RICE UNIVERSITY

The role of environmental variation and host gene flow on the vertical transmission and population prevalence of heritable symbionts

by

Michelle E. Sneck

A THESIS SUBMITTED
IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE

Doctor of Philosophy

APPROVED, THESIS COMMITTEE:

Dr. Tom E.X. Miller, Chair
Goodwin Assistant Professor

Dr. Scott P. Egan
Assistant Professor

Dr. Michael H. Kohn
Associate Professor

Dr. Bonnie Bartel
Ralph and Dorothy Looney Professor

HOUSTON, TEXAS
August 2017

RICE UNIVERSITY

The role of environmental variation and host gene flow on the vertical transmission and population prevalence of heritable symbionts

by

Michelle E. Sneck

A THESIS SUBMITTED
IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE

Doctor of Philosophy

APPROVED, THESIS COMMITTEE

Dr. Tom E.X. Miller, Chair
Goodwin Assistant Professor

Dr. Scott P. Egan
Assistant Professor

Dr. Michael H. Kohn
Associate Professor

Dr. Bonnie Bartel
Ralph and Dorothy Looney Professor

HOUSTON, TEXAS
August 2017

ABSTRACT

The role of environmental variation and host gene flow on the vertical transmission and population prevalence of heritable symbionts

by

Michelle Elizabeth Sneck

Heritable microbial symbionts, vertically transmitted from maternal host to offspring, have made an indelible contribution to the ecology and evolution of life on earth. For instance, the fixation of symbionts in hosts led to pivotal biological shifts, such as the evolution of vascular plants and eukaryotic cells. Vertically transmitted symbionts are often specialized to host genotypes and confer fitness benefits to hosts, including protection against abiotic and biotic stress. Despite their ubiquity and strong influence on hosts, our understanding of what drives the prevalence and persistence of heritable symbionts lags behind that of macro-organisms. Two factors are theorized to determine equilibrium frequencies of heritable symbionts: 1) symbiont vertical transmission rates, and 2) the relative fitness of symbiotic and non-symbiotic hosts. Therefore, characterizing when and how these factors vary in host populations are necessary first steps to predicting the population dynamics of heritable symbionts. Here, I used large-scale field surveys, greenhouse and common garden experiments, as well as demographic modeling approaches to test the hypothesis that outcrossing (i.e., gene flow) between genetically distant hosts disrupts symbiosis. Specifically, host outcrossing is hypothesized to create genetic incompatibilities between sexually reproducing hosts and their specialized clonal symbionts, which may reduce both vertical transmission rates and

symbiont mediated mutualistic benefits. First, I found that symbiont prevalence in one host species negatively associated with drought, while symbiont genotype explained residual variation in vertical transmission rates. These results suggest that symbiont genotype, and to a lesser extent, climate variables play roles in shaping symbiont population dynamics, but substantial variability was unexplained. Second, I manipulated gene flow between hosts along a gradient of genetic distances and determined that symbiont vertical transmission was robust to host outcrossing, which remained high for several host generations. Lastly, I quantified the net effect of host outcrossing on symbiont population dynamics. Contrary to my hypothesis, host outcrossing did not disrupt mutualistic benefits of symbiosis, and instead, buffered hosts against deleterious effects of outbreeding depression. Together, my work provides strong evidence that host outcrossing does not disrupt symbiosis, and alternatively demonstrates that heritable symbionts are important players in the population dynamics of outcrossing hosts.

Acknowledgments

First and foremost, I'd like to thank my dissertation advisor, Tom Miller. His unending support, empathy, kindness, and respect were pivotal to my success as an early-career scientist, and I am incredibly proud to be one of his first graduate students. The Miller lab's past and present members (Andrew Bibian, Brad Ochocki, Michelle Downey, Theresa Bohner, Emily Shultz, Marion Donald, Aldo Compagnoni, and Kory Kolis) have provided essential emotional, physical, and intellectual support throughout my years at Rice. I must also thank my dissertation committee, Doctor: Michael Kohn, Volker Rudolf, Scott Egan, and Bonnie Bartel. Each member provided a unique and important perspective to my dissertation. I also want to thank the BioSciences department, including graduate students, faculty, and staff for providing a supportive and collegiate environment, and for endorsing important outreach and professional development initiatives. Moreover, I want to extend an all-encompassing thank you to the undergraduate assistance I have received over the years. I want to extend special gratitude to two high school students, Philip Tan and Tommy Villalva Jr., who tirelessly aided with lab work and field work via the RSTEM REHSS program. Last, but of course not least, I would like to thank my family, Mitch, Kim, Matthew, Susan, Ronald and Briana for their loving support, and Michael Crepinsek for his incredible partnership. I would like to dedicate this work to my older brother Matthew Martin Sneck, whose memory fuels my commitment to excellence in all of life's pursuits.

Table of Contents

Acknowledgments iv

Table of Contents v

List of Figures vii

List of Tables x

List of Equations xi

Variation in the prevalence and transmission of heritable symbionts across host populations in heterogeneous environments 1

1.1. Introduction 2

1.2. Methods 8

1.2.1 Study system 8

1.2.2 Field sampling 9

1.2.3 Environmental data collection 10

1.2.4 Quantifying population-level endophyte prevalence and individual-level transmission 11

1.2.5 Molecular techniques to estimate endophyte genotype 12

1.2.6 Statistical analyses 14

1.3. Results 17

1.3.1 Potential drivers of population-level endophyte prevalence 17

1.3.2 Potential drivers of vertical transmission 19

1.3.3 Association between vertical transmission and endophyte genotype 20

1.3.4 Correlation between endophyte prevalence and transmission 22

1.4. Discussion 23

Does host outcrossing disrupt compatibility with heritable symbionts? 28

2.1. Introduction 29

2.2. Methods 34

2.2.1 Study system and plant material 34

2.2.2 Greenhouse crossing experiment 36

2.2.3 Common garden experiment 38

2.2.4 Data analyses 40

2.3. Results 42

2.3.1 Effectiveness of greenhouse crossing experiment 42

2.3.2 Vertical transmission rates in response to host outcrossing 43

2.3.3 Association between endophyte growth and vertical transmission rate 46

2.3.3 The influence of host outcrossing on E+ offspring fitness 47
2.4. Discussion 49
The effect of host outcrossing on symbiont population dynamics 54
Introduction 55
3.1. Methods 60
3.2.1 Study system and plant material 60
3.2.2 Greenhouse crossing experiment 62
3.2.2 Common garden 63
3.2.4 Vital rate estimation 65
3.2.5 Matrix projection model 67
3.2.6 Imperfect symbiont vertical transmission 68
3.2.7 The effect of host genetic distance on symbiont population dynamics 69
3.2. Results 70
3.3.1 The effect of endophytes on host vital rates 70
3.3.2 Effect of host outcrossing on symbiosis 72
3.3.3 The effect of host outcrossing on symbiont population dynamics 75
3.4 Discussion 77
Retrospective and Future Directions 83
References 90
Appendices 113

List of Figures

Figure 1.1 – Map of collection locations. Open triangles indicate sites where E. virginicus was collected alone, open circles indicate sites where E. canadensis was collected alone, and closed diamonds indicate where species were found co-occurring. Numbers next to symbols correspond with site number and name in Appendix A. 9

Figure 1.2 – Histograms of E. virginicus (black bars) and E. canadensis (grey bars) population-level endophyte prevalence (a) and individual-level endophyte transmission rates of endophyte positive (E+) hosts (b) across all sampled populations. 17

Figure 1.3 – Generalized linear mixed effect model estimates from Table 1.1 showing best fit patterns (solid line) of population-level endophyte frequencies (a & b) and individual-level vertical transmission rates (c & d) across annual mean maximum temperature (°C) or mean annual drought (SPEI) for E. virginicus and E. canadensis hosts. The null model (intercept) is indicated by dotted lines (panels b, c, & d). Hatched circles in panel b and d indicate endophyte vertical transmission rates from site number 2 (Flagstaff: Appendix A) that were not included in final model estimates (Table 1.1). 20

Figure 1.4 – Box and whisker plots of residual variance in endophyte vertical transmission explained by endophyte genotype (Appendix B). Residual variance was extracted from abiotic models with the most statistical support (Table 1.1) for both host species (a & b) and restricted to genotyped endophytes (N = number of endophytes per genotype). Endophyte genotypes were defined by their unique alkaloid genetic profile (Appendix B). In E. virginicus hosts, endophytes with genotype 2 transmitted at a significantly higher rate than endophytes with genotype 1 ($z = -2.77$, $P = 0.007$). 22

Figure 1.5 – Correlation between population-level mean endophyte prevalence and mean endophyte transmission observed in E. virginicus (open triangles) and E. canadensis (open circles) host. The dashed line represents a 1:1 relationship. 23

Figure 2.1 – Boxplots of estimated mean genetic distance between outcrossed host parents from each cross type within populations (intra-), between populations (inter-) and between species (hybrid). Letters indicate significant difference between means. 42

Figure 2.2 – The endophyte vertical transmission rate in F_1 (a) and F_2 (b) host generations as a function of mean genetic distance between parents and cross type (c & d). Fitted lines in panels (a) and (c) are parameters from the top AIC models (Table 2.1). The dotted red line in panel (a) is the plotted parameters from the genetic distance canddiate model[1] (Table 2.1). Intra-population crosses are indicated by open circles and fitted parameters are plotted with a solid line. Inter-population crosses are indicated by

triangles a perforated line. Lastly, hybrid crosses are indicated by squares a dotted line. In panel c, the null model is plotted with a perforated line. 45

Figure 2.3 – Panel (a) displays the continuous relationship between endophyte vertical transmission rate into F_2 generation seeds and mean endophyte hyphal density (hyphae per μm^3) within F_1 adult plants. Panel (b) is a boxplot of mean endophyte density in both inter-population and hybrid cross types. 46

Figure 2.4 – Relationship between F_1 seed germination success rate and mean genetic distance between parents 47

Figure 2.5 – Relationship between mean fertility of F_1 adult hosts and mean genetic distance between parents (a) and cross type (b). The fitted solid line in panel (a) is plotted parameters from the top AIC model (Table 2.1). 48

Figure 3.1 – Histograms displaying the frequency of genetic distances represented in the common garden experiment for both E^+ (a) and E^- (b) hosts. Colors indicate different cross types: within a population (dark gray: intra-), between populations (light gray: inter-), and between species (purple: hybrid). 64

Figure 3.2 – Fitted demographic functions (i.e., posterior means) for Elymus virginicus in survival and growth at low (a and c) and high (b and d) genetic distances. The genetic distance plotted for each vital rate is indicated by GD, or the mean genetic distance above (high) and below (low) a genetic distance of 8 for each parameter. Best fit lines and observed data for endophyte-symbiotic (solid line) and non-symbotic populations (dotted line) are filled (E^+) and open (E^-) points, respectively. Shaded regions (E^+ = blue; E^- = gray) indicate the 95% Bayesian Credible Interval. 71

Figure 3.3 – Fitted demographic functions (i.e., posterior means) for Elymus virginicus in the probability of production seeds (a and b), the total number of floweirng tillers (c and d), and the number of seeds produced per flowering tiller (e and f) at high and low genetic distances. The genetic distance plotted for each vital rate is indicated by GD, or the mean genetic distance above (high) and below (low) a genetic distance of 8 for each parameter. Best fit lines and observed data for endophyte-symbiotic (solid line) and non-symbotic (dotted line) populations are filled (E^+) and open (E^-) points, respectively. Shaded regions (E^+ = blue; E^- = gray) indicate the 95% Bayesian Credible Interval. 73

Figure 3.4 – Fitted demographic functions for endophyte vertical transmission rates (a) and seed germination rates ± SD (b) for outcrossed Elymus virginicus hosts. The blue line in panel (a) represents the 95% Bayesian Credible Interval. 74

Figure 3.5 – (a) Projected geometric popluation growth rates for symboitic (filled circle) and non-symbiotic (open circle) host populations across mean genetic distance between parents and (b) predicted population-level equilibrium symbiont prevalence across host genetic distances. 75

Figure 3.6 – Relative growth rates (λ) for symboitic (E^+) and non-symbiotic (E^-) host populations based on 200 samples from vital rate parameters posteriors. Each bar represente the frequency of potential outcomes from left to right: E^+ λ is always smaller than E^- λ across genetic distances; E^+ λ is always larger than E^- λ across genetic distances; E^- λ is larger at high genetic distances, and E^+ λ is larger at high genetic distances. 76

List of Tables

Table 1.1 – AIC_c model rankings for *Elymus virginicus* (EV) and *E. canadensis* (EC). Model fit diagnostics include delta AIC_c (ΔAIC_c) and AIC weight (AIC_cwt), which measure model support relative to all other candidate models. D is a measure of the proportional reduction in deviance when the predictor variable with the most statistical support is added to the null model, or the amount of deviance explained by the focal parameter (see Methods for additional details)..... 18

Table 2.1 – AIC model rankings. Model fit diagnostics include delta AIC_c (ΔAIC_c) and AIC weight (AIC_cwt), which measure model support relative to all other candidate models. 44

List of Equations

Equation 3.1 – Demographic fertility function..... 67

Equation 3.2 – Demographic growth and survival function..... 68

Equation 3.3 – Megamatrix accounting for imperfect endophyte transmission..... 69

Chapter 1

Variation in the prevalence and transmission of heritable symbionts across host populations in heterogeneous environments

This chapter has been edited, reformatted, and reprinted from: Sneck M. E., J. A. Rudgers, Y. A. Young, and T.E.X Miller 2017. Variation in the prevalence and transmission of heritable symbionts across host populations in heterogeneous environments. *Microbial Ecology* doi:10.1007/s00248-017-0964-4

Heritable microbes are abundant in nature and influential to their hosts and the communities in which they reside. However, drivers of variability in the prevalence of heritable symbionts and their rates of transmission are poorly resolved, particularly across host populations experiencing variable biotic and abiotic environments. To fill these gaps, I surveyed 25 populations of two native grasses (*Elymus virginicus* and *E. canadensis*) across the southern Great Plains (US). Both grass species host heritable endophytic fungi (genus *Epichloë*) and can hybridize where their ranges overlap. From a subset of hosts, I characterized endophyte genotype using genetic loci that link to

bioactive alkaloid production. First, I found mean vertical transmission rates and population-level prevalence were positively correlated, specifically for *E. virginicus*. However, both endophyte prevalence and transmission varied substantially across populations and did not strongly correlate with abiotic variables, with one exception: endophyte prevalence decreased as drought stress decreased for *E. virginicus* hosts. Second, I evaluated the potential influence of biotic factors and found that, after accounting for climate, endophyte genotype explained significant variation in symbiont inheritance. I also contrasted populations where host species co-occurred in sympatry versus allopatry. Sympatry could potentially increase interspecific hybridization, but this variable did not associate with patterns of symbiont prevalence or transmission success. My results reveal substantial variability in symbiont prevalence and transmission across host populations and identify symbiont genotype, and to a lesser extent, the abiotic environment as sources of this variation.

1.1. Introduction

Nearly all multicellular organisms host a rich diversity of symbiotic microbes, many of which are vertically transmitted from maternal host to offspring (Clay 1993, Hilgenboecker et al. 2008, Funkhouser and Bordenstein 2013). Inherited microbes often benefit their hosts in exchange for nutrients, protection, and regeneration (Schardl et al. 2013b), and this exchange may be mutually beneficial because vertical transmission couples host and symbiont fitness (Sachs et al. 2004). For example, in both plants and arthropods, heritable microbial symbionts can increase resistance to environmental stress (Worchel et al. 2013, Giauque and Hawkes 2013, Ren et al. 2014), competitive ability

(Miller and Rudgers 2014) and defense against enemies (Haine 2008, Panaccione et al. 2014). Some heritable microbes, particularly fungi, improve plant fitness by producing bioactive chemicals (Schardl et al. 2013a). Additionally, they may also mitigate the effects of global climate change (Kivlin et al. 2013) and environmental degradation on host populations (Malinowski and Belesky 2000, Marquis et al. 2014). The influence of heritable microbes extends beyond individual hosts to alter community composition and ecosystem processes (Rudgers et al. 2004, Faeth and Shochat 2010). Therefore, understanding factors that influence the ecological dynamics of microbial symbionts informs predictions of their effects on host populations, communities, and ecosystems. Despite a surge of recent interest in microbial symbioses, understanding patterns of symbiont prevalence across natural host populations has remained an elusive goal (Semmartin et al. 2015).

Vertical transmission links symbiont fitness to host fitness via host reproduction (Ewald 1987). This connection is hypothesized to select for a tightly coevolved mutualism that should persist at high frequencies in host populations (Ewald 1987, Sachs et al. 2004). However, across a diversity of host taxa, heritable microbes persist at frequencies that are variable and often intermediate, including endophytic fungi (*Epichloë* spp.) in plant hosts (Afkhami and Rudgers 2008), bacteria (*Wolbachia* spp.) in arthropod hosts (Tsuchida et al. 2002, Hilgenboecker et al. 2008), and some components of the human microbiome (Kraal et al. 2014). Variable frequencies of heritable symbionts are partly driven by changes in host and symbiont relationships that shift with ecological context (Chamberlain et al. 2014), which makes predicting the prevalence of heritable symbionts difficult. However, field observations of symbiont prevalence across host taxa

and populations have begun to reveal some consistent patterns (Tsuchida et al. 2002, Frade et al. 2007, Giauque and Hawkes 2013, Semmartin et al. 2015). Many studies have observed that symbiont prevalence varies systematically along environmental gradients such as elevation (Bazely et al. 2007, Ranelli et al. 2015), the presence of pathogens (Pérez et al. 2013), ocean depth (Frade et al. 2007) and ecosystem productivity (Semmartin et al. 2015). The context-dependent nature of host fitness benefits is a potential driver of the observed gradients in symbiont prevalence, wherein the benefits of the symbiosis increase with greater environmental stress (Clay 1988, Oliver et al. 2005, Haine 2008). For instance, Oliver *et al*. discovered that the prevalence of a facultative bacterial symbiont, *Hamiltonella defensa,* increased in insect hosts exposed to parasitoid wasps, but decreased when parasitoids were absent (Oliver et al. 2005, 2008).

While the fitness benefits of symbiosis are undoubtedly important determinants of symbiont prevalence, theory predicts an important, additional role of the vertical transmission rate (fraction of host offspring that inherit a symbiont) (Gundel et al. 2008). Estimates of individual vertical transmission rates have received relatively little attention compared to population-level symbiont frequency (Gundel et al. 2011b, 2016). In the few symbioses where vertical transmission has been quantified, it is often imperfect (<100 % of offspring inherit the symbiont) (Hilgenboecker et al. 2008, Afkhami and Rudgers 2008). Imperfect transmission has important implications for symbiont dynamics: even if symbionts benefit hosts in many contexts, they may be eliminated from host populations if their fitness benefits are not sufficiently strong to compensate for imperfect transmission (Yule et al. 2013, Gibert et al. 2015, Bibian et al. 2016). Therefore, a positive correlation between symbiont prevalence and transmission supports the

hypothesis that transmission plays a part in determining symbiont frequencies (Gundel et al. 2008)

Despite a potentially critical role of symbiont transmission in shaping symbiont frequencies, we lack a basic understanding of how the transmission process varies with biotic or abiotic context. If transmission tracks large-scale environmental variables, heritable symbionts may be vulnerable to changing climate regimes. Therefore, climate-driven fluctuations in vertical transmission success could affect the population dynamics of both symbiotic partners (Gundel et al. 2008). Few studies have quantified vertical transmission in response to environmental variables, yielding inconsistent results. For example, experimental studies examining the relationship between cool-season grasses and Epichloid fungi have found short-term responses of vertical transmission to environmental stress, thereby indicating that symbiont transmission is plastic (Gundel et al. 2011b, García Parisi et al. 2012), while others found no environmental effects (Davitt et al. 2011, Gibert and Hazard 2013). Simulated grazing and mechanical disturbance limited vertical transmission of *Epichloë occultans* in multiple accessions of annual ryegrass (*Lolium multiflorum*) (García Parisi et al. 2012), but drought treatments failed to alter transmission success of *Epichloë amarillans* in *Agrostis hyemalis* (Davitt et al. 2011). Although small-scale manipulations are valuable, they are limited by the number and breadth of environmental variables and symbiont genotypes that can be investigated. Therefore, estimates of vertical transmission across broad environmental gradients are a worthwhile complement to study how the biogeographic context correlates with symbiont inheritance at the landscape level.

Besides the abiotic environment, biotic factors could also act as a source of variation in symbiont transmission. One such factor is symbiont genotype (Schardl et al. 2013a). For instance, specific genotypes of *Epichloë* endophytes produce up to four classes of bioactive alkaloids (peramine, ergot alkaloids, lolines, and indole-diterpenes) known to impact host fitness (Schardl et al. 2013b, Saikkonen et al. 2016). This alkaloid gene diversity can be partially explained by modes of fungal reproduction. Many *Epichloë* species reproduce asexually via vertical transmission (Selosse and Schardl 2007), but some can reproduce sexually, through formation of stroma ("choke disease") followed by fertilization of opposite mating-types (*MTA* or *MTB*) (Clay and Schardl 2002). Although *Epichloë* species can reproduce both asexually and sexually, interspecific heteroploids that retained multiple genomes following a hybridization event are exclusively vertically transmitted (Moon et al. 2004). Hybrid endophytes can gain alkaloid genes from both ancestors, potentially increasing both benefits to host fitness (Saari and Faeth 2012) and ecological dominance over non-hybrid endophytes (Hamilton et al. 2009, Saari and Faeth 2012, Saari et al. 2014). Also, hybrid endophytes could vertically transmit at higher rates than non-hybrids, another mechanism that may promote their high natural abundance (Moon et al. 2004, Selosse and Schardl 2007). However, comparisons of vertical transmission rates between hybrid and non-hybrid endophytes are few (Sullivan and Faeth 2008, Jia et al. 2016).

An additional biotic factor that could affect context-dependent outcomes of symbiosis is the sympatry (same geographic area) or allopatry (different geographic area) of related host species. Sympatry increases the potential for gene flow between host species (i.e., interspecific hybridization) (Saikkonen 2004, Gundel et al. 2010). Host

outcrossing events are hypothesized to result in genotype mismatches that reduce vertical transmission for symbionts because most are asexual and exhibit a high degree of specialization to specific host species and genotypes (Christensen et al. 1997, Gibert and Hazard 2013). In both arthropods and plants, symbiotic bacteria or cellular organelles (e.g., chloroplasts) can interact with host genetic background in complex ways (Herre et al. 1999). To the best of my knowledge, no empirical evaluation of this hypothesis exists. Evidence that symbiont prevalence and/or vertical transmission is lower in sympatry vs. allopatry suggests there are biotic costs of co-occurring with close relatives, whereas higher estimates suggests biotic context increases the benefits of symbiosis.

Here I report both population-level prevalence and individual-level vertical transmission rates of heritable symbionts across the broad geographic distribution of two hybridizing host species (Saha et al. 2009). I focused on vertically transmitted fungal endophytes (*Epichloë* spp.) hosted by two native North American grass species (*Elymus virginicus* and *E. canadensis*) across strong temperature and precipitation gradients throughout the southern Great Plains. Seed-transmitted fungal endophytes, inherited from mother to offspring, occur in up to 30 % of grass species (Leuchtmann 1992) as well as in some legumes, morning glories, and sedges (Panaccione et al. 2014). The symbiosis is facultative for the plant but obligate for the endophytes. For a subset of symbiotic hosts, I quantified fungal genetic variation at loci associated with biosynthetic secondary metabolite pathways (Schardl et al. 2013b, Saikkonen et al. 2013), which predict the suite of bioactive alkaloids shown to have context-dependent effects on host fitness (Shymanovich et al. 2015). I compared symbiont prevalence and vertical transmission rate between populations that differed in host species sympatry (potential for inter-

specific gene flow) to evaluate the hypothesis that the biotic environment can act as a source of variation in symbiont population dynamics. The sampling efforts for symbiont prevalence and vertical transmission reported here– 25 native populations and 848 individuals surveyed – is among the most thorough efforts to-date for documenting variation that occurs within grass-endophyte symbioses (White 1987, Vinton et al. 2001, Ranelli et al. 2015, Żurek et al. 2016).

Specifically, I asked:

(1) Are population-level endophyte prevalence and individual-level vertical transmission rate associated with abiotic variation (temperature, precipitation, or drought)?

(2) After accounting for abiotic variation, is individual-level vertical transmission rate associated with endophyte genotype?

(3) When hybridizing host species occur in sympatry, is there lower population-level endophyte prevalence or individual-level vertical transmission rate?

(4) Does vertical transmission positively co-vary with symbiont prevalence across host populations?

1.2. Methods

1.2.1 Study system

I focused on two perennial grasses, *Elymus canadensis* (Canada wild rye) and *E. virginicus* (Virginia wild rye). *E. virginicus* is abundant in eastern N. America and *E. canadensis* in western N. America, but they overlap throughout the Midwest and southern Great Plains. These species readily self-pollinate, out-cross with conspecifics, and also

hybridize (Sanders and Hamrick 1980). Gene flow tends to be uni-directional, primarily from *E. canadensis* to *E. virginicus* (Saha et al. 2009). Both grasses host systemic fungal endophytes, *Epichloë* species (Clavicipitaceae) (Leuchtmann et al. 2014).

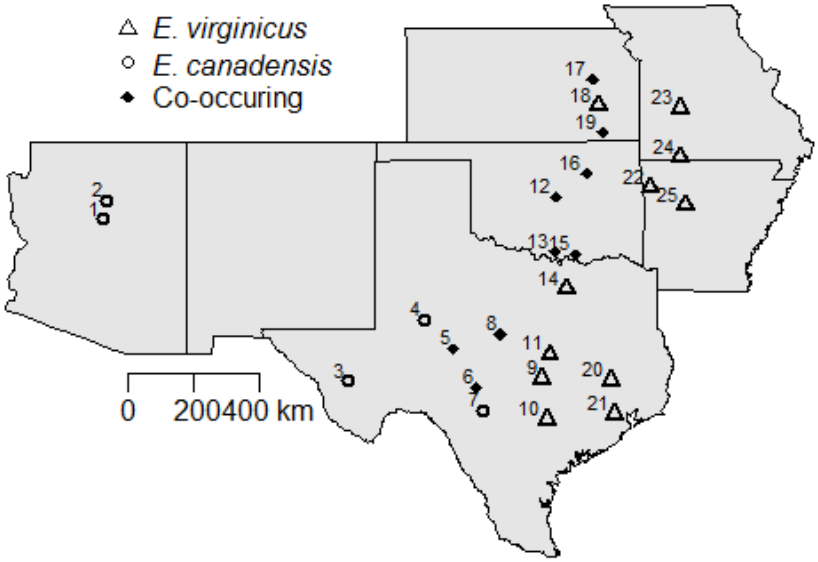

Figure 1.1 – Map of collection locations. Open triangles indicate sites where *E. virginicus* was collected alone, open circles indicate sites where *E. canadensis* was collected alone, and closed diamonds indicate where species were found co-occurring. Numbers next to symbols correspond with site number and name in Appendix A.

1.2.2 Field sampling

I surveyed populations of both host species across their distribution in the southern Great Plains (Figure 1.1). Collections maximized replication of host allopatry/sympatry as well as the broad range of environmental variation (mean annual precipitation: min = 355.4 mm; max = 1254.6 mm; mean annual maximum temperature: min = 15.6 °C; max = 27.6 °C). I characterized populations as *E. virginicus* alone (*N* = 11 populations), *E. canadensis* alone (*N* = 5), or both host species co-occurring in sympatry (*N* = 9) (Figure 1.1; Appendix A). Seed collections were made in 2013 after peak flowering (June – early December), when most plants had mature seeds (Supplementary Table 1). I collected ~30 individual plants (mean = 33.72; min = 16; max = 53) per population, and ~40 seeds from fully ripened or senescing inflorescences per plant (min.

of 2) in order to account for possible tiller-to-tiller variation in endophyte occurrence and transmission.

1.2.3 Environmental data collection

To investigate the potential role of abiotic variation in symbiont prevalence and transmission, I focused on three environmental variables: temperature, precipitation, and drought (an integrated measure of the other two variables). I chose maximum temperature because endophyte survival (Rolston et al. 1986) and host fitness benefits (Schardl et al. 2004) can be contingent upon high temperatures. I examined mean annual precipitation (mm) because it has been shown to co-vary with endophyte prevalence in other systems (Semmartin et al. 2015). I constrained calculations to the five-year period preceding the sampling year (2008-2012) to reflect recent climate conditions. Expanded (2000-2012) and shortened (2011-2012) time series were also tested to account for the influence of the longer-term average or more recent weather (Long et al. 2013); these analyses yielded qualitatively similar results (not shown). Environmental variables were calculated over 12 months, because use of yearly values received higher statistical support (minimum Akaike Information Criterion, AIC_c) than those constrained to the growing season (February – April) or reproductive season (April – July).

I used temperature and precipitation data from the PRISM Climate Group (Oregon State University, http://prism.oregonstate.edu, accessed August 2013) to calculate mean annual precipitation and mean maximum monthly temperature (°C). For the latter, monthly maximum temperatures were averaged over 12 months and then averaged across the 5-year window. Two alternative temperature metrics (mean annual

temperature and Growing Degree Days (Salazar-Gutierrez et al. 2013)) received less statistical support than mean maximum temperature and were therefore excluded from candidate models. As a measure of drought, I used the Standardized Precipitation-Evaporation Index (SPEI), which accounts for the duration and severity of water loss compared to water availability (Vicente-Serrano et al. 2010). Mean annual SPEI, integrated over 12 months, was calculated as the difference between monthly precipitation and potential evapotranspiration (using functions hargreaves and spei in R package SPEI, (Beguería and Vicente-Serrano 2013)). High SPEI estimates indicate low drought stress. Average monthly climate estimates for each sampling population encompassed a wide gradient of drought severity from no drought to moderate drought for both species (Appendix A).

1.2.4 Quantifying population-level endophyte prevalence and individual-level transmission

My main response variables were endophyte prevalence of each population (proportion of plants that were endophyte-symbiotic [E+]) and endophyte transmission of each individual (proportion of seeds from E+ maternal plants that were also E+). To estimate both variables, I focused on endophyte presence in host seeds. Previous studies have shown that vertically transmitted endophytes are most frequently lost during the maternal plant-to-seed transition (Afkhami and Rudgers 2008, Yule et al. 2013, Gibert et al. 2015). Therefore, a plant was designated E- (non-symbiotic) if none of its seeds contained fungal hyphae and E+ if any of its seeds contained fungal hyphae. This approach may underestimate endophyte prevalence because false negatives are possible in cases where transmission is low. I used microscopy to visually inspect 5 host seeds per

plant for presence/absence of hyphae in the seed coat and/or aleurone layer (White 1987, Miller and Rudgers 2014). Briefly, seeds were soaked in a 5% NaOH solution overnight, then squashed, stained with aniline blue, and examined with a light microscope under 200X magnification. The stain adheres to fungal hyphae, which are detectable regardless of seed or fungal viability. For an additional 15 seeds, I supplemented microscopy (which is time and labor intensive) with the immunoblot test kit (Agrinostics Ltd Co., Watkinsville, GA) whereby an antibody that narrowly targets endophyte proteins is used in conjunction with a chromagen to detect endophyte presence. Both microscopy and immunoblot techniques used for endophyte detection have been shown to produce similar estimates of endophyte presence or absence (Hiatt et al. 1999). I verified this relationship by correlating E+ prevalence estimated from microscopy with that from immunoblot assays (*E. virginicus*: $N = 453$, $r = 0.63$, $P < 0.001$; *E. canadensis*: $N = 237$, $r = 0.75$ $P < 0.001$). Lower correlations between microscopy and immunoblot results, particularly for *E. virginicus* hosts, was likely driven by small sample sizes of microscopy screenings (5 seeds), where instances of low to intermediate transmission went undetected. I aimed for ≥ 20 total seeds (microscopy + immunoblot) per plant for endophyte presence and transmission data, but a subset of plants had insufficient seeds to meet this target (mean = 16.1; min = 1; max = 33). In total, I assayed 13647 seeds from 848 host individuals from 25 populations (Appendix A).

1.2.5 Molecular techniques to estimate endophyte genotype

To determine endophyte genotypes for a subset of host populations (*Elymus virginicus*: $N = 9$; *E. canadensis*: $N = 3$; sympatry: $N = 5$), I germinated multiple seeds from 196 individual field-collected host plants in a greenhouse at Rice University during

spring 2014. My sampling scheme allowed us to determine the alkaloid genotype of vertically transmitted endophytes from a single maternal host by analyzing the genotypes of multiple offspring. Co-infections of multiple endophytes in the same host are rare (Wille et al. 2002), therefore all offspring derived from a single host are expected to have the same endophyte genotype. Genomic DNA from multiple offspring per maternal host (mean = 3.7; min = 1; max = 9; N = 545) was isolated from ~10 mg of lyophilized plant tissue using MagAttract 96 DNA plant core Kit (QIAGEN Inc., Valencia, CA) and analyzed following Takach *et al.* (Takach et al. 2012). In total, PCR assays included a third of all E+ maternal plants examined in this study (EV: N = 87; EC: N = 48) 17 populations. Endophyte DNA was amplified with a multiplex approach using 18 markers (Charlton et al. 2014), which infer both the production of four major alkaloid classes (peramine, ergot alkaloids, lolines, and indole-diterpenes) and endophyte mating type (*MTA* or *MTB*). Hybrid samples with the same mating types (*MTA*, *MTA* and *MTB*, *MTB*) cannot be distinguished from non-hybrid endophytes with my methods. Samples were scored for presence/absence of each gene marker. In total, seven unique genotypes were identified (numbered arbitrarily 1-7), whereby individuals with the same genetic profile were considered the same genotype. For a subset of endophytes (N = 11), individuals derived from the same maternal host revealed different genotypes, possibly due to collection contamination or co-infection. In these cases, I defaulted to the most common genotype observed among related individuals. Analyses using the alternative genotype(s) produced qualitatively similar results.

1.2.6 Statistical analyses

I used generalized linear mixed models (glmer in R package lme4; (Bates et al. 2014)), AIC-based model selection, and multi-model inference to associate endophyte prevalence and transmission with abiotic and biotic factors. In preliminary analyses, there were clear host species differences in endophyte prevalence and transmission rates. Therefore, I analyzed the host species separately to reduce candidate model complexity. Population endophyte prevalence was treated as a binomial response variable with the total sampled hosts as the number of trials and the total E+ hosts as the number of successes. Transmission was modeled similarly, but with multiple observations per population and the number of trials given by total seeds assayed per plant and successes given by total E+ seeds per plant. Both models included the random effect of population in addition to any fixed-effect predictor variables (below). To test for assumption violations of binomial models I used the sum of squared Pearson residuals divided by the residual degrees of freedom ($\hat{c}$). Values of $\hat{c}$ greater than 1 indicate overdispersion (c_hat in R package AICcmodavg, (Mazerolle 2016)). Overdispersion in the vertical transmission data (EV $\hat{c}$ = 7.2; EC $\hat{c}$ = 5.1) was corrected by nesting an individual random effect within the population random effect (EV $\hat{c}$ = 0.13; EC $\hat{c}$ = 0.0048). I did not detect overdispersion in the endophyte prevalence data and therefore only included the population random effect (EV $\hat{c}$ = 0.24; EC $\hat{c}$ = 0.15). To determine the influence of host sympatry, I created a binary variable accounting for the presence/absence of congeners as a proxy for biotic interactions (e.g., hybridization and competition).

In total, four candidate model sets were constructed corresponding to two response variables (endophyte prevalence and transmission) for each host species (EV

and EC) (Table 1.1). Each candidate model set tested the influence of temperature, precipitation, and drought (SPEI), and host sympatry as predictor variables. Sympatric host populations were sampled across varying environments, which allowed us to test for additive and interactive effects between each environmental predictor variable and sympatry respectively. Interactions between temperature and precipitation were not included because drought is a composite measure of the two; therefore, models with drought would receive highest support if interactions between temperature and precipitation were important. All candidate model sets included a null model representing random population variance, for a total of eleven models (Table 1.1).

Model selection was conducted using the second-order bias corrected Akaike Information Criterion (AIC_c) that ranks the relative support for each candidate model (aictab in R package AICcmodavg; (Mazerolle 2016)). The difference between the best model and all other models (ΔAIC_c) and the conditional probability for each model ($AIC_c wt$) were also calculated. To determine the amount of deviance explained by the highest-ranked model compared to the null, I calculated the proportional reduction in deviance (methods detailed in (Dalgleish et al. 2011)). This quantity (D) determines the strength of association between response and predictor variables (1 = perfect prediction; 0 = no association) (Zheng 2000).

Following model selection, I aimed to determine if endophyte genotype explained remaining residual variance in endophyte vertical transmission rates after accounting for other sources of variation. To accomplish this, residuals from the best model were used as the response variable (Table 1.1) in models testing the categorical effect of endophyte genotype against a null model using likelihood ratio tests. I did not include endophyte

genotype as a covariate in the original model selection because endophytes were genotyped from a subset of collected plants. In these models, each endophyte genotype (Appendix B) was given a unique categorical dummy variable (1-7, corresponding to the seven genotypes I detected).

Lastly, I calculated the Spearman correlation coefficient to determine the relationship between endophyte population prevalence and mean vertical transmission by population for each species individually.

1.3. Results

1.3.1 Potential drivers of population-level endophyte prevalence

Across populations, endophyte prevalence in *E. canadensis* (mean = 91.1%) was on average greater than *E. virginicus* (mean = 53.6%) (Figure 1.2a). For *E. virginicus*, model selection indicated that endophyte prevalence was lower under greater drought stress ($AIC_c wt$ = 0.47, D = 0.042), and increased from 47 % in dry sites (SPEI < 0) to 79% in mesic sites (SPEI > 0) (Figure 1.3a). *E. canadensis* had high endophyte prevalence across environments (prevalence: dry = 94%, mesic = 86%) (Figure 1.3a), and the null model received the most support (Table 1.1). Figure 1.3b shows *E. canadensis*

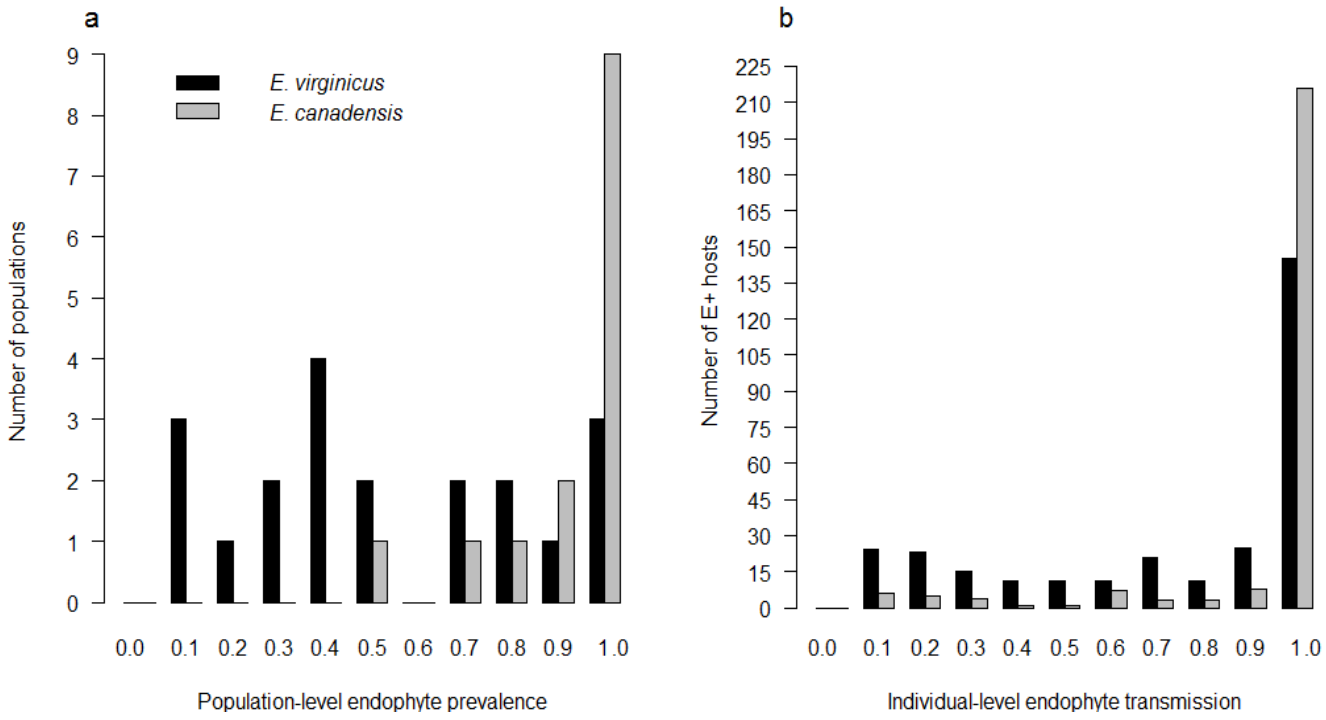

Figure 1.2 – Histograms of *E. virginicus* (black bars) and *E. canadensis* (grey bars) population-level endophyte prevalence (a) and individual-level endophyte transmission rates of endophyte positive (E+) hosts (b) across all sampled populations.

endophyte prevalence in relation to mean maximum temperature, which was the most supported environmental variable ($\Delta AIC_c = 0.56$).

Table 1.1 – AIC_c model rankings for *Elymus virginicus* (EV) and *E. canadensis* (EC). Model fit diagnostics include delta AIC_c (ΔAIC_c) and AIC weight ($AIC_c wt$), which measure model support relative to all other candidate models. *D* is a measure of the proportional reduction in deviance when the predictor variable with the most statistical support is added to the null model, or the amount of deviance explained by the focal parameter (see *Methods* for additional details).

	Population-level endophyte prevalence					Individual-level endophyte transmission				
Host	Model	AIC_c	ΔAIC	$AIC_c wt$	*D*	Model	AIC_c	ΔAIC	$AIC_c wt$	*D*
EV	SPEI[1,2]	133.66	0.00	0.47	0.042	null[1]	1206.99	0.00	0.27	
	tmax	136.02	2.36	0.14		tmax[2]	1207.55	0.56	0.20	0.0012
	SPEI + sympatry	136.45	2.79	0.12		SPEI	1208.63	1.64	0.12	
	null	136.48	2.82	0.11		ppt	1208.73	1.74	0.11	
	ppt	137.95	4.29	0.05		sympatry	1209.04	2.05	0.10	
	tmax + sympatry	139.05	5.39	0.03		tmax + sympatry	1209.58	2.59	0.07	
	sympatry	139.22	5.56	0.03		SPEI + sympatry	1210.67	3.67	0.04	
	SPEI * sympatry	139.45	5.79	0.03		ppt + sympatry	1210.77	3.77	0.04	
	ppt + sympatry	140.99	7.33	0.01		tmax * sympatry	1211.62	4.62	0.03	
	tmax * sympatry	142.20	8.55	0.01		SPEI * sympatry	1212.58	5.59	0.02	
	ppt * sympatry	144.14	10.48	0.00		ppt * sympatry	1212.85	5.85	0.01	
EC	null[1]	51.46	0.00	0.34		null[1]	263.26	0.00	0.27	
	tmax	53.00	1.54	0.16	0.039	sympatry	264.72	1.45	0.13	0.002
	sympatry	53.02	1.56	0.15		tmax [2]	264.84	1.58	0.12	0.002
	ppt	53.24	1.79	0.14		SPEI	265.19	1.92	0.10	
	SPEI	53.41	1.95	0.13		ppt	265.32	2.06	0.10	
	tmax + sympatry	55.85	4.40	0.04		tmax + sympatry	265.64	2.37	0.08	
	ppt + sympatry	56.88	5.42	0.02		ppt + sympatry	266.40	3.14	0.06	
	SPEI + sympatry	57.01	5.55	0.02		SPEI + sympatry	266.66	3.39	0.05	
	tmax * sympatry	60.26	8.80	0.00		tmax * sympatry	266.94	3.67	0.04	
	ppt * sympatry	61.88	10.42	0.00		SPEI * sympatry	268.20	4.93	0.02	
	SPEI * sympatry	62.06	10.60	0.00		ppt * sympatry	268.49	5.22	0.02	

1.3.2 Potential drivers of vertical transmission

E. canadensis (*N* = 257) had higher transmission rates (mean = 85.7%; max = 100%; min = 0.05%) than *E. virginicus* (*N* = 313; mean = 71.1%; max = 100%, min = 0.05 %) (Figure 1.2b). For both species, abiotic factors explained little variation in transmission rates. For *E. virginicus*, the null model ($AIC_c wt$ = 0.27) and the model containing temperature ($AIC_c wt$ = 0.20) received similar statistical support (Table 1.1), but temperature explained just a small fraction of the substantial variability in transmission (*D* = 0.0012) (Figure 1.3c). For *E. canadensis*, vertical transmission rates were consistently high, but declined slightly at low maximum temperatures (16 °C). Models containing temperature alone ($AIC_c wt$ = 0.54) and temperature plus sympatry ($AIC_c wt$ = 0.23) received the most statistical support. These models indicated that transmission rates increased with maximum temperatures and in sympatry. However, after removing the population at the extreme end of the temperature gradient, (Appendix A, site 2), the null model emerged as the best ($AIC_c wt$ = 0.27), indicating that the outlier was driving both the sympatry and temperature effects. Without the outlier, vertical transmission remained high across both the temperature gradient and sympatric/allopatric populations (Fig 1.3d, dotted line).

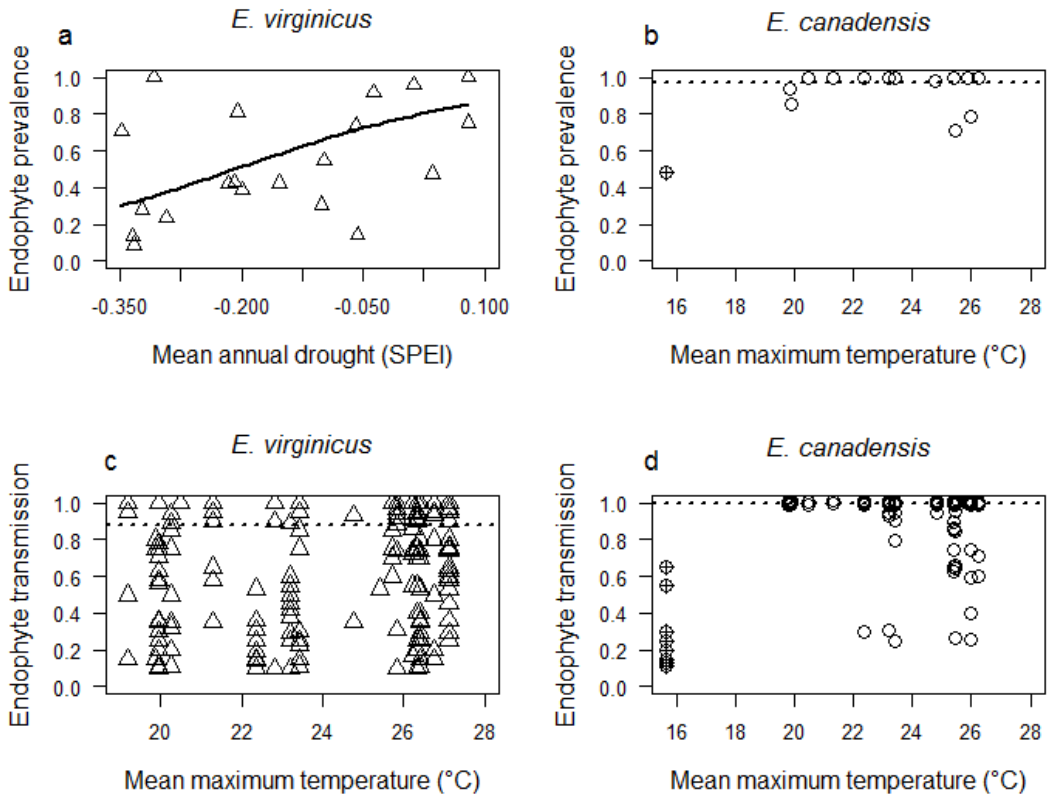

Figure 1.3 – Generalized linear mixed effect model estimates from Table 1.1 showing best fit patterns (solid line) of population-level endophyte frequencies (a & b) and individual-level vertical transmission rates (c & d) across annual mean maximum temperature (°C) or mean annual drought (SPEI) for *E. virginicus* and *E. canadensis* hosts. The null model (intercept) is indicated by dotted lines (panels b, c, & d). Hatched circles in panel b and d indicate endophyte vertical transmission rates from site number 2 (Flagstaff: Appendix A) that were not included in final model estimates (Table 1.1).

1.3.3 Association between vertical transmission and endophyte genotype

I identified seven unique endophyte genotypes over 136 host plants (Appendix B). All endophyte genotypes were positive for *PER* (peramine) markers, but genotypes varied in presence/absence of *LOL* (loline) and *EAS* (ergot alkaloid) loci. Overall, I found more endophyte genotypes in *E. canadensis* than in *E. virginicus*.

Mating type varied across samples and revealed hybrid endophytes bearing both *MTA* and *MTB* markers (Genotypes 3, 5 and 7; Appendix B). These hybrid endophytes

occurred primarily in *E. canadensis* and had more alkaloid markers than non-hybrids. Putatively sexually reproducing, non-hybrid endophytes (with only *MTA* or *MTB*) had fewer alkaloid markers and were vertically transmitted at lower average rates (76%) than hybrids (93.9%).

For both host species, endophyte genotype explained significant variation in residuals extracted from top supported vertical transmission models (Table 1.1) compared to the null (Likelihood ratio tests EV: $\chi^2 = 12.1$, $P = 0.007$; EC: $\chi^2 = 17.40$, $P < 0.0001$). Although many endophyte genotypes lacked sufficient replication to statistically compare mean transmission rates, post-hoc tests revealed that in *E. virginicus*, genotype 2 transmitted at a significantly higher rate (mean = 89.7%) than genotype 1 (mean = 64.5%) ($z = -2.49$, $P = 0.0128$) (Figure 1.4a). Genotype 1 has genes associated with peramine alkaloid production known to specifically target invertebrates (Schardl et al. 2014). In contrast, genotype 2 has genes for the production of both peramine and ergot alkaloids, which may defend against a wider range of vertebrate and invertebrate herbivores (Schardl et al. 2014). In *E. canadensis*, where sample sizes were smaller, post-hoc tests failed to detect significant pairwise differences, although the genotype with the highest vertical transmission was on average 35.5% greater than the genotype with the lowest. (Figure 1.4b).

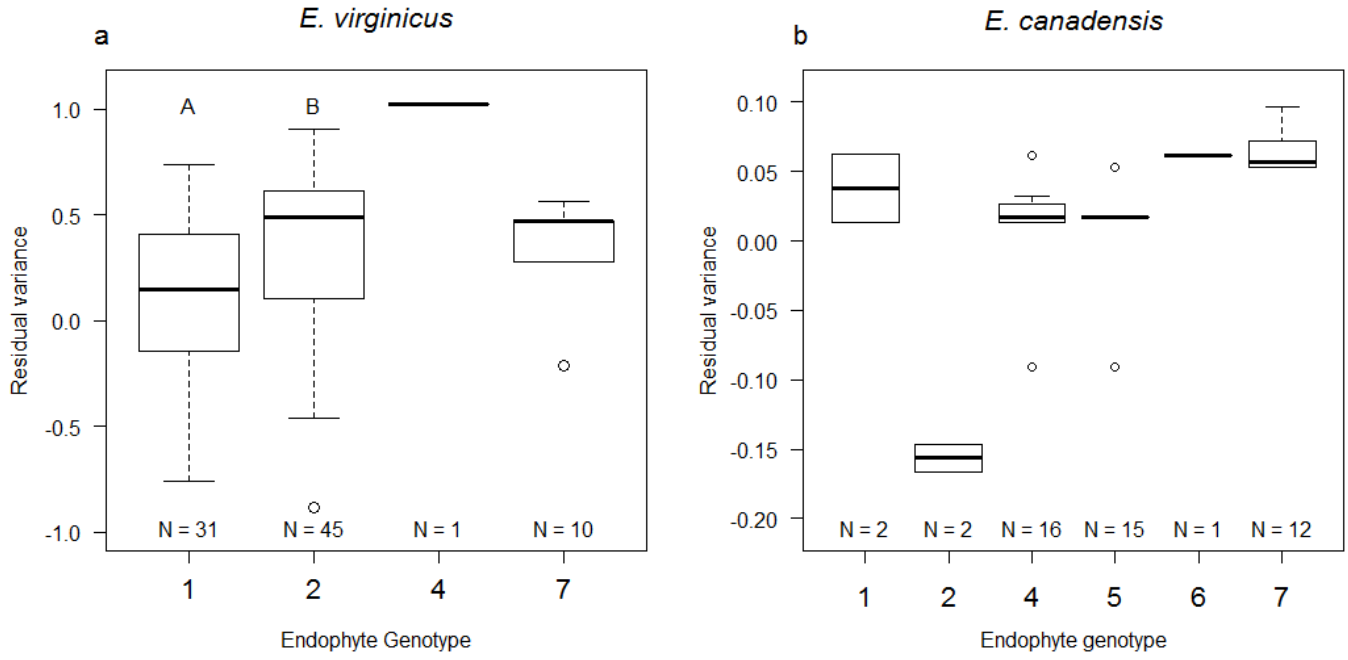

Figure 1.4 – Box and whisker plots of residual variance in endophyte vertical transmission explained by endophyte genotype (Appendix B). Residual variance was extracted from abiotic models with the most statistical support (Table 1.1) for both host species (a & b) and restricted to genotyped endophytes (*N* = number of endophytes per genotype). Endophyte genotypes were defined by their unique alkaloid genetic profile (Appendix B). In *E. virginicus* hosts, endophytes with genotype 2 transmitted at a significantly higher rate than endophytes with genotype 1 ($z = -2.77$, $P = 0.007$).

1.3.4 Correlation between endophyte prevalence and transmission

For *E. virginicus*, populations with high mean endophyte prevalence had higher mean vertical transmission success ($\rho = 0.659$, $S = 454$, $P = 0.0021$, Figure 1.5). In *E. canadensis*, there was less variability in prevalence and transmission and no significant correlation between the two (EV; EC: $\rho = 0.241$, $S = 345.14$, $P = 0.406$).

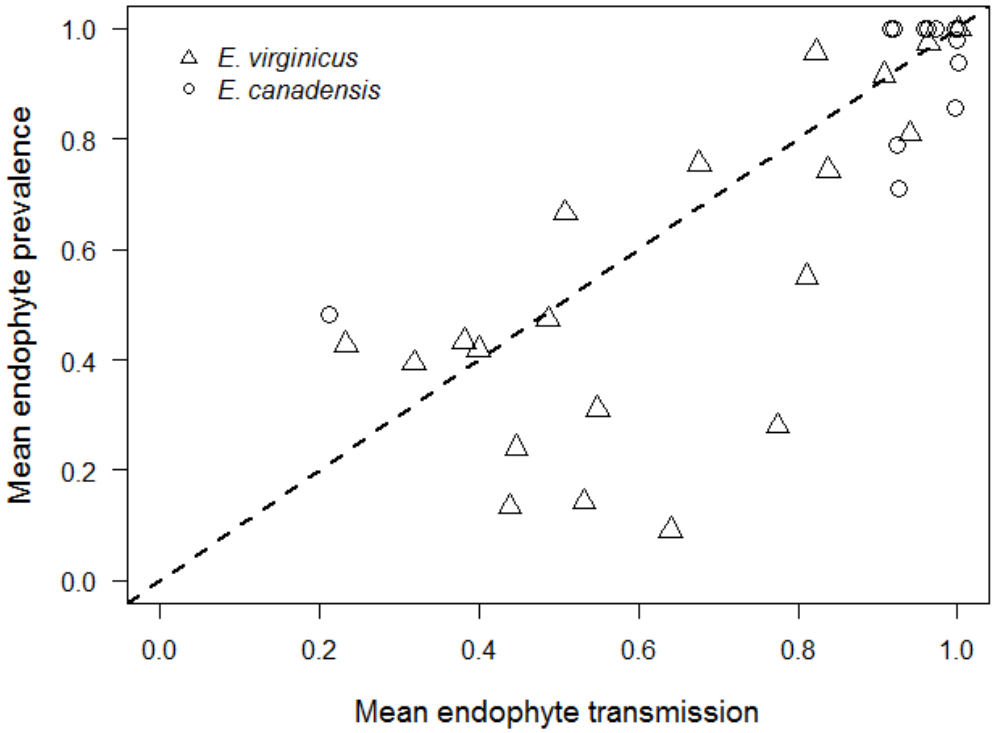

Figure 1.5 – Correlation between population-level mean endophyte prevalence and mean endophyte transmission observed in *E. virginicus* (open triangles) and *E. canadensis* (open circles) host. The dashed line represents a 1:1 relationship.

1.4. Discussion

Vertical transmission is an important determinant of heritable symbiont prevalence in host populations (Gundel et al. 2008, 2011b) but has received less empirical attention than the fitness effects of symbionts. To my knowledge, mine is the first study to examine both population-level symbiont prevalence and individual-level vertical transmission across strong environmental gradients. This approach enabled us to determine how symbiont prevalence and transmission associate with abiotic and biotic factors. I found that both endophyte prevalence and vertical transmission varied substantially between and (for transmission) within populations (Figure 1.2) and weakly

associated with large-scale abiotic variables (Figure 1.3). However, I did find one exception to this pattern- endophyte prevalence in *E. virginicus* significantly declined with greater drought stress (lower SPEI). Furthermore, I uncovered novel evidence that biotic context, specifically endophyte genotype, plays a role in determining symbiont inheritance (Figure 1.4). In contrast, sympatry, a proxy for hybridization potential, did not associate with symbiont prevalence or transmission. Lastly, vertical transmission may be a key constraint to symbiont prevalence in some host species, as evidenced by the strong positive correlation between vertical transmission and endophyte prevalence in *E. virginicus* (Gundel et al. 2011b) (Figure 1.5). A perfect correlation between endophyte prevalence and transmission is also predicted to occur when endophytes provide very strong fitness benefits, suggesting endophytes could act as mutualists in *E. virginicus* populations (Gundel et al. 2008). Together, my results propose endophyte genotype, and to a lesser extent climate variables play roles in shaping endophyte population dynamics, but substantial variability remains unexplained.

Previous surveys have detected influences of environmental conditions on endophyte prevalence (Bazely et al. 2007, Afkhami 2012), but have paid less attention to endophyte transmission (Gundel et al. 2009, Gibert and Hazard 2013, Gundel et al. 2016). My results extend this work by providing new evidence that prevalence of heritable endophytes and transmission from parent to offspring vary substantially across host individuals and populations (Figure 1.2) and in few cases, correlate with local climate. Other surveys found that endophyte prevalence either increased (Iannone et al. 2015) or declined (Victoria Novas et al. 2007) with greater aridity, thereby suggesting that host-endophyte relationships vary in their responses to abiotic stressors. In my study,

endophyte prevalence, but not transmission, decreased with increasing drought severity in *E. virginicus* (Figure 1.3a). In contrast, for *E. canadensis*, endophyte prevalence and transmission did not closely associate with abiotic factors (Figure 1.3b and d). As a whole, these results are surprising given that endophytes are classically hypothesized to increase host fitness in response to abiotic stress, particularly drought (Clay 1988, Schardl et al. 2004), and therefore should reach high prevalence in drought-stressed environments over time (Gundel et al. 2008). However, experimental studies have revealed that endophytes are not universally beneficial under abiotic stress (Cheplick 2004). For example, Rudgers and Swaffor (2009) demonstrated that *Elymus virginicus* hosting *Epichloë elymi* experienced more aboveground growth than endophyte-free hosts in response to daily watering, but this fitness boost was reduced by half in severe drought. This result suggests that drought could diminish rather than enhance benefits of symbiosis. Note that symbiont prevalence and transmission are not direct measures of mutualism (Gundel et al. 2016). Without manipulating symbiont presence, I cannot determine if or when endophytes act as mutualists in this system.

After accounting for abiotic influences, my work reveals a previously undocumented association between vertical transmission and endophyte genotype (Figure 1.4). Here, I present endophyte genotypes comprised of multiple genetic loci that informed two traits: 1) potential alkaloid production and 2) hybrid origin. Together, these traits may explain host-level differences in endophyte prevalence and transmission observed in the field. First, the seven endophyte genotypes described here (Appendix B), and elsewhere (Young et al. 2009, Charlton et al. 2012, Takach and Young 2014, Charlton et al. 2014), correspond to bioactive alkaloids produced by endophytic fungi *in*

planta. Epichloë alkaloids can influence host fitness by deterring herbivores (Saikkonen et al. 2013), increasing host resistance to pathogens (Pérez et al. 2013), and altering soil microbial composition (Rojas et al. 2016). It is possible that endophytes equipped with a diverse arsenal of alkaloids that increase host fitness may also be selected for increased transmission rates. My results are consistent with this hypothesis: endophytes in *E. canadensis* were more prevalent and transmitted at higher rates (Figure 1.2b), but also possessed more genetic loci for bioactive alkaloids compared to *E. virginicus* (Table 1.1 and Figure 1.4). Also, similar to previous observations (Charlton et al. 2012), endophytes at high prevalence in *E. canadensis* were also of hybrid origin (presence of both mating types) and therefore likely incapable of sexual reproduction and horizontal transmission (Selosse and Schardl 2007, Charlton et al. 2012, Faeth et al. 2017). In contrast, symbionts with mixed transmission modes (i.e., both vertical and horizontal) often occur at lower prevalence than exclusively vertically transmitted symbionts, presumably due to weaker fitness feedbacks with their partner (Afkhami and Rudgers 2008, Rudgers and Swafford 2009). Although I did not observe sexual stromata in any sampled population, sexual reproduction may occur, particularly in *E. virginicus* hosts, because both mating-types were present (a requirement for a heterothallic species) in eight of the 15 plant populations.

Host species co-occurrence explained little variation in either symbiont prevalence or vertical transmission (Table 1.1). Sympatry is a pre-requisite for interspecific gene flow and may also allow for biotic interactions such as competition (Wu et al. 2016) or increased exposure to shared enemies (Ness et al. 2011). I hypothesized that lower symbiont prevalence and transmission in sympatry vs. allopatry

could reflect costs of co-occurring with close relatives. My results do not support this hypothesis. However, molecular evidence of contemporary plant hybridization is necessary to demonstrate that interspecific gene flow was occurring in the sympatric populations I sampled. Future studies could inform this hypothesis by manipulating host outcrossing rates or measuring the strength of intra- vs. interspecific competition (Miller and Rudgers 2014), then quantifying endophyte vertical transmission.

Given the lack of strong evidence for abiotic drivers, what determines variability in symbiont vertical transmission at the landscape level? Here, I suggest some potential mechanisms. First, temporally or spatially fluctuating fitness benefits could maintain variability in transmission rates (Saikkonen et al. 2010a), particularly if, as my data suggest, endophyte genotypes that possibly differ in fitness benefits also differ in vertical transmission success. Explicit measures of both the fitness benefits and transmission rates of endophyte genotypes are necessary to address this hypothesis. Second, coarse-grained environmental variables may not strongly influence vertical transmission in this system, but instead, transmission could fluctuate temporally and spatially with factors such as herbivory. Although I cannot explain much of the variability in symbiont vertical transmission, my data suggest that for some host species, individual-level symbiont transmission constrains symbiont prevalence at the population level (Figure 1.5). Better understanding the sources of variation in individual-level transmission may therefore be the key to understanding larger scale patterns of endophyte distribution and abundance.

Chapter 2

Does host outcrossing disrupt compatibility with heritable symbionts?

Vertically transmitted symbionts are common in macro-organisms and can benefit hosts by increasing defense against biotic and abiotic stress. Because the fitness of partners is coupled via the process of vertical transmission, these symbionts often become specialized on host species or genotypes during long-term co-cladogenesis. However, high levels of specialization could create genetic incompatibilities between symbionts and novel host genotypes, generating costs to the symbiont when hosts outcross or hybridize. Incompatibilities between hosts and symbionts could come in several forms. First, symbionts may fail to colonize novel hosts that are genetically distant from their maternal host. Second, if transmission is successful, the symbiont may not grow in the novel host, which could influence symbiont transmission into subsequent host generations. Lastly, host outcrossing may impact the fitness of symbiotic partners. Here, I conducted a direct test of these hypotheses by manipulating outcrossing events between

genetically distant populations and species of cool-season grasses (family Poaceae) that host systemic vertically transmitted fungal endophytes (genus *Epichloë*). From these crosses, I measured endophyte vertical transmission across two host generations (F_1 and F_2). Host outcrossing was measured in two ways: 1) eight neutral microsatellite markers were used to estimate the genetic distance between mated hosts and 2) cross type, which distinguished intra-population, inter-population, and inter-specific crosses (hybridization). I found that both genetic distance and cross type influenced endophyte transmission into the first offspring generation (F_1), with overall transmission increasing with greater genetic distance between parents. However, I found that endophytes grew equally well in adult F_1 hosts and transmitted at high rates into the F_2 generation. Differences between symbiont transmission into the F_1 and F_2 host generations may be a function of environmental factors and maternal effects. Lastly, I found strong fitness consequences of outcrossing for hosts and the symbionts they harbor as seed germination increased but adult fertility declined with greater genetic distance between hosts. My results provide experimental evidence that host outcrossing does not disrupt compatibility with heritable symbionts.

2.1. Introduction

Most multicellular organisms host heritable microbes that can be vertically transmitted from maternal host to offspring (Funkhouser and Bordenstein 2013). Vertical transmission intimately links host and symbiont fitness via host reproduction and is expected to favor the evolution of mutualism (Ewald 1987, Douglas 1998, Sachs et al. 2004). Indeed, heritable microbes can benefit hosts by increasing defense against biotic

and abiotic stress in exchange for protection and regeneration (Oliver et al. 2005, Singh et al. 2011, Pérez et al. 2013).

An important consequence of vertical transmission is the evolution of specialized host-symbiont relationships that remain stable over macroevolutionary timescales (Bennett and Moran 2015, Cruaud and Rasplus 2016). As a result of long-term evolutionary dynamics, vertically transmitted symbionts specialize with host species or host genotypes, and consequently may become incompatible with genetically novel hosts (Goodrich et al. 2016, Chong and Moran 2016). Much of the evidence for genetic incompatibility between novel host-symbiont pairs comes from experimental interspecific cross-inoculations that result in a breakdown in compatibility- largely in lab-based insect systems. For instance, symbionts experimentally introduced to novel host species experienced low vertical transmission rates, while hosts had shortened lifespans, and reduced fertility (Christensen 1995, McGraw et al. 2002, Kageyama et al. 2006). This loss of compatibility is likely due to a decoupling of physio-chemical signaling between partners (Eaton et al. 2010, Oldroyd 2013). While these experiments inform hypotheses about the evolution of symbiosis, most prior studies have not investigated the consequences of novel host-symbiont interactions that occur naturally or on ecologically relevant timescales (Saikkonen et al. 2010b, Gundel et al. 2010).

One mechanism hypothesized to create genetic incompatibilities between symbiotic partners is genetic exchange between hosts, such as outcrossing or hybridization, which generates novel host genotypes each generation (Saikkonen 2004, Gundel et al. 2012, Gibert and Hazard 2013). Specifically, incompatibility between host and symbiont may arise from their contrasting reproductive modes: heritable symbionts

predominantly reproduce asexually during transmission from mother to offspring, but many hosts readily outcross (Saikkonen 2004). This mismatch means that symbionts encounter a different genetic background in the outcrossed host offspring they colonize compared to the genetic background of the maternal host from which they came, thereby creating the opportunity for host-symbiont incompatibilities (Bergstrom and Lachmann 2003, Jaenike 2012). However, it remains unclear if and how host outcrossing influences symbiotic relationships.

I hypothesize that host outcrossing could affect host-symbiont compatibility in at least two ways. First, the symbiont could experience a reduction in vertical transmission by failing to colonize outcrossed offspring that differ from the maternal genotype, with vertical transmission to offspring hypothesized to decline as genetic distance between parental (P_1) hosts increases (Gundel et al. 2011b, 2012a). Second, if the symbiont successfully transmits, it may experience aberrant growth in the novel host that could influence host fitness. For instance, bacteria (*Buchnera aphidicola*) titer abundance in pea aphid hosts varied in response to host genotype and reduced host reproductive rates at higher abundances (Chong and Moran 2016). Similarly, multiple strains of a heritable endophytic fungus (*Epichloë coenophiala*) either did not grow *in planta* or failed to persist after transplantation into novel adult hosts (Leuchtmann 1992, Leuchtmann and Clay 1993, Christensen 1995). Also, timing of symbiont colonization may determine when incompatibilities arise. For example, if symbiont vertical transmission occurs before host outcrossing, such as in some *Epichloë* endophyte-grass systems, the symbiont colonizes sporophytic maternal seed tissues before cross-fertilization (Majewska-Sawka and Nakashima 2004, Zhang et al. 2017). If this is the case, symbionts first encounter

novel host genotypes upon host germination, growth, and reproduction, which may result in poor symbiont growth in F_1 adults and/or low transmission to F_2 offspring.

Prior research has provided evidence to suggest that host genotypes determine symbiont transmission (Gibert and Hazard 2013) and that changes in transmission are potentially due to host-symbiont incompatibility (Saikkonen et al. 2010b, Gundel et al. 2012). However, additional work is needed on several fronts. First, past studies regarded gene flow as a quantitative metric (outcrossed or not), when in reality it is a continuum ranging from genetic exchange within a population to between species. A gradient of genetic distances is essential for testing predictions about the functional relationship, linear or otherwise, between host genetic distance and symbiont transmission (Gundel et al. 2010). Second, neutral markers used to measure genetic distance likely fail to account for the effect of outcrossing on locally adapted symbiotic partners (Gomulkiewicz et al. 2000). Outcrossing between populations or species exposed to different selective regimes (e.g., climate, predators, pathogens) can have strong, often contrasting, consequences for organisms (Lenormand 2002). For example, outcrossing can either facilitate (Aitken and Whitlock 2013, Tigano and Friesen 2016) or stymie (Rhymer and Simberloff 1996) local adaptation depending upon factors such as specific selection regimes and a population's demographic history (Garant et al. 2007). Given that symbionts can facilitate host adaptation to different environments (Redman et al. 2002, Richier 2005, Rodriguez et al. 2008, Byler et al. 2013), whether outcrossed hosts are from the same population, different populations or different species, could interact or operate independently with host genetic distance to determine host-symbiont compatibility (Gomulkiewicz et al. 2000, Cheplick and Faeth 2009).

Here, I used the symbiotic relationship between primarily vertically transmitted (via seeds) fungal endophytes in the genus *Epichloë* (Clavicipitaceae) and two species of cool-season grasses (*Elymus virginicus* and *Elymus canadensis*) to investigate the effect of host outcrossing on symbiont vertical transmission rates, symbiont density, and multiple components of host fitness. (Dewey 1983, Schardl et al. 2004). This system is particularly well-suited to answer questions regarding the effect of host outcrossing on symbiosis because both hosts can self-pollinate, outcross with conspecifics, and also hybridize inter-specifically (Church 1958).

I addressed the following specific questions: 1) Does host outcrossing influence endophyte vertical transmission rates across multiple host generations (in both F_1 and F_2 generation)? 2) Does endophyte density in F_1 plants respond to host genetic background and positively correlate with vertical transmission rates into the F_2 generation? 3) Does host outcrossing affect offspring fitness at multiple life stages (e.g., F_1 seed germination and adult fertility) and is host fitness associated with endophyte density? To answer these questions, I manipulated gene flow between parental (P_1) grasses of varying genetic distances to generate outcrossed seeds (F_1 generation) and quantified both endophyte vertical transmission rates into and germination success of F_1 seeds. I hypothesized that endophyte vertical transmission rate would decrease as the genetic distance between parents increased. Then, F_1 seedlings were transplanted into a common garden experiment, where I quantified fungal endophyte density *in planta*. Reduced *in planta* fungal growth in hybrid F_1 offspring relative to those generated from intrapopulation crosses would be consistent with incompatibility between symbiotic partners, and could explain variation in endophyte vertical transmission into F_2 seeds because fungal hyphae

must attain a high enough density inside the plant to successfully transmit to host seeds (Christensen 1995, Schardl et al. 2004). I estimated endophyte vertical transmission rates into F_2 seeds to determine if *in planta* symbiont growth positively correlated with symbiont transmission success. Lastly, I determined if outcrossing influenced fertility of hosts and thus opportunities for symbiont transmission by quantifying seed production success of symbiotic F_1 hosts.

2.2. Methods

2.2.1 Study system and plant material

To act as the parental (P_1) generation, I collected ~40 seeds from each of 69 individual plants from across natural populations of cool-season grasses, *Elymus virginicus* (N = 9 populations) and *Elymus canadensis* (N = 5 populations), throughout the Southern Great Plains in spring 2013). These collections ranged from southern Arizona to eastern Kansas and encompassed a strong aridity and temperature gradient (for collection methods see Sneck et al. 2017). Both *Elymus* harbor fungal endophytes within the genus *Epichloë*, which grow asymptomatically in above-ground host tissues. The symbiotic populations included in this study have likely responded to disparate selection regimes because *Epichloë* endophytes are more common in some environments over others (Sneck et al. 2017) and can buffer hosts against environmental stress (Saikkonen et al. 2016). Controlling gene flow between a pollen donor and recipient is possible because *Elymus* anthers emerge prior to stigmas. *E. virginicus* was chosen as the focal host species and primary pollen recipient to reflect previously described gene flow

patterns between sympatric *E. virginicus* and *E. canadensis* populations (Nelson and Tyrl 1978, Saha et al. 2009).

I pooled all collected seeds from an individual plant into a single maternal family. To screen for endophyte-positive plants (E+), 20 seeds per maternal family were surface sterilized in 5% bleach, cold stratified in 10% agarose in Parafilm-sealed petri dishes at 4°C for 2 weeks, and then germinated in the greenhouse at Rice University early spring 2014 and 2015 in 74-well potting trays (Grower's Supply, Dyersville, IA) with peat-based potting soil (Pro-mix, Premier Tech, Quakertown, PA). I checked for endophyte presence in at least two tillers (above-ground branch) using light microscopy under 200X magnification, where fungal hyphae stained with aniline blue are visible in thin sections of the inner leaf sheath (Bacon and White 1994). All E+ seedlings were transplanted into potting soil in 1.8 L pots (Kord Regal Standard Pots), fertilized as needed, and vernalized outside the greenhouse for ~2 months (winter 2014 and 2015) to promote flowering**.** In total, 107 E+ plants were included in the P_1 generation.

Estimating genetic distance between P_1 plants - To determine if endophyte vertical transmission and host fitness were a function of genetic distance between outcrossed P_1 plants, I estimated the mean genetic distance between each maternal family using eight previously developed microsatellite markers that revealed a total of 33 polymorphic alleles (Saha et al. 2009). Specifically, multiple individual plants from the same maternal family (median = 2 min = 1, max = 6) were genotyped and the genetic distance between each family was calculated as the total number of non-identical alleles across all microsatellite loci (min = 1, max = 18), where higher numbers indicate greater genetic distance (Huff et al. 1993). This approach to estimating genetic distance is widely

used for polyploid organisms (Falush et al. 2007, Pfeiffer et al. 2011, Schreier et al. 2013)**.** Given that multiple plants from the same maternal family were genotyped, I observed slight variations in the genetic distance between any two families. To accommodate this variation, I calculated the mean genetic distance across all members of the maternal family. I also genotyped the endophyte, which enabled us to account for effects of endophyte genotype on vertical transmission. (*For detailed methods see* Appendix C.) In addition, I determined the genotype of *Epichloë* sp. in each maternal family by amplifying 18 genetic markers using a multiplex approach described in Sneck et al. 2017. Endophyte genotypes, mainly comprised of two similar profiles, were defined based on genetic loci associated with both alkaloid production and mating type (Charlton et al. 2014) (Appendix C).

2.2.2 Greenhouse crossing experiment

To manipulate gene flow between P_1 plants, I made three types of experimental crosses (N = 160 total crosses): crosses within the same population (intra: N = 22), from different populations (inter: N = 85), or from different species (hybrid: N = 53). I used single plants as both pollen donors and recipients, with recipients treated as blocks (N = 72 maternal blocks) and cross types assigned to individual inflorescences within a plant, which typically had 10-20 inflorescences. To prevent self-pollination, recipient inflorescences were emasculated with fine-tipped forceps then placed in micro-perforated plastic bags (Perf-o-film®, Penn Jersey Paper, Philadelphia, PA). Within 1-3 d of emasculation, I added a pollen donor inflorescence to the bag and agitated it to facilitate pollination (Dewey 1971). Donor inflorescences with intact anthers were removed from

donor plants, and placed in 14-ml water-filled centrifuge tubes attached to a bamboo rod in the pollen recipient pot.

To confirm treatment effectiveness, I assessed seed production and genotyped offspring. Anther removal adequately reduced self-pollination because emasculated inflorescences without a pollen donor produced significantly fewer seeds (N = 30, mean = 2.6, min = 0, max = 18) than bagged, un-manipulated inflorescences (N = 41, mean = 15.51, min = 0, max = 67, t = -6.76, $P < 0.0001$). However, hand pollination techniques were not as efficient as natural pollination because seed production was significantly higher in bagged, un-manipulated inflorescences (i.e., naturally selfed, N = 16, mean = 35.31, min =1, max = 85) compared to experimentally selfed (N = 17, mean = 9.4, min = 0, max = 31, t = 3.72, P = 0.002) inflorescences on the same plant. To verify that treatments produced offspring of the intended parentage, I used the microsatellite markers described above to genotype a subset of experimentally crossed (N = 33) and naturally self-fertilized offspring (N = 7).

Endophyte transmission and germination of the F_1 generation - To determine if outcrossing altered endophyte transmission into F_1 seeds, I harvested mature seeds from pollen recipient inflorescences during summer 2014 (N = 124 crosses) and 2015 (N = 36 crosses). Seeds (N = 1013) were surface sterilized with 5% bleach, suspended in 10% agar within individual petri dishes sealed with Parafilm, and cold stratified at 4°C for 2 weeks. Then, petri dishes were placed under 32-Watt aquarium lights and exposed to 10 h of light per day. Endophyte status of seedlings was determined nondestructively in at least two tillers using light microscopy. I also assayed seeds that failed to germinate after two months by first soaking them in 5% NaOH solution overnight, then squashing and

staining. The stain binds to fungal hyphae, which is visible in the seed coat and/or aleurone layer at 200X magnification (White 1987), regardless of seed or fungal viability.

2.2.3 Common garden experiment

I estimated endophyte hyphal density in F_1 adults and endophyte transmission into F_2 offspring in a semi-natural setting using E+ F_1 offspring from the three cross types: intra: $N = 17$, inter: $N = 68$, and hybrid: $N = 58$ (total =143). I transplanted F_1 offspring into 6 L plastic pots in potting soil (Pro-mix, Premier Tech, Quakertown, PA) during early November 2015 and vernalized outdoors. In February 2016, pots were sunk into 20-cm deep holes at 1 m spacing at a field site in Houston, TX (29.65N, -95.44W). To aid plant establishment, each pot received 16 g of Osmocote® fertilizer (The Scotts Company, Maryville, OH) and was watered daily for one week. Ambient vegetation was mowed as needed to reduce light competition. Unlike my greenhouse experiment, these plants were strictly selfed to control for pollen donor identity. I imposed selfing by bagging three immature inflorescences with micro-perforated bags. Bagged inflorescences produced fewer seeds (mean = 13.2) than unbagged (mean = 24), suggesting that plants allocated more resources to unbagged tillers ($t = -3.65$, $P = 0.0004$).

Endophyte growth in F_1 adult plants - Hyphal density was estimated in a subset of E+ common garden plants (inter: $N = 36$; hybrid $N = 20$) in June 2016, during peak plant reproduction, when fungal endophytes are at high densities (Schardl et al. 2004). I was unable to sample intrapopulation crosses due to tissue senescence later in the season. Endophyte presence was detected in multiple non-destructively sampled tillers per plant

(*N* tillers = 63, mean tillers sampled per plant = 2.5, min = 1, max = 4 using light microscopy). Peel samples were taken from the same tillers from which endophyte transmission was estimated (*detailed below*). In addition to tiller-to-tiller variation within plants, I accounted for within-tiller hyphal density variation by analyzing multiple leaf sheath views per tiller, captured with ZEISS Axiocam ERc 5s microscope camera (median views per tiller =2, min = 1, max = 17). Number of views per tiller was marginally negatively correlated with hyphal density (correlation = -0.23, t_{61} = -1.87, P = 0.066). Hyphal density was calculated as the mean hyphal length per tiller pooled over all tiller views, or the mean number of scaled pixels (288 pixels = 10 µm) occupied by hyphae visible within 200X magnification field of view (image processing software ImageJ: (Schneider et al. 2012).

Endophyte transmission success into the F_2 generation - In August 2016, I harvested F_2 seeds from bagged inflorescences to estimate vertical transmission rates with a high-throughput antibody immunoblot membrane that narrowly targets *Epichloë* endophyte proteins (Agrinostics Ltd Co., Watkinsville, GA) in this system (Sneck et al. 2017). I assayed multiple seeds per inflorescence (median seeds per inflorescence = 10, min = 1, max =16) and multiple inflorescences per plant (mean seeds per plant = 14, min = 1, max = 67) for a total of 1622 seeds assayed. Lastly, I used the number of bagged seeds to correlate endophyte density with host fitness at the individual tiller level.

Adult fertility of F_1 plants - We also collected one non-bagged inflorescence per plant to determine how original cross type affected F_1 seed production (which I knew to be affected by bagging).

2.2.4 Data analyses

I compared four candidate model sets of generalized linear mixed effects models to estimate the influence of host outcrossing on vertical transmission in the F_1 and F_2 generation and E+ host offspring performance at seed and adult life stages (Table 2.1). Transmission and seed germination was treated as binomial response variables, with the total number of trials given by the total seeds assayed per plant (glmer in R version 3.4.0, Package lme4). Fertility, the total number of seeds produced by an unbagged tiller, was treated as a continuous negative binomial response (package glmmADMB in R). Host outcrossing was represented by two predictor variables, genetic distance and cross type, which are distinct because genetic distance was based narrowly on a set of neutral loci and thus, cross type may capture additional genomic differences between individuals potentially due to different selective regimes. I treated genetic distance (GD) as a continuous estimate of genetic differences between parents for both F_1 and F_2 generations (*for details see* Appendix C).

To test for a quadratic relationship between genetic distance and each response variable, a quadratic term was included; however, it received little statistical support based upon Akaike Information Criterion values (AIC_c) (not shown) and was removed from analyses. Additionally, all models included maternal and paternal plant identity as random effects. All possible combinations of additive and interactive relationships between each predictor variable were included in the four candidate model sets including a null model representing only random variance associated with paternal and maternal identity, for a total of five models per candidate model set. When appropriate, I determined that all models conformed to assumptions of binomial models and did not

show significant overdispersion by calculating $\hat{c}$, the sum of squared Pearson residuals divided by the residual degrees of freedom.

Model selection was conducted to rank the relative support for each model within their respective candidate model sets using the second-order bias corrected Akaike Information Criterion (AIC_c) model selection techniques (aictab in R package AICcmodavg). The difference between the best model and all other candidate models was calculated using delta AIC_c (ΔAIC_c), where the best model receives a ΔAIC_c value of zero. To calculate the relative statistical support for each candidate model, I calculated the AIC_c weight ($AIC_c wt$), with higher values indicating more support from the data. Statistical significance of top AIC-ranked models was estimated using likelihood ratio tests (mixed in R package afex).

To determine the association between endophyte density and transmission success, I calculated the Pearson's correlation coefficient between endophyte density in F_1 adult plants and endophyte vertical transmission rate into F_2 seeds at both the individual tiller and plant level. Also, I calculated the correlation between endophyte density and seed production at the tiller level. Lastly, I tested for differences in endophyte density with host genetic distance and between cross types.

2.3. Results

2.3.1 Effectiveness of greenhouse crossing experiment

I predicted that, if experimental crosses were effective, outcrossed offspring would be equally genetically distant from their maternal and paternal parents, and more genetically distant from their maternal parent than self-pollinated offspring. Indeed, the genetic distance to maternal vs. paternal plants was not significantly different (t_{59} = 1.72 P = 0.091), while outcrossed offspring were on average more genetically distant from maternal plants than self-fertilized offspring (t_{12} = 3.95, P = 0.002) (Appendix C1, Figure C1). Additionally, genetic distance between cross types tracked genetic distance of the microsatellite markers in ways I intended: hybrid cross types were significantly more

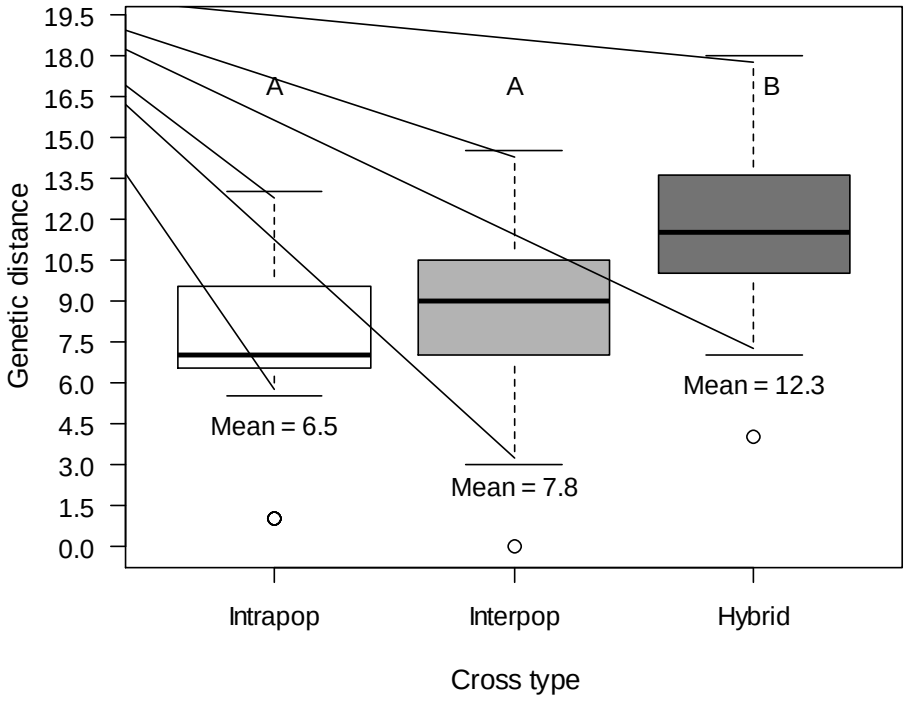

Figure 2.1 – Boxplots of estimated mean genetic distance between outcrossed host parents from each cross type within populations (intra-), between populations (inter-) and between species (hybrid). Letters indicate significant difference between means.

genetically distant than intra- ($t_{17} = 5.62$, $P < 0.001$) and interpopulation crosses ($t_{58} = 6.87$, $P < 0.001$), but genetic distances of inter- and intrapopulation crosses were not significantly different from each other ($t_{16} = 1.34$, $P = 0.18$) (Figure 2.1).

2.3.2 Vertical transmission rates in response to host outcrossing

I found mixed support for the hypothesis that endophyte vertical transmission rates decline as genetic distance between parental hosts increases (Figure 2.2). For the F_1 generation, there were differences in transmission between crosses within and between host populations and species that could not be fully explained by the microsatellite loci alone (Figure 2.2b red line, Table 2.1). Specifically, I found statistical support that transmission rates differed between cross types and with mean genetic distance between parents ($AIC_c wt$ = 87%, likelihood ratio test: $\chi^2 = 12.24$; df = 2, $P = 0.002$, Table 2.1) (Figure 2.2a). Mean endophyte transmission for both intra- and inter-population crosses increased from 61 - 85% at low genetic distances (GD = 3.5 – 7) to 95 - 96% at high genetic distances (GD = 13 – 14.5) respectively. For hybrid crosses, transmission rates remained high at low genetic distances (mean = 100%, GD = 4 - 8) but declined at the highest genetic distances (mean = 62%, GD = 15 – 16). Considering all F_1 cross types together, endophyte transmission increased across genetic distances (dotted red line Figure 2.2a, Table 2.1). In contrast, for the F_2 generation, the null model received the most statistical support ($AIC_c wt$ = 50%), which included only variation associated with maternal and paternal identities (Table 2.1, Fig. 2.2c hatched line). Overall, endophyte vertical transmission rates in both generations were high (F_1: mean = 81%, min = 0%, max = 100%) with mean transmission rates greater into the F_2 generation (F_2: mean = 96%, min = 0%, max = 100%) (Figure 2.2).

Table 2.1 – AIC model rankings. Model fit diagnostics include delta AIC_c (ΔAIC_c) and AIC weight ($AIC_c wt$), which measure model support relative to all other candidate models.

Response	Model	ΔAIC	AIC*wt*
E+ transmission F_1	cross type * genetic distance	0.00	0.87
	cross type	5.90	0.05
	null	6.07	0.04
	genetic distance[1]	7.10	0.02
	cross type + genetic distance	7.71	0.02
E+ transmission F_2	null	0.00	0.50
	genetic distance	2.14	0.17
	cross type	2.27	0.16
	cross type + genetic distance	2.68	0.13
	cross type * genetic distance	5.39	0.03
Seed germination F_1	cross type * genetic distance	0.00	0.83
	cross type	5.26	0.06
	cross type + genetic distance	5.67	0.05
	genetic distance	5.83	0.05
	null	8.46	0.01
Adult fertility F_1	genetic distance	0.00	0.35
	cross type + genetic distance	0.80	0.23
	cross type	0.80	0.23
	cross type * genetic distance	2.80	0.12
	null	3.53	0.06

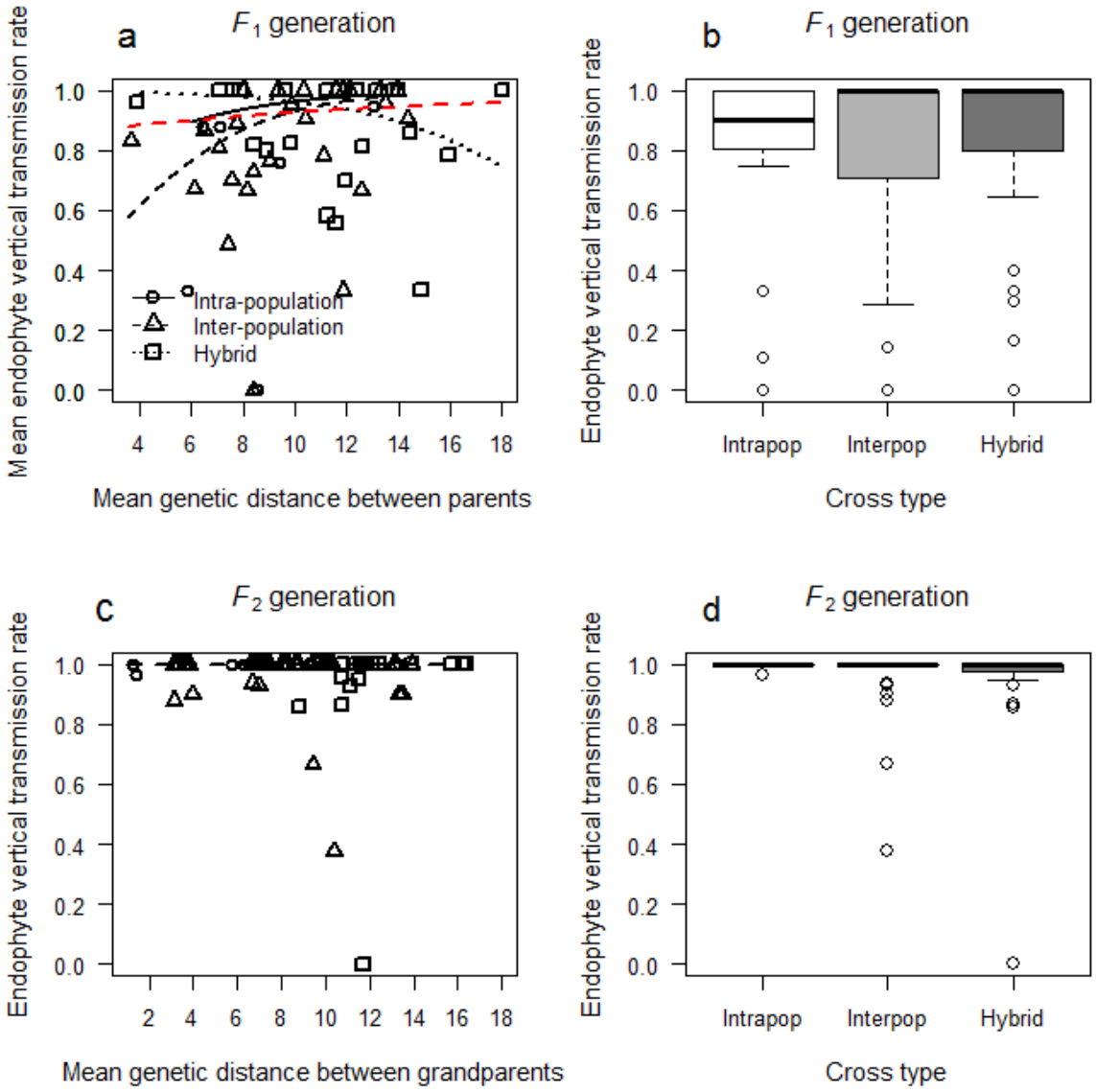

Figure 2.2 – The endophyte vertical transmission rate in F_1 (a) and F_2 (b) host generations as a function of mean genetic distance between parents and cross type (c & d). Fitted lines in panels (a) and (c) are parameters from the top AIC models (Table 2.1). The dotted red line in panel (a) is the plotted parameters from the genetic distance canddiate model[1] (Table 2.1). Intra-population crosses are indicated by open circles and fitted parameters are plotted with a solid line. Inter-population crosses are indicated by triangles a perforated line. Lastly, hybrid crosses are indicated by squares a dotted line. In panel c, the null model is plotted with a perforated line.

2.3.3 Association between endophyte growth and vertical transmission rate

Endophyte hyphal density within individual tillers was not significantly associated with vertical transmission into the F_2 generation (-0.08, t_{61} = -0.636, P = 0.53) (Figure 2.3a). Additionally, endophyte hyphal density at the tiller-level was not significantly correlated with transmission at the whole plant-level because tiller transmission and plant-level transmission were tightly positively correlated (Pearson's correlation coefficient = 0.94, t_{61} = 21, P < 0.0001). Endophyte density in F_1 adults did not respond to host genetic background as endophyte density was not significantly correlated with genetic distance (correlation coefficient = 0.14, t_{61} = 1.12, P = 0.26), nor were there significant differences in endophyte density between cross types (t_{32} = 0.35, P

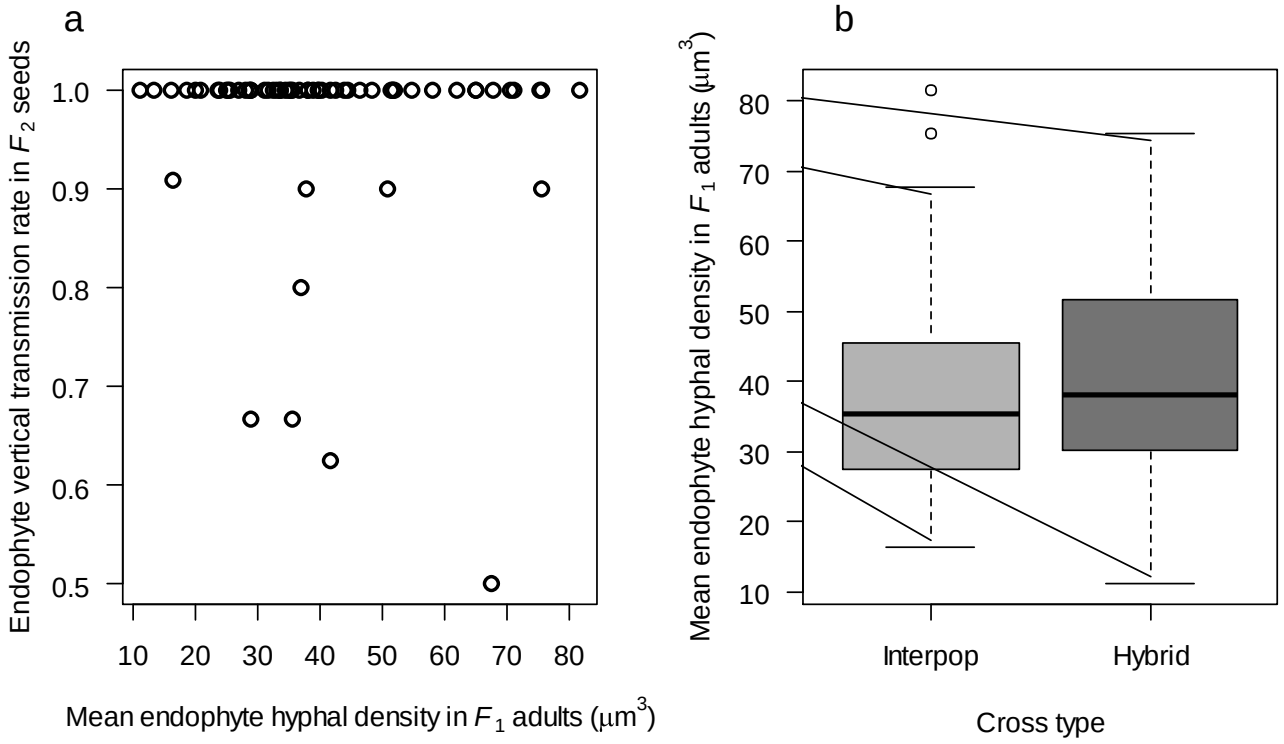

Figure 2.3 – Panel (a) displays the continuous relationship between endophyte vertical transmission rate into F_2 generation seeds and mean endophyte hyphal density (hyphae per μm^3) within F_1 adult plants. Panel (b) is a boxplot of mean endophyte density in both inter-population and hybrid cross types.

= 0.73) (Figure 2.3b). Lastly, endophyte density was not correlated seed production at the tiller level (correlation coefficient = 0.081, $t_{61} = 0.63$, $P = 0.5$)

2.3.3 The influence of host outcrossing on E+ offspring fitness

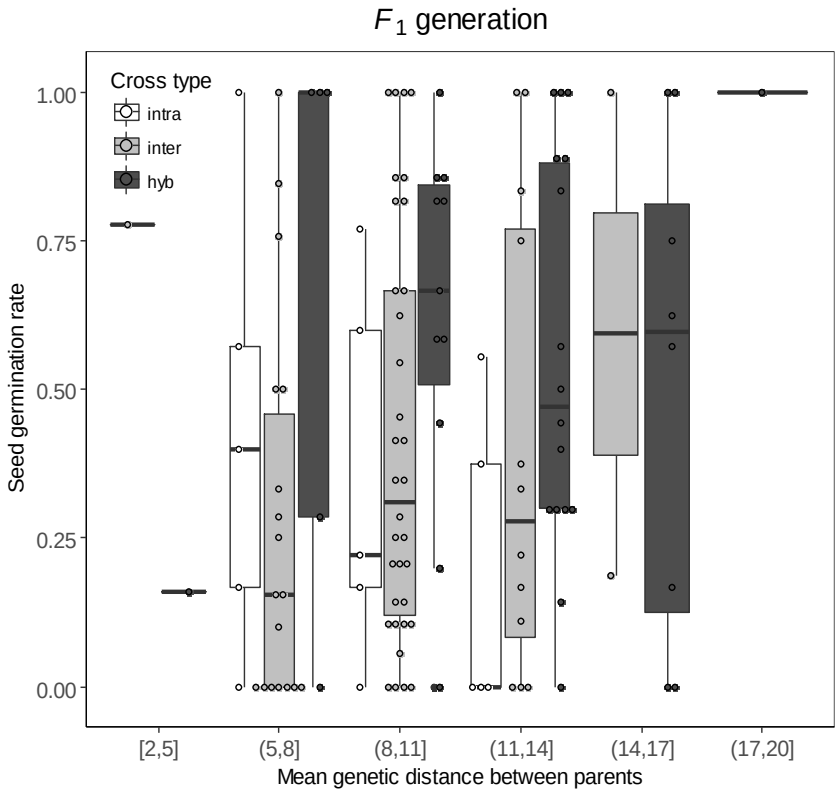

Figure 2.4 – Relationship between F_1 seed germination success rate and mean genetic distance between parents.

F_1 Seed germination: F_1 seed germination depended upon both the genetic distance between parents and cross type, as I found strong statistical support for an interactive effect of cross type and the genetic distance ($AIC_c wt = 83\%$, $\chi^2 = 10.20$, df= 2, $P = .006$, Table 1). Each cross type experienced different effects of genetic distance on germination, which declined with greater genetic distance for both hybrid and intrapopulation offspring and increased for interpopulation offspring. Post-hoc comparisons revealed that, on average, hybrid offspring germinated at the highest rates (mean = 58.6%) compared to both intra-population (mean = 32.2%, $z = 4.047$, $P < 0.0001$) and inter-population offspring (mean = 38.3%, $z = 4.027$, $P < 0.0001$) (Figure 2.4).

F_1 Adult fertility: Fertility (number of seeds produced) declined with increasing genetic distance (AIC_c*wt* = 35%) and also depended upon cross type (AIC_c*wt* = 23%), where hybrids experienced the lowest mean fertility rates (63%) compared to both intra- (92%) and inter-population (90%) F_1 adults (Figure 2.5). Although a single model did not receive the most AIC_c*wt* support, the top three models all contained genetic distance and cross type as predictor variables ($< \Delta AIC_c = 2$) (Table 2.1).

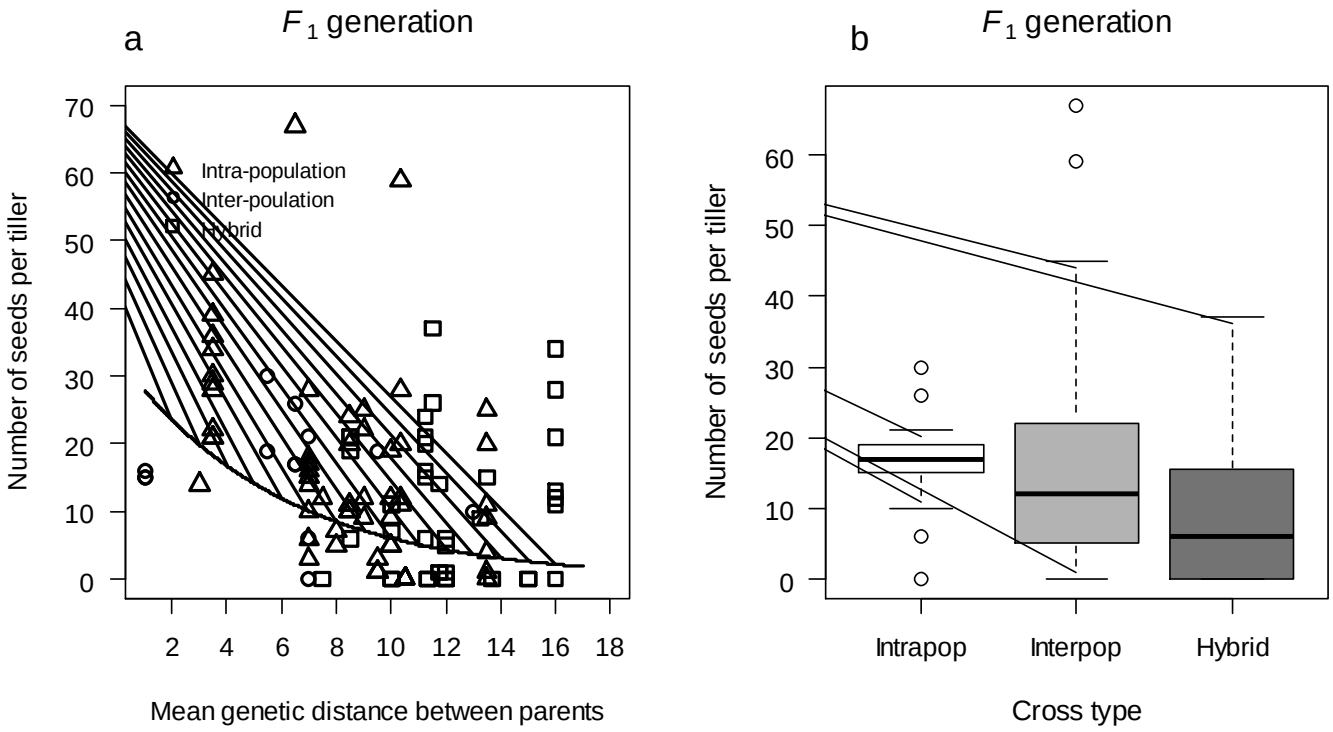

Figure 2.5 – Relationship between mean fertility of F_1 adult hosts and mean genetic distance between parents (a) and cross type (b). The fitted solid line in panel (a) is plotted parameters from the top AIC model (Table 2.1).

2.4. Discussion

Host outcrossing is hypothesized to create genetic incompatibilities between symbiotic partners that could reduce symbiont vertical transmission rates as well as alter both symbiont growth and host fitness (Saikkonen 2004, Cheplick and Faeth 2009). Importantly, the degree to which host outcrossing disrupts host-symbiont interactions may be a function of the genetic distance or the cross type of outcrossed hosts (Gundel et al. 2010). As a novel test of this hypothesis, I manipulated gene flow between two grass species that harbor vertically transmitted endophytic fungi and measured symbiont vertical transmission rates into multiple host generations, symbiont growth within outcrossed hosts, and several metrics of host fitness.

First, I found mixed support for the hypothesis that symbiont vertical transmission rates decline with increasing genetic distance between outcrossed hosts. In the F_1 generation, mean transmission rates were driven by two interacting factors, 1) cross type: whether mated P_1 hosts were from the same population (intra-), different populations (inter-), or different species (hybrid), and 2) the genetic distance between them (Figure 2.2a, b). Although this interaction received strong support ($AIC_c wt$ = 87%), differences in endophyte transmission between cross types may be a statistical artifact given most data clustered at or near 100% transmission (Figure 2.2a). If endophyte transmission does respond to host cross type, this suggests that host-symbiont incompatibilities likely arise after cross-pollination and/or symbionts may preferentially transmit into some host genotypes over others. Overall, these data demonstrate that endophyte transmission is robust to host outcrossing, but host-symbiont compatibility may weaken at the extreme ends (low and high) of genetic distance (Gundel et al. 2012). Given that *Elymus* spp. have

diverged relatively recently and continue to experience interspecific gene flow, selection may have favored generalist endophytes that occupy a diversity of host genotypes (Leuchtmann and Clay 1993, Saha et al. 2009, Sun 2014). Additionally, when considering the influence of genetic distance alone, a positive relationship between endophyte vertical transmission and genetic distance emerged (Figure 2.2a red dotted line). This trend is contrary to hypotheses regarding host-symbiont compatibility (Saikkonen 2004, Gundel et al. 2012), but may reflect empirical work demonstrating endophyte transmission rates are higher in hosts with higher fitness (Gundel et al. 2011a). Therefore, it is possible that endophytes selectively transmit into seeds with ideal genetic backgrounds, especially if offspring sired by parents of moderate to high genetic distances are more fit, as some of my data suggest (Figure 2.4 and 2.5). However, which symbiotic partner controls symbiont transmission, the host or the symbiont, is still an open question in this system (Gundel et al. 2017).

In the F_2 host generation, endophyte vertical transmission rates remained high (mean = 96%) across genetic distances for all cross types (Figure 2.2c, d) compared to the F_1 generation, which experienced an increase in vertical transmission with host genetic distance. There could be several reasons for different transmission rates between host generations. First, the F_1 generation was produced by experimental outcrossing treatments, which I know reduced seed set compared to self-fertilized individuals in the greenhouse. Therefore, experimental treatments may have induced pollen limitation. In contrast, the F_2 generation was produced by self-fertilization in the common garden, where pollen was likely more abundant. Given that endophytes can manipulate host reproduction by allocating resources to maternal (seed) over paternal (pollen) functions

(Gorischek et al. 2013), it is possible that endophyte transmission also responds to pollen load. Targeted studies controlling pollen grain count, viability, and donor identity are required to test this prediction. Second, endophytes transmitting into F_1 seeds in the greenhouse were exposed to a different environment than F_2 seeds in the common garden. In the greenhouse, water availability and soil nutrients were controlled, whereas F_1 adults were exposed to ambient common garden conditions. Although past research did not detect differences in *Epichloë* endophyte transmission due to host resource availability (Davitt et al. 2011), studies in other systems have shown changes in symbiont frequency in response to the presence or absence of herbivores (Clay et al. 2005) and parasites (Oliver et al. 2008). Therefore, exposure to differing biotic and abiotic environments may have driven the differences in endophyte transmission between greenhouse and common garden hosts. Note that physical environment was confounded with maternal environment between the F_1 and F_2 generation in this study, but in context of past research, I hypothesize that, in addition to the genetic background of hosts, symbiont transmission may be sensitive to environmental conditions.

Another potential consequence of host-symbiont incompatibility is reduced symbiont growth in novel hosts. I hypothesized that endophyte density would be lowest in hosts with the most genetically distant parents. Moreover, limited symbiont growth could lead to changes in endophyte vertical transmission rates (Gundel et al. 2011b). Contrary to this prediction, I found that endophyte hyphal density did not respond to host genetic distance or cross type, with endophytes growing equally well in inter-population (mean density = 41.5 hyphae per μm^3) and hybrid cross types (mean density = 39.8 μm^3) (Figure 2.3b). This observation contrasts with previous inoculation experiments that

observed reduced or abnormal growth of symbionts in novel hosts (Christensen 1995, Chong and Moran 2016), but provides an additional line of evidence that *Epichloë* endophytes can be compatible with diverse *Elymus* genetic backgrounds.

Even though symbionts can occupy and grow in genetically distant hosts, I found that outcrossing can have contrasting fitness consequences for both symbiotic partners. For instance, seed germination rates were determined by an interaction between genetic distance and cross type, with hybrid seeds experiencing the highest germination rates (Figure 2.4). This trend suggests that outcrossing between hosts, even hybridization, can be beneficial to symbiotic pairs by potentially providing additional genetic variation (i.e. heterosis) (Ellstrand et al. 2013). On the other hand, I observed strong fitness costs to host outcrossing. Host fertility (seed production) decreased as genetic distance between parents increased (Figure 2.5a), with hybrids producing the fewest seeds (Figure 2.5b). Therefore, even if symbiont transmission remains high, the fitness consequences of outcrossing on hosts presents strong tradeoffs for the symbionts they harbor. This finding is particularly relevant for symbiotic hosts susceptible to pulsed outcrossing events, such as hybrid zones or broadcast spawning events. Previous experimental crosses between *Elymus virginicus* and *E. canadensis* also produced infertile hybrids without controlling for endophyte presence, which suggests that host outcrossing influences host fitness regardless of host-symbiont compatibility (Church 1958, Nelson and Tyrl 1978). Future studies should investigate the role of host outcrossing on both symbiotic and non-symbiotic hosts, which would be a critical control to determine the direct and indirect effects of outcrossing on host fitness and host-symbiont compatibility.

In conclusion, this study provides novel experimental evidence that outcrossing between genetically distant hosts does not disrupt host-symbiont compatibility via reductions in symbiont transmission into and occupation of genetically novel hosts. Surprisingly, host outcrossing may increase rather than decrease symbiont vertical transmission rates, which is a factor theorized to drive symbiont prevalence in host populations (Saikkonen et al. 2002, Gundel et al. 2008, Yule et al. 2013, Bibian et al. 2016). I also demonstrate that host outcrossing can have strong and contrasting fitness effects for hosts and the symbionts they harbor. Therefore, I posit that gene flow between hosts could play a role in determining the prevalence of heritable symbionts in natural host populations and should be incorporated into future population models used to characterize the outcome of symbiotic interactions.

Chapter 3

The effect of host outcrossing on symbiont population dynamics

Heritable symbionts, passed from maternal host to offspring, are both ecologically and evolutionarily influential. Despite their widespread importance, we lack a basic understanding of factors that determine symbiont prevalence in natural host populations. Outcrossing between genetically distant hosts is hypothesized to influence symbiont population dynamics by creating genetic incompatibilities between specialized symbiotic pairs, which could reduce both symbiont transmission and the fitness benefits of symbiosis. Using the grass-fungal endophyte system (genus *Epichloë*), I tested this hypothesis by comparing the fitness (vital rates) of experimentally outcrossed symbiotic (E^+) and non-symbiotic (E^-) hosts of known genetic distances in a common garden setting. I also quantified endophyte vertical transmission rates in all outcrossed E^+ hosts. Vital rate measurements from the common garden were used to parameterize a size-structured population model, which both accounted for imperfect symbiont inheritance

and predicted symbiont equilibrium frequencies at different levels of host genetic distance. In contrast to my hypothesis, I uncovered several lines of evidence that host outcrossing does not disrupt symbiosis. First, endophyte vertical transmission and E^+ host fitness remained relatively high across host genetic distances. Second, the population model predicted that symbiont prevalence would increase, rather than decrease, in genetically distant host populations. This pattern is largely explained by greater E^+ host survival and fertility (probability of producing offspring) at high genetic distances compared to E^- hosts, thereby demonstrating that symbionts can buffer hosts against outbreeding depression. Together, these findings suggest that symbiotic relationships are robust to host outcrossing and that symbionts potentially play a role in mitigating the deleterious fitness consequences of outcrossing between genetically distant host populations (e.g., hybridization).

Introduction

Organisms across taxa are increasingly seen as indivisible from the diverse microbial symbionts they host, many of which vertically transmit from maternal host to offspring. Symbiont inheritance tightly connects host and symbiont fitness via host reproduction and is theorized to select for mutualistic partnerships (Ewald 1987, Sachs et al. 2011). In fact, beneficial vertically transmitted symbionts are widely observed in nature, whereby hosts gain increased defense against pathogens (Pérez et al. 2013), parasites (Oliver et al. 2005), and environmental stress (Malinowski and Belesky 2000) in exchange for symbiont reproduction and dispersal. Vertically transmitted symbionts can also influence host evolution by facilitating the occupation of novel niches (*reviewed in*

(Brucker and Bordenstein 2012). For instance, acquiring heritable bacteria (genus *Buchnera*) enabled sap-feeding insects to dominate vascular plants as a food source ~160-280 million years ago (Moran et al. 1993, Zientz et al. 2004). However, despite the ecological and evolutionary importance of vertically transmitted symbionts, much is unknown about factors that constrain symbiont persistence in natural host populations (Borer et al. 2013).

Due to positive fitness feedbacks between symbiotic partners, theory suggests beneficial vertically transmitted microbes should reach fixation in host populations (Clay 1990). However, heritable symbionts typically persist at intermediate levels, with anywhere between 0 and 100% of hosts in a population harboring the symbiont (insects: (Hilgenboecker et al. 2008); plants: Sneck et al. 2017; animals: (Usher et al. 2001)). This pattern has made predicting equilibrium symbiont frequencies difficult. However, theoretical and empirical models have identified two main drivers of symbiont frequencies in host populations: 1) the rate of symbiont vertical transmission from host to offspring, and 2) the relative fitness of symbiotic (E^+) vs. non-symbiotic (E^-) hosts (Ravel et al. 1997, Saikkonen et al. 2002, Gundel et al. 2008, Gibert and Hazard 2013, Bibian et al. 2016). Therefore, identifying factors that modify these two processes is key to predicting equilibrium symbiont frequencies in host populations.

One factor hypothesized to alter both symbiont vertical transmission rates and symbiont effects on host fitness is outcrossing between genetically distant hosts (Cheplick and Faeth 2009, Gundel et al. 2010). Specifically, outcrossing between host populations or host species produces novel genotypes that could become genetically incompatible with specialized clonal symbionts (Saikkonen 2004, Bennett and Moran

2015). Specifically, vertically transmitted symbionts occupy particular host genotypes and have few opportunities to outcross compared to hosts (Saikkonen 2004, Gundel et al. 2012, Gibert and Hazard 2013). These two factors: 1) symbiont host specificity and 2) contrasting rates of sexual reproduction, set the stage for genetic incompatibilities to arise (Bergstrom and Lachmann 2003, Jaenike 2012).

The effects of host outcrossing on symbiotic partner compatibility could manifest in several ways. First, the symbiont may experience a reduction in vertical transmission by failing to colonize outcrossed offspring due to physiological constraints (Bright and Bulgheresi 2010, Eaton et al. 2010, Oldroyd 2013). Symbiont transmission failure resulting from novel host-symbiont pairings has been documented in both lab-based cross-inoculations (do Valle Ribeiro 1993, Christensen 1995, Brem and Leuchtmann 2002, Kageyama et al. 2006) and field-based experimental gene flow studies (Saikkonen et al. 2010). Second, if the symbiont is successfully transmitted, mutualistic benefits to the out-crossed host may be reduced or the symbiont could even become costly to the host (Christensen et al. 1997, Chong and Moran 2016). For instance, bacteria (*Buchnera aphidicola*) abundance varied by host genotype and reduced host fitness in novel pea aphid hosts (Chong and Moran 2016).

To predict the influence of host outcrossing on heritable symbionts, it is important to consider the direct effects of host outcrossing on host fitness, independent of disruptions to host-symbiont compatibility. On the one hand, outcrossing could increase heterozygosity and its potential fitness benefits (e.g., heterosis) (Holt and Gomulkiewicz 1997, Charlesworth and Charlesworth 1999). On the other hand, host outcrossing may reduce host fitness if alleles are transferred between locally adapted populations or

species that experience different selective regimes (e.g., outbreeding depression) (Lynch 1991, Keller and Waller 2002, Edmands and Timmerman 2003). Given the counter-acting forces of heterosis and outbreeding depression, one hypothesis is that host fitness is maximized at an intermediate genetic distances of outcrossed individuals (Gundel et al. 2010).

Additionally, outcrossing between hosts may have fitness consequences for the symbionts they harbor. Heterosis in hosts may cause symbionts to experience higher fitness, but this will depend upon the degree of compatibility with the outcrossed hosts. An interaction between symbiotic presence or absence and host outcrossing can arise if genetic incompatibilities increase with genetic distance and reduce fitness benefits of the symbiosis (Gundel et al. 2010). Therefore, in combination with potential reductions in transmission efficiency due to physiological incompatibilities between novel host-symbiont combinations, host outcrossing could limit population-level symbiont frequencies.

Few studies have experimentally tested the effects of host outcrossing on individual-level host-symbiont compatibility, and none have evaluated its effects on population-level symbiont dynamics. Recent observational and empirical studies have shown host genetic background alters symbiont transmission, potentially due to host-symbiont incompatibility (Saikkonen et al. 2010b, Gundel et al. 2011a, Gibert and Hazard 2013). However, most studies regard gene flow as a quantitative metric (outcrossed or not), when in reality gene flow occurs on a gradient from genetic exchange within a population to between species (hybridization) and can have strong demographic consequences particularly in plant systems (Ellstrand 2014, Gompert and Buerkle 2016).

To my knowledge, no previous studies have considered a continuous genetic gradient to assess the potential non-linear relationships between host outcrossing and symbiotic interactions. Furthermore, the connection between experimental estimates of host and symbiont incompatibility to population-level patterns of symbiont prevalence has yet to be made. This connection is the critical link for determining if host outcrossing can promote intermediate symbiont frequencies.

Here I test for the influence of quantitative variation in host outcrossing on the dynamics of vertically transmitted symbionts in natural host populations. To accomplish this, a direct comparison between symbiotic (E^+) and non-symbiotic (E^-) hosts of similar genetic background is essential, and this comparison must be replicated across genetic distances of parent hosts. I conducted this comparison by experimentally manipulating outcrossing events between cool-season (Poaceae, sub-family Pooideae) grasses of known genetic distances that host vertically transmitted systemic endophytic fungi in the genus *Epichloë* (Schardl et al. 2004). Then, in a common garden setting, I measured both endophyte vertical transmission rates and vital rates of E^+ and E^- outcrossed hosts (e.g., survival, growth, and fertility) over two reproductive seasons and asked: 1) How do endophytes impact host vital rates, and 2) How does host outcrossing affect symbiosis? With these vital rates, I also parameterized a size-structured matrix population model that accounted for imperfect endophyte transmission and host genetic background to determine: 3) Do individual host-level endophyte effects scale up to modify endophyte persistence?

3.1. Methods

3.2.1 Study system and plant material

To act as parent hosts, I collected ~40 seeds from each of 60 individual plants from across natural populations of cool-season grasses, *Elymus virginicus* (*N* = 9 populations) and *Elymus canadensis* (*N* = 5 populations), throughout the Southern Great Plains in spring 2013. These collections included grass populations from northern Arizona to eastern Kansas (*for collection methods see* Sneck et al. 2017). Both *Elymus* species harbor systemic vertically transmitted fungal endophytes within the genus *Epichloë* that can buffer hosts against environmental stress (Saikkonen et al. 2016). This system allowed us to take advantage of intermediate transmission, where E^+ hosts produce both E^+ and E^- seeds (Afkhami and Rudgers 2008, Sneck et al. 2017). This means that, for a given genetic distance between recipient (maternal) and donor (paternal) plants, the maternal hosts can produce both E^+ and E^- offspring that we presume differ only in the presence of the symbiont. I controlled gene flow between pollen donor and recipient by removing anthers prior to stigma emergence as described in Chapter 2. *E. virginicus* was the focal host species and primary pollen recipient to reflect patterns of gene flow previously described between sympatric *E. virginicus* and *E. canadensis* populations (Nelson and Tyrl 1978, Saha et al. 2009).

To propagate hosts, I followed protocols in Chapter 2. Briefly, I pooled all collected seeds from an individual plant into a single maternal family. Twenty seeds per maternal family were germinated in 74-well potting trays (Grower's Supply, Dyersville, IA) with peat-based potting soil (Pro-mix, Premier Tech, Quakertown, PA) in the

greenhouse at Rice University early spring 2014 and 2015 and non-destructively screened for endophytes using light microscopy (Bacon and White Jr 1994). Additionally, endophyte negative (E^-) hosts from the same maternal family or original population collection were propagated to serve as close proxies to full-sibling E^+ vs. E^- comparisons. To promote flowering, seedlings were transplanted into potting soil in 1.8 L pots (Kord Regal Standard Pots), fertilized as needed, and vernalized outside the greenhouse for ~2 months (winter 2014 and 2015). In total, my experimental crosses included 45 E^+ and 37 E^- individual parents. The genetic distance between each maternal family was estimated using eight previously developed neutral markers (Saha et al. 2009). For an in-depth description of molecular and computational methods used to estimate genetic distance, see Appendix C. To estimate the genetic distance between crossed plants, multiple individual plants from the same maternal family (median = 2 min = 1, max = 6) were genotyped and the genetic distance between each individual family was calculated in a distance matrix as the total number of non-identical alleles at each microsatellite locus, with higher numbers indicating greater genetic distance. Multiple plants from the same maternal family were genotyped, which resulted in slight variations in genetic distance between any two families. To accommodate this variation, I calculated the average of genetic distances between each family, which I refer to as genetic distance or mean genetic distance between parents. Additionally, I genotyped the *Epichloë* endophytes spp. within each maternal family (Charlton et al. 2012). All endophytes in this study are made up of two similar genetic profiles (Appendix B).

3.2.2 Greenhouse crossing experiment

I manipulated host outcrossing by conducting experimental gene flow treatments between plants from relatively low to high genetic distances, which fell into three categories: intra-population (*N* = 31), inter-population (*N* = 125), and inter-specific or hybrid (*N* = 83) crosses (*N* =240 total crosses). Individual plants acted as both pollen donor and recipients, with maternal pollen recipients treated as blocks. In other words, each cross involving an individual maternal genotype were considered within the same maternal block (*N* blocks = 71). *Elymus virginicus* produces ~10-20 reproductive tillers per plant, each with a single inflorescence that contains many florets. This allowed us to manipulate crossing events at the individual tiller level within a maternal block. I prevented self-pollination of recipient tillers by first removing immature anthers from all florets, which were then placed in micro-perforated plastic bags (Perf-o-film®, Penn Jersey Paper, Philadelphia, PA). Within 1-3 d of emasculation, I added a pollen donor inflorescence to the bag and agitated to facilitate pollination (Dewey 1971). Donor inflorescences with intact anthers were removed from donor plants, and placed in 14-ml water-filled centrifuge tubes attached to a bamboo rod in the pollen recipient pot. My previous study determined that these outcrossing techniques are effective in several ways (Chapter 2). First, I showed that emasculated plants produced significantly fewer seeds than bagged unmanipulated inflorescences. Second, I used the microsatellite markers described above to genotype a subset of experimentally crossed and naturally self-fertilized offspring, which demonstrated that 1) outcrossed offspring are more genetically distant from their maternal plant than self-fertilized offspring and 2) genetically

intermediate compared to both parents (*for molecular results see* Appendix C and Figure C1).

I propagated E^+ and E^- outcrossed hosts for the common garden experiment by harvesting mature seeds from pollen recipient inflorescences during summer 2014 (*N* = 61 crosses) and 2015 (*N* = 179 crosses). To determine the effect of outcrossing on seed germination rates and for use in the demographic model detailed below, I surface sterilized seeds (*N* = 1013) with 5% bleach, suspended in 10% agar within individual petri dishes sealed with Parafilm, and cold stratified at 4°C for 2 weeks. Then, petri dishes were placed under 32-Watt aquarium lights and exposed to 10 h of light per day. Germinated seedlings were transplanted into potting soil in 1.8 L pots (Kord Regal Standard Pots) in the greenhouse and their endophyte status was determined nondestructively in at least two tillers using light microscopy (Bacon and White Jr 1994).

3.2.2 Common garden

In order to quantify the effect of host outcrossing on symbiosis, I aimed to compare the fitness of symbiotic vs. non-symbiotic hosts with similar genetic backgrounds in a single environment. However, due to high endophyte transmission into seedlings, few E^+ and E^- full-sibling pairs (*N* = 44) were generated for this comparison. Therefore, the common garden experiment included E^+ and E^- paired from crosses between plants with the same parents (*N* = 4 plants), maternal family (*N* = 11), paternal family (*N* = 28), population (*N* = 63), or no direct familial comparison (*N* = 71). A total of 141 E^+ hosts and 80 E^- hosts of varying genetic backgrounds were transplanted into 6 L plastic pots in potting soil (Pro-mix, Premier Tech, Quakertown, PA) during early

November 2015 and vernalized outdoors (Figure 3.1). In February 2016, pots were sunk into 20-cm deep holes at 1 m spacing at a field site in Houston, TX (29.65N, -95.44W). To aid plant establishment, each pot received 16 g of Osmocote® fertilizer (The Scotts Company, Maryville, OH) and was watered daily for one week. Ambient vegetation was mowed as needed to reduce light competition. Unlike my greenhouse experiment, plants were strictly selfed to control for pollen donor identity. I imposed selfing by bagging three immature inflorescences with micro-perforated bags April 2016. The seeds produced via selfing were only used to estimate endophyte vertical transmission rate.

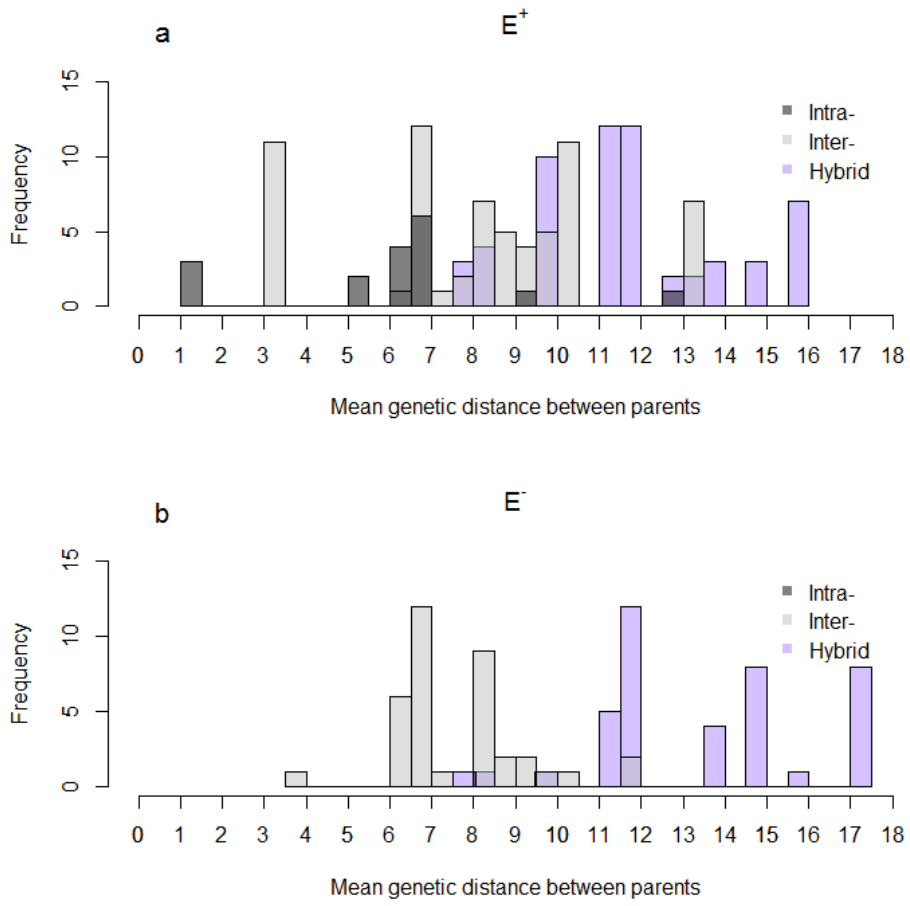

Figure 3.1 – Histograms displaying the frequency of genetic distances represented in the common garden experiment for both E^+ (a) and E^- (b) hosts. Colors indicate different cross types: within a population (dark gray: intra-), between populations (light gray: inter-), and between species (purple: hybrid).

In summer 2016 and 2017 I collected demographic data during peak seed production and before tiller senescence. For each individual plant, I tracked survival as well as counted the total number of vegetative and reproductive tillers to estimate plant size and reproductive effort, respectively. In July 2016, I collected mature seeds from bagged inflorescences to estimate endophyte vertical transmission with a high-throughput antibody immunoblot membrane that targets *Epichloë* endophyte proteins (Agrinostics Ltd Co., Watkinsville, GA). I assayed multiple seeds per inflorescence for both E^+ and E^- plants (median seeds per inflorescence = 10, min = 1, max =16) and multiple inflorescences per plant (mean seeds per plant = 14, min = 1, max = 67) for a total of 1622 seeds assayed. However, bagged inflorescences produced fewer seeds (mean = 13.2) than un-bagged (mean = 24), suggesting that plants allocated more resources to un-bagged tillers (t = - 3.65, P = 0.0004). Therefore, to accurately estimate seed production at the tiller level, I collected one additional non-bagged inflorescence from each plant (N = 2558 seeds). Lastly, in June 2017 I re-affirmed endophyte status and retention in two vegetative tillers from each plant with immunoblot techniques.

3.2.4 Vital rate estimation

I used the two data collection seasons to fit statistical models for seven individual-host level processes (vital rates) that determine population dynamics for symbionts as well as symbiotic (E^+) and non-symbiotic (E^-) hosts: survival (from time t to t +1), growth (size t to size t + 1), probability of producing seeds, number of flowering tillers, fertility of seed producing flowering tillers (total # of seeds per tiller), seed germination, and endophyte vertical transmission rate. The first five vital rates were dependent upon a three-way interaction between presence or absence of symbionts and two continuous factors: log_e

(size *t*) and genetic distance (total number of allelic differences between crossed hosts). Seed germination and endophyte transmission were dependent upon host genetic distance alone. To account for variation in host vital rates unrelated to symbionts, each model also included host maternal and paternal identity as random effects.

Global models for all vital rates, with fully interacting predictor variables (i.e., size *t* * endophyte status * genetic distance), were estimated using generalized linear mixed effects models using a Markov chain Monte Carlo (MCMC) method (R package MCMCglmm, (Hadfield 2010). This approach avoids computational issues associated with Information Criterion model averaging particularly in response to model selection uncertainty and models with complex interactions (Cade 2015). Unless otherwise stated, default priors were used for all models: a Wishart probability distribution for (co)variances and a normal distribution for fixed effects. Specifically, I estimated the probability of seed production and plant survival as Bernoulli responses (0 = produced no seeds or 1 = produced at least one seed) using the "categorical" distribution type and its recommended prior covariance structures (Hadfield 2010). I estimated the probability of seed germination and endophyte transmission as binomial ("multinomial", k = 2) responses, with the total number of trials given by the total seeds assayed per plant. Lastly, I modeled host growth, number of flowering tillers, and number of seeds per flowering tiller using a Poisson distribution with an individual random effect, which approximates a negative binomial distribution. Model convergence was assessed by Gelman-Rubin diagnostics on three separate Markov chains, which quantifies within and between chain variances (settings: number of iterations = 10^5, burn-in =3000, thinning =100) (Gelman and Rubin 1992).

3.2.5 Matrix projection model

To estimate endophyte equilibrium frequencies in response to host genetic distance, I used the vital rate parameters from each linear model to parameterize a matrix projection model (MPM) (Caswell 2001). The population vector included 84 discrete sizes (corresponding to the observed size range of 1 to 84 tillers) from which I calculated the geometric population growth rate (λ) of E^+ and E^- hosts. Similar to previous studies, I have taken a matrix model approach because my data was discrete (size = # of tillers), so constructing a matrix model with as many stages as those observed in the data both improves its predictive ability (Tenhumberg et al. 2009) and avoids placing individuals into arbitrary size classes. Additionally, I followed Compagnoni et al. (2016), and parameterized the MPM with generalized linear statistical models described above, which enabled us to populate a high-dimensional projection matrix with few parameters.

While size was modeled as a discrete variable in the growth sub-model, the predictor used in vital rate functions was log_e(number of $tillers_t$) in addition to the scalar genetic distance (*GD*). The number of 1 tiller sized seedlings in year $t + 1$ produced by adult hosts across size classes was given by:

Equation 3.1– Demographic fertility function

$$\mathrm{n}(1)_{t+1} = \sum_{x=L}^{x=U} P(log_e(x))_{GD} F(log_e(x))_{GD} Ss(log_e(x))_{GD} \mathrm{n}(x)_t \ \varepsilon_{GD}$$

To calculate the number of seedlings per flowering tiller of an *x*-sized plant at a given genetic distance, the probability of producing seeds (*P*) was multiplied by the total

number of flowering tillers (*F*), and by the number of seeds produced per flowering tiller (*Ss*). Viable flowering tillers were then multiplied by the probability of seedling germination, ϵ. The dynamics of adult plants across the 84 discrete size classes were given by:

Equation 3.2– Demographic growth and survival function

$$\mathrm{n}(y)_{t+1} = \sum_{x=L}^{x=U} S\left(log_e(x)\right)_{GD} G\left(y, log_e(x)\right)_{GD} \mathrm{n}(x)_t$$

The first term represents growth from size *x* to *y*, $G\left(y, log_e(x)\right)_{GD}$, conditioned on the probability of survival at size *x* and host genetic distance, $S\left(log_e(x)\right)_{GD}$. Together, these demographic functions constitute the fertility (B = Equation 3.1) and growth/survival (P = Equation 3.2) components of my matrix projection model. Construction and analysis of the MPM occurred in R v. 3.2.4.

3.2.6 Imperfect symbiont vertical transmission

Many vertically transmitted symbionts, including Epichloae endophytes, are lost during transmission from maternal host to offspring (Hilgenboecker et al. 2008, Afkhami and Rudgers 2008). Similar to past studies, I included the possibility of symbiont loss in my demographic model by pairing E^+ and E^- matrix projection models into a single 'megamatrix' that includes transitions between size classes and endophyte status (Yule et al. 2013, Chung et al. 2015). This 'megamatrix' included four submatrices that capture: the probability of successful vertical transmission (E^+ to E^+) represented by τ, endophyte

loss (E^+ to E^-) represented by 1 - τ, persistence of non-symbiotic hosts (E^- to E^-), and the transition of non-symbiotic hosts to symbiotic (E^- to E^+) (Gundel et al. 2008). The effect of imperfect vertical transmission on host plant population growth and equilibrium endophyte frequency were modeled using transmission rates observed in the common garden, which were very high (mean = 96.5%). I did not observe endophyte horizontal transmission in my common garden, which requires the formation of sexual conidia (i.e. choke disease). Therefore, for the E^+ and E^- transition, I populated both the fertility (B) and growth (P) portions of the E^- to E^+ submatrix with 0's. The combined E^+ and E^- models takes the form:

Equation 3.3 – Megamatrix structure accounting for imperfect endophyte transmission

$$\begin{pmatrix} E^-(y)_{t+1} \\ E^+(y)_{t+1} \end{pmatrix} = \begin{pmatrix} B^- + P^- & (1-\tau)\,B^- + P^- \\ 0 & \tau B^+ + P^+ \end{pmatrix} \begin{pmatrix} E^-(x)_t \\ E^+(x)_t \end{pmatrix}$$

3.2.7 The effect of host genetic distance on symbiont population dynamics

Lastly, I determined equilibrium endophyte frequencies in hosts at varying genetic distances. To do this, I used the 'megamatrix' structure described above and the mean posterior predictions from the vital rate models to calculate λ and the equilibrium frequencies for E^+ and E^- hosts across the observed distribution of genetic distances, which was represented by a vector of values ranging from 6 to 16. Truncation of the genetic distance vector was motivated by unrealistic vital rate predictions at extreme ends of the genetic distance gradient where replication was low. I then calculated the endophyte equilibrium frequency across host genetic distances using the stable stage

distribution (sum of right eigenvectors) for both E^+ and E^- hosts (Stubben and Milligan 2007).

3.2. Results

3.3.1 The effect of endophytes on host vital rates

The effects of endophyte symbiosis differed between host vital rates. Endophytes benefitted hosts by enhancing both survival and growth, especially for large plants (Figure 3.2). For instance, mortality rates were twice as high for non-symbiotic hosts (37%) compared to symbiotic hosts (18%) across all host sizes. In addition, endophytes enhanced flowering tiller production (E^+: mean flowering tillers = 8, max = 28; E^-: mean = 6, max = 14) with greater flowering in larger hosts. However, on average, symbiotic hosts produced fewer seeds per tiller (mean E^+ seeds per tiller = 16.4, min = 1, max = 67) compared to non-symbiotic hosts (E^-: = 21.8, min =1, max = 68) (Figure 3.4e and f).

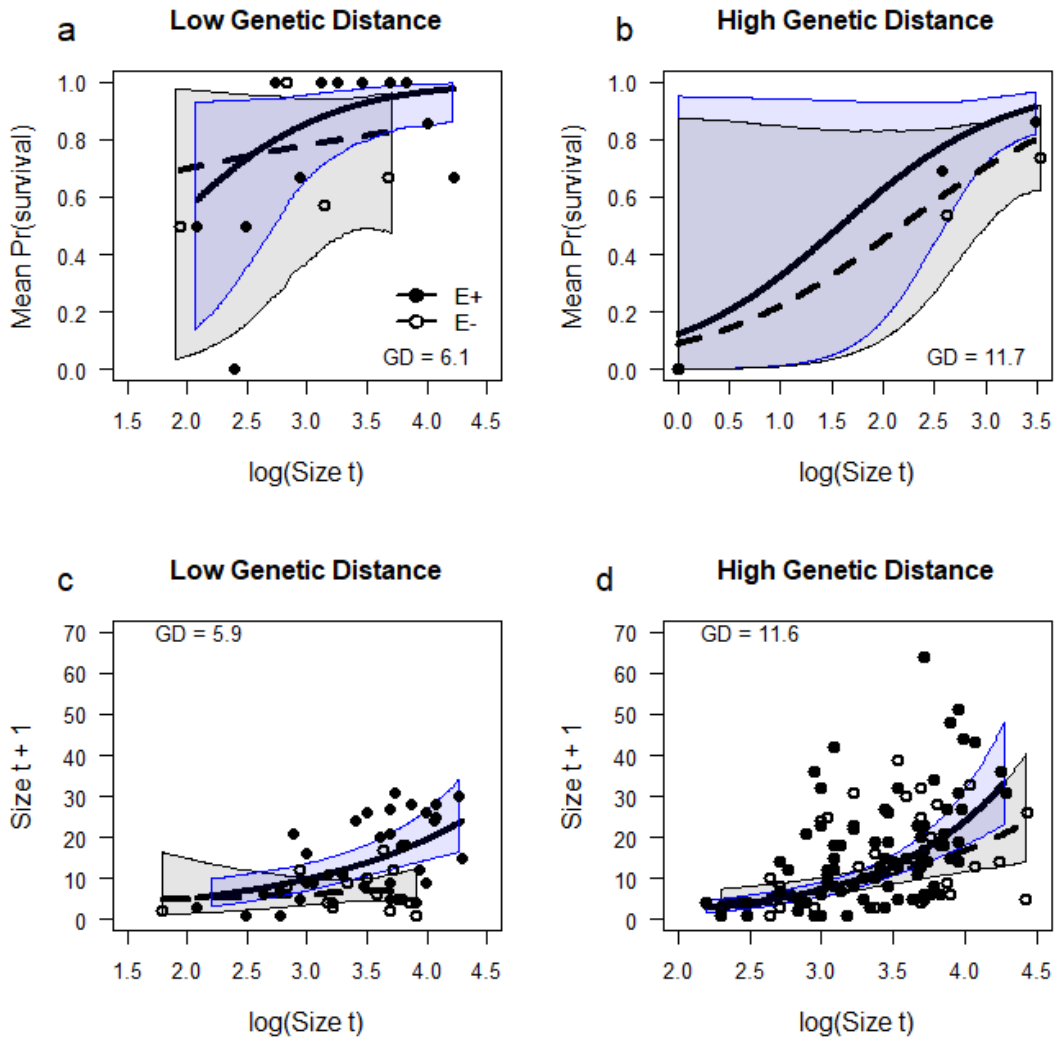

Figure 3.2 – Fitted demographic functions (i.e., posterior means) for *Elymus virginicus* in survival and growth at low (a and c) and high (b and d) genetic distances. The genetic distance plotted for each vital rate is indicated by GD, or the mean genetic distance above (high) and below (low) a genetic distance of 8 for each parameter. Best fit lines and observed data for endophyte-symbiotic (solid line) and non-symbotic populations (dotted line) are filled (E^+) and open (E^-) points, respectively. Shaded regions (E^+ = blue; E^- = gray) indicate the 95% Bayesian Credible Interval.

3.3.2 Effect of host outcrossing on symbiosis

The relative advantage of symbiotic hosts across genetic distances differed between vital rates. For instance, small to moderate sized symbiotic hosts experienced reduced growth (Figure 3.2c and d) flowering, and seed production (Figure 3.3c, d, e, and f) at high genetic distances compared to non-symbiotic hosts. In contrast, endophytes increased host survival (Figure 3.2b) and fertility (Figure 3.3b) in symbiotic hosts relative to non-symbiotic hosts across genetic distances and host sizes. Specifically, both host types successfully produced seeds at low genetic distances (Figure 3.3a); however, at high genetic distances, non-symbiotic hosts experienced a greater reduction in mean fertility (-37%) compared to symbiotic hosts (-24%) (Figure 3.3b). Regardless of endophyte status, several vital rates displayed evidence of outbreeding depression, where fitness is expected to decrease with increasing genetic distance between parental hosts. For example, several metrics of host fitness declined in response to outcrossing between parents at high compared to low genetic distances, including reduced survival rates (Figure 3.2b), greater rates of infertility (Figure 3.3b), and smaller seed sets (Figure 3.3f).

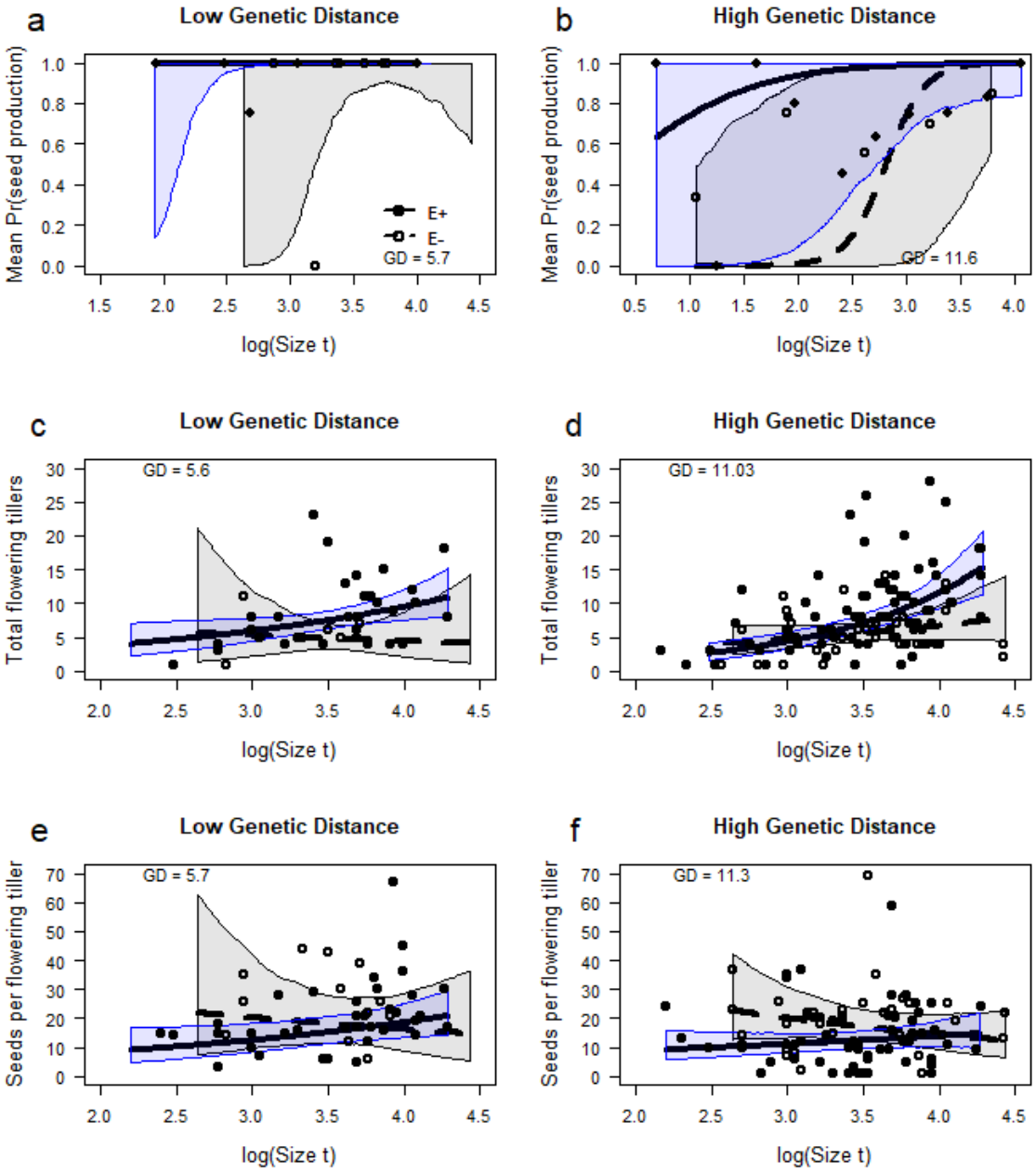

Figure 3.3 – Fitted demographic functions (i.e., posterior means) for *Elymus virginicus* in the probability of production seeds (a and b), the total number of floweirng tillers (c and d), and the number of seeds produced per flowering tiller (e and f) at high and low genetic distances. The genetic distance plotted for each vital rate is indicated by GD, or the mean genetic distance above (high) and below (low) a genetic distance of 8 for each parameter. Best fit lines and observed data for endophyte-symbiotic (solid line) and non-symbotic (dotted line) populations are filled (E^+) and open (E^-) points, respectively. Shaded regions (E^+ = blue; E^- = gray) indicate the 95% Bayesian Credible Interval.

Endophyte transmission and seed germination - In addition to size-dependent vital rates, I also estimated endophyte transmission and seed germination in response to host outcrossing, which were included in the population model (similar transmission and germination results reported in Chapter 2, Figure 2.2c and Figure 2.4, respectively**).** Here, I found that endophyte transmission was close to perfect (mean = 96%, min = 0%, max = 100%) (Figure 3.4a) and seed germination rates increased with genetic distance between parents (Figure 3.4b).

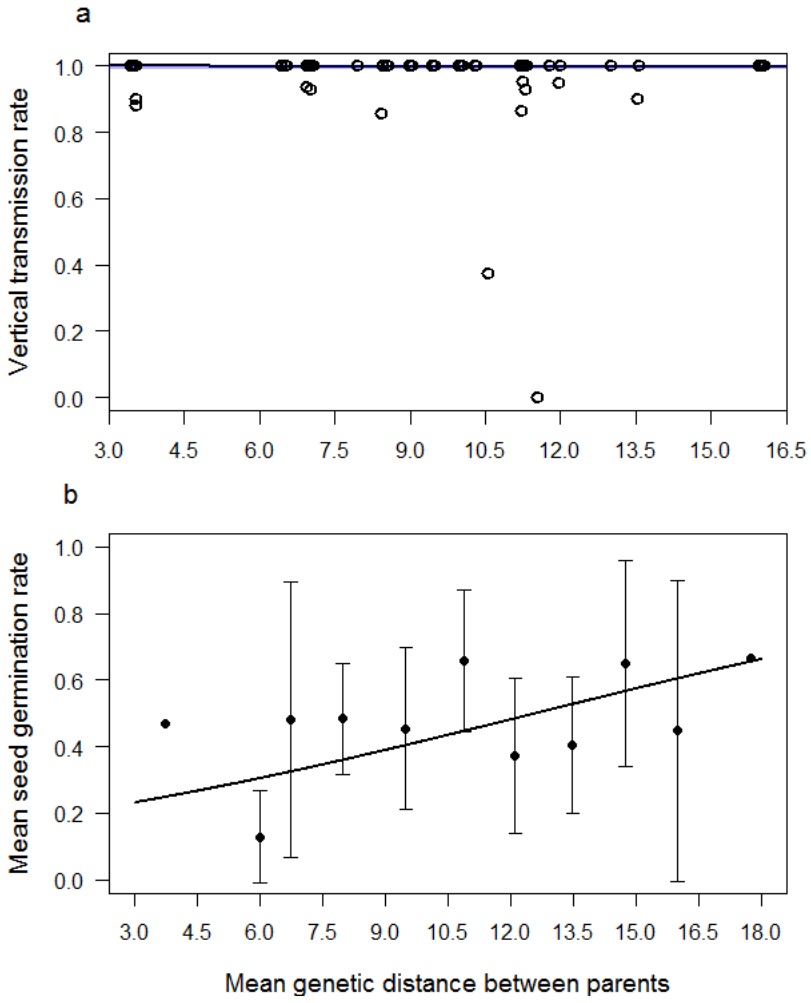

Figure 3.4 – Fitted demographic functions for endophyte vertical transmission rates (a) and seed germination rates ± SD (b) for outcrossed *Elymus virginicus* hosts. The blue line in panel (a) represents the 95% Bayesian Credible Interval.

3.3.3 The effect of host outcrossing on symbiont population dynamics

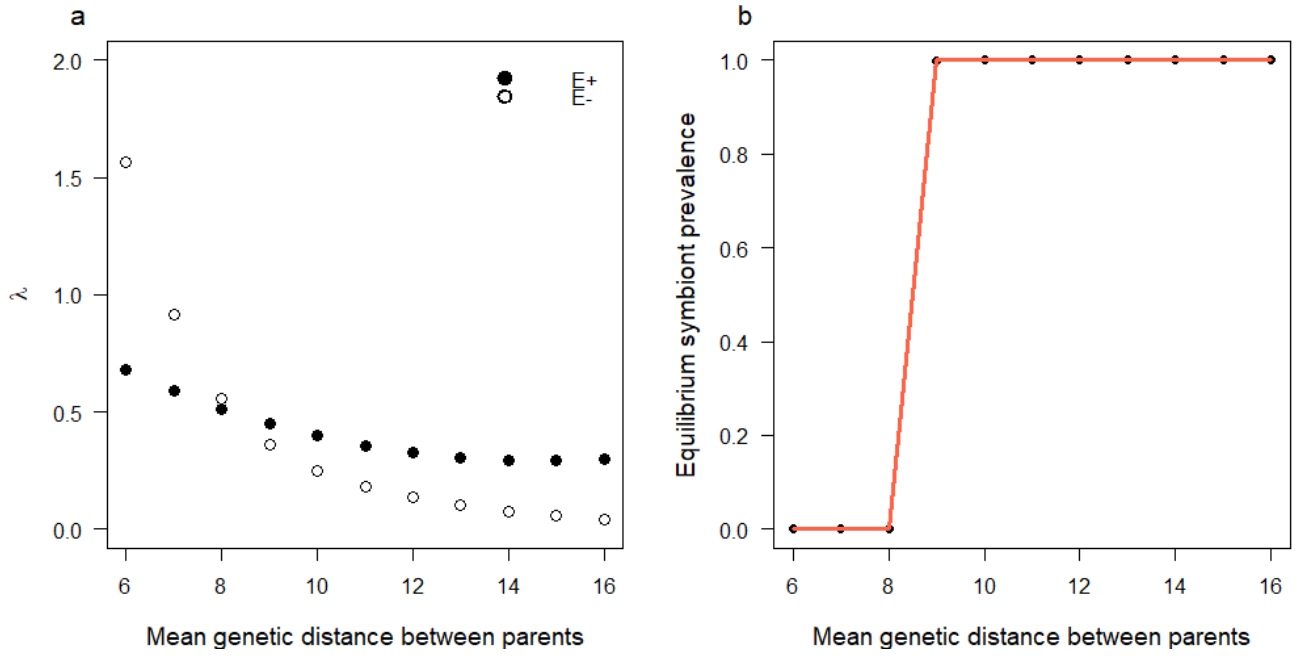

Figure 3.5 – (a) Projected geometric popluation growth rates for symboitic (filled circle) and non-symbiotic (open circle) host populations across mean genetic distance between parents and (b) predicted population-level equilibrium symbiont prevalence across host genetic distances.

The geometric population growth rate (λ) for the symbiotic (E^+) host population decreased from 0.68 to 0.30, with the highest growth rates predicted for hosts at lower genetic distances (Figure 3.5a). I observed a similar trend for the non-symbiotic host population (E^-), where λ decreased from 1.6 to 0.04 as host genetic distance increased. Although on average both common garden populations were predicted to decline ($\lambda < 1.0$), I observed a genetic distance dependent flip in dominance such that lager λ values shifted from non-symbiotic to symbiotic host populations (Figure 3.5b). To determine if this trend was consistent despite variation observed in the vital rate models, I tallied the number of shifts from E^+ $\lambda < E^-$ λ at low genetic distances to E^+ $\lambda > E^-$ λ at high genetic distances by re-running the MPM 200x's populated by samples from the vital rate

parameter posteriors. I found that symbiotic hosts were predicted to have greater lambda values than non-symbiotic hosts twice as often in populations at high genetic distances (Figure 3.6)

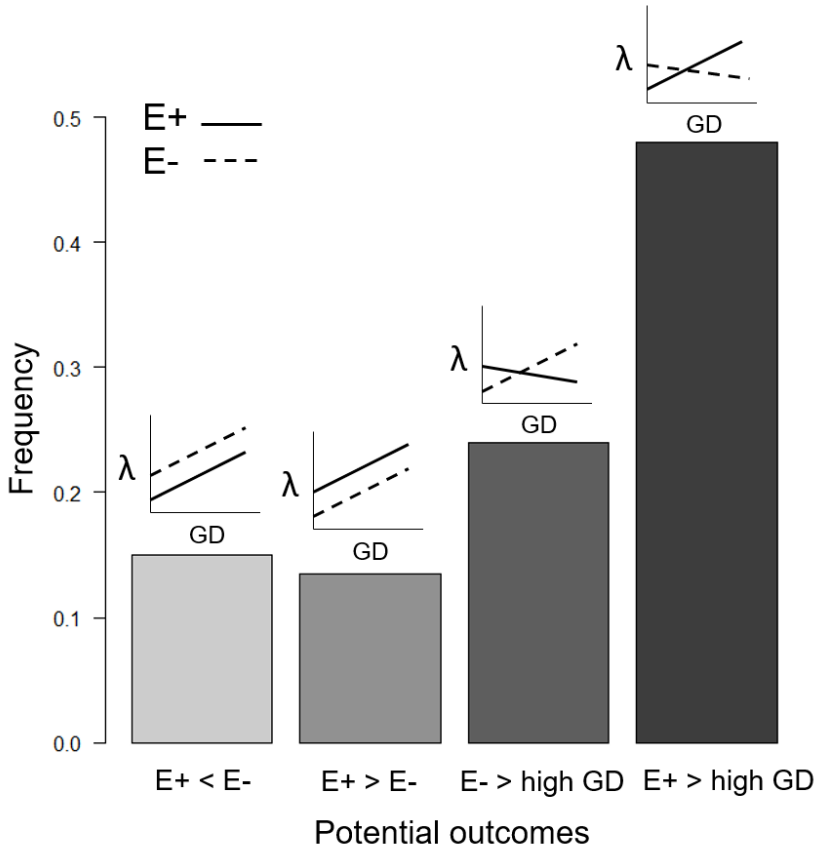

Figure 3.6 – Relative growth rates (λ) for symboitic (E^+) and non-symbiotic (E^-) host populations based on 200 samples from vital rate parameters posteriors. Each bar representes the frequency of potential outcomes from left to right: E^+ λ is always smaller than E^- λ across genetic distances; E^+ λ is always larger than E^- λ across genetic distances; E^- λ is larger at high genetic distances, and E^+ λ is larger at high genetic distances.

3.4 Discussion

Ecological theory maintains that vertical transmission and the relative fitness of symbiotic vs. non-symbiotic hosts interact to determine the population dynamics of heritable symbionts (Saikkonen et al. 2002, Gundel et al. 2008). Outcrossing between genetically distant hosts is predicted to modify both of these factors by creating genetic incompatibilities between hosts and specialized symbionts that could limit both symbiont transmission and reduce mutualistic benefits (Saikkonen 2004, Cheplick and Faeth 2009, Gundel et al. 2012). This study, to the best of my knowledge, is the first to directly test for the effect of host outcrossing on individual symbiotic interactions and how they scale up to determine symbiont prevalence at the population level (Figure 3.5). To accomplish this, I manipulated outcrossing events between hosts along a gradient of genetic distances ranging from relatively low (within populations) to high (between species), and compared the vital rates of closely related outcrossed symbiotic (E^+) and non-symbiotic hosts (E^-) in a common garden setting. Similar to past studies, this work provides evidence that endophytes provide fitness benefits to hosts by increasing host survival, growth, and aspects of host reproduction particularly in larger hosts (Yule et al. 2013, Chung et al. 2015). However, my results do not support the hypothesis that host outcrossing creates incompatibilities between symbiotic partners. Specifically, I found that both fitness benefits of symbionts (Figures 3.2 and 3.3) and symbiont vertical transmission from adult to offspring (Figure 3.4a) did not strongly decline with increasing genetic distance

between outcrossed hosts. In fact, the relative fitness of symbiotic hosts increased with increasing genetic distance for several vital rates (Figures 3.2b and 3.3b), which likely contributed to higher population growth rates for symbiotic hosts relative to non-symbiotic hosts at high genetic distances (Figure 3.5). This result provides novel experimental evidence that host outcrossing can modify the relative fitness of symbiotic and non-symbiotic hosts and therefore may play an important role in determining symbiont prevalence in host populations. Regardless of host symbiotic status, I also found strong support for decreases (i.e., outbreeding depression) in host fitness in response to host outcrossing.

Similar to past research investigating the demographic consequences of heritable symbionts, I found that symbionts were both costly and beneficial to hosts (Oliver et al. 2008, Stanley H. Faeth 2009, Rudgers et al. 2012). My unique contribution to this literature is the inclusion of genetic distance as a continuous predictor of host-symbiont interactions. Here, I demonstrated that mutualistic benefits of endophytes on individual hosts were dependent upon host genetic distance, and to some extent, host size. For example, endophytes increased host survival, growth, and flowering tiller production, especially in large hosts at low genetic distances (Figures 3.2 and 3.3). In contrast, endophytes reduced overall seed production in hosts across sizes and genetic backgrounds (Figure 3.3e and f). This cost of symbiosis likely contributed to predicted higher population growth rates (λ) of non-symbiotic plants at lower genetic distances, which indicates that endophytes would not persist in hosts with low genetic distances. However, λ for symbiotic plants increased and even eclipsed λ for E^- plants at higher

genetic distances (genetic distance = ~9) (Figure 3.5). To demonstrate the validity of this finding and to account for statistical uncertainty, I repeatedly sampled from the posterior distribution of each parameter in the vital rate function and re-calculated λ 200 times for both E^+ and E^- plants across genetic distances. With this, I provide strong evidence that population growth rates of symbiotic hosts are twice as likely to be larger than that of non-symbiotic hosts at high genetic distances (Figure 3.6).

It is important to note that my demographic model included a reduced distribution of host genetic distances, which was motivated by vital rate estimates that were unsupported by my raw data for E^- hosts at low genetic distances. This choice illuminates several limitations of this study. First, due to high endophyte transmission and retention, it was difficult to propagate E^- hosts to act as a rigorous control for symbiont effects. Therefore, data for E^+ hosts outnumbered E^- hosts, sometimes more than 2:1 depending upon vital rate. Second, non-familial E^+ and E^- comparisons were included in this study. I accounted for this variance by including paternal and maternal identity as random effects in the vital rate models, but there may be additional non-target variance for which I cannot account. Third, vital rates shown to influence host-symbiont dynamics were not considered in this study, such as seedling establishment rate (Chung et al. 2015). Despite these limitations, my work strongly demonstrates that host outcrossing does not disrupt symbiosis in this system.

Additionally, I found that endophyte vertical transmission remained high across host genetic distances. This fails to support the hypothesis that host outcrossing limits symbiont transmission due to host-symbiont genetic incompatibilities. This finding also

deviates from previous large scale surveys of natural host populations, where intermediate vertical transmission rates are more the rule than the exception (Hilgenboecker et al. 2008, Afkhami and Rudgers 2008). There are several potential reasons for near perfect endophyte transmission in this study. First, in a previous study, I quantified endophyte transmission across *Elymus virginicus* populations throughout the southern Midwest (USA) and demonstrated that endophyte vertical transmission was strongly correlated to both environmental variables (e.g. drought) and endophyte genotype (Sneck et al. 2017). In contrast, hosts in my common garden were exposed to approximately identical environmental conditions and harbored one of two very similar endophyte genotypes, thereby eliminating several known factors that influence symbiont heritability. Second, I imposed self-fertilization by bagging inflorescences used to estimate endophyte transmission rates, which likely reduced pollen diversity. My previous work suggests that endophytes may respond to pollen donor identity and that vertical transmission may be more variable when genetic distance between maternal plant and pollen donor is high (Figure 2.2a). Lastly, high endophyte transmission rates provide the opportunity to compare my results to theoretical predictions, which posit that small changes in vertical transmission rates can strongly influence symbiont population dynamics, especially when symbionts provide weak fitness benefits (Saikkonen et al. 2002, Gundel et al. 2008). On the other hand, if transmission is high, equilibrium symbiont frequencies are predicted to be limited by the relative fitness of symbiotic and non-symbiotic hosts. Here, I present a novel context in which endophytes become costly-within hosts of relatively low genetic distance. Moreover, I show that these costs are

strong enough to potentially eliminate endophytes, even when transmission is extremely high.

I predicted that host outcrossing could have fitness consequences for hosts regardless of symbiont presence and that host fitness may be maximized at intermediate genetic distances. Here, I show that hosts experienced reduced fitness (i.e., outbreeding depression) in response to host outcrossing and fitness did not peak at intermediate levels of genetic distance. For instance, seed germination rates increased with increasing genetic distance between hosts (Figure 3.4b). However, I did not track the relative germination success of symbiotic vs. non-symbiotic seeds, which can influence symbiont population dynamics (Bibian et al. 2016). In contrast, host survival (Figure 3.2b) and fertility (Figure 3.3b) declined dramatically in hosts at high genetic distances. This finding supports past studies that widely documented reduced fitness in highly outcrossed organisms (e.g., hybrids) (Edmands 1999, Oakley et al. 2015). For instance, Church demonstrated that artificial *Elymus virginicus* x *Elymus canadensis* hybrids grew vigorously, but produced few seeds (Church 1958). Together, my work suggests that outcrossing can have contrasting effects on important vital rates that influence host population dynamics.

I conclude that, via changes in several key vital rates, host outcrossing has the potential to modify endophyte persistence in host populations. Specifically, I demonstrate that endophytes may mitigate the deleterious effects of outcrossing by buoying survival and fertility in genetically distant hosts. This finding is particularly relevant to efforts that have championed the use of symbionts to increase host population growth in response to habitat fragmentation and climate change (Gundel et al. 2013, Hamilton et al. 2016). As

one of the predicted consequences of environmental perturbation is altered or increased rates of gene flow between shifting populations (Edmands 2006, Parmesan 2006), symbionts may act as demographic remediators by reducing potentially strong negative effects of gene flow between genetically disparate host populations.

Retrospective and Future Directions

One of the central motivations of ecology is to understand factors that determine the prevalence and persistence of organisms across time and space. Over the past several decades, ecologists have focused primarily on describing the populations of macro-organisms, while microscopic organisms have received relatively little attention (Semmartin et al. 2015). Recently, the telescope, or microscope in this case, has been flipped around- ecologists such as myself are becoming increasingly aware that microscopic organisms (e.g., fungi, viruses, bacteria) are important components of natural systems, especially those that live symbiotically within a host (Rudgers et al. 2004, Faeth and Shochat 2010). One particularly powerful symbiotic interaction is between endophytic fungi (genus *Epichloë*) found in the above-ground tissues of cool-season grasses (Schardl et al. 2004). These fungi have been the focus of my dissertation. They can produce powerful bioactive alkaloids that deter vertebrate and invertebrate herbivores (Young et al. 2009), increase host resistances to environmental stress (Cheplick 2004), and even manipulate host reproduction (Gorischek et al. 2013).

Similar to other widespread microbial symbionts (e.g., *Wolbachia* and *Buchnera*), epichloid fungi are primarily asexual and passed vertically from mother to offspring. As a consequence of long-term evolutionary relationships with particular hosts, heritable symbionts are often specialized to specific host genotypes and completely dependent upon hosts for reproduction (Ewald 1987). Past research has demonstrated that artificial host-symbiont interactions via cross-inoculation in lab-based systems have resulted in

incompatible partnerships (Christensen 1995, Chong and Moran 2016). An additional way that novel host-symbiont interactions are theorized to occur is through host gene flow (e.g., outcrossing between species), where host offspring are genetically disparate from the maternal genotype (Gundel et al. 2012). Host-symbiont incompatibility, as a result of host outcrossing, may manifest as a reduction in both symbiont vertical transmission and symbiont mediated host fitness benefits. Importantly, both symbiont vertical transmission rates and symbiotic host fitness are theorized to, independently or in concert, determine symbiont prevalence in host populations (Saikkonen et al. 2002, Gundel et al. 2008). Therefore, the key to predicting symbiont population dynamics is identifying factors that influence both symbiont transmission and symbiont host fitness. Here, I have used experimental field-based and mathematical modeling approaches to answer the questions: **Do novel host-symbiont combinations generated via host outcrossing change symbiotic interactions (transmission and fitness), and if so, how do these changes scale up to determine symbiont prevalence in natural host populations?** Each of my chapters utilizes the symbiotic relationship between fungal endophytes and their grass hosts.

In order to answer these questions, I needed a basic understanding of how often symbionts in natural host populations transmit and where they occur in space, which became the focus of Chapter one of my dissertation. Surprisingly, little is understood about how endophyte transmission rates correlate with macro-environmental factors such as precipitation, drought, and temperature. Additionally, this is the first study to determine if variation in endophyte transmission is associated with endophyte genotype

(Schardl et al. 2013b). During an extensive fieldtrip throughout the southern Midwest of the United States, I collected seeds from natural populations of two host grasses (*Elymus virginicus* and *E. canadensis*) that both house fungal endophytes within the genus *Epichloë* (Figure 1.1). From these collections, I determined that the individual-level endophyte transmission rates and population-level endophyte prevalence varied across space and with host identity (Figure 1.2). In addition, I found that endophyte prevalence in *E. virginicus* hosts declined with higher drought (Figure 1.3), which aligned with previous experimental studies (Rudgers and Swafford 2009). However, endophyte prevalence and transmission remained high across nearly all *E. canadensis* populations, and did not strongly associate with abiotic factors (Figure 1.3). The most striking result from this work was that biotic context, specifically endophyte genotype, likely plays a stronger role in determining symbiont inheritance than abiotic context (Figure 1.4). Moreover, my results pointed to the primacy of endophyte transmission as a major constraint to symbiont prevalence. This finding led me to investigate the role of host outcrossing on symbiont transmission.

Chapter two of my dissertation details how I experimentally manipulated outcrossing events between pollen recipient and pollen donors in a greenhouse and common garden setting. From these mating events, I measured endophyte transmission from symbiotic mothers to outcrossed siblings. This work is the first empirical test of the hypothesis that host outcrossing disrupts symbiont vertical transmission rates. With the generous help of collaborators at the Noble Research Institute, I used molecular techniques to quantify the genetic distance between each mated host pair (Saha et al.

2009). This study provided multiple unique scientific contributions. First, I quantified symbiont transmission rates across a known gradient of genetic distances between hosts, from low to high. Second, I accounted for cross-generational effects by investigated symbiont transmission into multiple host generations. Therefore, for the first time, this empowered me to determine the functional relationship between host genotype and symbiont transmission. After two summers of experimental mating and seed harvesting, I discovered that endophyte vertical transmission across host generations was incredibly robust to host outcrossing (Figure 2.2). Moreover, endophyte transmission did not decline with increasing genetic distance between mated pairs. In fact, contrary to my hypothesis, I observed that transmission rates slightly increased from low genetic distance to high genetic distance. However, this study did reveal that host outcrossing can be costly for both the host and the symbiont. Specifically, I observed that host offspring generated by more genetically distant parents experienced lower fertility (Figure 2.5), with some offspring failing to produce a single seed. This observation inspired the field-based experiment and subsequent mathematical model to quantify the net effect of host outcrossing on symbiont population dynamics.

Lastly, Chapter three elegantly pulled all of the various loose strings of my dissertation together. In my previous chapters I had observed unexplained variation in symbiont transmission and prevalence across natural host populations, which suggested that changes in either transmission or host fitness determine whether symbionts reach high or low prevalence within a population. Additionally, I had experimentally demonstrated that endophyte transmission is robust to host outcrossing, but strongly

affected host fitness. In order to determine the net effect of host outcrossing on symbiont population dynamics, I needed to compare the fitness of symbiont positive (E+) and symbiont negative (E-) hosts across genetic distances. Chapter three takes this next logical step by asking: how does host outcrossing influence both symbiont transmission and host fitness to ultimately determine population-level symbiont prevalence? To answer this question, I took a mathematical modeling approach and used data gathered form an experimental common garden to parameterize a size-structured mega-matrix population model (Yule et al. 2013, Chung et al. 2015, Bibian et al. 2016, Compagnoni et al. 2016). This model accounted for both imperfect endophyte transmission and host genetic background. Contrary to previous hypotheses, I found that host outcrossing actually increased endophyte population growth rates (lambda), and predicted endophytes would become fixed in hosts at greater genetic distances (Figure 3.5). A surprising implication of this work is the finding that symbionts buffer hosts against the deleterious effects of outbreeding depression. For instance, E+ hosts experienced increased survival and seed production compared to E- hosts at high genetic distances, which could have wide-reaching implications for plant breeding and habitat restoration.

While my work rigorously addressed a previously un-tested hypothesis, there remains additional avenues to explore. First, taken together, the chapters of my dissertation allude to a grander conclusion that symbiont prevalence is the product of complex biotic and abiotic interactions. For instance, it is likely that interactions between host genotype, symbiont genotype, and the environment determine when and where endophytes are found. My work was limited to a single common garden, where each

plant had ample water, few herbivores, and little competition. It is likely that in the crucible of a more natural environment, small vital rate differences between symbiotic and non-symbiotic hosts greatly impact symbiont population growth. Moreover, I was unable to control endophyte genotype due to difficulties with endophyte elimination/inoculation. To address these shortcomings, future work should compare E+ and E- hosts of varying genetic distances across environments or exposed to differing levels of abiotic or biotic stress. Importantly, all symbiotic hosts should be occupied by a single endophyte genotype. I hypothesize that certain "ideal" combinations of host and symbiont genotypes will dominate in a given environment. In fact, this may be why I observed highly variable endophyte prevalence and transmission rates across natural landscapes, where perhaps only the best host-symbiont combinations persist. Second, I used a relatively crude measure of genetic distance between mated host plants, which was estimated with 8 previously generated neutral markers (Saha et al. 2009). These markers cannot be used to determine heterozygosity and had to be reduced to a binary present or absent phenotype (Falush et al. 2007, Pfeiffer et al. 2011, Schreier et al. 2013). In the future, genomic approaches, unshackled by the limitations of site-specific markers, should be used to provide more robust host genotypes that can be used to calculate basic population genetic metrics (e.g., Fst, AMOVA). Also, endophytes were identified by alkaloid gene profiles (Takach and Young 2014), and therefore could not assign unique and phylogenetically supported endophyte identities. Since I completed my molecular work, genetic and genomic studies involving a vast diversity of endophyte genotypes have been conducted (Schardl et al. 2014). Using these published approaches, a more

fine-grained description of both host and symbiont genotype will only further refine our understanding of host-symbiont interactions.

References

Afkhami, M. E. 2012. Fungal endophyte–grass symbioses are rare in the California floristic province and other regions with Mediterranean-influenced climates. Fungal Ecology 5:345–352.

Afkhami, M. E., and J. A. Rudgers. 2008. Symbiosis Lost: Imperfect Vertical Transmission of Fungal Endophytes in Grasses. The American Naturalist 172:405–416.

Agapow, P.-M., and A. Burt. 2001. Indices of multilocus linkage disequilibrium. Molecular Ecology Notes 1:101–102.

Aitken, S. N., and M. C. Whitlock. 2013. Assisted gene flow to facilitate local adaptation to climate Change. Annual Review of Ecology, Evolution, and Systematics 44:367–388.

Bacon, C. W., and J. F. White Jr. 1994. Stains, media, and procedures for analyzing endophytes. Biotechnology of endophytic fungi of grasses 47:56.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2014. Fitting linear mixed-effects models using lme4. arXiv:1406.5823.

Bazely, D., J. P. Ball, M. Vicari, A. J. Tanentzap, M. Bérenger, T. Rakocevic, and S. Koh. 2007. Broad-scale geographic patterns in the distribution of vertically-transmitted, asexual endophytes in four naturally-occurring grasses in Sweden. Ecography 30:367–374.

Beguería, S., and S. M. Vicente-Serrano. 2013. SPEI: Calculation of the Standardized Precipitation-Evaporation Index.

Bennett, G. M., and N. A. Moran. 2015. Heritable symbiosis: The advantages and perils of an evolutionary rabbit hole. Proceedings of the National Academy of Sciences 112:10169–10176.

Bergstrom, C. T., and M. Lachmann. 2003. The Red King effect: when the slowest runner wins the coevolutionary race. Proceedings of the National Academy of Sciences 100:593–598.

Bibian, A. J., J. A. Rudgers, T. E. Miller, W. M. Mooij, and A. A. Winn. 2016. The role of host demographic storage in the ecological dynamics of heritable symbionts. The American Naturalist 188:446–459.

Borer, E. T., L. L. Kinkel, G. May, and E. W. Seabloom. 2013. The world within: Quantifying the determinants and outcomes of a host's microbiome. Basic and Applied Ecology 14:533–539.

Brem, D., and A. Leuchtmann. 2002. Intraspecific competition of endophyte infected vs uninfected plants of two woodland grass species. Oikos 96:281–290.

Bright, M., and S. Bulgheresi. 2010. A complex journey: transmission of microbial symbionts. Nature Reviews Microbiology 8:218–230.

Brown, A. H. D., M. W. Feldman, and E. Nevo. 1980. Multilocus structure of natural populations of *Hordeum spontaneum*. Genetics 96:523–536.

Brucker, R. M., and S. R. Bordenstein. 2012. Speciation by symbiosis. Trends in Ecology & Evolution 27:443–451.

Byler, K. A., M. Carmi-Veal, M. Fine, and T. L. Goulet. 2013. Multiple symbiont acquisition strategies as an adaptive mechanism in the coral *Stylophora pistillata*. PLoS ONE 8:e59596.

Cade, B. S. 2015. Model averaging and muddled multimodel inferences. Ecology 96:2370–2382.

Caswell, H. 2001. Matrix population models: Construction, Analysis, and Interpretation. Sinauer Associates, Sunderland, MA.

Chamberlain, S. A., J. L. Bronstein, and J. A. Rudgers. 2014. How context dependent are species interactions? Ecology Letters 17:881–890.

Charlesworth, B., and D. Charlesworth. 1999. The genetic basis of inbreeding depression. Genetical Research 74:329–340.

Charlton, N. D., K. D. Craven, M. E. Afkhami, B. A. Hall, S. R. Ghimire, and C. A. Young. 2014. Interspecific hybridization and bioactive alkaloid variation increases diversity in endophytic *Epichloë* species of *Bromus laevipes*. FEMS Microbiology Ecology 90:276–289.

Charlton, N. D., K. D. Craven, S. Mittal, A. A. Hopkins, and C. A. Young. 2012. *Epichloë canadensis*, a new interspecific Epichloid hybrid symbiotic with Canada wildrye (*Elymus canadensis*). Mycologia 104:1187–1199.

Cheplick, G. P. 2004. Recovery from drought stress in *Lolium perenne* (Poaceae): are fungal endophytes detrimental? American Journal of Botany 91:1960–1968.

Cheplick, G. P., and S. H. Faeth. 2009. Ecology and Evolution of the Grass-Endophyte Symbiosis. Oxford University Press, New York, New York.

Chong, R. A., and N. A. Moran. 2016. Intraspecific genetic variation in hosts affects regulation of obligate heritable symbionts. Proceedings of the National Academy of Sciences 113:13114–13119.

Christensen, M. J. 1995. Variation in the ability of *Acremonium* endophytes *of Lolium perenne, Festuca arundinacea* and *F. pratensis* to form compatible associations in the three grasses. Mycological Research 99:466–470.

Christensen, M. J., O.-P. Ball, R. J. Bennett, and C. L. Schardl. 1997. Fungal and host genotype effects on compatibility and vascular colonization by *Epichloë festucae*. Mycological research 101:493–501.

Chung, Y. A., T. E. X. Miller, and J. A. Rudgers. 2015. Fungal symbionts maintain a rare plant population but demographic advantage drives the dominance of a common host. Journal of Ecology 103:967–977.

Church, G. L. 1958. Artificial hybrids of *Elymus virginicus* with *E. canadensis*, *Interruptus*, *Riparius*, and *Wiegandii*. American Journal of Botany 45:410.

Clay, K. 1988. Fungal Endophytes of Grasses: A Defensive Mutualism between Plants and Fungi. Ecology 69:10.

Clay, K. 1993. The ecology and evolution of endophytes. Agriculture, Ecosystems & Environment 44:39–64.

Clay, K., J. Holah, and J. A. Rudgers. 2005. Herbivores cause a rapid increase in hereditary symbiosis and alter plant community composition. Proceedings of the National Academy of Sciences of the United States of America 102:12465–12470.

Clay, K., and C. Schardl. 2002. Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. The American Naturalist 160:S99–S127.

Compagnoni, A., A. J. Bibian, B. M. Ochocki, H. S. Rogers, E. L. Schultz, M. E. Sneck, B. D. Elderd, A. M. Iler, D. W. Inouye, H. Jacquemyn, and others. 2016. The effect of demographic correlations on the stochastic population dynamics of perennial plants. Ecological Monographs 86:480–494.

Cruaud, A., and J.-Y. Rasplus. 2016. Testing cospeciation through large-scale cophylogenetic studies. Current Opinion in Insect Science 18:53–59.

Dalgleish, H. J., D. N. Koons, M. B. Hooten, C. A. Moffet, and P. B. Adler. 2011. Climate influences the demography of three dominant sagebrush steppe plants. Ecology 92:75–85.

Davitt, A. J., C. Chen, and J. A. Rudgers. 2011. Understanding context-dependency in plant–microbe symbiosis: The influence of abiotic and biotic contexts on host fitness and the rate of symbiont transmission. Environmental and Experimental Botany 71:137–145.

Dewey, D. R. 1971. Synthetic Hybrids of *Hordeum bogdanii* with *Elymus canadensis* and *Sitanion hystrix*. American Journal of Botany 58:902.

Dewey, D. R. 1983. Historical and current taxonomic perspectives of *Agropyron*, *Elymus*, and related genera. Crop Science 23:637–642.

Douglas, A. E. 1998. Host benefit and the evolution of specialization in symbiosis. Heredity 81:599–603.

Dufresne, F., M. Stift, R. Vergilino, and B. K. Mable. 2014. Recent progress and challenges in population genetics of polyploid organisms: an overview of current state-of-the-art molecular and statistical tools. Molecular Ecology 23:40–69.

Eaton, C. J., M. P. Cox, B. Ambrose, M. Becker, U. Hesse, C. L. Schardl, and B. Scott. 2010. Disruption of signaling in a fungal-grass symbiosis leads to pathogenesis. Plant Physiology 153:1780–1794.

Edmands, S. 1999. Heterosis and outbreeding depression in interpopulation crosses spanning a wide range of divergence. Evolution 53:1757–1768.

Edmands, S. 2006. Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management: Relative risk of inbreeding and outbreeding. Molecular Ecology 16:463–475.

Edmands, S., and C. C. Timmerman. 2003. Modeling factors affecting the severity of outbreeding depression. Conservation Biology 17:883–892.

Ellstrand, N. C. 2014. Is gene flow the most important evolutionary force in plants? American Journal of Botany 101:737–753.

Ellstrand, N. C., P. Meirmans, J. Rong, D. Bartsch, A. Ghosh, T. J. de Jong, P. Haccou, B.-R. Lu, A. A. Snow, C. N. Stewart Jr., J. L. Strasburg, P. H. van Tienderen, K. Vrieling, and D. Hooftman. 2013. Introgression of crop alleles into wild or weedy populations. Pages 325–345 *in* Futuyma, DJ, editor. Annual review of Ecology, Evolution, and Systematics, VOL 44.

Ewald, P. W. 1987. Transmission modes and the evolution of parasitism-mutualism continuum. Annals of the New York Academy of Sciences 503:295–306.

Faeth, S. H., M. Oberhofer, S. Saari, K. E. Haskins, and T. Shymanovich. 2017. Does hybridization of endophytic symbionts in a native grass increase fitness in resource-limited environments? Ecology 98:138–149.

Faeth, S. H., and E. Shochat. 2010. Inherited microbial symbionts increase herbivore abundances and alter arthropod diversity on a native grass. Ecology 91:1329–1343.

Falush, D., M. Stephens, and J. K. Pritchard. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. Molecular Ecology Notes 7:574–578.

Frade, P. R., F. De Jongh, F. Vermeulen, J. Van Bleijswijk, and R. P. M. Bak. 2007. Variation in symbiont distribution between closely related coral species over large depth ranges: coral symbiont distribution over large depths. Molecular Ecology 17:691–703.

Funkhouser, L. J., and S. R. Bordenstein. 2013. Mom knows best: The universality of maternal microbial transmission. PLoS Biology 11:e1001631.

Garant, D., S. E. Forde, and A. P. Hendry. 2007. The multifarious effects of dispersal and gene flow on contemporary adaptation. Functional Ecology 21:434–443.

GarcíA Parisi, P. A., C. Casas, P. E. Gundel, and M. Omacini. 2012. Consequences of grazing on the vertical transmission of a fungal *Neotyphodium* symbiont in an annual grass population: Grazing effect on fungal endophyte transmission. Austral Ecology 37:620–628.

Gelman, A., and D. B. Rubin. 1992. Inference from Iterative Simulation Using Multiple Sequences. Statistical Science 7:457–472.

Giauque, H., and C. V. Hawkes. 2013. Climate affects symbiotic fungal endophyte diversity and performance. American Journal of Botany 100:1435–1444.

Gibert, A., and L. Hazard. 2013. Genetically based vertical transmission drives the frequency of the symbiosis between grasses and systemic fungal endophytes. Journal of Ecology 101:743–752.

Gibert, A., D. Magda, and L. Hazard. 2015. Interplay between endophyte prevalence, effects and transmission: Insights from a natural grass population. PLOS ONE 10:e0139919.

Gompert, Z., and C. A. Buerkle. 2016. What, if anything, are hybrids: enduring truths and challenges associated with population structure and gene flow. Evolutionary Applications 9:909–923.

Gomulkiewicz, R., J. N. Thompson, R. D. Holt, S. L. Nuismer, and M. E. Hochberg. 2000. Hot spots, cold spots, and the geographic mosaic theory of coevolution. The American Naturalist 156:156–174.

Goodrich, J. K., E. R. Davenport, J. L. Waters, A. G. Clark, and R. E. Ley. 2016. Cross-species comparisons of host genetic associations with the microbiome. Science 352:532–535.

Gorischek, A. M., M. E. Afkhami, E. K. Seifert, and J. A. Rudgers. 2013. Fungal symbionts as manipulators of plant reproductive biology. The American Naturalist 181:562–570.

Gundel, P. E., W. B. Batista, M. Texeira, M. A. Martinez-Ghersa, M. Omacini, and C. M. Ghersa. 2008. *Neotyphodium* endophyte infection frequency in annual grass populations: relative importance of mutualism and transmission efficiency. Proceedings of the Royal Society B: Biological Sciences 275:897–905.

Gundel, P. E., L. A. Garibaldi, M. A. Martínez-Ghersa, and C. M. Ghersa. 2011a. *Neotyphodium* endophyte transmission to *Lolium multiflorum* seeds depends on the host plant fitness. Environmental and Experimental Botany 71:359-366

Gundel, P. E., L. A. Garibaldi, P. M. Tognetti, R. Aragón, C. M. Ghersa, and M. Omacini. 2009. Imperfect vertical transmission of the endophyte *Neotyphodium* in exotic grasses in grasslands of the flooding Pampa. Microbial Ecology 57:740–748.

Gundel, P. E., J. G. N. Irisarri, L. Fazio, C. Casas, and L. I. Pérez. 2016. Inferring field performance from drought experiments can be misleading: The case of symbiosis between grasses and *Epichloë* fungal endophytes. Journal of Arid Environments. 132:60-62.

Gundel, P. E., M. A. Martínez-Ghersa, M. Omacini, R. Cuyeu, E. Pagano, R. Ríos, and C. M. Ghersa. 2012. Mutualism effectiveness and vertical transmission of symbiotic fungal endophytes in response to host genetic background: Grass-fungus mutualism and host genetic background. Evolutionary Applications 5:838–849.

Gundel, P. E., M. Omacini, V. O. Sadras, and C. M. Ghersa. 2010. The interplay between the effectiveness of the grass-endophyte mutualism and the genetic variability of

the host plant: Endophyte-grass mutualism and genetic variability. Evolutionary Applications 3:538–546.

Gundel, P. E., L. I. Pérez, M. Helander, and K. Saikkonen. 2013. Symbiotically modified organisms: nontoxic fungal endophytes in grasses. Trends in plant science 18:420–427.

Gundel, P. E., J. A. Rudgers, and C. M. Ghersa. 2011b. Incorporating the process of vertical transmission into understanding of host-symbiont dynamics. Oikos 120:1121–1128.

Gundel, P. E., J. A. Rudgers, and K. D. Whitney. 2017. Vertically transmitted symbionts as mechanisms of transgenerational effects. American Journal of Botany 104:787–792.

Hadfield, J. D. 2010. MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmm R Package. Journal of Statistical Software 33:1–22.

Haine, E. R. 2008. Symbiont-mediated protection. Proceedings of the Royal Society B: Biological Sciences 275:353–361.

Hamilton, C. E., J. D. Bever, J. Labbé, X. Yang, and H. Yin. 2016. Mitigating climate change through managing constructed-microbial communities in agriculture. Agriculture, Ecosystems & Environment 216:304–308.

Hamilton, C. E., S. H. Faeth, and T. E. Dowling. 2009. Distribution of hybrid fungal symbionts and environmental stress. Microbial ecology 58:408–413.

Hayden, M. J., T. M. Nguyen, A. Waterman, and K. J. Chalmers. 2008. Multiplex-Ready PCR: A new method for multiplexed SSR and SNP genotyping. BMC Genomics 9:80.

Herre, E. A., N. Knowlton, U. G. Mueller, and S. A. Rehner. 1999. The evolution of mutualisms: exploring the paths between conflict and cooperation. Trends in Ecology & Evolution 14:49–53.

Hiatt, E. E., N. S. Hill, J. H. Bouton, and J. A. Stuedemann. 1999. Tall fescue endophyte detection: commercial immunoblot test kit compared with microscopic analysis. Crop Science 39:796–799.

Hilgenboecker, K., P. Hammerstein, P. Schlattmann, A. Telschow, and J. H. Werren. 2008. How many species are infected with *Wolbachia*? – a statistical analysis of current data: *Wolbachia* infection rates. FEMS Microbiology Letters 281:215–220.

Holt, R. D., and R. Gomulkiewicz. 1997. How does immigration influence local adaptation? A reexamination of a familiar paradigm. The American Naturalist 149:563–572.

Huff, D. R., R. Peakall, and P. E. Smouse. 1993. RAPD variation within and among natural populations of outcrossing buffalograss (*Buchloe dactyloides* (Nutt.) *Engelm*). Theoretical and Applied Genetics 86:927–934.

Iannone, L. J., J. G. N. Irisarri, P. D. Mc Cargo, L. I. Pérez, and P. E. Gundel. 2015. Occurrence of *Epichloë* fungal endophytes in the sheep-preferred *grass Hordeum comosum* from Patagonia. Journal of Arid Environments 115:19–26.

Jaenike, J. 2012. Population genetics of beneficial heritable symbionts. Trends in Ecology & Evolution 27:226–232.

Jia, T., M. Oberhofer, T. Shymanovich, and S. H. Faeth. 2016. Effects of hybrid and non-hybrid *Epichloë* endophytes and their associated host genotypes on the response of a native grass to varying environments. Microbial Ecology 72:185–196.

Kageyama, D., H. Anbutsu, M. Watada, T. Hosokawa, M. Shimada, and T. Fukatsu. 2006. Prevalence of a non-male-killing *Spiroplasma* in natural populations of *Drosophila hydei*. Applied and Environmental Microbiology 72:6667–6673.

Kamvar, Z. N., J. F. Tabima, and N. J. Grünwald. 2014. *Poppr* : an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2:e281.

Keller, L. F., and D. M. Waller. 2002. Inbreeding effects in wild populations. Trends in Ecology & Evolution 17:230–241.

Kivlin, S. N., S. M. Emery, and J. A. Rudgers. 2013. Fungal symbionts alter plant responses to global change. American Journal of Botany 100:1445–1457.

Kraal, L., S. Abubucker, K. Kota, M. A. Fischbach, and M. Mitreva. 2014. The Prevalence of Species and Strains in the Human Microbiome: A Resource for Experimental Efforts. PLoS ONE 9:e97279.

Lenormand, T. 2002. Gene flow and the limits to natural selection. Trends in Ecology & Evolution 17:183–189.

Leuchtmann, A. 1992. Systematics, distribution, and host specificity of grass endophytes. Natural Toxins 1:150–162.

Leuchtmann, A., C. W. Bacon, C. L. Schardl, J. F. White, and M. Tadych. 2014. Nomenclatural realignment of *Neotyphodium* species with genus *Epichloë.* Mycologia 106:202–215.

Leuchtmann, A., and K. Clay. 1993. Nonreciprocal compatibility between *Epichloë typhina* and four host grasses. Mycologia:157–163.

Long, D., B. R. Scanlon, L. Longuevergne, A. Y. Sun, D. N. Fernando, and H. Save. 2013. GRACE satellite monitoring of large depletion in water storage in response to the 2011 drought in Texas: GRACE-based drought monitoring. Geophysical Research Letters 40:3395–3401.

Lynch, M. 1991. The genetic interpretation of inbreeding depression and outbreeding depression. Evolution 45:622–629.

Majewska-Sawka, A., and H. Nakashima. 2004. Endophyte transmission via seeds of *Lolium perenne* L.: immunodetection of fungal antigens. Fungal Genetics and Biology 41:534–541.

Malinowski, D. P., and D. P. Belesky. 2000. Adaptations of endophyte-infected cool-season grasses to environmental stresses: mechanisms of drought and mineral stress tolerance. Crop Science 40:923–940.

Marquis, M., I. Del Toro, and S. L. Pelini. 2014. Insect mutualisms buffer warming effects on multiple trophic levels. Ecology 95:9–13.

Mazerolle, M. J. 2016. AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c).

McGraw, E. A., D. J. Merritt, J. N. Droller, and S. L. O'Neill. 2002. *Wolbachia* density and virulence attenuation after transfer into a novel host. Proceedings of the National Academy of Sciences 99:2918–2923.

Miller, T. E. X., and J. A. Rudgers. 2014. Niche Differentiation in the Dynamics of Host-Symbiont Interactions: Symbiont Prevalence as a Coexistence Problem. The American Naturalist 183:506–518.

Moon, C. D., K. D. Craven, A. Leuchtmann, S. L. Clement, and C. L. Schardl. 2004. Prevalence of interspecific hybrids amongst asexual fungal endophytes of grasses: Hybrid fungal endophytes. Molecular Ecology 13:1455–1467.

Moran, N. A., M. A. Munson, P. Baumann, and H. Ishikawa. 1993. A molecular clock in endosymbiotic bacteria is calibrated using the insect hosts. Proceedings of the Royal Society of London B: Biological Sciences 253:167–171.

Nelson, E. N., and R. J. Tyrl. 1978. Hybridization and introgression between *Elymus canadensis* and *Elymus virginicus* (Poaceae). Pages 32–34 Proc. Oklahoma Academy of Science 58:32-34.

Ness, J. H., E. J. Rollinson, and K. D. Whitney. 2011. Phylogenetic distance can predict susceptibility to attack by natural enemies. Oikos 120:1327–1334.

Oakley, C. G., J. A. Agren, and D. Schemske. 2015. Heterosis and outbreeding depression in crosses between natural populations of *Arabidopsis thaliana.* Heredity 115:73.

Oldroyd, G. E. D. 2013. Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. Nature Reviews Microbiology 11:252–263.

Oliver, K. M., J. Campos, N. A. Moran, and M. S. Hunter. 2008. Population dynamics of defensive symbionts in aphids. Proceedings of the Royal Society B: Biological Sciences 275:293–299.

Oliver, K. M., N. A. Moran, and M. S. Hunter. 2005. Variation in resistance to parasitism in aphids is due to symbionts not host genotype. Proceedings of the National Academy of Sciences of the United States of America 102:12795–12800.

Panaccione, D. G., W. T. Beaulieu, and D. Cook. 2014. Bioactive alkaloids in vertically transmitted fungal endophytes. Functional Ecology 28:299–314.

Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. Annual Review of Ecology, Evolution, and Systematics 37:637–669.

Peakall, R., and P. E. Smouse. 2006. genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288–295.

Pérez, L. I., P. E. Gundel, C. M. Ghersa, and M. Omacini. 2013. Family issues: fungal endophyte protects host grass from the closely related pathogen *Claviceps purpurea*. Fungal Ecology 6:379–386.

Pfeiffer, T., A. M. Roschanski, J. R. Pannell, G. Korbecka, and M. Schnittler. 2011. Characterization of microsatellite loci and reliable genotyping in a polyploid plant, *Mercurialis perennis* (Euphorbiaceae). Journal of Heredity 102:479–488.

Ranelli, L. B., W. Q. Hendricks, J. S. Lynn, S. N. Kivlin, and J. A. Rudgers. 2015. Biotic and abiotic predictors of fungal colonization in grasses of the Colorado Rockies. Diversity and Distributions 21:962-976.

Ravel, C., Y. Michalakis, and G. Charmet. 1997. The Effect of Imperfect Transmission on the Frequency of Mutualistic Seed-Borne Endophytes in Natural Populations of Grasses. Oikos 80:18.

Redman, R. S., K. B. Sheehan, R. G. Stout, R. J. Rodriguez, and J. M. Henson. 2002. Thermotolerance generated by plant/fungal symbiosis. Science 298:1581–1581.

Ren, A., M. Wei, L. Yin, L. Wu, Y. Zhou, X. Li, and Y. Gao. 2014. Benefits of a fungal endophyte in *Leymus chinensis* depend more on water than on nutrient availability. Environmental and Experimental Botany 108:71–78.

Rhymer, J. M., and D. Simberloff. 1996. Extinction by hybridization and introgression. Annual Review of Ecology and Systematics 27:83–109.

Richier, S. 2005. Symbiosis-induced adaptation to oxidative stress. Journal of Experimental Biology 208:277–285.

Rodriguez, R. J., J. Henson, E. Van Volkenburgh, M. Hoy, L. Wright, F. Beckwith, Y.-O. Kim, and R. S. Redman. 2008. Stress tolerance in plants via habitat-adapted symbiosis. The ISME journal 2:404–416.

Rojas, X., J. Guo, J. W. Leff, D. H. McNear, N. Fierer, and R. L. McCulley. 2016. Infection with a shoot-specific fungal endophyte (*Epichloë*) alters tall fescue soil microbial communities. Microbial Ecology 72:197–206.

Rolston, M. P., M. D. Hare, K. K. Moore, and M. J. Christensen. 1986. Viability of *Lolium* endophyte fungus in seed stored at different moisture contents and temperatures. New Zealand Journal of Experimental Agriculture 14:297–300.

Rudgers, J. A., J. M. Koslow, and K. Clay. 2004. Endophytic fungi alter relationships between diversity and ecosystem properties. Ecology Letters 7:42–51.

Rudgers, J. A., T. E. Miller, S. M. Ziegler, and K. D. Craven. 2012. There are many ways to be a mutualist: endophytic fungus reduces plant survival but increases population growth. Ecology 93:565–574.

Rudgers, J. A., and A. L. Swafford. 2009. Benefits of a fungal endophyte in *Elymus virginicus* decline under drought stress. Basic and Applied Ecology 10:43–51.

Saari, S., and S. H. Faeth. 2012. Hybridization of *Neotyphodium* endophytes enhances competitive ability of the host grass. New Phytologist 195:231–236.

Saari, S., S. Richter, M. Robbins, and S. H. Faeth. 2014. Bottom-up regulates top-down: the effects of hybridization of grass endophytes on an aphid herbivore and its generalist predator. Oikos 123:545–552.

Sachs, J. L., C. J. Essenberg, and M. M. Turcotte. 2011. New paradigms for the evolution of beneficial infections. Trends in Ecology & Evolution 26:202–209.

Sachs, J. L., U. G. Mueller, T. P. Wilcox, and J. J. Bull. 2004. The evolution of cooperation. The Quarterly Review of Biology 79:135–160.

Saha, M. C., C. A. Young, and A. A. Hopkins. 2009. Genetic variation within and among wildrye populations from the Southern Great Plains. Crop Science 49:913.

Saikkonen, K. 2004. Evolution of endophyte plant symbioses. Trends in Plant Science 9:275–280.

Saikkonen, K., P. E. Gundel, and M. Helander. 2013. Chemical ecology mediated by fungal endophytes in grasses. Journal of Chemical Ecology 39:962–968.

Saikkonen, K., D. Ion, and M. Gyllenberg. 2002. The persistence of vertically transmitted fungi in grass metapopulations. Proceedings of the Royal Society B: Biological Sciences 269:1397–1403.

Saikkonen, K., S. Saari, and M. Helander. 2010a. Defensive mutualism between plants and endophytic fungi? Fungal Diversity 41:101–113.

Saikkonen, K., P. R. Wäli, and M. Helander. 2010b. Genetic compatibility determines endophyte-grass combinations. PloS one 5:e11395.

Saikkonen, K., C. A. Young, M. Helander, and C. L. Schardl. 2016. Endophytic *Epichloë* species and their grass hosts: from evolution to applications. Plant Molecular Biology 90:665–675.

Salazar-Gutierrez, M. R., J. Johnson, B. Chaves-Cordoba, and G. Hoogenboom. 2013. Relationship of base temperature to development of winter wheat. International Journal of Plant Productivity 7:741–762.

Sanders, T. B., and J. L. Hamrick. 1980. Variation in the breeding system of *Elymus canadensis*. Evolution:117–122.

Schardl, C. L., S. Florea, J. Pan, P. Nagabhyru, S. Bec, and P. J. Calie. 2013a. The Epichloae: alkaloid diversity and roles in symbiosis with grasses. Current Opinion in Plant Biology 16:480–488.

Schardl, C. L., A. Leuchtmann, and M. J. Spiering. 2004. Symbioses of grasses with seedborne fungal endophytes. Annual Review of Plant Biology 55:315–340.

Schardl, C. L., C. A. Young, N. Moore, N. Krom, P.-Y. Dupont, J. Pan, S. Florea, J. S. Webb, J. Jaromczyk, J. W. Jaromczyk, M. P. Cox, and M. L. Farman. 2014. Genomes of plant-associated Clavicipitaceae. Pages 291–327 Advances in Botanical Research. Elsevier.

Schardl, C., C. Young, J. Pan, S. Florea, J. Takach, D. Panaccione, M. Farman, J. Webb, J. Jaromczyk, N. Charlton, P. Nagabhyru, L. Chen, C. Shi, and A. Leuchtmann. 2013b. Currencies of mutualisms: Sources of alkaloid genes in vertically transmitted Epichloae. Toxins 5:1064–1088.

Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9:671–675.

Schreier, A. D., B. Mahardja, and B. May. 2013. Patterns of population structure vary across the range of the White Sturgeon. Transactions of the American Fisheries Society 142:1273–1286.

Selosse, and C. L. Schardl. 2007. Fungal endophytes of grasses: Hybrids rescued by vertical transmission? An evolutionary perspective. The New Phytologist 173:452–458.

Semmartin, M., M. Omacini, P. E. Gundel, and I. M. Hernández-Agramonte. 2015. Broad-scale variation of fungal-endophyte incidence in temperate grasses. Journal of Ecology 103:184–190.

Shymanovich, T., S. Saari, M. E. Lovin, A. K. Jarmusch, S. A. Jarmusch, A. M. Musso, N. D. Charlton, C. A. Young, N. B. Cech, and S. H. Faeth. 2015. Alkaloid variation among Epichloid endophytes of Sleepygrass (*Achnatherum robustum*) and consequences for resistance to insect herbivores. Journal of Chemical Ecology 41:93–104.

Singh, L. P., S. S. Gill, and N. Tuteja. 2011. Unraveling the role of fungal symbionts in plant abiotic stress tolerance. Plant Signaling & Behavior 6:175–191.

Sneck, M. E., J. A. Rudgers, C. A. Young, and T. E. X. Miller. 2017. Variation in the Prevalence and Transmission of Heritable Symbionts Across Host Populations in Heterogeneous Environments. Microbial Ecology:1–14.

Stanley H. Faeth. 2009. Asexual fungal symbionts alter reproductive allocation and herbivory over time in their native perennial grass hosts. The American Naturalist 173:554–565.

Stubben, C. J., and B. G. Milligan. 2007. Estimating and analyzing demographic models using the popbio Package in R. Journal of Statistical Software 22.

Sullivan, T. J., and S. H. Faeth. 2008. Local adaptation in *Festuca arizonica* infected by hybrid and nonhybrid *Neotyphodium* endophytes. Microbial Ecology 55:697–704.

Sun, G. 2014. Molecular phylogeny revealed complex evolutionary process in *Elymus* species: Complex evolutionary process in *Elymus* species. Journal of Systematics and Evolution 52:706–711.

Sun, G.-L., B. Salomon, and R. von Bothmer. 1997. Analysis of tetraploid *Elymus* species using wheat microsatellite markers and RAPD markers. Genome 40:806–814.

Takach, J. E., S. Mittal, G. A. Swoboda, S. K. Bright, M. A. Trammell, A. A. Hopkins, and C. A. Young. 2012. Genotypic and chemotypic diversity of *Neotyphodium* endophytes in tall fescue from Greece. Applied and Environmental Microbiology 78:5501–5510.

Takach, J. E., and C. A. Young. 2014. Alkaloid genotype diversity of tall fescue endophytes. Crop Science 54:667.

Tenhumberg, B., A. J. Tyre, and R. Rebarber. 2009. Model complexity affects transient population dynamics following a dispersal event: a case study with pea aphids. Ecology 90:1878–1890.

Tigano, A., and V. L. Friesen. 2016. Genomics of local adaptation with gene flow. Molecular Ecology 25:2144–2164.

Tsuchida, T., R. Koga, H. Shibao, T. Matsumoto, and T. Fukatsu. 2002. Diversity and geographic distribution of secondary endosymbiotic bacteria in natural populations of the pea aphid, *Acyrthosiphon pisum*. Molecular Ecology 11:2123–2135.

Usher, K. M., J. Kuo, J. Fromont, and D. C. Sutton. 2001. Vertical transmission of cyanobacterial symbionts in the marine sponge *Chondrilla australiensis* (*Demospongiae*). Hydrobiologia 461:9–13.

do Valle Ribeiro, M. A. 1993. Transmission and survival of Acremonium and the implications for grass breeding. Agriculture, Ecosystems & Environment 44:195–213.

Vicente-Serrano, S. M., S. Beguería, and J. I. López-Moreno. 2010. A multi-scalar drought index sensitive to global warming: The Standardized Precipitation Evapotranspiration Index. Journal of Climate 23:1696–1718.

Victoria Novas, M., M. Collantes, and D. Cabral. 2007. Environmental effects on grass-endophyte associations in the harsh conditions of south Patagonia: Environment affects endophytes incidence in native grasses. FEMS Microbiology Ecology 61:164–173.

Vinton, M. A., E. S. Kathol, K. P. Vogel, and A. A. Hopkins. 2001. Endophytic fungi in Canada wild rye in natural grasslands. Journal of range management:390–395.

White, J. 1987. Widespread distribution of endophytes in the Poaceae. Plant Disease 71:340–342.

Wille, P., T. Boller, and O. Kaltz. 2002. Mixed inoculation alters infection success of strains of the endophyte *Epichloë bromicola* on its grass host *Bromus erectus*. Proceedings of the Royal Society of London B: Biological Sciences 269:397–402.

Worchel, E. R., H. E. Giauque, and S. N. Kivlin. 2013. Fungal symbionts alter plant drought response. Microbial Ecology 65:671–678.

Wu, L., A. Ren, Y. Jing, Y. Zhou, X. Wang, J. Qin, and Y. Gao. 2016. Endophytic benefit for a competitive host is neutralized by increasing ratios of infected plants. Acta Oecologica 70:112–120.

Young, C. A., B. A. Tapper, K. May, C. D. Moon, C. L. Schardl, and B. Scott. 2009. Indole-diterpene biosynthetic capability of *Epichloë* endophytes as predicted by *ltm* gene analysis. Applied and Environmental Microbiology 75:2200–2211.

Yule, K. M., T. E. X. Miller, and J. A. Rudgers. 2013. Costs, benefits, and loss of vertically transmitted symbionts affect host population dynamics. Oikos:1512–1520.

Zhang, W., S. D. Card, W. J. Mace, M. J. Christensen, C. R. McGill, and C. Matthew. 2017. Defining the pathways of symbiotic *Epichloë* colonization in grass embryos with confocal microscopy. Mycologia 109:153–161.

Zheng, B. 2000. Summarizing the goodness of fit of generalized linear models for longitudinal data. Statistics in Medicine 19:1265–1275.

Zientz, E., T. Dandekar, and R. Gross. 2004. Metabolic interdependence of obligate intracellular bacteria and their insect hosts. Microbiology and Molecular Biology Reviews 68:745–770.

Żurek, G., B. Wiewióra, M. Żurek, and R. Łyszczarz. 2016. Environmental effect on *Epichloë* endophyte occurrence and ergovaline concentration in wild populations of forage grasses in Poland. Plant and Soil 410:383-399.

Appendices

Appendix A – 2013 collection site location, coordinates, and environmental details for *Elymus canadensis* (EC) and *E. virginicus* (EV). Site number corresponds to site collection locations, including state parks (SP), displayed in Fig 1.1 and referenced in Table 2.1. In sites where both species were present (Both), the two entries for the number of plants collected (*N*), endophyte prevalence (E+), and mean vertical transmission rate (VT) ± associated variance correspond to each species respectively (EC;EV). In addition to precipitation (ppt.) and temperature (temp.), The Standardized Precipitation-Evapotranspiration Index (SPEI) ere included as predictors of endophyte prevalence and transmission.

Site	Site name	Species	*N*	E+ (%)	Mean VT rate	Ppt. (mm)	Temp. (°C)	SPEI	Lat.	Long.	Elev (m)	Sampling Date
1	Slide Rock SP, AZ	EC	16	94	100 ± 0	569.10	19.80	0.06	34.92	-111.73	1835	10/14/13
2	Flagstaff, AZ[1]	EC	29	48	21 ± 3.3	511.55	15.66	0.09	36.23	-111.58	1639	10/14/13
3	Davis Mountains SP, TX	EC	46	98	99 ± .006	355.45	24.80	0.19	30.61	-103.89	1508	8/7/2013
4	Big Spring, TX	EC	33	79	92 ± 4.0	388.03	25.97	0.06	32.23	-101.49	753	8/8/2013
5	San Angelo SP, TX	Both	5;28	100;97	100 ± 0; 97 ± 3.4	480.17	25.85	0.08	31.47	-100.54	602	8/8/2013
6	South Llano SP, TX	Both	18;24	100;92	96 ± 1.3; 91 ± 4.2	497.72	26.23	-0.04	30.45	-99.81	547	8/6/2013
7	Lost Maples SP, TX	EC	39	100	98 ± 0.78	581.55	25.41	0.01	29.83	-99.59	655	8/5/13
8	Lake Brownwood SP, TX	Both	38;7	71;14	92 ± 2.8; 53 ± 0	623.38	25.43	-0.06	31.86	-99.03	448	8/8/13
9	McKinney Falls SP, TX	EV	35	74	84 ± 10	707.32	26.74	-0.06	30.18	-97.72	174	6/5/13
10	Palmetto SP, TX	EV	46	96	83 ± 6.1	714.01	27.12	0.01	29.59	-97.58	100	8/5/13
11	Mother Neff SP, TX	EV	20	55	81 ± 8.5	822.70	25.73	-0.10	31.32	-97.47	223	6/4/13
12	Lake Thunderbird SP, OK	Both	9;21	100;43	92 ± 5.4; 23 ± 2.2	827.52	22.36	-0.15	35.23	-97.27	331	7/17/13
13	Lake Murray SP, OK	Both	22;30	100;43	92 ± 4.9; 38 ± 13	856.94	23.42	-0.21	34.07	-97.10	231	7/16/13
14	Lake Lewisville, TX	EV	22	9	64 ± 17	799.94	24.77	-0.33	33.07	-96.95	512	12/6/13
15	Lake Texoma SP, OK	Both	20;31	100;42	96 ± 2.4; 40 ± 4.6	913.48	23.21	-0.22	33.98	-96.63	192	7/16/13
16	Keystone SP, OK	Both	4;27	100;28	100 ± 0; 78 ± 6.3	874.98	21.30	-0.32	36.14	-96.27	253	7/17/13
17	Fall River SP, KS	Both	7;30	85;13	100 ± 0; 44 ± 15	1008.03	19.89	-0.33	37.65	-96.09	295	7/18/13
18	Toronto SP, KS	EV	27	67	51 ± 16	1033.10	19.93	-0.35	37.75	-95.94	288	7/18/13
19	Elk City SP, KS	Both	7;12	100;100	100 ± 0; 100 ± 0	1156.65	20.49	-0.31	37.25	-95.78	246	7/18/13

20	Huntsville SP, TX	EV	38	47	49 ± 11	973.30	26.40	0.04	30.62	-95.54	100	8/5/13
21	Rice University, TX	EV	41	76	67 ± 13	1122.22	26.33	0.08	29.71	-95.40	15	6/26/13
22	Devils Den SP, AK	EV	29	31	55 ± 10	1351.55	20.27	-0.10	35.78	-94.25	424	7/19/13
23	Pomm De Terre SP, MO	EV	25	24	45 ± 19	1277.94	19.18	-0.29	37.88	-93.32	263	7/19/13
24	Table Rock SP, MO	EV	33	39	32 ± 6.5	1262.00	19.93	0.06	36.58	-93.31	297	7/19/13
25	Bona Dea SP, AK	EV	21	81	94 ± 4.8	1453.07	22.80	-0.21	35.30	-93.16	134	7/20/13

[1]Collection location removed from data predicting endophyte vertical transmission in *E. canadensis* hosts

Appendix B – Genetic diversity of endophytes occupying *Elymus virginicus* (EV) and *E. canadensis* (EC) hosts based upon successful amplification of markers associated with alkaloid biosynthesis genes, and their alkaloid predictions[1]. When endophytes from both host species were assayed (Both) from a single location[2], the number of hosts assayed (Plant *N*) are included for each species respectively (EV;EC). Although we tested for the presence of loci within the *IDT* and *LTM* genes, none were detected and therefore not presented here.

					Mating type (*MT*)[5]			Peramine			*LOL*				*EAS*				
E+ genotype	Predicted alkaloids[3]	Pop[2]	Plant *N*[4]	Host	*A*	*B*	*AB*	*perA* A2	*perA* T2	*per A* R	*lolC*	*lolA*	*lolO*	*lolP*	*dmaW*	*easC*	*easA*	*cloA*	*lpsB*
1	PER	5	1	EV		+		+	+	+									
		6	3;1	Both		+		+	+	+									
		9	3	EV		+		+	+	+									
		11	2	EV		+		+	+	+									
		13	6;1	Both	+	+		+	+	+									
		16	2	EV	+	+		+	+	+		(+)							
		17	1	EV	+			+	+	+									
		18	3	EV	+	+		+	+	+									
		19	3	EV	+			+	+	+									
		22	2	EV	+			+	+	+									
		23	2	EV	+			+	+	+									
		25	1	EV	+			+	+	+		(+)							
2	PER /CC	5	10	EV	+	+		+	+	+					+	+			
		6	4;2	Both	+	+		+	+	+					+	+		(+)	
		9	7	EV	+	+		+	+	+					+	+			
		10	12	EV	+	+		+	+	+					+	+			
		11	2	EV	+	+		+	+	+					+	+			
		15	2	EV		+		+	+	+					+	+			
		16	2	EV		+		+	+	+					+	+			
		22	1	EV	+			+	+	+	(+)				+	+			
		25	5	EV		+		+	+	+					+	+			
3	PER/ AcAP	18	1	EV	+		+	+	+	+	+	+	(del)[6]						
4	PER/ AcAP/ CC	3	4	EC	+			+	+	+	+	+	(del)[6]		+	+			
		6	8	EC	+			+	+	+	+	+	(del)[6]		+	+			
		12	3	EC	+			+	+	+	+	+	(del)[6]		+	+			
		13	1	EC	+			+	+	+	+	+	(del)[6]		+	+			
5	PER/ NANL/ CC	1	2	EC			+	+	+	+	+	+	+		+	+			
		3	12	EC			+	+	+	+	+	+	+		+	+			
		13	1	EC			+	+	+	+	+	+	+		+	+		(+)	
6	PER/ NANL/ EC	12	1	EC		+		+	+	+	+	+	+		+	+	+	+	
7	PER/ NANL/ ERV	1	9	EC		+	+	+	+	+	+	+	+		+	+	+	+	+

12	3	EC			+	+	+	+	+	+	+		+	+	+	+	+			
18	2	EV	+			+	+	+	+	+	+		+	+	+	+	+			
19	6	EV	+			+	+	+	+	+	+		+	+	+	+	+			
25	2	EV		+		+	+	+	+	+	+		+	+	+	+	+			

[1] +, marker detected; (+) marker detected in a subset of samples. Marker descriptions and sizes found in Charlton *et al.* [63]
[2] Collection population (pop) numbers corresponding to Fig. 1 and Supplemental Table 1
[3] Predicted chemotype based upon genotype prediction conventions. Chemotypes: PER, peramine; CC, chanoclavine; AcAP, 1-acetamidopyrrolizidine; NANL, *N*-acetylnorloline; ERV, ergovaline
[4] Number of endophytes corresponding to chemotype from each species respectively (EV ; EC)
[5] Mating-type genotypes are defined based upon inheritance of *MTA* and *MTB* loci. Nonhybrid endophytes are either *MTA* or *MTB* but hybrid endophytes can be *MTA MTB* (can be identified by PCR) or *MTA MTA* or *MTB MTB* (cannot be identified by PCR)
[6] (del) marker indicates a deletion at that loci that results in the gene being nonfunctional.

Appendix C – Molecular techniques to quantify genetic distance between outcrossed parents (P_1 generation)

Tissue sampling, PCR protocol, and processing microsatellite markers

We used a multiplex approach with nine fluorescently labeled simple sequence repeat (SSR) microsatellite markers to estimate genetic distance between outcrossed P_1 parents and to confirm the effectiveness of the greenhouse crossing experiment (Hayden et al. 2008, Saha et al. 2009) (Table C1 & Figure C1). Genomic DNA was extracted from ~10 mg of freshly frozen and lyophilized plant tissue using MagAttract 96 DNA plant core Kit (QIAGEN Inc., Valencia, CA) and analyzed following Takach *et al.* (Takach et al. 2012). PCR was performed in a total volume of 10 µl containing 20 ng of template DNA, 1.0 unit of Promega GoTaq™ DNA Polymerase (Promega Corp., Madison, WI), 2 mM of dNTPs, 2 µl of 5X Colorless GoTaq™ Buffer, 10 µM of the reverse and M13 dye primer, and 5 µM of the forward M13 fluorescently tagged primer. The PCR cycling parameters were: an initial denaturation of 3 min at 95°C, then 6 cycles of 94°C for 45 s, 68°C for 5 min, and 72°C for 60 s followed by 8 cycles of 94°C for 45 s, 58°C for 2 min, and 72°C for 30 s. Then, 25 cycles at 94°C for 45 s, 50°C for 2 min, and 72°C for 30 s followed by a final extension of 72°C for 7 min. To confirm amplification, we ran each PCR product on a 1.5% agarose gel visualized with ethidium bromide and a UV light. Then, 1 µl of amplified PCR product per SSR marker was suspended in Hi-Di Formamide with 0.5 µl LIZ 500 ladder (Applied Biosystems, Farmingham, MA) and then separated on an Applied Biosystems 3730 capillary sequencer. Raw data were analyzed using GENEMAPPER 4.0 and Peak Scanner 2 (Applied Biosystems). Kentucky 31 (*Festuca arundinacea*) of known endophyte status were used as positive and negative

controls.

Estimating genetic distance between P_1 parents

Both P_1 parent species (*Elymus virginicus* and *E. canadensis*) are allopolyploids, with genomes from *Pseudoroegneria* and *Critesion* species (Dewey 1983, Sun et al. 1997). However, estimating genotypes from allopolyploid organisms is challenging due to potential amplification of multiple genomes from the two progenitor species, which makes assigning heterozygotes impossible without knowing the origin of each amplified sequence (Dufresne et al. 2014). Therefore, to avoid taxonomic issues common to polyploids, analyses were performed using data formats that assume uniform ploidy across individuals.

First, to measure linkage disequilibrium among loci, we calculated a modified index of association robust to small sample sizes ($\bar{r}_d$) (Brown et al. 1980, Agapow and Burt 2001) between the amplified microsatellite loci in the R package *poppr* (Kamvar et al. 2014). Higher measures of $\bar{r}_d$ indicate greater linkage between loci. We uncovered a pair of significantly linked loci ($\bar{r}_d = 0.0418$, $P = .001$). Removing one of two loci from the dataset significantly reduced linkage disequilibrium ($\bar{r}_d = 0.0299$, $P = 0.061$). In total, we identified 29 alleles across the eight remaining loci (mean = 3.75 alleles per locus). We then generated a saturating genotype accumulation curve, which quantifies the amount of power within the data to discriminate between unique individuals given a random sub-sample of *n* loci (command locus_table and genotype_curve in *poppr* (Kamvar et al. 2014). Then, we calculated a pairwise, individual-by-individual (*N* x *N*) genetic distance matrix using the binary data set in GenAlEx v. 6.502 (Peakall and

Smouse 2006), which tallies the total number of differences between two genetic profiles (Huff et al. 1993). When multiple individuals per maternal line were genotyped, genetic distance was averaged over all estimates. Using this distance matrix, we conducted a distance-based cluster analysis (PCoA, GenAlEx v. 6.502), which revealed genetic separation between *Elymus virginicus* individuals as well as between *E. virginicus* and *E. canadensis* host species (Figure C3).

Estimating endophyte genotype

Extracted DNA from multiple offspring per P_1 hosts (mean = 3.7, min =1, max = 9, (N = 545) was isolated using techniques described in Sneck et al. 2017. Endophyte DNA was isolated from plant DNA with a multiplex approach using 18 markers, which infer both the major alkaloid classes (peramine, ergot alkaloids, lolines, and indole-diterpenes) and endophyte mating type (*MTA* or *MTB*) (Charlton et al. 2014). Only two similar endophyte genotypes were found in P_1 plants, one that likely produces the alkaloid peramine (PER) and the other likely produces both peramine and chanoclavine (PER/CC) (Sneck et al. 2017). Endophyte genotype did not explain remaining variation in residuals extracted from the top supported models (Table 2.1) compared to a null model using likelihood ratio tests in both the F_1 (χ^2 = 0.505, P = 0.918) and F_2 offspring generations (χ^2 = 0.4.03, P = 0.817). Therefore, the endophyte vertical transmission rates observed in this study are likely a product of outcrossing treatment and not endophyte genotype.

Table C1 – Microsatellite markers used to estimate genetic distance between outcrossed parents modified from Saha et al. 2009. Primer 61 was dropped from final analyses due to linkage with primer 59. An annealing temperature of 58°C was used for all primers.

Name	Forward primer	Reverse primer	Expected size	Repeats	Allele per locus	Gene diversity[1]
19	TGGATTTGCAATTAGCCTCA	GCTCGTGTATGGCCTTCAAT	176-530	ta	2	0.39
22	ATGATGTCCGAGGAGGAGAA	CATCATGATCCAGTGCCTTG	184-266	agg	3	0.63
32	ACGGTCTGTACCGTGGATGT	GCTGTAGACTCAGCCGAACC	288-330	ctg	4	0.57
50	GATGGACGAAGGCTTCTTTG	AGCCGAACCTGAACTCAGAC	177-287	cag	4	0.72
59	TTTGCACTCTCGGACCTAGC	CGGTACACCTTCTGCACCTT	288-290	ga	4	0.73
61	GTCGCCGGAGAAGAGAAGAG	AACGCTAGCCGTGATGACTT	127-142	ag	4	0.59
78	TCCTAAGCAGAGCTCGATCC	GAGGTTGGCGAACTTCCTC	164-216	ga	4	0.72
113	CAATGGTGGTGCAAGAAATG	AGAGAGCAAGGAGGAAGAAACC	153-248	ct	5	0.71
142	ACTTGCCGGAGAAGAAGCTC	ATACAGGAGGAGGAGGAGCAG	185-304	aga	4	0.73

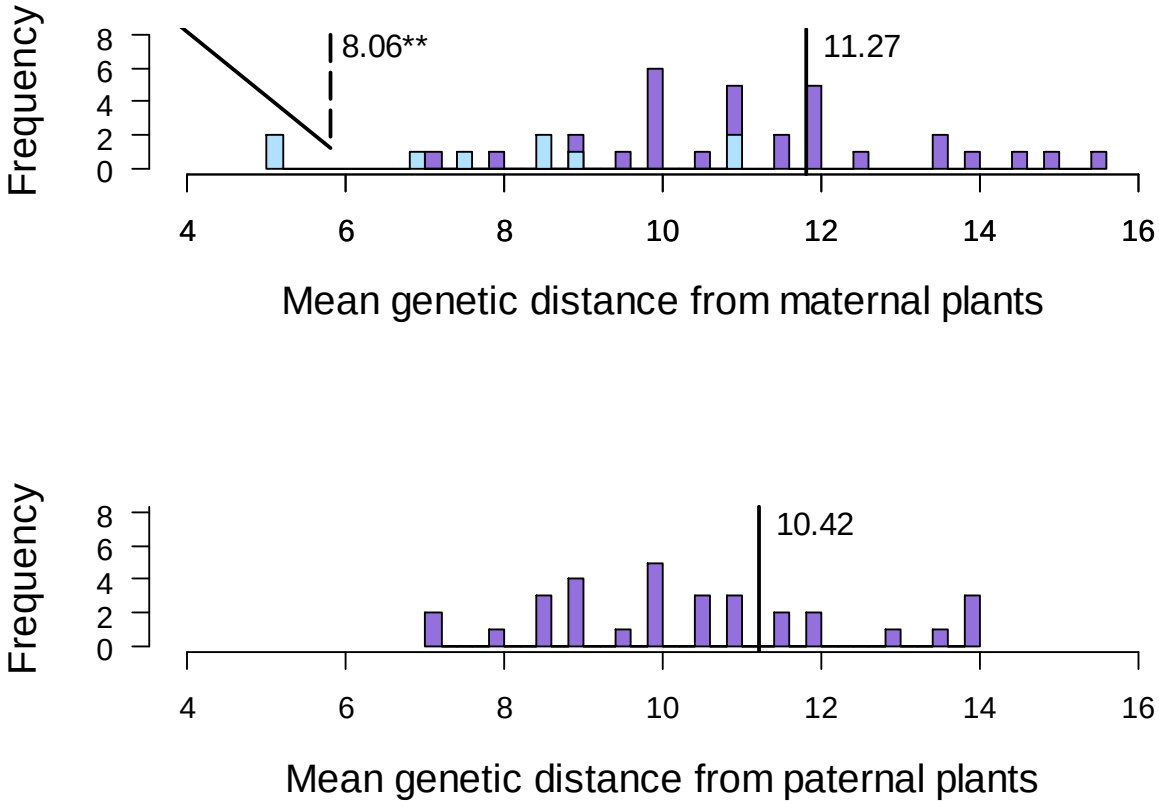

Figure C1 – To demonstrate the effectiveness of experimental crosses between P_1 plants, several outcrossed and self-pollinated individuals were genotyped using eight microsatellite markers. We found that outcrossed offsrping were genetically distant from both parents (purple) (t = 1.72, P = 0.091). Also, compared to self-fertilized offspring (blue), outcrossed offspring were significantly genetically distant from maternal plants (t = 3.95, P = 0.002). These data provide evidence that our experimental crosses successfully altered the genetic background of outcrossed offspring. Solid (outcrossed) and perforated (selfed) vertical bars and adjacent numbers indicate the mean genetic distance of each group.

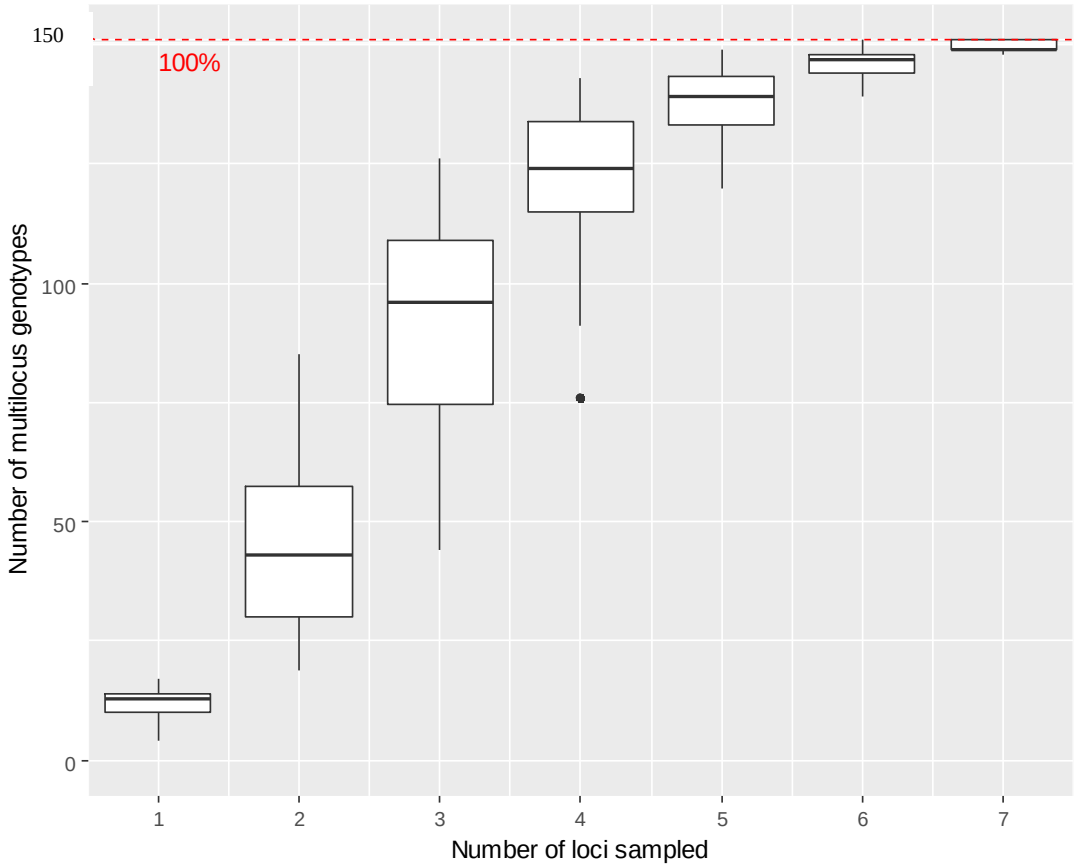

Figure C2 – Genotype accumulation curve of the eight microsatellite loci used to quantify genetic distance between outcrossed parents. Boxplots show the number of unique genotypes estimated at each number of sampled loci. The saturating curve indicates that the number of loci used were sufficient to discriminate between individuals. Samples were bootstrapped 1000 times to create this distribution.

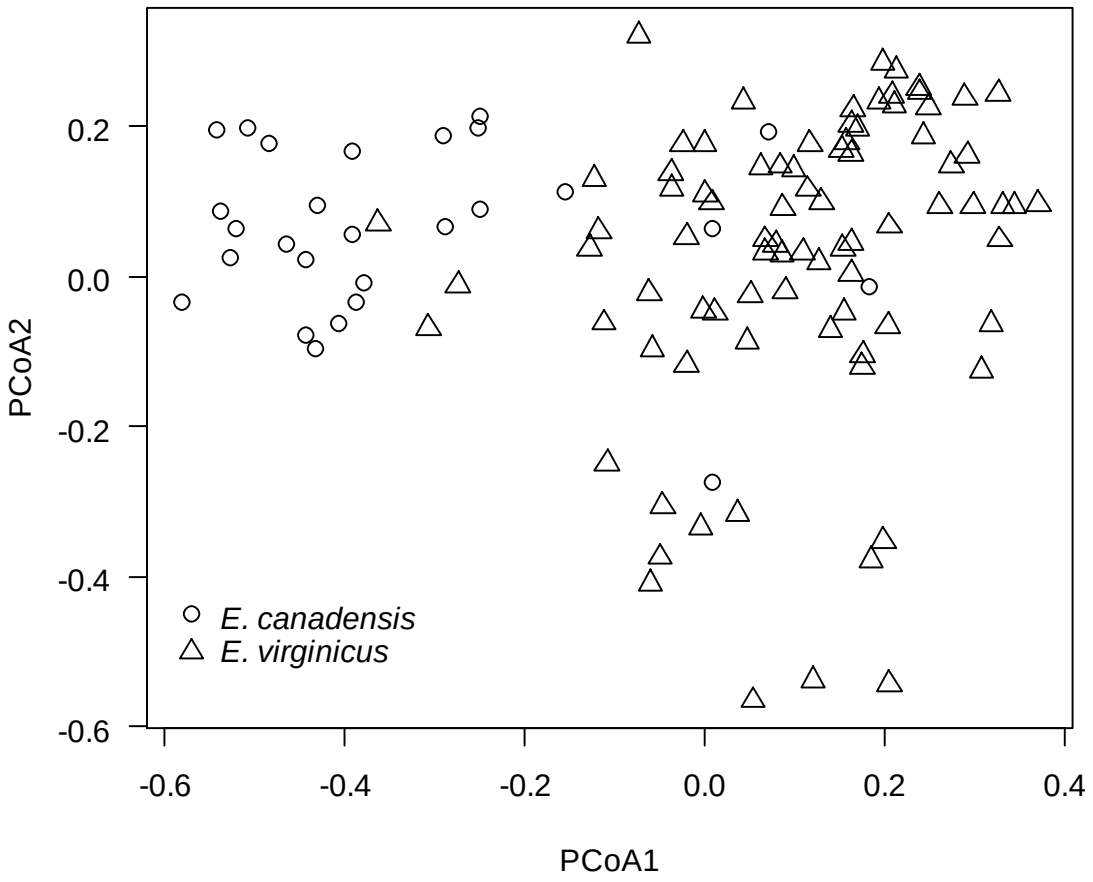

Figure C3 – Principle Coordinate Analysis (PCoA) representing the genetic distance between P_1 individuals within a host species and between host species. This PCoA displays the relationship between collected individuals based upon multivariate genetic distances estimated from eight microsatellite loci, wherein spatial distance between points (spread between Principle Coordinates 1 and 2) is analogous to estimated genetic distance.