

Variation in the Prevalence and Transmission of Heritable Symbionts Across Host Populations in Heterogeneous Environments

Michelle E. Sneck¹ · Jennifer A. Rudgers² · Carolyn A. Young³ · Tom E. X. Miller¹

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Abstract 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, we surveyed 25 populations of two native grasses (*Elymus virginicus* and *Elymus canadensis*) across the southern Great Plains (USA). Both grass species host heritable endophytic fungi (genus *Epichloë*) and can hybridize where their ranges overlap. From a subset of hosts, we characterized endophyte genotype using genetic loci that link to bioactive alkaloid production. First, we 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, we evaluated the potential

influence of biotic factors and found that, after accounting for climate, endophyte genotype explained significant variation in symbiont inheritance. We also contrasted populations where host species co-occurred in sympatry vs. allopatry. Sympatry could potentially increase interspecific hybridization, but this variable did not associate with patterns of symbiont prevalence or transmission success. Our 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.

Keywords Vertical transmission · *Epichloë* spp. · Endophytic fungi-grass symbiosis · Plant-microbe · Symbiont prevalence · Hybridization

Introduction

Nearly all multicellular organisms host a rich diversity of symbiotic microbes, many of which are vertically transmitted from maternal host to offspring [1–3]. Inherited microbes often benefit their hosts in exchange for nutrients, protection, and regeneration [4], and this exchange may be mutually beneficial because vertical transmission couples host and symbiont fitness [5]. For example, in both plants and arthropods, heritable microbial symbionts can increase resistance to environmental stress [6–8], competitive ability [9], and defense against enemies [10, 11]. Some heritable microbes, particularly fungi, improve plant fitness by producing bioactive chemicals [12]. Additionally, they may also mitigate the effects of global climate change [13] and environmental degradation on host populations [14, 15]. The influence of heritable microbes extends beyond individual hosts to alter community composition and ecosystem

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✉ Michelle E. Sneck
mes8@rice.edu

¹ Department of BioSciences, Program in Ecology and Evolutionary Biology, Rice University, 6100 Main Street, Houston, TX 77005, USA

² Department of Biology, University of New Mexico, Albuquerque, NM 87131, USA

³ Samuel Roberts Noble Foundation, Inc, Ardmore, OK 73401, USA

processes [16, 17]. 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 [18].

Vertical transmission links symbiont fitness to host fitness via host reproduction [19]. This connection is hypothesized to select for a tightly co-evolved mutualism that should persist at high frequencies in host populations [5, 19]. 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 [20], bacteria (*Wolbachia* spp.) in arthropod hosts [2, 21], and some components of the human microbiome [22]. Variable frequencies of heritable symbionts is partly driven by changes in host and symbiont relationships that shift with ecological context [23], 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 [6, 18]. Many studies have observed that symbiont prevalence varies systematically along environmental gradients such as elevation [25, 26], the presence of pathogens [27], ocean depth [24], and ecosystem productivity [18]. 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 [10, 28, 29]. 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 [29, 30].

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) [31]. Estimates of individual vertical transmission rates have received relatively little attention compared with population-level symbiont frequency [32, 33]. In the few symbioses where vertical transmission has been quantified, it is often imperfect (<100% of offspring inherit the symbiont) [2, 20]. 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 [34–36]. Therefore, a positive correlation between symbiont prevalence and transmission supports the hypothesis that transmission plays a part in determining symbiont frequencies [31].

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 [31]. 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 [32, 37], while others found no environmental effects [38, 39]. Simulated grazing and mechanical disturbance limited vertical transmission of *Epichloë occultans* in multiple accessions of annual ryegrass (*Lolium multiflorum*) [37], but drought treatments failed to alter transmission success of *Epichloë amarillans* in *Agrostis hyemalis* [39]. 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 [12]. 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 [4, 40]. This alkaloid gene diversity can be partially explained by modes of fungal reproduction. Many *Epichloë* species reproduce asexually via vertical transmission [41], but some can reproduce sexually, through formation of stroma (“choke disease”) followed by fertilization of opposite mating types (*MTA* or *MTB*) [42]. Although *Epichloë* species can reproduce both asexually and sexually, interspecific heteroploids that retained multiple genomes following a hybridization event are exclusively vertically transmitted [43]. Hybrid endophytes can gain alkaloid genes from both ancestors, potentially increasing both benefits to host fitness [44] and ecological dominance over nonhybrid endophytes [44–46]. Also, hybrid endophytes could vertically transmit at higher rates than nonhybrids, another mechanism that could promote their high natural abundance [41, 43]. However, comparisons of vertical transmission rates between hybrid and nonhybrid endophytes are few [47, 48].

An additional biotic factor that could affect context-dependent outcomes of symbiosis is the sympatry or allopatry of related host species. Sympatry increases the potential for gene flow between host species (i.e., interspecific

hybridization) [49, 50]. Host outcrossing events are hypothesized to result in genotype mismatches that reduce vertical transmission for symbionts because they are largely asexual and exhibit a high degree of specialization to specific host species and genotypes [38, 51]. In both arthropods and plants, symbiotic bacteria or cellular organelles (e.g., chloroplasts) can interact with host genetic background in complex ways [52]. To the best of our 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, we report both population-level prevalence and individual-level vertical transmission rates of heritable symbionts across the broad geographic distribution of two hybridizing host species [53]. We focused on vertically transmitted fungal endophytes (*Epichloë* spp.) hosted by two native North American grass species (*Elymus virginicus* and *Elymus 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 [54] as well as in some legumes, morning glories, and sedges [11]. The symbiosis is facultative for the plant but obligate for the endophytes. For a subset of symbiotic hosts, we quantified fungal genetic variation at loci associated with biosynthetic secondary metabolite pathways [4, 55], which predict the suite of bioactive alkaloids shown to have context-dependent effects on host fitness [56]. We compared symbiont prevalence and vertical transmission rate between populations that differed in host species sympatry (potential for interspecific 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 [26, 57–59].

Specifically, we 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?

Materials and Methods

Study System

We focused on two perennial grasses, *E. canadensis* (Canada wildrye) and *E. virginicus* (Virginia wildrye). *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 [60]. Gene flow tends to be uni-directional, primarily from *E. canadensis* to *E. virginicus* [53]. Both grasses host systemic fungal endophytes, *Epichloë* species (Clavicipitaceae) [61].

Field Sampling

We surveyed populations of both host species across their distribution in the southern Great Plains (Fig. 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). We characterized populations as *E. virginicus* alone ($N = 11$ populations), *E. canadensis* alone ($N = 5$), or both host species co-occurring in sympatry ($N = 9$) (Fig. 1; Table 1). Seed collections were made in 2013 after peak flowering (June–early December), when most plants had mature seeds (Supplementary Table 1). We 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.

Environmental Data Collection

To investigate the potential role of abiotic variation in symbiont prevalence and transmission, we focused on three environmental variables: temperature, precipitation, and drought (an integrated measure of the other two). We chose maximum temperature because endophyte survival [62] and host fitness benefits [63] can be contingent upon high temperatures. We examined mean annual precipitation (mm) because it has been shown to co-vary with endophyte prevalence in other systems [18]. We constrained calculations to the 5-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 [64]; 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

Fig. 1 Map of collection sites. 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 Supplementary Table 1

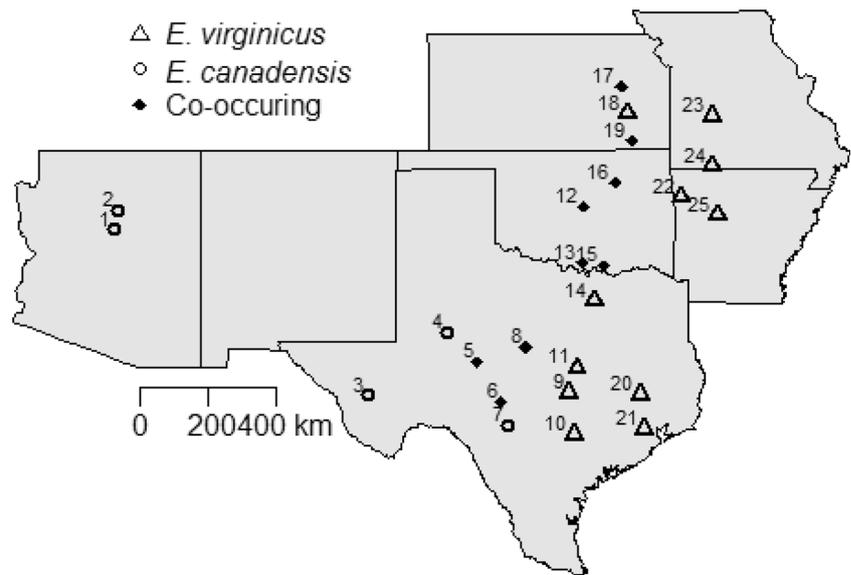


Table 1 AIC_c model rankings for *Elymus virginicus* (EV) and *E. canadensis* (EC)

Population-level endophyte prevalence						Individual-level endophyte transmission					
Host	Model	AIC _c	ΔAIC	AICwt	D	Model	AIC _c	ΔAIC	AICwt	D	
EV	SPEI ^a	133.66	0.00	0.47	0.042	Null ^a	1206.99	0.00	0.27		
	tmax	136.02	2.36	0.14		tmax ^b	1207.55	0.56	0.20	0.0012	
	SPEI + sympatry		136.45	2.79	0.12		SPEI	1208.63	1.64	0.12	
			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 r	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 ^a	51.46	0.00	0.34		Null ^a	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 ^b	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		

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 “Materials and Methods” for additional details)

^a Parameter estimates from this model plotted in Fig. 3

^b Residuals extracted from these models for endophyte genotype analyses

the growing season (February–April) or reproductive season (April–July).

We 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 [65]) received less statistical support than mean maximum temperature and were therefore excluded from candidate models. As a measure of drought, we used the Standardized Precipitation-Evaporation Index (SPEI), which accounts for the duration and severity of water loss compared with water availability [66]. 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 [67]). 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 (Supplemental Table 1).

Quantifying Population-Level Endophyte Prevalence and Individual-Level Transmission

Our 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, we 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 [20, 34, 35]. Therefore, a plant was designated E− (nonsymbiotic) 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. We used microscopy to visually inspect five host seeds per plant for presence/absence of hyphae in the seed coat and/or aleurone layer [9, 57]. Briefly, seeds were soaked in a 5% NaOH solution overnight, then squashed, stained with aniline blue, and examined with a light microscope under $\times 200$ magnification. The stain adheres to fungal hyphae, which are detectable regardless of seed or fungal viability. For an additional 15 seeds, we 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 [68]. We verified this 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, were likely driven by small sample sizes of microscopy screenings (five seeds), where instances of low to intermediate transmission went undetected. We 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, we assayed 13,647 seeds from 848 host individuals from 25 populations (Supplementary Table 1).

Molecular Techniques to Estimate Endophyte Genotype

To determine endophyte genotypes for a subset of host populations (*E. virginicus*: $N = 9$; *E. canadensis*: $N = 3$; sympatry: $N = 5$), we germinated multiple seeds from 196 individual field-collected host plants in a greenhouse at Rice University during spring 2014. Our 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 [69]; therefore, all offspring derived from a single host should 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. [70]. 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 [71], 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*, and *MTB*) cannot be distinguished from nonhybrid endophytes with our 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, we defaulted to the most common genotype observed among related individuals. Analyses using the alternative genotype(s) produced qualitatively similar results.

Statistical Analyses

We used generalized linear mixed models (glmer in R package lme4) [72], 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, we 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 we used the sum of squared Pearson residuals divided by the residual degrees of freedom (c). Values of c greater than 1 indicate overdispersion (c_{hat} in R package AICcmodavg) [73]. Overdispersion in the vertical transmission data (EV $c = 7.2$; EC $c = 5.1$) was corrected by nesting an individual random effect within the population random effect (EV $c = 0.13$; EC $c = 0.0048$). We did not detect overdispersion in the endophyte prevalence data and therefore only included the population random effect (EV $c = 0.24$; EC $c = 0.15$). To determine the influence of host sympatry, we 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). 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 11 models (Table 1).

Model selection was conducted using the second-order bias corrected AIC_c that ranks the relative support for each candidate model (aictab in R package AICcmodavg) [73]. The difference between the best model and all other models (ΔAIC_c) and the conditional probability for each model (AIC_cwt) were also calculated. To determine the amount of deviance

explained by the highest-ranked model compared with the null, we calculated the proportional reduction in deviance (methods detailed in [74]). This quantity (D) determines the strength of association between response and predictor variables (1 = perfect prediction; 0 = no association) [75].

Following model selection, we 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) in models testing the categorical effect of endophyte genotype against a null model using likelihood ratio tests. We 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 (Table 2) was given a unique categorical dummy variable (1–7, corresponding to the seven genotypes we detected).

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

Results

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%) (Fig. 2a). For *E. virginicus*, model selection indicated that endophyte prevalence was lower under greater drought stress (AIC_cwt = 0.47, $D = 0.042$) and increased from 47% in dry sites (SPEI < 0) to 79% in mesic sites (SPEI > 0) (Fig. 3a). *E. canadensis* had high endophyte prevalence across environments (prevalence: dry = 94%, mesic = 86%) (Fig. 3b), and the null model received the most support (Table 1). Figure 3b shows *E. canadensis* endophyte prevalence in relation to mean maximum temperature, which was the most supported environmental variable ($\Delta\text{AIC}_c = 0.56$).

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%) (Fig. 2b). For both species, abiotic factors explained little variation in transmission rates. For *E. virginicus*, the null model (AIC_cwt = 0.27) and the model containing temperature (AIC_cwt = 0.20) received similar statistical support (Table 1), but temperature explained just a small fraction

Table 2 (continued)

E+ genotype	Predicted alkaloids ^b	Pop ^a	Plant N ^c	Host	Mating type (MT) ^d			Peramine			LOL			EAS			
					A	B	AB	perA A2	perA T2	perA R	lolC	lolA	lolO	lolP	dmaW	easC	easA
		18	2	EV	+			+	+	+	+	+	+	+	+	+	+
		19	6	EV	+			+	+	+	+	+	+	+	+	+	+
		25	2	EV		+		+	+	+	+	+	+	+	+	+	+

When endophytes from both host species were assayed (both) from a single location², 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

^a“+” marker detected, “(+)” marker detected in a subset of samples (marker descriptions and sizes found in Charlton et al. [63])

^aCollection population (pop) numbers corresponding to Fig. 1 and Supplemental Table 1

^bPredicted chemotype based upon genotype prediction conventions. Chemotypes: peramine (PER), chanoclavine (CC), 1-acetamidopyrrolizidine (AcAP), N-acetylornitine (NANL), and ergovaline (ERV)

^cNumber of endophytes corresponding to chemotype from each species, respectively (EV; EC)

^dMating-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)

^e(del) marker indicates a deletion at that loci that results in the gene being nonfunctional

of the substantial variability in transmission ($D = 0.0012$) (Fig. 3c). For *E. canadensis*, vertical transmission rates were consistently high but declined slightly at low maximum temperatures (16 °C). Models containing temperature alone ($AIC_{cwt} = 0.54$) and temperature plus sympatry ($AIC_{cwt} = 0.23$) 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 (Supplementary Table 1, site 2), the null model emerged as the best ($AIC_{cwt} = 0.27$), 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. 3d, dotted line).

Association Between Vertical Transmission Rate and Endophyte Genotype

We identified seven unique endophyte genotypes over 136 host plants (Table 2). All endophyte genotypes were positive for peramine (PER) markers, but genotypes varied in presence/absence of loline (LOL), and ergot alkaloid (EAS) loci (Table 2). Overall, we 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; Table 2). These hybrid endophytes occurred primarily in *E. canadensis* and had more alkaloid markers than nonhybrids. Putatively sexually reproducing, nonhybrid 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) compared with 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%) ($z = -2.49$, $P = 0.0128$) (Fig. 4a). Genotype 1 has genes associated with peramine alkaloid production known to specifically target invertebrates. 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. 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 (Fig. 4b).

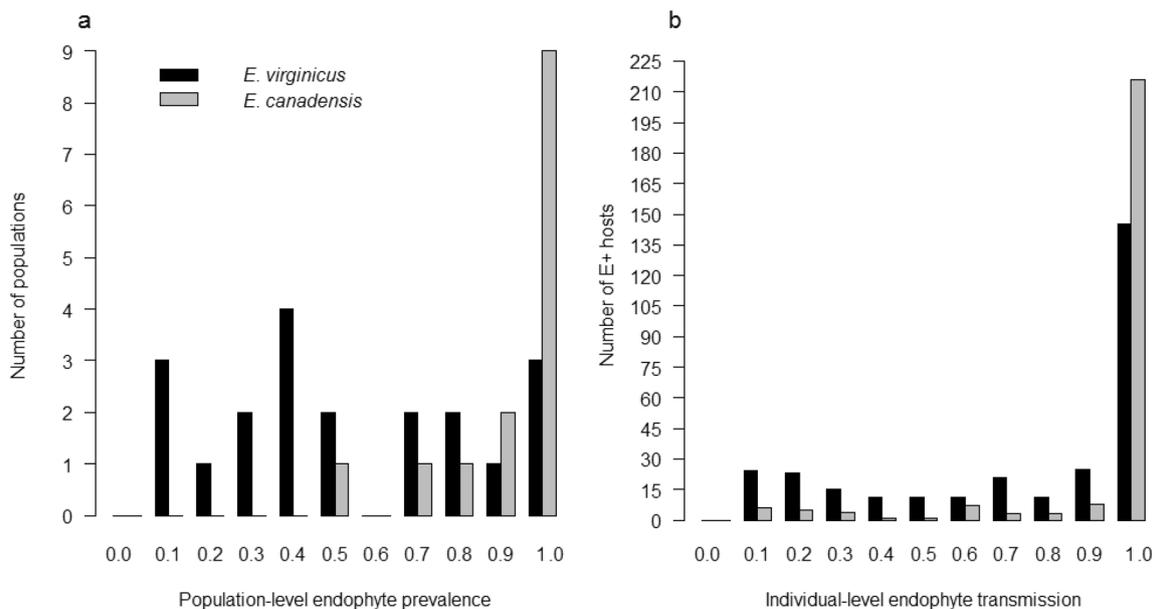


Fig. 2 Histograms of *E. virginicus* (black bars) and *E. canadensis* (gray bars) population-level endophyte prevalence (a) and individual-level endophyte transmission rates of endophyte positive (*E+*) hosts (b) across all sampled populations

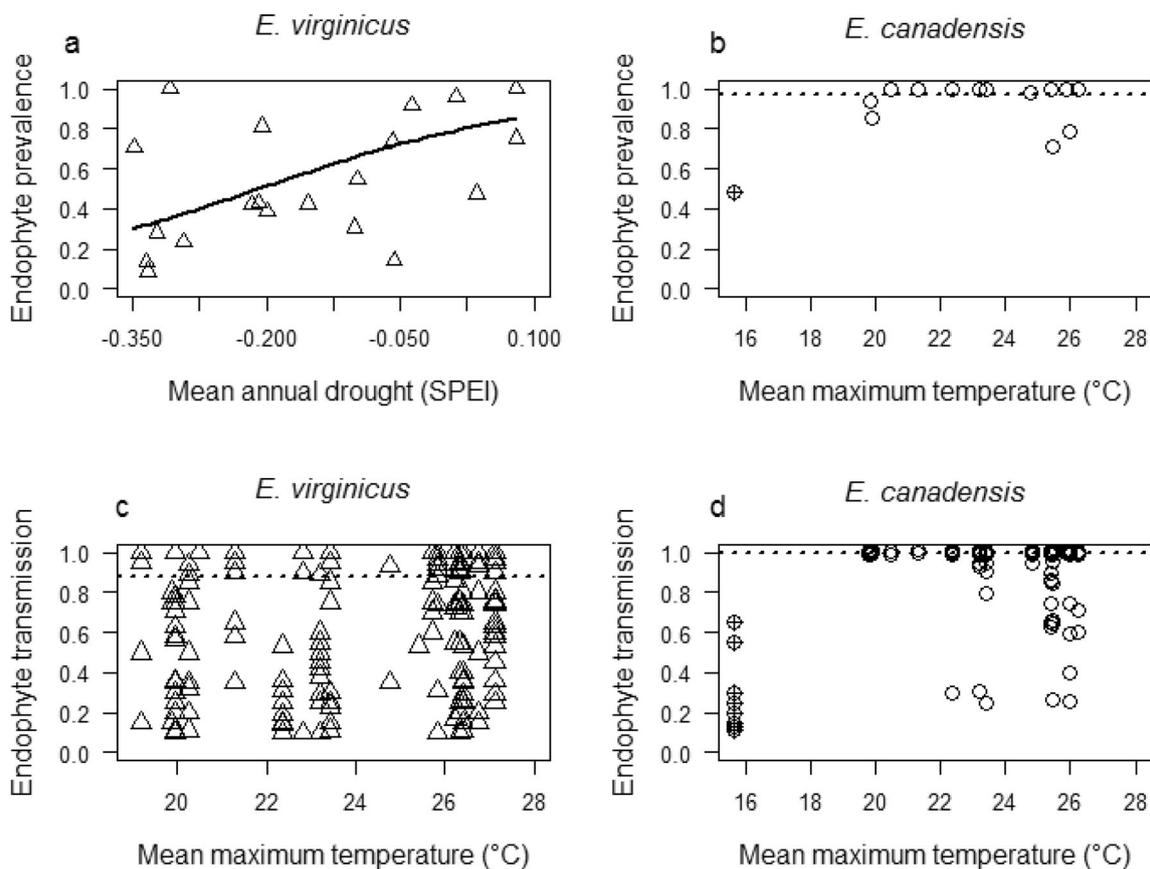


Fig. 3 Generalized linear mixed effect model estimates from Table 1 showing best fit patterns (solid line) of population-level endophyte frequencies (a, b) and individual-level vertical transmission rates (c, d) across annual mean maximum temperature (°C) or mean annual drought (SPEI) for *E. virginicus* and *E. canadensis* hosts. The null model

(intercept) is indicated by dotted lines (b–d). Hatched circles in (b) and (d) indicate endophyte vertical transmission rates from site number 2 (flagstaff: Supplementary Table 1) that were not included in the final model estimates (Table 1)

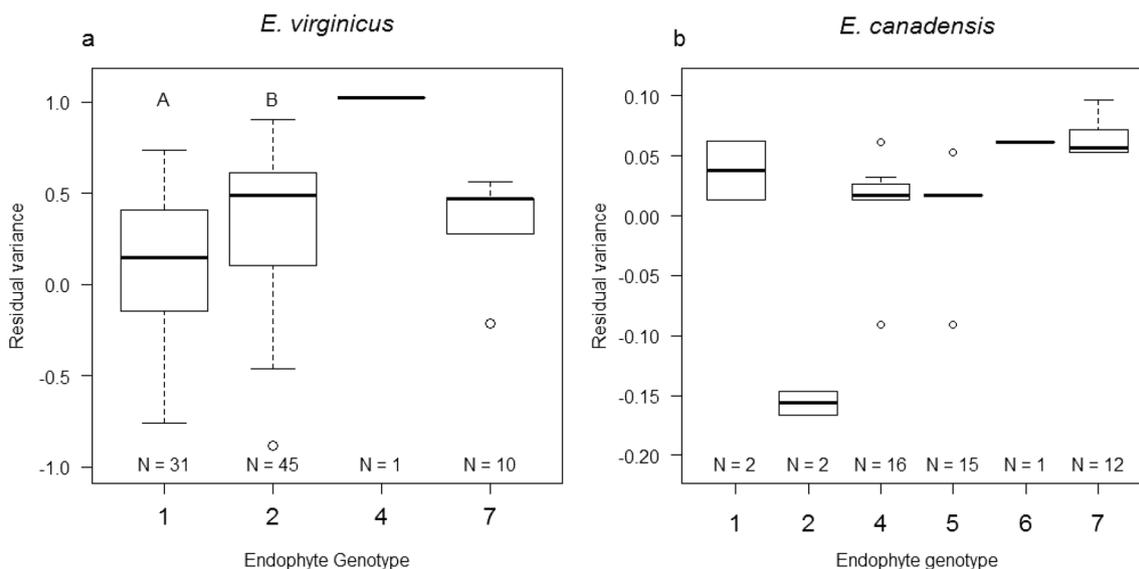


Fig. 4 Box and whisker plots of residual variance in endophyte vertical transmission explained by endophyte genotype (Table 2). Residual variance was extracted from abiotic models with the most statistical support (Table 1) for both host species (a, b) and restricted to genotyped

endophytes (*N* number of endophytes per genotype). Endophyte genotypes are defined by their unique alkaloid genetic profile (Table 2). In *E. virginicus* hosts, endophytes with genotype 2 transmitted at a significantly higher rate than endophytes with genotype 1 ($z = -2.77, P = 0.007$)

Correlation Between Endophyte Prevalence and Transmission

For *E. virginicus*, populations with high mean endophyte prevalence had higher mean vertical transmission success ($\rho = 0.659, S = 454, P = 0.0021$, Fig. 5). In *E. canadensis*, there was less variability in prevalence and transmission and no significant correlation between the two (EV; EC: $\rho = 0.241, S = 345.14, P = 0.406$).

Discussion

Vertical transmission is an important determinant of heritable symbiont prevalence in host populations [31, 32] but has received less empirical attention than the fitness effects of symbionts. To our knowledge, ours 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. We found that both endophyte prevalence and vertical transmission varied substantially between and (for transmission) within populations (Fig. 2) and weakly associated with large-scale abiotic variables (Fig. 3). However, we did find one exception to this pattern—endophyte prevalence in *E. virginicus* significantly declined with greater drought stress (lower SPEI). Furthermore, we uncovered novel evidence that biotic context, specifically endophyte genotype, plays a role in

determining symbiont inheritance (Fig. 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* [32] (Fig. 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 [31]. Together, our results propose endophyte genotype, and to a

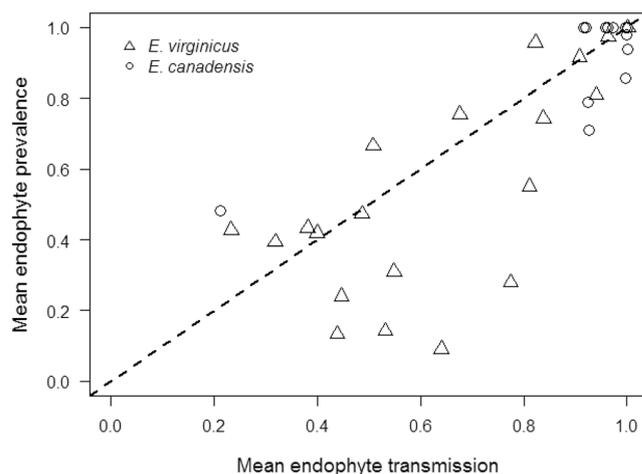


Fig. 5 Correlation between population-level mean endophyte prevalence and mean endophyte transmission observed in *E. virginicus* (open triangles) and *E. canadensis* (open circles) host. The dashed line represents the 1:1 relationship

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 [25, 76] but have paid less attention to endophyte transmission [33, 38, 77]. Our 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 (Fig. 2) and in few cases, correlate with local climate. Other surveys found that endophyte prevalence either increased [78] or declined [79] with greater aridity, thereby suggesting that host-endophyte relationships vary in their responses to abiotic stressors. In our study, endophyte prevalence, but not transmission, decreased with increasing drought severity in *E. virginicus* (Fig. 3a). In contrast, for *E. canadensis*, endophyte prevalence and transmission did not closely associate with abiotic factors (Fig. 3b, 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 [28, 63], and therefore should reach high prevalence in drought-stressed environments over time [31]. However, experimental studies have revealed that endophytes are not universally beneficial under abiotic stress [80]. For example, Rudgers and Swafford [81] demonstrated that *E. 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 [33]. Without manipulating symbiont presence, we cannot determine if or when endophytes act as mutualists in this system.

After accounting for abiotic influences, our work reveals a previously undocumented association between vertical transmission and endophyte genotype (Fig. 4). Here, we present endophyte genotypes composed 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 (Table 2), and elsewhere [71, 82–84], corresponds to bioactive alkaloids produced by endophytic fungi *in planta*. *Epichloë* alkaloids can influence host fitness by deterring herbivores [55], increasing host resistance to pathogens [27], and altering soil microbial composition [85]. It is possible that endophytes equipped with a diverse arsenal of alkaloids that increase host fitness may also be selected for increased transmission rates. Our results are consistent with this hypothesis: endophytes in *E. canadensis* were more prevalent and

transmitted at higher rates (Fig. 2b) and also possessed more genetic loci for bioactive alkaloids compared with *E. virginicus* (Table 1; Fig. 4). Also, similar to previous observations [84], 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 [41, 84, 86]. 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 [20, 81]. Although we 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 8 of the 15 plant populations.

Host species co-occurrence explained little variation in either symbiont prevalence or vertical transmission (Table 1). Sympatry is a pre-requisite for interspecific gene flow and may also allow for biotic interactions such as competition [87] or increased exposure to shared enemies [88]. We proposed that lower symbiont prevalence and transmission in sympatry vs. allopatry could reflect costs of co-occurring with close relatives. Our 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 we sampled. Future studies could inform this hypothesis by manipulating host outcrossing rates or measuring the strength of intra- vs. interspecific competition [9], 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, we suggest some potential mechanisms. First, temporally or spatially fluctuating fitness benefits could maintain variability in transmission rates [89], particularly if, as our 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 we cannot explain much of the variability in symbiont vertical transmission, our data suggest that for some host species, individual-level symbiont transmission constrains symbiont prevalence at the population level (Fig. 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.

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