

Niche Differentiation in the Dynamics of Host-Symbiont Interactions: Symbiont Prevalence as a Coexistence Problem

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ABSTRACT: Heritable symbioses can have important ecological effects and have triggered important evolutionary innovations. Current predictions for long-term symbiont prevalence are based on their fitness benefits and vertical transmission rates but ignore nonlinear competitive feedbacks among symbiotic and symbiont-free hosts. We hypothesized that such feedbacks function as stabilizing mechanisms, promoting coexistence of host types and maintaining intermediate symbiont frequency at the population scale. Using a model grass/endophyte symbiosis, we manipulated competition within and between endophyte-symbiotic (E+) and endophyte-free (E-) hosts and fit competition models to experimental data. We show for the first time that symbiont-structured competition can generate stable coexistence of E+ and E- hosts, even under perfect vertical transmission. Niche differentiation was the key to coexistence, causing hosts of each type to limit themselves more strongly than each other. These results establish roles for nonlinear competitive dynamics and niche differentiation in the ecology and evolution of heritable symbionts.

Keywords: competitive coexistence, ecological niche, host-symbiont interactions, vertical transmission.

Introduction

Most multicellular organisms host microbial symbionts, and many of these are transmitted vertically, from parents to offspring. Vertically transmitted symbioses are widespread across the tree of life, including the heritable microbes common in terrestrial arthropods (Engelstädter and Hurst 2009) and marine invertebrates (e.g., Sharp et al. 2007) as well as the rich microbiomes of vertebrates (Ley et al. 2008). Heritable symbionts can affect the fitness and population dynamics of their hosts (Rudgers et al. 2012; Yule et al. 2013) and the communities and ecosystems in which they reside (Clay and Holah 1999; Knowlton and Rohwer 2003). They

are also responsible for some of the most important evolutionary innovations in the history of life, such as the chloroplasts of plants and mitochondria of eukaryotes (Margulis 1996). Understanding the dynamics of microbial symbionts on ecological timescales can provide insight into the conditions that lead to persistence of novel symbioses and, ultimately, tight integration of symbiont and host genomes, as in the case of eukaryotic organelles.

Vertical transmission couples the fitnesses of host and symbiont and is therefore expected to favor the evolution of host-symbiont mutualism (Ewald 1987; Sachs et al. 2004). Due to this fitness feedback mechanism, symbionts that have positive effects on their hosts are expected to become fixed in host populations, and parasitic symbionts are expected to be eliminated (e.g., Clay 1993). Despite these expectations, evidence for intermediate symbiont frequency is widespread (Tsuchida et al. 2002; Davidson and Stahl 2006; Rudgers et al. 2009; Iannone et al. 2011). For example, the population-level frequency of the vertically transmitted bacterium *Wolbachia* follows a bimodal distribution across species of arthropod host, with peaks at 0% and 100% but a broad range of infection levels between these extremes (Hilgenboecker et al. 2008). Similarly, for the vertically transmitted fungal endophytes of many grasses, intermediate symbiont frequencies seem to be more the rule than the exception (reviewed in Cheplick and Faeth 2009; Rudgers et al. 2009). Variable and often intermediate symbiont frequencies have contributed to confusion over whether vertically transmitted symbionts function primarily as mutualists, as predicted by evolutionary theory, or as parasites (Faeth 2002; Faeth et al. 2004). A better mechanistic understanding of the factors that determine symbiont frequency in host populations is important for resolving this debate.

A leading hypothesis to explain intermediate symbiont frequency is imperfect vertical transmission, whereby some

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offspring of symbiotic parents fail to inherit the symbiont. Theoretical models show that imperfect transmission can stabilize intermediate infection frequencies, even when symbionts confer a strong fitness advantage, because there is a persistent source of nonsymbiotic hosts each generation (Turelli 1994; Ravel et al. 1997; Gundel et al. 2008). Empirical studies have confirmed that imperfect symbiont transmission is widespread in plant and animal hosts (e.g., Hurst et al. 2001; Moran and Dunbar 2006; Afkhami and Rudgers 2008; Gundel et al. 2011) and contributes to intermediate symbiont frequencies in natural populations (Gibert and Hazard 2013; Yule et al. 2013). Like most symbiosis theory, the imperfect transmission hypothesis assumes linear population dynamics, whereby fitness and transmission parameters are unaffected by population density. In this article, we explore an alternative hypothesis that is rooted in nonlinear dynamics, approaching symbiont frequency as a problem of competitive coexistence of symbiotic and nonsymbiotic hosts.

As host populations grow, symbiotic and nonsymbiotic individuals experience density-dependent competition with themselves and with each other. Ecological theory tells us that the relative strengths of these two classes of competition—within and between species or, in this case, host “types” (symbiotic and symbiont-free)—determines the potential for stable coexistence of competitors (Chesson 2000; Chase and Leibold 2003). Our understanding of how competitive interactions contribute to long-term symbiont frequency is limited because no previous studies have employed experimental designs that allow rigorous estimation of density-dependent competition within and between host types. Such approaches provide contact between experimental results and theoretical models of competition and are therefore essential for inferring long-term competitive outcomes. These approaches are also important for quantifying the two main components of competition: equalizing mechanisms, which reduce the mean fitness differences between competitors and promote neutral coexistence, and stabilizing mechanisms, such as niche differences between competitors (Chesson 2000).

Niche differences are critical stabilizing forces because they concentrate within-type competition relative to between-type competition, providing an advantage to low-frequency competitors, and can thus offset competitive asymmetry due to mean fitness differences (Harpole and Suding 2007; Levine and HilleRisLambers 2009; Adler et al. 2010). Thus far, predictions for how competition should influence symbiont frequency have focused on the mean fitness effects of symbionts (Clay 1993; Gundel et al. 2008; Rudgers et al. 2012), including the dependence of fitness effects on environmental variables such as herbivore pressure (Clay et al. 2005). However, symbionts can have important effects on the physiology and ecology of their hosts

and available evidence suggests that they can modify niche characteristics. For example, symbiosis with vertically transmitted microbes can alter host responses to abiotic stress (Malinowski and Belesky 2000; Davitt et al. 2011; Saari and Faeth 2012), modify interactions with natural enemies (Haine 2008; Brownlie and Johnson 2009), and provide access to novel resources (Joy 2013) and means of nutrient acquisition (Stewart et al. 2005; Kaiser et al. 2010; Feldhaar 2011). We hypothesized that niche differences could offset a fitness difference between symbiotic and nonsymbiotic intraspecific competitors and promote their stable coexistence. We tested this hypothesis by fitting competition models to data from experimental populations in which we manipulated the strength of density-dependent competition within and between host types.

Our work focused on the heritable symbiosis between the fungal endophyte *Epichloë amarillans* (Clavicipitaceae) and its native grass host, *Agrostis hyemalis* (Poaceae). Epichloid fungal endophytes, primarily in the genera *Neotyphodium* and *Epichloë*, are estimated to occur in up to 30% of grass species (Leuchtman 1992). In cases where grass and endophyte fitnesses are tightly coupled via exclusively vertical transmission, these symbionts are thought to function primarily as mutualists (e.g., Rudgers et al. 2012), although interaction outcomes may depend upon environmental context (Davitt et al. 2011; Miranda et al. 2011). Epichloid endophytes produce alkaloids that can confer host resistance to various stresses, including herbivory (Scharld et al. 2004). Despite potential for benefits of endophyte symbiosis, evidence from natural populations indicates that intermediate endophyte frequencies are widespread (Rudgers et al. 2009). In *A. hyemalis*, for example, population-level endophyte frequency ranges from 0% to 100% with a mean of 69% symbiotic individuals (Rudgers et al. 2009).

We integrated density manipulations of endophyte-symbiotic (E+) and endophyte-free (E-) hosts with classic models of competition to ask (1) What are the roles of equalizing (fitness differences) and stabilizing (niche differences) mechanisms in the competitive dynamics of E+ and E- hosts? (2) Are observed niche differences sufficient to maintain intermediate endophyte frequencies in host populations? Finally, (3), because imperfect vertical transmission is known to occur in this and other symbioses, providing an alternative mechanism to achieve intermediate symbiont frequencies, we asked, What are the relative contributions of imperfect vertical transmission and niche differences to equilibrium endophyte frequency?

Material and Methods

Focal Species

Agrostis hyemalis (winter bentgrass) is a native, cool-season (C₃) grass that occurs throughout the eastern and mid-

western United States and as far west as South Dakota and Texas. *Agrostis hyemalis* occurs in disturbed roadsides, prairies, and forest understories and can exhibit an annual, biennial, or short-lived perennial life history (Gould 1975). Throughout its range, *A. hyemalis* hosts *Epichloë amarilans*, a predominantly asexual symbiont that is vertically transmitted from maternal plants to offspring via seeds. Horizontal (contagious) transmission, indicated by fungal stromata on the plant inflorescence (Clay and Schardl 2002), was rarely observed in natural populations we sampled (T. E. X. Miller, personal observation) and did not occur in this experiment or in the field population from which the seeds were derived. The rate of vertical transmission (proportion of seeds from E+ maternal plants that are E+) is often imperfect, ranging from 0.3 to 1.0 under field conditions (Yule et al. 2013).

Seed Propagation and Scoring

The plants used in our experiment were derived from seeds collected from a natural population at the Stephen F. Austin Experimental Forest near Nacogdoches, Texas (31°29'58"N, 94°46'01"W). Endophyte frequency in this population is high (96% E+). Seeds were collected from approximately 100 individuals and a subset were heat treated in a 62°C water bath for 6 min, which kills the endophyte but not the plant (Davitt et al. 2011). Heat treatment allowed us to decouple endophyte presence/absence from maternal plant genetic background, which are coinherited in naturally occurring lineages. E- and E+ seeds were then reared through two generations in the greenhouse to minimize potential nontarget effects of heat treatment.

We screened our seed stocks for the presence of fungal hyphae in the aleurone layer by soaking 12–24 h in a 5% NaOH solution, staining with aniline blue (Bacon and White 1994), and examining seed squashes under $\times 200$ –400 magnification on a bright-field compound microscope (Zeiss Axiostar Plus). After two generations in the greenhouse, the E- seed stock remained completely nonsymbiotic (0% E+, $n = 25$ seeds), consistent with exclusively vertical transmission. However, due to imperfect vertical transmission, our E+ seed stock was less than completely symbiotic (92% E+, $n = 100$ seeds).

Experimental Design

We conducted a greenhouse-based response surface experiment to quantify competition within and between E+ and E- plants and its influence on long-term symbiont frequency. Response surface designs have proved powerful for studies of interspecific competition (Inouye 2001) but have not been used to study intraspecific competition

structured by presence/absence of a symbiont. Our response surface crossed starting densities of E+ and E- seeds, each ranging from 1 to 300, for a total of 66 treatments (fig. A1; figs. A1–A3 available online). Each combination of E+ and E- was replicated 3 or 4 times. For data analysis, the realized densities in the initial (t) generation were adjusted from the stocking densities to reflect the impurity of the E+ seed stock (fig. A1). For example, a treatment consisting of 100 seeds from the E+ stock and 0 seeds from the E- stock has expected starting densities of 92 E+ and 8 E- seeds.

For each replicate population, the assigned numbers of E+ and E- seeds were counted and added to a square (12.7 cm \times 12.7 cm \times 10.2 cm) plastic pot filled with peat-based potting soil (Pro-mix, Premier Tech, Quakertown, PA). All seeds were added November 6, 2009. Eight populations of the total 264 had no seedlings emerge within 1 month and were replaced. In January 2010, we counted all plants in the populations. Experimental populations were maintained at 18.3°C under ambient light (0.9–1.5 lumen/cm² at midday) and kept in flats that were filled with 5 cm of water twice per week (mean \pm SD soil volumetric water content: 31.7% \pm 15.6%). Plants flowered from late March through mid-June of 2009, and we collected inflorescences bearing mature seeds every 1–2 days during the flowering period. The mean \pm SE germination rate was 0.46 \pm 0.015. Because our population-dynamic models project a seed-to-seed transition (see “Candidate Models”), imperfect germination was included in our estimates of reproductive rate.

From each experimental population, we collected two types of data: (1) individual-level vertical transmission rate for E+ plants and (2) population-level densities of E+ and E- seeds in generation $t + 1$. For vertical transmission rate, we collected the first mature (“primary”) inflorescence from each population, counted total number of spikelets on the inflorescence, and scored 36 randomly selected seeds (or all seeds, if total was < 36) for fungal hyphae following the methods described above. *Agrostis hyemalis* produces a single seed per spikelet; spikelet counts therefore provide an upper bound on seed production (spikelets can be counted more reliably than the 0.5-mm-long seeds). Because endophyte symbiosis can advance flowering under some conditions (Davitt et al. 2011), we assumed that, in a mixed population, the first flowering plant was likely to be E+. The resulting seed scores allowed us to test this assumption (maternal plants with zero E+ seeds were assumed to be E- and excluded from the transmission rate data set).

All subsequent (“secondary”) inflorescences were collected as they matured. Once flowering and fruiting were complete, the total number of spikelets across all secondary inflorescences was counted. To estimate population-level

endophyte frequency in generation $t + 1$, we scored 36 seeds that were randomly selected from the pool of seeds produced across all secondary inflorescences. Unlike the primary inflorescence data, endophyte frequency in seeds from the secondary inflorescences results from the combined effects of the relative abundances of E+ and E- plants in generation t , their relative rates of seed production, and the transmission rates of E+ plants. For each population, we calculated total density of E- and E+ seeds in generation $t + 1$ as

$$\begin{aligned} E_{t+1}^- &= s'(1 - f') + s''(1 - f''), \\ E_{t+1}^+ &= s'f' + s''f'', \end{aligned} \quad (1)$$

where s and f represent spikelet counts and endophyte frequency, respectively, and primes represent the primary or secondary seed batches.

There were 33 populations in which plants established but died before flowering; these were retained as zeros in the analysis. For 21 populations that were 100% E- or 100% (actually 92%) E+, generation $t + 1$ seeds from secondary inflorescences were not available for scoring because they were used for a separate experiment immediately after harvest. For these populations, we used the seed scores from the primary inflorescence to represent both the vertical transmission rate and the population-level symbiont frequency (assuming that, in a population of uniform endophyte status, these should be equal, on average). We examined the consequences of this assumption by rerunning all analyses with these 21 populations entirely excluded. We found that our qualitative results were unaltered and proceeded to use the complete data set. In total, there were 246 populations for which we had complete information about seed densities at the beginning (E_t^-, E_t^+) and end (E_{t+1}^-, E_{t+1}^+) of the experiment, representing one complete generation of the plant (and endophyte) life cycle. Of these, we excluded three outliers with unusually high seed production (>2,000 seeds greater than the next-greatest value) that destabilized model fits and led to problems with parameter convergence. Raw data are deposited in the Dryad Digital Data Repository: <http://dx.doi.org/10.5061/dryad.s147c> (Miller and Rudgers 2013).

Candidate Models

We fit a set of candidate models (table 1) to test the contributions of equalizing mechanisms (fitness differences), stabilizing mechanisms (niche differences), and imperfect vertical transmission to equilibrium endophyte frequency. As a theoretical framework for our model selection, we used the Hassell-Comins competition model (Hassell and Comins 1976), a discrete-time version of Lotka-Volterra competition for an annual life cycle. We explored the fits

of six competing model structures (table 1) that crossed three hypotheses for competition with two hypotheses for vertical transmission (perfect or imperfect). Models 1 and 2 represent competitive equivalence of E+ and E- hosts: all parameters are shared between host types and intra- and intertype competitors are equivalent. Under these null models of competition, symbiont frequency in generation $t + 1$ should match symbiont frequency in generation t if transmission is perfect (model 1) or decline at a rate determined entirely by the vertical transmission rate τ (model 2). Models 3 and 4 allow fitness differences to contribute to changes in symbiont frequency between generations under perfect transmission (model 3) or in combination with imperfect transmission (model 4). Models 5 and 6 include fitness differences plus the potentially stabilizing effect of niche differences via the parameters α^- and α^+ . Condition $\alpha^- = \alpha^+ = 1$ would indicate that host types are interchangeable in their competitive effects (as in models 3 and 4), whereas $\alpha^- < 1$, for example, would indicate that E- plants are more strongly affected by competition with other E- plants than they are affected by E+ plants. Condition $\alpha^- < 1$, $\alpha^+ < 1$ is a necessary but not sufficient condition for stable coexistence (Hassell and Comins 1976). These model forms specify an annual life history (no survivors between generations), consistent with our experimental system: more than 99% of the total plants in our greenhouse experiment (5,629/5,683) died after flowering. The models also exclude the possibility of a persistent seed bank, a potentially important component of competition between annual plants (e.g., Levine and HilleRisLambers 2009). We were comfortable excluding seed bank dynamics from the candidate models because our ongoing studies of *A. hyemalis* seed banks indicate that seed viability declines rapidly under field conditions and equally so for E+ and E- seeds (A. J. Bibian, unpublished data).

Model Fitting, Model Selection, and Parameter Estimation

We fitted each candidate model to experimental data by minimizing the sum of the negative log likelihood over the parameters (Burnham and Anderson 2002) using the "optim" function in R, version 2.15.1 (R Core Development Team 2012). We tested a range of parameter values to insure that our optimization procedure identified global minima. We quantified model fit using Akaike's Information Criterion (AIC), from which we calculated Δ AIC (the difference between each AIC value and the minimum of the candidate set) and AIC weights (proportional weight of evidence in favor of each model). While seed density is a discrete variable, our approximations for E_{t+1}^- and E_{t+1}^+ involved weighting seed counts by estimated symbiont frequencies, which led to noninteger density estimates. Be-

Table 1: Model selection analysis for the recruitment of E+ and E− seeds in experimental populations

Model	Equation	Competitive difference	Vertical transmission	No. of parameters	ΔAIC	AIC weight
1	$E_{t+1}^- = \frac{\lambda E_t^-}{1 + [b(E_t^- + E_t^+)]^\gamma},$ $E_{t+1}^+ = \frac{\lambda E_t^+}{1 + [b(E_t^- + E_t^+)]^\gamma}$	None	Perfect	5	10.4	<.01
2	$E_{t+1}^- = \frac{\lambda[E_t^- + E_t^+(1-\tau)]}{1 + [b(E_t^- + E_t^+)]^\gamma},$ $E_{t+1}^+ = \frac{\lambda E_t^+ \tau}{1 + [b(E_t^- + E_t^+)]^\gamma}$	None	Imperfect	6	12.4	<.01
3	$E_{t+1}^- = \frac{\lambda^- E_t^-}{1 + [b^-(E_t^- + E_t^+)]^\gamma},$ $E_{t+1}^+ = \frac{\lambda^+ E_t^+}{1 + [b^+(E_t^- + E_t^+)]^\gamma}$	Fitness difference	Perfect	8	2.8	.12
4	$E_{t+1}^- = \frac{\lambda^- E_t^-}{1 + [b^-(E_t^- + E_t^+)]^\gamma} + \frac{\lambda^+ E_t^+(1-\tau)}{1 + [b^+(E_t^- + E_t^+)]^\gamma},$ $E_{t+1}^+ = \frac{\lambda^+ E_t^+ \tau}{1 + [b^+(E_t^- + E_t^+)]^\gamma}$	Fitness difference	Imperfect	9	4.8	.05
5	$E_{t+1}^- = \frac{\lambda^- E_t^-}{1 + [b^-(E_t^- + \alpha^- E_t^+)]^\gamma},$ $E_{t+1}^+ = \frac{\lambda^+ E_t^+}{1 + [b^+(E_t^- + \alpha^+ E_t^+)]^\gamma}$	Fitness + niche difference	Perfect	10	.0	.49
6	$E_{t+1}^- = \frac{\lambda^- E_t^-}{1 + [b^-(E_t^- + \alpha^- E_t^+)]^\gamma} + \frac{\lambda^+ E_t^+(1-\tau)}{1 + [b^+(E_t^- + \alpha^+ E_t^+)]^\gamma},$ $E_{t+1}^+ = \frac{\lambda^+ E_t^+ \tau}{1 + [b^+(E_t^- + \alpha^+ E_t^+)]^\gamma}$	Fitness + niche difference	Imperfect	11	.7	.34

Notes: Models represent competing hypotheses for the roles of fitness differences, niche differences, and imperfect vertical transmission in symbiont dynamics. The ΔAkaike Information Criterion (AIC) and AIC weight quantify empirical support for each model. Dynamics are governed by the low-density reproductive rate (λ), the per capita effect of competitors on reproduction (b), the scaled effect of intertype competitors relative to intratype competitors (α), and the form of the density-dependent response (γ). For symbiont-structured competition with fitness and niche differences, these parameters are specific to symbiotic (+) and nonsymbiotic (−) hosts. With fitness differences only, $\alpha^- = \alpha^+ = 1$. The number of parameters includes those shown plus two parameters, not shown, that define the standard deviation of residual error as a linear function of initial seed density.

cause we wished to retain the information in the noninteger values, we used a normal likelihood function, which was the continuous distribution that best fit the seed data. For all candidate models, we fitted the standard deviation as a linear function of initial population density, which, in preliminary analyses, provided a much better fit than a constant standard deviation (variance in seed production declined with initial density; see “Results”).

We used a bootstrapping approach to quantify the uncertainty associated with parameter estimates for the best-fitting model. For each of 1,000 bootstrap replicates, we randomly selected 75% of the complete data set and fitted the model to the data subset, as above, selecting a different 75% each iteration. The distributions of the maximum likelihood parameter values across bootstrap replicates reflect uncertainty in the parameter estimates, given the data. This approach had the advantage of preserving correlations among parameters, an important property for our simulation work (see “Simulation Experiments”).

Direct Estimation of Frequency-Dependent Population Growth

To assess potential for coexistence directly from the recruitment data, we examined per capita rates of increase of E+ and E− hosts in relation to their frequency in saturated populations (total seed densities of 25–100). We chose this density range to insure strong total density dependence (fig. 1) and to maximize representation of both host types at low and high frequencies (fig. A1). We used linear regression to test for negative relationships, which would indicate that the greatest potential for increase in a competitive environment is associated with rarity, a hallmark of niche differentiation.

Direct Estimation of Vertical Transmission Rate

The model selection analysis provided an indirect test of symbiont transmission, asking whether imperfect vertical transmission left a detectable fingerprint on the population

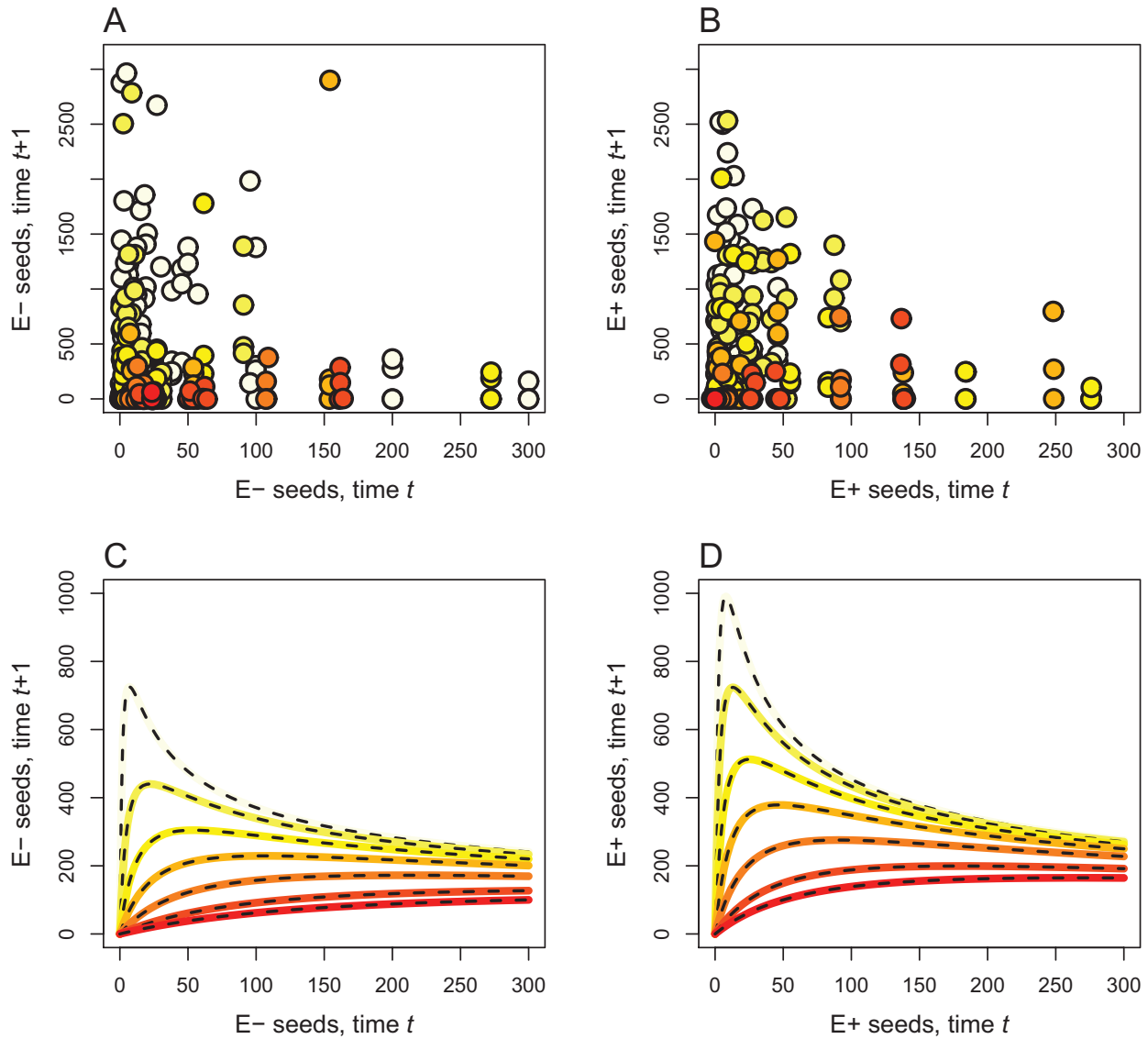


Figure 1: A, B, Density-dependent recruitment of endophyte-free (A) and endophyte-symbiotic (B) seeds. C, D, Best-fit competition model with fitness and niche differences between E+ and E- hosts (model 5 in table 1), corresponding to the data in A and B. From lightest to darkest, shading corresponds to 2.5, 10, 25, 50, 100, 200, and 300 competitors of the other type (E+ in A, C and E- in B, D); in A and B, these values are the midpoints of data ranges.

dynamics. In addition, our experimental methods (scoring seeds from primary inflorescences) provided direct estimates of vertical transmission across replicate populations. We quantified the vertical transmission rate for all populations that had at least five E+ seeds from the primary inflorescence. We chose the cutoff of five seeds to exclude rare false positives from E- maternal plants, which would bias the estimated transmission rate (fig. A2).

Simulation Experiments

We conducted simulation experiments to quantify the relative contributions of niche differences (α^- and α^+ were set to their maximum likelihood estimates or set equal to 1, respectively) and symbiont transmission (τ was drawn from the distribution of observed values or set equal to 1) to the equilibrium symbiont frequency. For each treat-

ment, we estimated the equilibrium frequency for each of the 1,000 parameter sets corresponding to the bootstrap replicates (see “Model Fitting, Model Selection, and Parameter Estimation”). Because we selected parameter sets and not individual parameter values, this approach allowed us to characterize the distribution of expected symbiont frequency given parameter uncertainty while accounting for correlations between parameters. (For example, λ and b tended to positively covary such that high or low values of both provided equally good fits to the data.) Analytical solutions for equilibrium symbiont frequency are possible only under perfect vertical transmission. We therefore estimated equilibrium frequency numerically after 500 time steps. We verified that 500 time steps were sufficient by comparing results to the analytical solution for treatments with perfect transmission.

Results

Intraspecific competition was structured by the presence/absence of fungal symbionts, and imperfect vertical transmission left little signature on the competitive dynamics (table 1). The best-fitting model indicated that both equalizing mechanisms (mean fitness differences) and stabilizing mechanisms (niche differences) contributed to competition between E+ and E− hosts but assumed perfect vertical transmission (model 5 in table 1). Including the possibility of imperfect vertical transmission did not substantially improve the fit, although this model was a close second, and we return to the contributions of imperfect transmission below. The next-best models included fitness differences but no niche differences. Null models, in which E+ and E− hosts were competitively equivalent, received virtually no support from the data (table 1).

Endophyte-symbiotic and endophyte-free hosts differed in their density-dependent competitive dynamics. Figure 1 shows observed E+ and E− recruitment in relation to initial densities (A , B) and predictions of the best-fit model (C , D). Population dynamics were overcompensatory for both host types: beyond the linear growth phase, increasing seed densities reduced recruitment (fig. 1A, 1B). Despite qualitative similarity in the shapes of E− and E+ recruitment functions (fig. 1C, 1D), there were strong differences between host types in their underlying dynamics, as indicated by the maximum likelihood parameter estimates: E− plants had an advantage over E+ plants in low-density reproductive rate (parameter λ , fig. 2A). However, this difference was offset by a greater negative effect of density dependent competition (parameter b) on E− plants (fig. 2B), indicating that E− plants could realize a fecundity advantage only under the very lowest-density conditions.

Effects of niche differentiation are represented by parameters α^- and α^+ (table 1). Maximum likelihood esti-

mates for the α 's indicate that competitive effects of E+ and E− hosts on each other were weaker than the effects of competition among hosts of the same type, consistent with niche differentiation (fig. 2C). However, niche differences were asymmetric, with E+ hosts more strongly buffered from competitive effects of E− hosts than E− hosts were buffered from competitive effects of E+ hosts (fig. 2C).

While the model selection analysis did not reveal a detectable signature of imperfect transmission on host population dynamics (table 1), our direct estimates confirmed that imperfect transmission did occur (fig. A2). The mean \pm SE vertical transmission rate of E+ plants across experimental populations was 0.95 ± 0.011 ($n = 135$).

Simulation experiments showed that when niche differences were removed ($\alpha^- = \alpha^+ = 1$) but mean fitness differences remained, endophytes became extinct $\sim 23\%$ of the time and went to fixation $\sim 73\%$ of the time in random samples from parameter uncertainty ranges, assuming perfect (100%) transmission (fig. 3A, filled bars). Thus, fitness differences associated with symbiosis gave a moderate advantage to E+ hosts. Coexistence was very rare ($<4\%$) when niche differences were removed. Including observed levels of imperfect transmission increased the likelihood of intermediate symbiont frequency to 16% and decreased the likelihood of endophyte fixation to 54% (fig. 3A, open bars). Including the observed niche differences made coexistence much more likely, even when vertical transmission was perfect (fig. 3B, filled bars). Stable intermediate endophyte frequencies (between 0.01 and 0.99) occurred in 56% of random samples from parameter ranges with perfect transmission. Finally, the combination of niche differentiation and imperfect transmission increased the likelihood of intermediate endophyte frequency to 67% (fig. 3B, open bars). When competitive exclusion occurred, it was almost always in favor of E+ hosts, reflecting the asymmetry in competitive buffering.

Stabilizing niche differentiation is predicted to generate a negative relationship between per capita growth rate and frequency, indicating the greatest potential for increase when rare (Harpole and Suding 2007; Levine and HilleRisLambers 2009). We used the raw data to examine per capita rates of increase for E+ and E− hosts in relation to their relative abundances in saturated populations (fig. A3). Consistent with the model-fitting results, we found that E+ per capita growth rates declined significantly with E+ frequency in saturated populations ($F_{1,79} = 6.1$, $P < .015$). E− growth rates also showed a negative slope with respect to E− frequency, but this slope was not statistically different from 0 ($F_{1,90} = 0.4$, $P < .8$). The difference in strength of these relationships for E+ and E− hosts is consistent with the asymmetry in niche differentiation in-

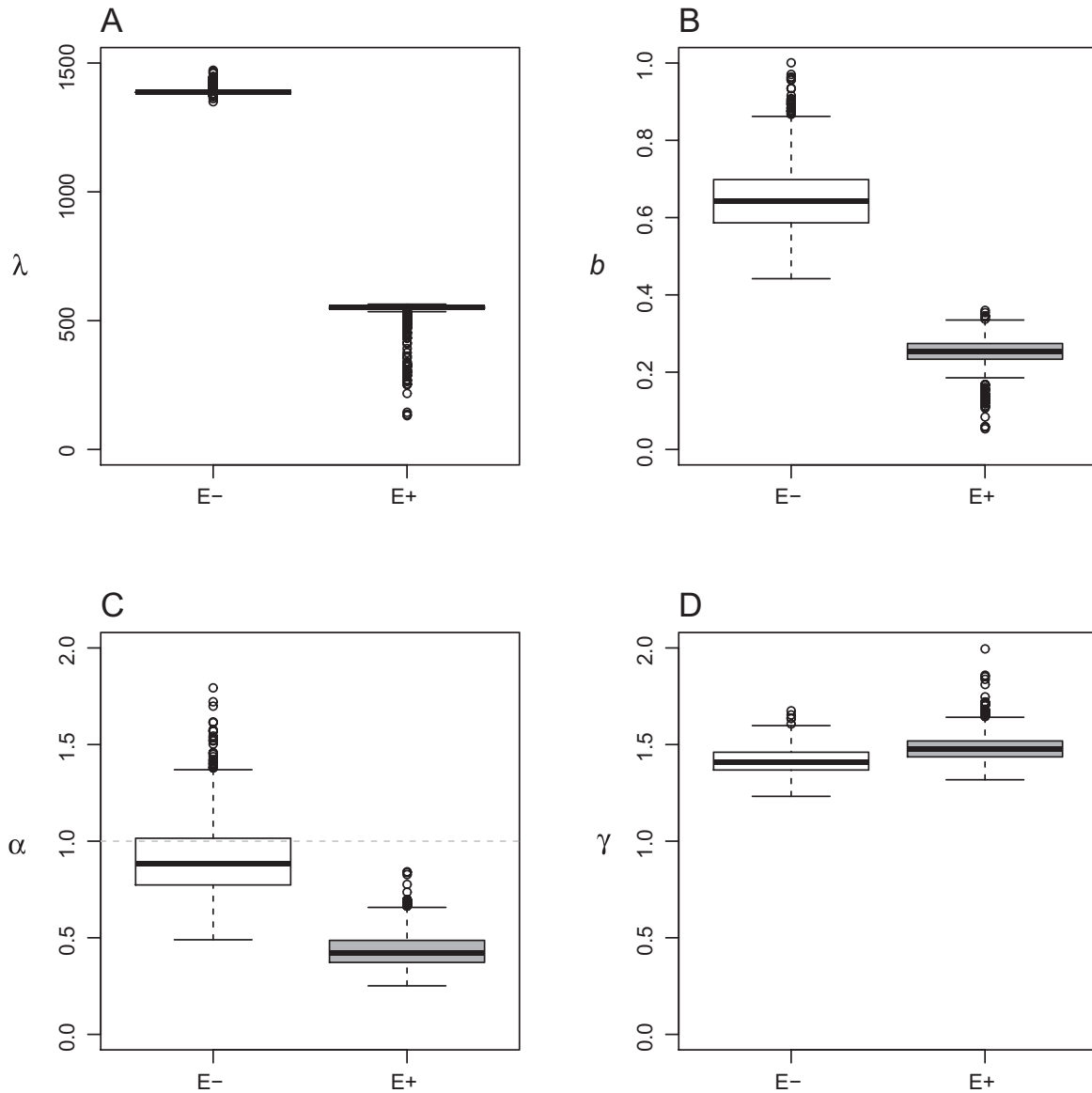


Figure 2: Maximum likelihood parameter estimates corresponding to model 5 (table 1). Boxplots show the distributions of parameter estimates from 1,000 bootstrap replicates (thick black lines are medians, boxes show the inner quartiles, whiskers extend to the fifth and ninety-fifth percentiles, and dots are outliers). Parameters are defined in table 1. Dashed gray line in C shows $\alpha = 1$.

indicated by their maximum likelihood parameter estimates (fig. 2C).

Discussion

Understanding the processes that determine the persistence and prevalence of vertically transmitted symbionts in host populations is a fundamental goal in the study of symbiosis. Explanations for variable and intermediate symbiont frequencies have primarily invoked mechanisms

based on linear (density-independent) population dynamics, notably imperfect transmission (Gundel et al. 2008) but also including migration (Saikkonen et al. 2002) and nonequilibrium dynamics (Clay 1993). Our results indicate that symbiont-structured, density-dependent competition is an important component of the ecological dynamics of host-symbiont interactions and merits greater attention. We show for the first time that symbiont-structured competition can generate locally stable intermediate symbiont frequencies, even under perfect vertical trans-

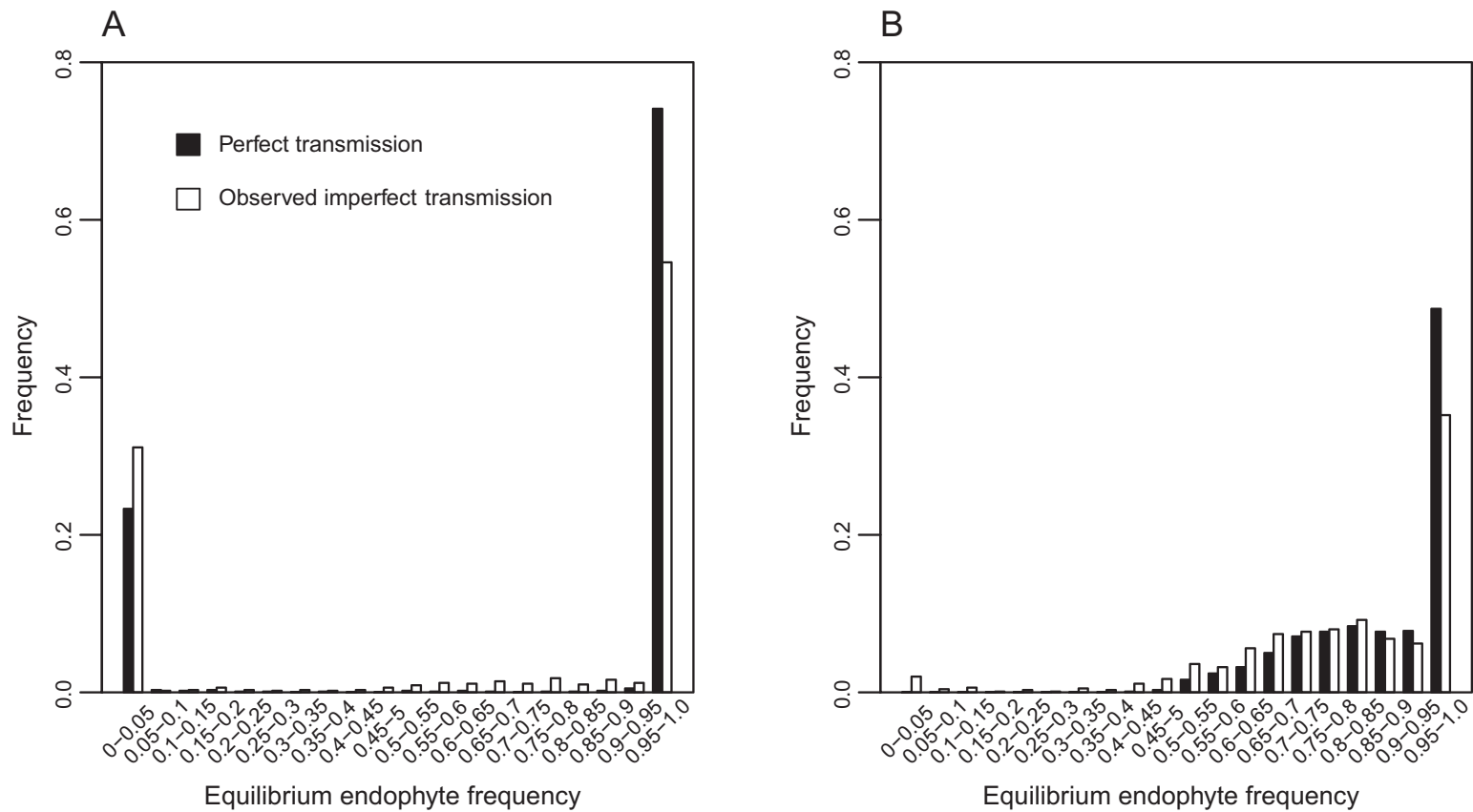


Figure 3: Histograms of equilibrium endophyte frequency in which parameter values were estimated from 1,000 bootstrap replicates (parameter distributions shown in fig. 2). Black bars show results assuming perfect vertical transmission of endophytes, and white bars show results based on observed transmission rates. *A*, Niche differences removed ($\alpha^- = \alpha^+ = 1$) with all other parameters drawn from uncertainty ranges. *B*, Observed niche differences included (α 's drawn from uncertainty ranges).

mission. Density-dependent competition was stronger within versus between symbiotic and symbiont-free hosts, indicative of niche differentiation. This classic mechanism of interspecific coexistence can similarly stabilize host populations at an intermediate symbiont frequency.

Niche differentiation was the key to stable coexistence of E+ and E− host types, as it is known to be for interspecific competitors (Levine and HilleRisLambers 2009; Adler et al. 2010). In fact, niche differentiation was a more important mechanism for generating intermediate frequencies of *Epichloë amarillans* in *Agrostis hyemalis* populations than was imperfect vertical transmission. The model that best fit our data included fitness and niche differences between E+ and E− hosts but not imperfect transmission (table 1). When we incorporated direct empirical estimates of imperfect transmission and sampled from the uncertainty ranges of our parameters, niche differences remained the primary driver of intermediate frequencies (fig. 3). Imperfect transmission certainly occurred and amplified the contribution of niche differentiation to intermediate symbiont frequency but was unlikely to generate much variation in symbiont frequencies in the absence of niche differences.

Given their potential to stabilize coexistence of symbiotic and symbiont-free hosts, niche characteristics warrant further study in host-symbiont dynamics. In this context, it is important to consider whether symbionts expand versus shift the ecological niche of their host. Niche expansion would mean that symbiont-free hosts occupy a subset of the broader niche space of symbiotic hosts. This would make the competitive buffering effect of niche differences asymmetric (symbiotic hosts would be more buffered from nonsymbiotic hosts than the reverse, as we found here) and possibly inadequate to stabilize coexistence. On the other hand, a niche shift would mean that symbiotic hosts occupy novel areas of niche space that are unavailable to symbiont-free hosts but abandon other areas of niche space still occupied by symbiont-free hosts. This latter scenario would be more likely to promote coexistence. To our knowledge, no previous studies have explicitly addressed the question of symbiont-mediated niche shift versus expansion; we think this would be a fruitful area for future work.

Our results are consistent with recent studies suggesting potential for symbionts to modify niche-related traits of their hosts (e.g., Feldhaar 2011; Saari and Faeth 2012). Even so, the magnitude of niche differentiation in our experiment was surprising given the rather homogenous greenhouse environment in which these populations were competing. While pinpointing the physiological traits that drive niche differences between E+ and E− plants was beyond the scope of this study, the grass-endophyte literature provides some clues. A previous greenhouse study

of the *A. hyemalis*/*E. amarillans* symbiosis revealed effects of endophytes on host phenology, with E+ plants flowering earlier than E− plants (Davitt et al. 2011). If other developmental processes are similarly shifted, temporal partitioning of the growing season could concentrate competition within host types. However, our data do not support the hypothesis of phenological shifts caused by endophyte symbiosis: populations that were entirely E+ or E− had statistically identical durations of time between seed addition and the onset of flowering ($t_{56.5} = 0.2, P < 0.84$), and between the onset and end of flowering ($t_{33.7} = -0.85, P < 0.4$). We also found no support for the hypothesis that endophyte symbiosis modified plant biomass allocation above- vs. belowground (analysis of root : shoot ratio: $t_{35.5} = -0.21, P < .8$). However, other root characteristics, such as rooting depth or root hair length, could affect resource partitioning (Malinowski et al. 1999), or host types could specialize on different forms of nutrients (e.g., McKane et al. 2002).

There has been much discussion over whether heritable microbes such as fungal endophytes function as mutualists or parasites of their hosts, fueled in part by their often variable and intermediate frequencies of occurrence (Faeth 2002; Cheplick and Faeth 2009). The idea that vertically transmitted symbionts with beneficial fitness effects should be maintained at high frequency in host populations is intuitively appealing. However, our results suggest that inferring symbiont effects on hosts from their population-level frequency can be a problematic exercise. Current theory for the maintenance of interspecific diversity, which highlights the independent roles of equalizing and stabilizing mechanisms (Chesson 2000), has not previously been considered in studies of host-symbiont interactions but is enlightening in this context. In the absence of stabilizing forces such as niche differences, the competitor with a fitness advantage excludes the other. However, niche differentiation can offset a fitness deficit and stabilize persistence of an inferior competitor. If these mechanisms operate similarly in intraspecific symbiont-structured competition, as our results demonstrate, then it is not possible to infer which host type has a fitness advantage from the frequency of the host type alone. For example, endophytes may confer a strong mean fitness advantage, but if they cause a niche shift of sufficient magnitude, they could persist in stable mixtures of E+ and E− hosts. Counterintuitively, strong host-symbiont mutualism may actually facilitate persistence of symbiont-free hosts if it is also associated with niche differentiation. Imperfect transmission (Gundel et al. 2008) and migration (Saikkonen et al. 2002) could further cloud connections between fitness effects of symbionts and local symbiont frequency, as others have recognized (Cheplick and Faeth 2009), and modeling their influence can provide additional insights. Thus,

observed symbiont frequencies are, at best, incomplete information about the fitness effects of symbionts on hosts and, at worst, completely mute on the issue.

When we removed niche differences in simulations of the *A. hyemalis*/*E. amarillans* symbiosis, we found that either host type could “win” in competition, although E+ hosts had a modest advantage. Thus, under our experimental conditions, the mean fitness effect of endophyte symbiosis was neutral to slightly positive. Interestingly, similar fitnesses arose from very different parameter values. E− plants exhibited a high intrinsic growth rate but also a high susceptibility to density dependence, whereas E+ plants had a lower growth rate but were also more weakly affected by density-dependent competition (fig. 2A, 2B). Our experimental conditions were relatively benign, with consistently high water availability throughout the experiment. Previous work with *A. hyemalis* has shown that endophyte symbiosis is more beneficial during drought years (Yule et al. 2013) and under low, but not high, water availability in the greenhouse (Davitt et al. 2011). We suspect that, under stressful field conditions, fitness benefits of endophyte symbiosis could be stronger, potentially strong enough to overwhelm the stabilizing effects of niche differences. Thus, we do not suggest that inferences from our greenhouse experiment provide direct understanding of symbiont dynamics in nature for this or other host species. Rather, the value of our work lies in the identification and quantification of a novel mechanism for a widespread pattern. The relative importance of this mechanism in natural populations remains a rich line of inquiry yet to be explored. Furthermore, fluctuating environments can alter competitive dynamics (Chesson 2000) and likely play an additional role in natural populations that was not captured in our competition experiment.

Conclusions

Density-dependent processes govern the dynamics of most populations yet have not been rigorously incorporated into understanding of host-symbiont interactions. We show that attention to density-dependent competition, including its decomposition into fitness and niche differences, is important for understanding the ecological dynamics of symbiosis. Our results indicate a need for further work on the generality and biological mechanisms of niche differentiation between symbiotic and symbiont-free hosts, particularly across the many diverse systems involving terrestrial arthropod and marine invertebrate hosts of heritable microbes.

More broadly, we argue that greater attention to nonlinear processes could advance understanding of the ecological dynamics of host-symbiont interactions and that

ecological dynamics of facultative interactions provide insight into the evolutionary histories of interactions that are now tightly integrated, such as the organelles of eukaryotic cells. Newly established symbioses face a high likelihood of extinction if symbiotic hosts do not receive strong fitness benefits or if they transmit symbionts imperfectly. Our results indicate that, in a density-dependent environment, stabilizing forces such as niche differences could promote symbiont persistence even when their fitness effects are roughly neutral. Niche differentiation may therefore be an important and understudied factor in the evolutionary trajectories of incipient symbioses.

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Fig. 1.

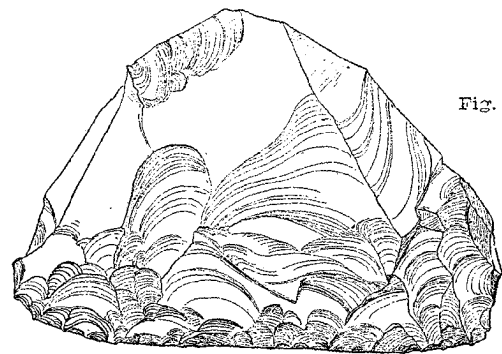


Fig. 2.

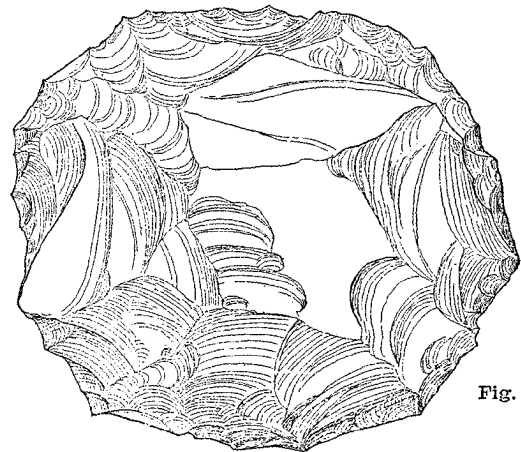


Fig. 3.

“After a short search a leaf-shaped implement of very fine workmanship was found; it is made of the black opaque obsidian [...]; an outline of this implement is given in Fig. 1. Having continued the search as long as the time at my command would permit, I was amply rewarded in the possession of ten more or less perfect implements. [...] One is somewhat pyramidal in shape, as shown in Fig. 2; the bottom is flat, the flaked surfaces extending from the base to the apex; it is [...] the only specimen in the collection that appears to have been in the least used; the sharp edge at the base is considerably worn; Fig. 3 is a top view of the same.” From “Notes on an Extensive Deposit of Obsidian in the Yellowstone National Park” by Wm. H. Holmes (*The American Naturalist*, 1879, 13:247–250).