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Author(s): Alexander M. Gorischek, Michelle E. Afkhami, Elizabeth K. Seifert, and Jennifer A. Rudgers

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Fungal Symbionts as Manipulators of Plant Reproductive Biology

Alexander M. Gorischek,¹ Michelle E. Afkhami,² Elizabeth K. Seifert,¹ and Jennifer A. Rudgers^{1,3,*}

1. Department of Ecology and Evolutionary Biology, Rice University, Houston, Texas 77005; 2. Department of Evolution and Ecology, University of California, Davis, California 95616; 3. Department of Biology, University of New Mexico, Albuquerque, New Mexico 87131

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ABSTRACT: Symbioses have shaped the evolution of life, most notably through the fixation of heritable symbionts into organelles. The inheritance of symbionts promotes mutualism and fixation by coupling partner fitness. However, conflicts arise if symbionts are transmitted through only one sex and can shift host resources toward the sex through which they propagate. Such reproductive manipulators have been documented in animals with separate sexes but not in other phyla or sexual systems. Here we investigated whether the investment in male relative to female reproduction differed between hermaphroditic host plants with versus without a maternally inherited fungal symbiont. Plants with the fungus produced more seeds and less pollen than plants lacking the fungus, resulting in an ~40% shift in functional gender and a switch from male-biased to female-biased sex allocation. Given the ubiquity of endophytes in plants, reproductive manipulators of hermaphrodites may be widespread in nature.

Keywords: mutualism, parasitism, epichloae, sex allocation, sex ratio, *Elymus*.

Introduction

In sexually reproducing organisms, selection should favor the balance of investments between male and female lineages that maximizes long-term reproductive success (de Jong and Klinkhamer 2005; Sinclair et al. 2012). However, uniparentally inherited symbionts may shift this balance to their own advantage by increasing investment toward the sex through which they propagate (Hamilton 1967; Turelli 1994). This shift may occur despite selection for mutualism caused by the coupling of host and symbiont fitness during the process of vertical transmission (Bull and Rice 1991; Herre et al. 1999; Sachs et al. 2004). For example, a symbiont transmitted exclusively through the maternal host lineage could gain a fitness advantage by directing host investment toward female offspring and

away from male offspring. Conflict arises because host genes are passed equally through successful male and female offspring, while symbiont genes are passed only through female offspring. Such conflicts, manifested as male killing, cytoplasmic incompatibility, feminization of male embryos, and induction of parthenogenesis, have been documented in species with separate sexes (gonochorists), particularly arthropods and crustaceans that host maternally transmitted *Wolbachia* bacteria (Werren et al. 2008; Engelstadter and Hurst 2009). Reproductive manipulation of hermaphrodites, which have both male and female sex organs, is likely facilitated by the plasticity of parental investment in male versus female gametes but remains unexplored (Schärer 2009).

Symbioses with fungal endophytes are pervasive in plants, and uniparental transmission through the maternal lineage is common (Bacon and White 2000; Rodriguez et al. 2009). Fungal endophytes reside in aboveground plant tissues and can impart drought tolerance, resistance to herbivores and pathogens, and enhanced competitiveness (Cheplick and Faeth 2009; Schardl 2010). Endophytes confer these benefits to the host plant but might also increase their own fitness by manipulating host resources away from pollen and toward seeds. Therefore, we evaluated the reproductive investments of symbiotic and symbiont-free *Elymus virginicus* (Virginia wild rye, Poaceae), a perennial grass that is widespread in North America and commonly hosts the endophytic fungus *Epichloë elymi* (Rudgers et al. 2009; Saha et al. 2009).

Methods

Design

We compared reproductive investments among three groups of *Elymus virginicus* plants: symbiotic offspring of symbiotic parents (abbreviated “++,” $n = 84$), symbiont-free offspring of symbiotic parents produced by im-

* Corresponding author; e-mail: jrudgers@unm.edu.

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perfect vertical transmission of the endophyte (first-generation symbiont-free “+–,” $n = 37$), and offspring of host lineages lacking the endophyte in the native field population (multigeneration symbiont-free “––,” $n = 48$).

Parental Generation

In July 2006, we collected seeds from 60 naturally occurring individuals of *E. virginicus* at the Stephen F. Austin Experimental Forest (Nacogdoches, TX), where the endophyte occurs in ~25% of host plant individuals. Seeds were germinated in wet sand at 5°C, then transplanted as seedlings into 115-mL pots (Conetainers, Stuewe and Sons, Canby, OR) filled with ProMix BX potting soil (Premier Horticulture, Quakertown, PA). We grew these plants in the greenhouse (~23°C, daily watering) for ~3 months, then produced clones of these plants consisting of 1–3 tillers each. On November 9 and 10, 2007, we planted 100% symbiotic or symbiont-free populations into a common garden at the forest. Each population consisted of 20 unique plant genotypes ($n = 5$ populations per endophyte status; 200 plants total).

Greenhouse Experiment

From June 26 to August 6, 2008, we collected seeds from experimental populations. Seeds were germinated in wet sand at 5°C, and seedlings were transplanted into 1-L pots of soil. We grew these progeny of our experimental field populations in the greenhouse (~23°C, daily watering) and treated them monthly with 2% insecticidal soap to control aphids (Garden Safe, Spectrum Brands, Atlanta).

Endophyte Detection

We scored all plants for endophyte presence using light microscopy to examine thin sections of inner leaf sheath stained with lactophenol cotton blue (Bacon and White 1994). The endophyte was identified as *Epichlöe elymi* (Clavicipitaceae) based on intron regions of the genes encoding β -tubulin (*tub2*) and translation elongation factor 1- α (*tef1*; Schardl 2010; C. Young and C. Schardl, unpublished data). *Epichlöe* can reproduce both sexually via stromata (fungal reproductive structures that sterilize inflorescences) and asexually via seed transmission; however, no stromata were produced in our natural population, experimental field plots, or greenhouse-grown plants, which is not uncommon in this host species (Saha et al. 2009).

Plant Investment

On average, flowering plants produced three inflorescences and 166 anthers. We collected anthers opportunistically from mature inflorescences and allowed them to dehisce onto sterile, plastic plates. Pollen grains from one mature anther per plant were captured photographically (Adobe Photoshop, ver. 9.0.2, Adobe Systems, Mountain View, CA) and counted using particle analysis (ImageJ, US National Institutes of Health, Bethesda, MD). We estimated total pollen production per plant as the product of the per-anther pollen count, the number of pollen-producing florets, and the number of anthers per floret (= 3 anthers). Seeds were collected upon maturation and weighed to the nearest 0.001 g. Plants were harvested on October 4, 2009, by clipping shoots at the soil surface and washing roots through a 1-mm sieve (US standard sieve no. 18, Soil Test, Lake Bluff, IL). Tillers and roots were dried to constant mass (60°C) and weighed to the nearest 0.001 g. Mature inflorescences were harvested separately for seed counting and were not included in vegetative biomass measurements. Of the 571 inflorescences produced by the experimental plants, 10 remained immature at the time of plant harvest; we estimated seed production for these immature inflorescences as the average number of seeds produced by the mature inflorescences from the same plant.

For each plant individual (i), functional gender was calculated following Lloyd (1980): $G_i = d_i/[d_i + l_i E]$, where d_i = the number of seeds, l_i = the number of pollen grains, and E = the equivalency factor used to adjust the fitness of pollen to that of seeds, $E = \sum d_i / \sum l_i$. For analysis of reproductive investments, we used one-way ANOVA with the fixed effect of endophyte status (++, +–, or ––) (SAS ver. 9.1, SAS Institute, Cary, NC), followed by post hoc Tukey HSD tests. Most variables were transformed to satisfy the assumptions of homogeneity of variance and normality (table 1; Dryad data repository, doi:10.5061/dryad.84c26).

Pollen and Seed Viability

Because seed or pollen viability may be affected by endophyte symbiosis, the realized functional gender (corrected by the viability of seeds and pollen) may differ from the seed to pollen ratio. We had two sources of information on seed viability. First, we collected a subset of seeds from the experimental field populations in 2009 (E+ [endophyte present] $n = 35$, E– [endophyte absent] $n = 64$ seeds). Seeds were surface sterilized in 50% bleach and then plated onto petri plates of 1% water agar (mean = 5.5 seeds per plate). For cold stratification, we sealed plates with Parafilm and refrigerated them at 4°C from November 18, 2009, to January 4, 2010. We then placed cold-

Table 1: Transformations, *F* ratios, degrees of freedom (df), and *P* values from one-way ANOVA for variables reported in figures 1 and 2 and in the text

Response, scale	Transformation	<i>F</i>	df	<i>P</i>
Lloyd's <i>G_i</i> :				
Per plant	None	30.84	2, 166	<.0001
Tillers:				
Per plant	$(x + 0.5)^{0.5}$	30.32	2, 230	<.0001
Per gram above-ground biomass	$\log(x + 0.1)$	14.86	2, 230	<.0001
Inflorescences:				
Per plant	$(x + 0.5)^{0.5}$	18.07	2, 230	<.0001
Per tiller	$\log(x + 0.1)$.32	2, 202	.7243
Florets:				
Per plant	$(x + 0.05)^{0.5}$	6.87	2, 230	.0013
Per inflorescence	$(x + 0.05)^{0.5}$	16.18	2, 202	<.0001
Pollen grains:				
Per plant	$x^{0.3}$	4.77	2, 166	.0097
Per bud	None	27.84	2, 166	<.0001
Seeds:				
Per plant	$(x + 0.05)^{0.5}$	13.5	2, 230	<.0001
Per bud	None	21.8	2, 195	<.0001
Seed mass:				
Per plant	$\log(x + .0001)$	6.3	2, 193	.0022
Per seed	None	1.35	2, 193	.2610
Biomass:				
Aboveground	None	5.93	2, 230	.0031
Belowground	None	6.78	2, 228	.0014
Total per plant	None	5.25	2, 228	.0059

stratified plates in the greenhouse to allow for germination. The germination status of each seed was scored weekly for 9 weeks; no new seedlings had germinated after week 6. We tested whether the endophyte status of the experimental plot was associated with an altered probability of germination (log linear model on binomial data) or rate of germination (log(no. of days to germination)) using general linear models (SAS ver. 9.1).

In addition to data from the laboratory, we recorded recruitment rates in the experimental field populations every year for 4 years (2009–2012). Each year, we estimated total seed production in the plot and marked every new seedling that recruited. The plots lacked a prior seed bank for *E. virginicus*; therefore, we could be confident that all recruits originated from the experimental plants. We calculated the recruitment rate (= no. of recruits per plot in year $t + 1$ /no. of seeds produced per plot in year t for each year ($t = 2008$ –2011)). We also calculated recruitment rate assuming a persistent seed bank (= no. of recruits per plot in year $t + 1$ /[no. of seeds produced per plot in all years prior to $t + 1$ - no. of recruits per plot in all years prior to $t + 1$]). We tested for an effect of endophyte status of the field population, year, and endophyte \times year on the recruitment rate ($n = 5$ plots per endophyte status)

using a general linear mixed model with plot as a random effect, nested within endophyte status (SAS ver. 9.1). Less conservative models that excluded the plot factor returned similar and did not reduce the fit of the model to the data (corrected Akaike Information Criterion < 2 in all cases), indicating that plot identity explained little variation in the data.

Data on pollen viability could refine estimates of functional gender; however, for several reasons, we did not pursue estimates of pollen viability in this study. In grasses, pollen viability often declines rapidly following anther dehiscence (Dafni and Firmage 2000). In some species of *Elymus* and the related genus *Leymus*, pollen loses viability after just 3–4 h (Cicin 1954; Huang et al. 2004). Second, available staining methods are often ineffective for assessments of grass pollen viability (Dafni and Firmage 2000; Wang et al. 2004). Third, if effects of endophyte symbiosis on pollen viability were to alter our conclusions, the endophyte must have opposing effects on pollen viability (which would have to increase) and pollen grain number (which was reduced). Because the endophyte does not occur in pollen grains, we suspect an endophyte-mediated increase in pollen viability is unlikely.

Results

Plants with the endophyte showed greater relative investment in female function than plants lacking the endophyte. To control for plant genetic background, we first compared naturally symbiotic plants to plants that lost their symbionts during imperfect maternal transmission of the endophyte (see Afkhami and Rudgers 2008; Gundel et al. 2011). Thus, both symbiotic plants and first-generation symbiont-free plants were progeny of the same pool of symbiotic parents ($++$ treatment vs. $+ -$ treatment). The functional gender of plants with the endophyte was 25% more female biased than first-generation symbiont-free plants. Furthermore, the functional gender of plants with the endophyte was female biased ($= 0.56$; 0.5 is equal investment, < 0.5 indicates male bias, > 0.5 indicates a female bias), whereas the functional gender of symbiont-free plants was male biased ($= 0.45$; fig. 1; table 1).

Shifts in the functional gender metric at the scale of the whole plant corresponded to differential allocation patterns during particular stages of plant reproductive development. Specifically, symbiotic plants matured 25% more seeds per floret (fig. 2A) but produced 44% less total pollen and 25% less pollen per floret than symbiont-free plants (fig. 2B). Plants with the endophyte also made fewer florets per inflorescence (fig. 2C), which contributed to the overall reduction in male function. The endophyte did not mediate a trade-off between the size and the number of seeds (Gundel et al. 2012) because endophyte presence

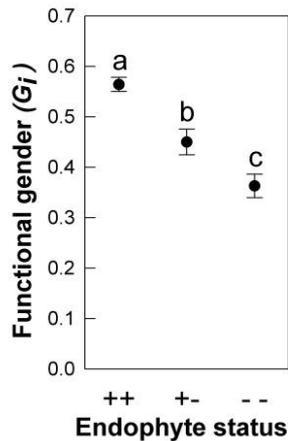


Figure 1: *Elymus virginicus* plants with the endophyte *Epichl e elymi* show more female-biased functional gender relative to plants without the endophyte ($F_{2,166} = 30.8$, $P < .0001$, $r^2 = 0.27$). Paired symbols indicate endophyte status, with symbiotic plants represented by “++,” plants that lost the endophyte during maternal transmission by “+−,” and multigeneration symbiont-free plant lineages by “−−.” The first sign represents the parent and the second the offspring, with “+” indicating endophyte symbiosis and “−” indicating a lack thereof. Circles with bars indicate means \pm SE. Different letters show significant differences following a post hoc Tukey HSD test.

did not significantly influence average seed mass (fig. 2D). Symbiotic plants produced 31% more inflorescences (fig. 2E) and 19% more vegetative tillers (fig. 2F) but made less total vegetative biomass (mean \pm SE, 1.98 ± 0.05 g) than symbiont-free plants (2.39 ± 0.13 g, Tukey HSD, $P < .05$). Vegetative biomass was not correlated with the functional gender of individual plants ($r^2 = 0.004$, $P = .43$), indicating that any effects of the symbiont did not manifest as a consequence of differences in plant size.

We also compared symbiotic plant lineages to lineages derived from symbiont-free maternal plants (i.e., multigeneration symbiont-free plants). Functional gender was 55% more female biased for symbiotic plants (fig. 1), reflecting 107% more total seeds (fig. 2A) and 25% fewer total pollen grains (fig. 2B). Plants from lineages with the endophyte matured 47% more seeds per floret (fig. 2A) and produced 29% fewer pollen grains per floret (fig. 2B) than plants from lineages lacking the endophyte. The endophyte was not associated with significant differences in pollen grain size (mean area captured photographically \pm SE, symbiotic = $1,400 \pm 30 \mu\text{m}^2$, multigeneration symbiont-free = $1,450 \pm 20 \mu\text{m}^2$, $F_{1,28} = 1.5$, $P > .23$). The vegetative biomass of symbiotic plants (mean \pm SE, 1.98 ± 0.05 g) did not differ significantly from that of multigeneration symbiont-free plants (mean \pm SE, 2.15 ± 0.10 g, Tukey HSD, $P > .05$); however, symbiotic

plants made 38% more florets, 81% more inflorescences, and 43% more vegetative tillers (fig. 2).

Both types of symbiont-free plants produced similar quantities of seeds per floret, pollen per plant, pollen per floret, inflorescences per tiller, and tillers per gram biomass (fig. 2), but multigeneration symbiont-free plants were functionally more male than first-generation symbiont-free plants (fig. 1) because they produced fewer total seeds (fig. 2A). Dissimilarities between the two types of symbiont-free plants may reflect underlying genetic variation between plant lineages or differences in maternal provisioning between symbiotic and symbiont-free parents. However, differences were not environmentally driven because all plants were propagated in a common environment for two generations.

Because seed and pollen viability may differ with endophyte symbiosis, the realized functional gender (corrected by viability of each) may differ from the seed to pollen ratio measured here. Although we were unable to quantify pollen viability, results for seed viability were consistent with the hypothesis that the endophyte increases plant allocation to female function. In the laboratory, seeds with the endophyte germinated, on average, 3 days faster than seeds without the endophyte ($++$ mean \pm SE = 13.9 ± 0.6 days to germinate, $--$ mean = 16.8 ± 0.6 days; $F_{1,87} = 9.7$, $P < .003$). However, over the course of 9 weeks, symbiont-free seeds were just as likely to germinate as symbiotic seeds ($++$ mean \pm SE = $89\% \pm 5\%$ of seeds germinated, $--$ mean = $91\% \pm 4\%$; $\chi^2 = 0.1$, $P = .748$). Experimental field populations with the endophyte had a higher rate of recruitment (back-transformed least squares mean [95% CI] = 0.47 [0.20, 0.84]) than symbiont-free populations (mean = 0.22 [0.05, 0.50]); however, this difference was not statistically significant ($F_{1,8} = 2.2$, $P = .18$). Symbiotic populations also had higher, but not significantly different, recruitment rates when a persistent seed bank was incorporated, which reduced the overall rate of recruitment by increasing the size of the seed pool (back-transformed mean recruitment rate [95% CI]; $E+$ = 0.07 [0.04, 0.11]; $E-$ = 0.05 [0.02, 0.09]; $F_{1,8} = 0.8$, $P = .411$). In field populations, we detected a strong effect of year for both recruitment models (without seed bank, $F_{1,24} = 4.4$, $P = .014$; with seed bank $F_{1,24} = 9.5$, $P < .001$), demonstrating that the models had sufficient statistical power. There were no statistically significant interactions between endophyte status and year (without seed bank, $F_{1,24} = 0.7$, $P = .593$; with seed bank $F_{1,24} = 1.2$, $P = .349$).

Discussion

The presence of a fungal endophyte in *Elymus virginicus* was associated with a significant shift in plant functional

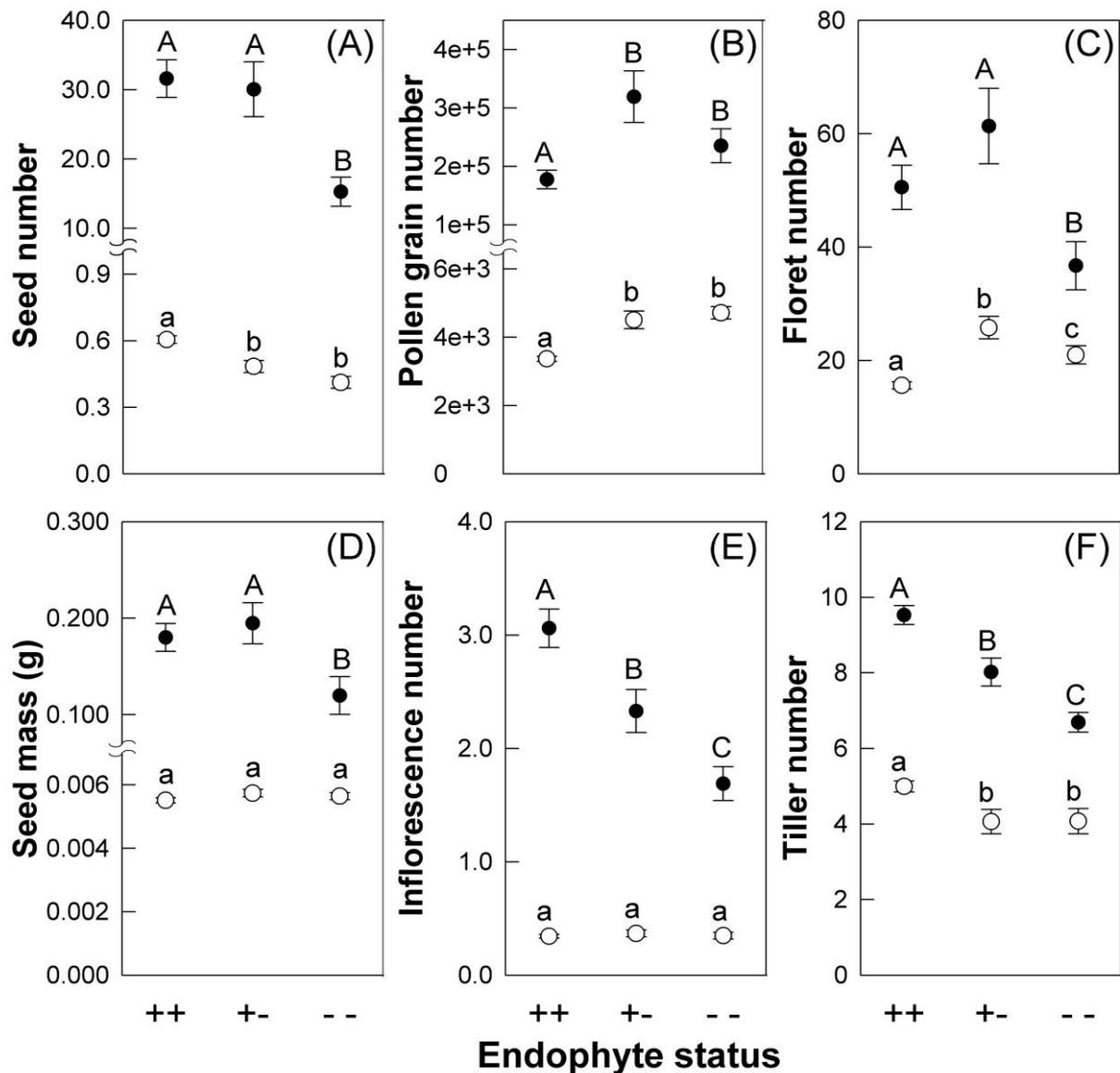


Figure 2: Effects of the *Epichloë elymi* endophyte on vegetative and reproductive allocation of *Elymus virginicus* plants. Paired symbols show endophyte status as described in figure 1. Symbols marked by different letters differed significantly following a post hoc Tukey HSD test. Filled circles and uppercase letters refer to per-plant means \pm SE. Open circles and lowercase letters refer to means \pm SE for seeds matured per floret (A), pollen produced per floret (B), florets per inflorescence (C), seed mass per seed (D), inflorescences per tiller (E), and tillers per gram aboveground biomass (F). Table 1 presents *F* ratios, *df*, and *P* values.

gender, characterized by reduced plant investment in pollen and increased investment in seeds. Environmental conditions, such as nutrients, water, and light availability, have been estimated to cause from 30% to upward of 250% difference in reproductive allocation in plants (Freeman et al. 1981; Vitale et al. 1987; Koelewijn and Hunscheid 2000; Stehlik et al. 2008; Friedman and Barrett 2011). Thus, the magnitude of change in *E. virginicus* functional

gender was well within the range of effects reported for other factors altering reproductive allocation in plants. Our results demonstrate that microbial symbionts deserve greater consideration in empirical tests of plant sex allocation. We also hope to spark the development of new theory predicting the evolutionary dynamics of reproductive manipulators in a broader range of organisms, including hermaphroditic hosts.

Mechanisms Underlying Reproductive Manipulation

Insights into the mechanisms and dynamics of symbiont-mediated reductions in pollen production may be gained from studies of nuclear-cytoplasmic male sterility (CMS) in plants, which has been documented in > 150 species and is a primary cause of gynodioecy (plant populations composed of females and hermaphrodites; Hanson 1991). CMS can occur when mitochondrial genes encode proteins that suppress pollen production, and male sterility has been associated with reactive oxygen species produced by mitochondria (Chase 2007; Peng et al. 2010). Reactive oxygen species are also generated by fungal endophytes (Tanaka et al. 2006; Eaton et al. 2010; Hamilton et al. 2012) and could be one mechanism underlying how endophytes influence pollen production. Further study of endophyte behavior in florets and ovules may shed new light on the mechanisms of interference with plant reproduction.

Maintenance of Variation in Symbiont Frequency

Here, we evaluated pollen and seed production for plants from symbiotic lineages, plants from symbiont-free lineages, and plants that had naturally lost their symbionts during imperfect vertical transmission in the previous generation. We suspect that symbiont-free lineages have persisted for a long time, as intermediate symbiont frequencies are not uncommon in grasses and often occur in *Elymus* species in particular (Rudgers et al. 2009). Although we do not know what mechanisms maintain variation in symbiont frequency in natural host populations, endophyte-mediated protection against herbivory does not appear to be a strong driver in this species (Crawford et al. 2010), nor does protection from drought (Rudgers and Swafford 2009). While the focus of empirical research has been on the fitness costs and benefits of symbiosis (Cheplick and Faeth 2009), symbionts can also be maintained at intermediate frequencies in host populations simply through high rates of imperfect vertical transmission, particularly if the fitness benefits conferred by symbionts are relatively small (Gundel et al. 2011; Yule et al., forthcoming). This may be the case in *E. virginicus*. In addition, models of CMS dynamics predict maintenance of the male sterility polymorphism due to negative frequency-dependent selection (Bailey and Delph 2007). If female plants have even a small fitness advantage, CMS will increase in the population until seed production becomes constrained by the pollen supply (Charlesworth 1981). Frequency-dependent selection constrained by the pollen supply may similarly explain why endophytes often occur in less than 100% of host individuals and vary widely in their prevalence

among host populations and species (Rudgers et al. 2009).

Limitations of This Study

Our results are consistent with the hypothesis that endophytes function as reproductive manipulators; however, it remains possible that differences in reproductive allocation are due, at least in part, to plant genotype. For example, the success of vertical transmission could depend on the genotype of the seed. Future work inoculating seedlings that are full sibs would further disentangle plant genotype and endophyte effects, although inoculation success could also be genotype specific. Alternatively, clones of symbiotic plants could be subjected to fungicide to eliminate the endophyte (e.g., Faeth 2009), provided that nontarget effects of the chemicals can be ruled out.

A second limitation is the lack of data on pollen viability. Ultimately, a combination of several approaches, for example, measures of respiration and pollen chemistry, viability staining, in vitro and in vivo germination estimates, and hand pollinations or paternity analysis to measure capacity to effect seed set, would refine estimates of plant functional gender (Dafni and Firmage 2000). Importantly, however, if the effects of endophyte symbiosis on pollen viability were to alter our conclusions, then the endophyte must reduce pollen grain number, as we have shown here, but increase pollen viability. Given the selective advantage to an endophyte of reducing plant investment in pollen, it seems more likely that the endophyte would reduce, rather than increase, pollen viability. Overall, due to the variety of factors that can influence pollen viability, we think that sophisticated estimates of pollen viability would have strengthened our conclusions to a relatively small degree.

Male-Biased Sex Allocation in Wind-Pollinated Plants

Reproductive manipulation is a strong hypothesis for the female-biased functional gender observed in symbiotic plants, but symbiont-free plants were male biased. Why? There are a number of factors that predict an optimal functional gender that deviates from equal investment. Functional gender varies with the degree and type of self-compatibility (Brunet 1992; de Jong et al. 1999), the number of available mates (Schärer 2009), the size of the floral display (Karron and Mitchell 2012), and the shapes of the fitness gain curves for pollen versus seeds (Charlesworth and Charlesworth 1981; Charnov 1982; Brunet 1992). Gain curves characterize the increase in male (or female) fitness as a function of increased plant investment in pollen (or seeds). For example, if the gain curve for pollen is linear, as is expected for wind-pollinated plants, and the female

gain curve is saturating (e.g., short seed dispersal distances increase local competition at high levels of seed investment), the optimal functional gender will be male biased (Charlesworth and Charlesworth 1981; Charnov 1982; de Jong et al. 2002). We have often observed tight clusters of seedlings germinating from *E. virginicus* inflorescences that fall next to parent plants; therefore, a saturating female gain curve is a possibility in this wind-pollinated species. In contrast to these gain curve differences, selfing should shift functional gender toward female investment, and the type of selfing (within the same flower or between flowers on the same individual) can influence the amount of bias (e.g., de Jong et al. 1999). Although the genus *Elymus* is known to be self-compatible and includes autogamous taxa (Love and Connor 1982; Jensen et al. 1990), we do not know how much or what type of selfing occurs in our *E. virginicus* population. Empirically, it is not uncommon for wind-pollinated plants to have male-biased functional gender (McKone et al. 1998; Friedman and Barrett 2011). Thus, it is possible that our observation of male-biased functional gender in symbiont-free plants reflects the optimal investment strategy to maximize plant fitness.

Future Directions for Research on Symbionts and Plant Reproductive Biology

In addition to the manipulation of functional gender detected here, plant reproductive biology may be altered by endosymbionts in other, possibly subtle, ways. For instance, symbiont-mediated modifications to inflorescence or floret architecture could alter the distance and rate of pollen dispersal and pollen capture. Although symbiont effects on reproductive architecture have yet to be explored, experiments have revealed important contributions of inflorescence architecture to pollination in grasses (Friedman and Harder 2004). The effects of symbionts on inflorescence height may be of particular interest because height influences both seed and pollen dispersal distances, which can then affect optimal functional gender (Sakai and Sakai 2003). In addition, symbionts could influence flowering phenology (J. Rudgers and A. Gorischek, unpublished data), inbreeding rates (Botham et al. 2009), the relative performance of male and female plants (Eppley et al. 2009), or plant mate choice, as in incompatibility-inducing *Wolbachia*. Altogether, such effects could alter the genetic structure and fitness of plant populations and possibly drive the evolution of plant sexual systems.

Applied Significance to Restoration and Agronomy

Many endophyte-hosting grasses, including *E. virginicus*, are widely used for ecological restoration (Sanderson et al. 2004). Furthermore, *Elymus* is a close relative of im-

portant crop species, including barley, wheat, and rye. The increased seed production of symbiotic plants demonstrated here, along with the enhanced vegetative growth, survival, herbivore resistance, and drought tolerance of symbiotic plants reported elsewhere (Cheplick and Faeth 2009; Rudgers and Swafford 2009), could boost establishment of host populations grown for food or restoration. Preservation of endophyte viability in seeds requires proper storage techniques (e.g., Welty et al. 1987), and failure to restore the symbiotic status of host populations could have negative consequences for restoration and revegetation efforts, as well as for crop production.

Conclusion

We have shown that, rather than being a passive occupant, the symbiotic fungus *Epichloë elymi* may actively manipulate its host plant, causing a distortion of reproductive investment away from pollen and toward seeds. Fungal and bacterial endophytes are nearly ubiquitous in plants (Bacon and White 2000; Rodriguez et al. 2009), and recent studies are turning up more and more cases of their maternal inheritance (e.g., Ralphs et al. 2011; Hardoim et al. 2012). Thus, these microbial symbionts are important to consider as agents of selection on plant reproductive strategies.

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