

Heritable symbionts alter host life history schedules

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Abstract

Introduction

3 Endosymbioses between micro- and macro-organisms are widespread in the natural world and have played a key role in the evolution of life. Many microbial symbionts are vertically transmitted from parent to offspring, such that survival and reproduction of the symbiont are tied to that
6 of the host. Both theory and empirical evidence have shown that predominantly vertically reproducing endosymbionts are selected to act as mutualists in order to increase the reproduction of their host, and therefore themselves. However, symbioses that are mutually beneficial in their net
9 effects may still involve conflict of interest. The stability of host-symbiont mutualism therefore requires mechanisms that resolve conflict or benefits that are sufficiently strong to compensate for it.

12 Microbial symbionts can induce diverse effects on age-specific survival and reproduction – parameters that define the host life history. Such life history modifications may or may not constitute host-symbiont conflict depending on how they influence net fitness of hosts. For
15 vertically-transmitted symbionts, selection may act to favor symbiont strategies that induce early host reproduction, which ensures symbiont fitness against the risk of host mortality or reproductive failure later in life.¹ For example, infection by the heritable bacterial symbiont *Wolbachia*
18 has been found to accelerate early host reproduction and modify age-specific survival rates in pharaoh ants (Singh and Linksvayer, 2020) and fruit flies (Fry et al., 2004). Shield bugs carry heritable extra-cellular bacterial symbionts in the mid-gut that reduce the pre-ovipositional period by 10 days, on average (Karamipour et al., 2021). Symbiont-driven shifts in host life history
21 strategies toward a faster pace of life may still be consistent with host-symbiont mutualism, as long as positive effects of symbionts compensate for any costs associated with deviation from
24 optimal host life history strategies. On the other hand, advantages of symbiosis may allow hosts to access higher-fitness strategies such that “deviations” of the life history schedule work in fa-

¹All else equal, a perfectly and exclusively heritable symbiont should have the same ESS life history strategy as its host, but imperfect transmission or retention may favor symbionts getting out earlier.

vor of both host and symbiont. Rarely are these alternative fitness implications known or tested
(Karamipour et al., 2021)

Life history manipulation is best studied in arthropod-microbe symbioses, but plants also harbor a rich consortium of microbial associates with the potential to alter life history schedules. Fungal endophytes in the genus *Epichloë* are obligate symbionts of cool-season grasses that are vertically-transmitted through host seed production; some *Epichloë* species also engage in horizontal transmission at the expense of host reproduction (Cheplick and Faeth, 2009; Clay and Schardl, 2002).

In this study, we leverage a unique, long-term symbiont removal experiment, combined with statistical and demographic modeling, to evaluate how endophyte symbiosis modifies the trajectory of mortality and reproduction of host grasses. Taxonomically replicated across seven host-symbiont pairs, our 14-year field experiment has longitudinally tracked replicated cohorts of recruitment in grass populations that were established either symbiotically with *Epichloë* fungal endophytes (S+) or with endophytes eliminated through heat treatment (S-). By tracking multiple host cohorts that recruit in different years, our study is uniquely able to isolate, for the first time, age-specific effects of endophyte symbiosis from the confounding influence of background inter-annual variation. We combined statistical estimation of age- and symbiont-specific reproduction and mortality schedules with matrix projection models to synthesize the overall influence of symbiosis on host fitness and life history metrics. Specifically, we asked the following:

- Are there age-specific effects of endophytes on host reproduction and mortality schedules?
- How do endophyte effects on reproduction and survival combine to influence host life history strategies?
- Are shifts in host life history consistent with overall host-symbiont mutualism?
- How consistent are the life history outcomes of symbiosis across host-symbiont taxonomic pairs, and can host and/or symbiont traits explain inter-specific variation?

Methods

Experimental design

Data collection

54 Data collection methods follow that of our previous study (Fowler et al., 2024).

Statistical modeling

We used the long-term demographic data to fit statistical models for age- and symbiont-dependent
 57 vital rates. Model-fitting was done in a hierarchical Bayesian framework using Stan (Carpenter
 et al., 2017) and the ‘rstan’ package (Stan Development Team, 2024) in R version 4.2.3 (R Core
 Team, 2023). Hierarchical modeling allowed us to maximize inference by “borrowing strength”
 60 across taxonomic, temporal, and spatial dimensions of the experimental design. We fit models
 for three vital rates: age-specific survival, age-specific fertility, and recruitment. For all models,
 we ran three chains for 10,000 iterations with a thinning rate of 2. We used vague priors except
 63 where noted.

For age-specific vital rates, age was modeled as a categorical factor rather than a continuous
 covariate; this allowed for maximally flexible age-dependence without having to assume a
 66 functional form of how vital rates varied with age. Due to mortality, sample sizes decreased
 with increasing age (Table). For each species we pooled advanced ages with fewer than 10 ob-
 servations each into a combined “old” age class. For example, if the oldest age with at least 10
 69 observations was 5 years old, everything older than 5 was pooled as age class “6+”. When S+
 and S- populations differed in the upper age that satisfied $n \geq 10$, we used the younger age of
 the two to define the “old” age group. This difference was most pronounced in *Poa alsodes*, where
 72 the oldest age with $n \geq 10$ was 1yo for S- and 4yo for S+ (Table). Therefore, age classes for the *P.*
alsodes vital rate models were 0, 1, and 2+. All other host species had more even age representa-

tion between S+ and S- and greater representation of later ages, with “old” age groups reaching
 75 4+ for *E. villosus* and *E. virginicus*, 5+ for *P. autumnalis*, 6+ for *A. perennans* and *F. subverticillata*,
 and 7+ for *P. sylvestris*.

Age-specific survival

78 The survival model included fixed effects of host species, age class, and endophyte status and
 random effects associated with year-to-year and plot-to-plot variation. Let y_{ijkl} be the survival
 status (1=alive, 0=dead) of individual i of species j in plot k and year l . We modeled these data
 81 as Bernoulli-distributed with probability of survival \hat{p}_{ijkl} given by:

$$\begin{aligned} \text{logit}(\hat{p}_{ijkl}) &= \alpha_{a[i]}^j + \beta_{a[i]}^j * e_k + \rho_k + \tau_l^j \\ \rho_k &\sim N(0, \sigma_{plot}^2) \\ \tau_l^j &\sim N(0, \sigma_{year}^2) \end{aligned}$$

Factor variable $a[i]$ is the age class of individual i in year l and e_k is the endophyte status (1=S+,
 0=S-) assigned to plot k , which we assume to apply equally to all individuals in plot k . Parameter
 84 α_a^j is the survival probability of species j age class a (on the logit scale) and parameter β_a^j is the
 effect of endophyte symbiosis on that survival rate. Host species share variance parameters (σ^2)
 for plot (ρ_k) and year (τ_l^j) effects. Year effects τ_l^j are explicitly associated with species j , allowing
 87 different species to experience good and bad years at different times (while sharing the same
 variance). Because each plot contains only one species, plot effects ρ_k are implicitly associated
 with species j . We used the weakly informative priors $\alpha_a^j \sim N(-1, 5)$, indicating that plants
 90 are more likely to die than survive, on average, with one standard deviation below and above
 the mean corresponding to 0.24% and 98% survival probabilities, respectively. These priors
 accommodated a wide range of potential model outcomes while pointing the algorithm in the
 93 right direction to aid convergence.

Age-specific fertility

The fertility model was similar in structure to the survival model except that the response variable was the count of inflorescences of individual i of species j in plot k and year l . We modeled these counts as Negative Binomially-distributed with mean μ_{ijkl} and species-specific overdispersion ϕ_j . $\log(\mu_{ijkl})$ was given by the same linear predict as $\text{logit}(\hat{p}_{ijkl})$ in Eq. ???. Overdispersion parameters were modeled as a Gamma-distributed species random effect $\phi_j \sim \text{Gamma}(\alpha^\phi, \beta^\phi)$ with cross-species mean $\frac{\alpha^\phi}{\beta^\phi}$. While the inflorescence counts were zero-heavy, posterior predictive checks confirmed that the negative binomial model could generate the observed frequencies of zeroes.

Recruitment rate

Finally, we modeled the number of recruits of species j observed in plot k and year l as Poisson-distributed with mean λ_{jkl} . We defined this mean as a function of the total number of inflorescences (inf) produced in plot k in the previous year $l - 1$, allowing for random plot and year effects as above:

$$\begin{aligned}\lambda_{jkl} &= \exp(\alpha^j + \beta^j * e_k + \rho_k + \tau_l^j) * inf_{k,l-1} \\ \rho_k &\sim N(0, \sigma_{plot}^2) \\ \tau_l^j &\sim N(0, \sigma_{year}^2)\end{aligned}$$

Species-specific parameters α^j control the rate at which last year's inflorescences generate this year's recruitment, and parameters β^j control endophyte effects on this rate. Note that the model is agnostic to *how* endophytes effect the inflorescence-to-recruitment rate (e.g., through effects on seed number per inflorescence, seed germination rate, or seedling survival). The covariate $inf_{k,l}$ was calculated for each plot by summing inflorescences across all individuals, including the original plants that are otherwise not included in these analyses, since their reproduction contributed

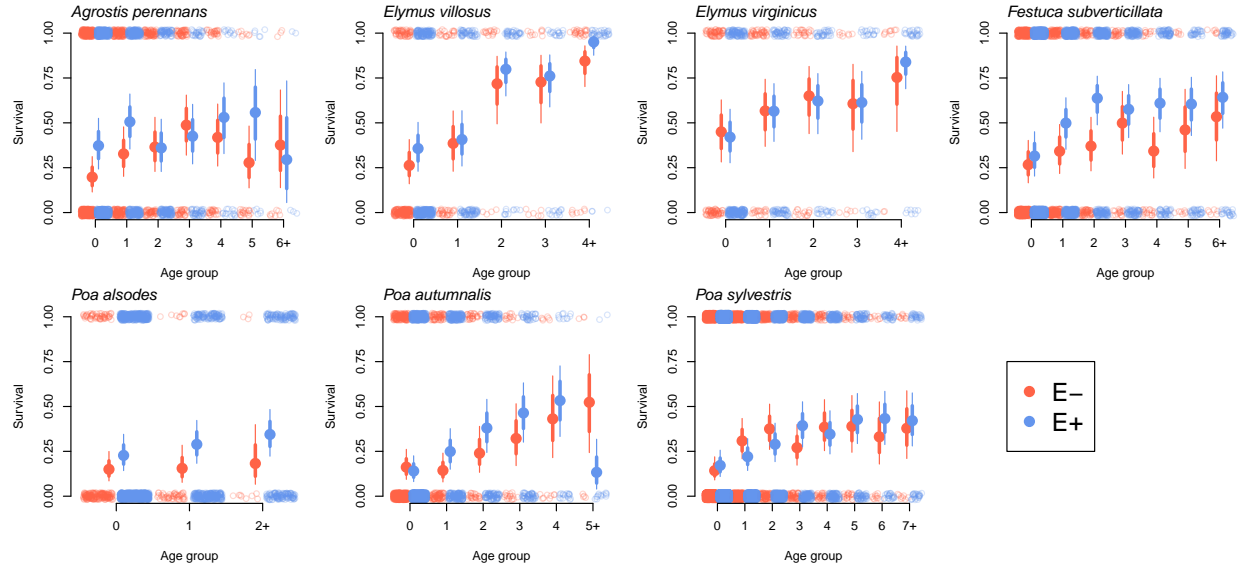


Figure 1:

to observed recruitment. We assume that recruitment occurs exclusively from seed production
 111 in the preceding year, i.e., no long-lived seed bank. Eq. ?? implies that if no inflorescences are
 produced in a plot, there should be no recruitment in the following year (mostly true??).

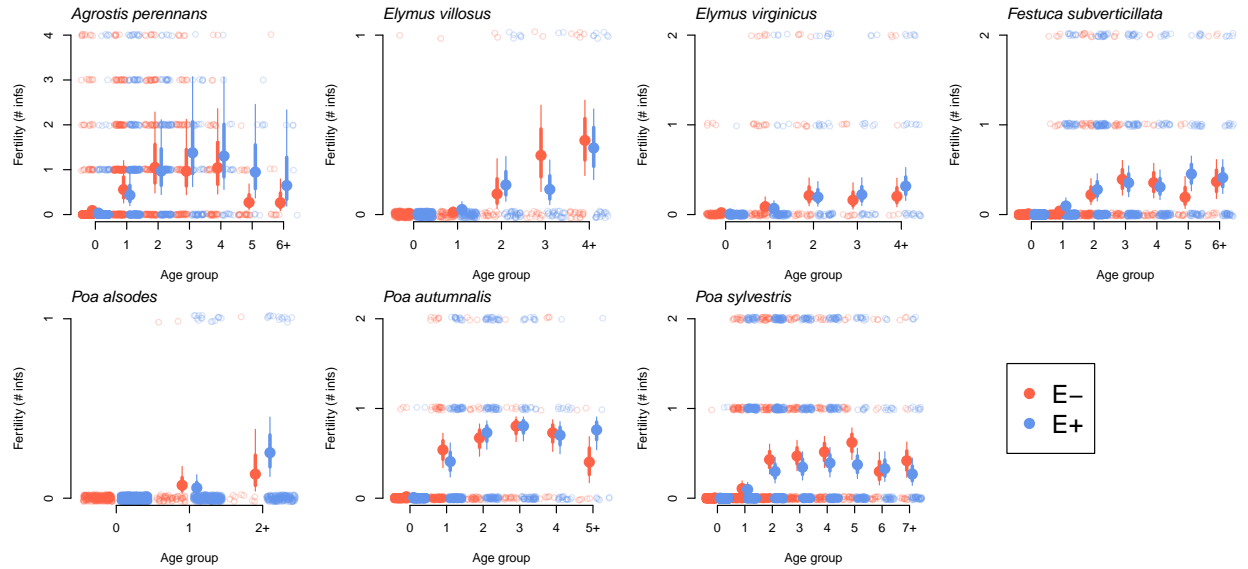


Figure 2:

Demographic modeling

Results

Discussion

Conclusion

Acknowledgments

Statement of Authorship

Data and Code Availability

Appendix A: Additional Methods and Parameters

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Endo	Age	AGPE	ELRI	ELVI	FESU	POAL	POAU	POSY
0	0	678	258	125	809	253	816	2516
0	1	256	50	63	253	58	160	527
0	2	135	19	36	106	8	66	229
0	3	83	15	12	47	3	36	131
0	4	50	9	5	30	0	18	70
0	5	26	8	3	13	0	5	39
0	6	7	7	3	6	0	3	19
0	7	1	5	0	5	0	2	9
0	8	1	4	0	2	0	1	4
0	9	1	0	0	0	0	0	3
0	10	0	0	0	0	0	0	1
0	11	0	0	0	0	0	0	1
1	0	770	385	273	1750	1135	1775	5473
1	1	341	108	97	515	392	389	1802
1	2	184	47	48	264	123	187	744
1	3	66	29	27	165	76	99	339
1	4	24	12	15	99	31	45	214
1	5	10	8	9	61	5	21	112
1	6	5	5	5	28	0	3	57
1	7	0	3	2	17	0	0	33
1	8	0	3	1	9	0	0	17
1	9	0	1	0	3	0	0	4
1	10	0	1	0	3	0	0	2
1	11	0	1	0	2	0	0	2
1	12	0	1	0	0	0	0	0
1	13	0	1	0	0	0	0	0
1	14	0	1	0	0	0	0	0

Table A1: Sample sizes (number of individuals) for each endophyte status (Endo), age (years), and host species.