibling gametophytes is genetically equivalent to selfing in seed plants, and so this prediction should be applicable for the maintenance of separate sexes in mosses. In *C. purpureus*, cumulative inbreeding depression was greater than 0.5, and so the consequences of inbreeding depression are severe. Thus, inbreeding depression may be important in the maintenance of separate sexes in mosses. In addition, it is likely that our estimate of inbreeding depression is an underestimate because other traits that were not measured also experience significant inbreeding depression. For example, Stark (2002a) showed significant variation in sporophyte abortion among families, which may result from inbreeding depression under stressful conditions. In the current study, sporophyte abortion was observed in several of the experimental treatments in both species but was not quantified. In addition, the benign environment experienced in greenhouse populations will also lower estimates of inbreeding depression (Dudash, 1990; Wolfe, 1993; Goodwillie, 2000). Selection for increased spore dispersal, which would reduce selfing between gametophytes, would be one consequence of such high levels of inbreeding depression.

If combined sexes are an inevitable consequence of polyploidy, then it is unlikely that inbreeding depression is a mechanism that completely prevents the evolution of combined sexes, because diploid progeny would be reproductively isolated from their haploid progenitors and selfing of surviving progeny would result in purging of genetic load (Klekowski, 1982). The evolutionary pathways between combined and separated sexes are unknown, but it is possible that even after purging by intragametophytic selfing, inbreeding depression may subsequently select for separate sexes. For example, spatial or temporal separation of the sexes is known in many cosexual moss species (Longton and Miles, 1982; Stark, 2002b), and these separations have often been interpreted as outcrossing mechanisms (Longton and Miles, 1982; Wyatt, 1994). Further estimates of selfing rates and inbreeding depression in other species of mosses are needed to fully test whether inbreeding depression can select or maintain separate sexes in mosses.

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