

The Role of Host Demographic Storage in the Ecological Dynamics of Heritable Symbionts

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ABSTRACT: Heritable symbioses are widespread and ecologically important. Many host organisms have complex life cycles that include diverse opportunities for symbionts to affect their host and be lost during development. Yet, existing theory takes a simplified view of host demography. Here, we generalize symbiosis theory to understand how demographic “storage” in the form of dormant or prereproductive life stages can modify symbiosis dynamics. Using grass-endophyte symbioses as context, we developed models to contrast the role of the seed bank (a storage stage) against the reproductive stage in symbiont persistence and prevalence. We find that the seed bank is as important as or more important than the reproductive stage in driving symbiont dynamics, as long as passage through the seed bank is obligate. Flexible entry to the seed bank substantially weakens its influence on symbiont persistence but can modify prevalence in counterintuitive ways. Our models identify a role for legacy effects, where hosts that lose symbionts retain their demographic influence. The retention of benefits via legacy effects can reduce symbiont prevalence and even cause prevalence to decline with increasing benefits to hosts because symbiont-free hosts carry those benefits. Our results resolve connections between individual-level host-symbiont interactions and population-level patterns, providing guidance for empirical studies.

Keywords: host-symbiont dynamics, demographic modeling, stage-structured populations, mutualism, *Epichloë*, fungal endophyte.

Introduction

Most multicellular organisms harbor symbiotic microbes that can have important effects on host fitness and population dynamics (Yule et al. 2013; Oliver et al. 2014) and the communities and ecosystems in which they reside (Knowlton and Rohwer 2003; Rudgers and Clay 2007; Jaenike and Brekke 2011). Vertical transmission is a key feature of many

ecologically important symbioses, including interactions between heritable bacteria and arthropods (Engelstädter and Hurst 2009; Feldhaar 2011), zooxanthellae and corals (Baird et al. 2009), chemosynthetic microbes and deep-sea animals (Dubilier et al. 2008), bacterial symbionts and sponges (Schmitt et al. 2012), fungal endophytes and grasses (Selosse and Schardl 2007), and gut microbiota and vertebrates (Ley et al. 2008). Given the ubiquity and significance of heritable symbionts, understanding the dynamics of their interactions with host organisms is an important area of inquiry.

Vertical transmission couples the fitness of the symbiont to that of the host and is expected to favor the evolution of host-symbiont mutualism due to positive fitness feedbacks (Ewald 1987; Sachs et al. 2004). Heritable symbionts that are beneficial are therefore expected to become fixed in host populations, whereas parasitic symbionts may be eliminated if opportunities for horizontal transfer are rare or absent (e.g., Clay 1993). Despite these expectations, intermediate prevalence of heritable symbionts in host populations (mixture of symbiotic and symbiont-free hosts) is more the rule than the exception (Schulthess and Faeth 1998; Hilgenboecker et al. 2008; Oliver et al. 2008; Erickson et al. 2012; Gibert and Hazard 2013; Miller and Rudgers 2014; Semmartin et al. 2015). Theoretical models have shown that imperfect vertical transmission, where some offspring of symbiotic parents fail to inherit the symbiont, can promote intermediate prevalence, even when symbionts confer benefits to their host (Turelli 1994; Ravel et al. 1997; Gundel et al. 2008; Hancock et al. 2011). Empirical evidence from a diversity of microbial symbioses indicates that imperfect vertical transmission is widespread (Darby and Douglas 2003; Narita et al. 2007; Afkhami and Rudgers 2008; Oliver et al. 2014; Gibert et al. 2015).

While theoretical and empirical work has emphasized the importance of vertical transmission (Gundel et al. 2011), other transitions in the host life cycle provide additional opportunities for symbiont loss. Host demographic “storage” in the form of dormant or prereproductive stages could lead

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to loss of the symbiont prior to host reproduction. We refer to the loss of symbionts during somatic development across life-history stages of an individual as “imperfect retention.” For example, arthropod hosts of heritable bacteria have complex life cycles that may include long-term egg dormancy and demographic transitions through prereproductive metamorphic stages; it is well documented that these hosts can lose inherited symbionts during prereproductive transitions (e.g., Darby and Douglas 2003; Prado et al. 2006; Geib et al. 2009). Similarly, in plant symbioses, sustained seed dormancy and transitions from seeds to seedlings provide opportunities for symbiont loss prior to flowering (e.g., Afkhami and Rudgers 2008; Gibert et al. 2015). Despite broad potential for imperfect retention of symbionts, most symbiosis theory either assumes that imperfect transmission from parent to offspring is the only pathway of symbiont loss or does not distinguish between failed transmission and failed retention (Ravel et al. 1997; Turelli 1994; Gundel et al. 2008; Hancock et al. 2011; Kwiatkowski and Vorburger 2012).

Host life-history complexity provides not only additional pathways of symbiont loss but also new pathways for symbiont effects on hosts. For example, effects of symbionts can vary in magnitude and even direction between host life stages (Rudgers et al. 2012; Yule et al. 2013; Chung et al. 2015; Gibert et al. 2015). No previous studies have considered whether interactions that occur during a storage stage (imperfect retention and effects on prereproductive vital rates) have similar or different consequences for symbiont dynamics than interactions during a reproductive stage (imperfect transmission and effects on host reproduction). Consequently, it remains unknown whether existing theory applies broadly to hosts with complex life histories. The combination of imperfect retention and symbiont effects on prereproductive vital rates sets the stage for legacy effects, where hosts that lose symbionts retain their demographic influence. The role of legacy effects in symbiosis is not well understood, though there is growing awareness that effects of past environments, including interactions with other species, can be important for organisms with complex life cycles (e.g., Thomas and Rudolf 2010; O’Connor et al. 2014).

Here, we develop general theory to understand how demographic storage influences the ecological dynamics of vertically transmitted symbionts. We employ the concept of seed banking as an extreme form of storage, but this theory applies generally to symbioses with any form of demographic stage structure. To facilitate interpretation, we ground our model in a particular ecological context: the widespread symbiosis between cool-season grasses and vertically transmitted fungal endophytes (Cheplick and Faeth 2009), an ecologically and economically important model system of heritable symbiosis in which demographic storage may play an important role.

Epicloid fungi are systemic symbionts of at least 80 genera of host grasses (Saikkonen et al. 2006). Fungal endophytes can have beneficial effects on various aspects of plant performance, including increased drought tolerance (Kannadan and Rudgers 2008; Davitt et al. 2011) and herbivore resistance (Müller and Krauss 2005; Rudgers and Clay 2007), but negative effects are also commonly reported (e.g., Faeth 2009; Rudgers et al. 2012). Most epicloid endophytes are predominantly or exclusively vertically transmitted, and imperfect vertical transmission is well documented (Afkhami and Rudgers 2008; Gibert and Hazard 2013). Experiments and large-scale surveys have sought to link endophyte occurrence and prevalence with their effects on host fitness (Rudgers et al. 2009; Iannone et al. 2012; Miller and Rudgers 2014; Santangelo et al. 2015; Semmartin et al. 2015). Such studies have revealed surprising patterns, including cases in which endophytes harm their host but occur at high frequency (e.g., Faeth and Sullivan 2003) and others in which endophytes provide strong benefits but occur at low frequency (e.g., Gibert and Hazard 2013). A theoretical framework that accommodates life-history complexity may help resolve connections between individual-level processes and population-level patterns.

Like many plants, grass life cycles often include potentially long-lived seed banks (Bakker et al. 1996), raising the possibility of imperfect retention during demographic storage. Indeed, it is well recognized that endophytes in seeds lose viability at a faster rate than do the seeds themselves (Rolston et al. 1986; Gundel et al. 2009). This observation suggests that passage through a seed bank should reduce endophyte prevalence and increase extinction risk. However, it may also be important to consider effects of endophytes on seed vital rates. For example, positive, neutral, and negative effects on seed survival and germination have all been reported, and effects are often dependent on environmental context (Clay 1987; Novas et al. 2003; Vila-Aiub et al. 2005; Faeth and Hamilton 2006; Gundel et al. 2006a, 2006b, 2009, 2010).

Our goal was to evaluate whether and how the introduction of a seed bank in the host life cycle modifies host-symbiont population dynamics. We contrast the dynamics that result from failed symbiont retention and demographic effects on seed vital rates against those that result from failed vertical transmission and effects on reproductive rates. We develop two variations on the theme of host demographic storage: obligate storage, where every seed must pass through a seed bank before recruiting as a reproductive adult, and partial storage, where some seeds recruit directly to the reproductive stage. We also include the possibility of legacy effects in a seed whose endophyte dies, which we show to be an important consideration. Our models bracket realistic variation in host life cycle complexity and therefore provide insight into when and how

storage stages matter for host-symbiont interactions and when and how they do not.

Model Development

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Because we are interested in populations structured by both demographic stage and symbiont status, matrix projection models provide a natural fit. We consider a plant population structured by two life stages (seeds in the seed bank and reproductive plants) with endophyte-symbiotic (E+) and nonsymbiotic (E-) states in each stage (fig. 1). Let the vector \mathbf{n} , represent the densities of E- seeds, E- plants, E+ seeds, and E+ plants in year t . Change in population size and composition is governed by a discrete-time transition matrix (\mathbf{A}) according to $\mathbf{n}_{t+1} = \mathbf{A}\mathbf{n}_t$. All of our analyses focus on two components of symbiont dynamics: whether symbionts will persist in the long term (“persistence”) and, if so, the frequency at which they will occur (“prevalence”). For simple transition matrixes, eigenanalysis provides all of the information necessary to infer persistence and prevalence (Caswell 2001; Gundel et al. 2008). For prevalence, we focus on the fraction of plants in the reproductive stage that are symbiotic, a more relevant quantity for field studies than the E+ fraction of the total (reproductive plant + seed

bank) population. We assume that the environment is constant, that aboveground plants are semelparous, and that symbiont transmission is exclusively vertical such that transitions from the E- state to the E+ state have a probability of 0 (fig. 1; in app. A we present analyses in which this assumption is relaxed; apps. A–D available online). The model also assumes density independence, although our results hold for density-dependent environments (app. B).

Obligate Seed Dormancy

We begin with the assumption that seed banking is obligate. All seeds enter the seed bank; if they survive, they recruit into the reproductive stage the following year. Symbiont loss may occur via imperfect transmission from maternal plants to seeds or via imperfect retention from the seed bank to reproductive plants (fig. 1). The transition matrix is

$$\mathbf{A} = \begin{bmatrix} 0 & f^- & 0 & f^+(1-\tau) \\ s^- & 0 & s^+(1-\rho) & 0 \\ 0 & 0 & 0 & f^+\tau \\ 0 & 0 & s^+\rho & 0 \end{bmatrix}. \quad (1)$$

Parameter f indicates per capita seed production by reproductive plants, and s indicates the probability of seed survival in the seed bank. Both demographic processes may be unique to E- and E+ hosts, indicated with superscripts - and +, respectively. The symbiont loss parameters are vertical transmission rate τ (probability that a seed from an E+ plant is also E+) and retention rate ρ (probability that a reproductive plant from an E+ seed is also E+).

We refer to E- seeds arising from E+ seeds that lose their symbiont as being converted. In defining the transition matrix, we are forced to consider whether legacy effects of former symbionts could modify the survival of converted seeds (represented by the effective survival rate s' in eq. [1]). Depending on the mechanism by which symbionts influence their hosts during storage, converted hosts might retain their E+ survival rate, assume the E- survival rate, or exhibit intermediate survival. We formulate the model to accommodate all possibilities, defining effective survival of converted seeds as a weighted average of E+ and E- rates:

$$s' = as^+ + (1-a)s^-. \quad (2)$$

In the analyses that follow, we consider two extremes that bracket scenarios in nature: converted seeds perfectly retain ($a = 1$) or completely lose ($a = 0$) the influence of their former symbiont.

To reduce dimensionality of the model and simplify the analysis, we define F and S as ratios of reproductive and seeds

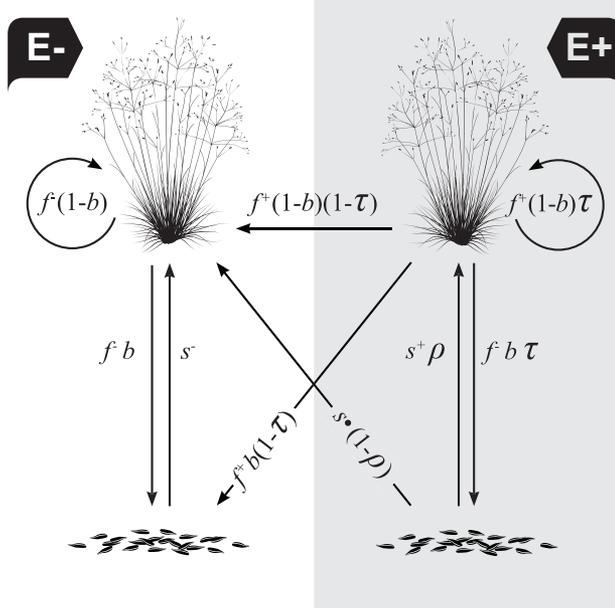


Figure 1: Schematic of theoretical model for the influence of demographic storage on host-symbiont population dynamics. The model is inspired by endophyte symbiosis in grasses with a seed bank. Hosts are structured by demographic state (reproductive plant or seed in bank) and endophyte state (E- or E+). Figure represents the obligate seed bank model for $b = 1$ and the partial seed bank model for $0 < b < 1$. Symbology of demographic transitions (arrows) corresponds to models (1) and (11). Illustration by Karina I. Helm.

survival rates, respectively, of E+ to E− hosts ($F = f^+/f^-$, $S = s^+/s^-$). F and S values of 1 represent a perfectly neutral symbiont, and values less than or greater than 1 represent costs or benefits, respectively. Long-term symbiont persistence requires that the E+ component of the population increases at a rate that is greater than that of the E− component. We solved for the parameter relationships that satisfy this condition and the long-run prevalence of symbionts as a function of host demography and symbiont loss (app. C).

Symbiont Persistence. We found that symbiont persistence requires

$$FS > \frac{1}{\tau\rho}. \quad (3)$$

We can glean several biological insights from this result. First, if symbionts are retained perfectly and have no effect on seed survival ($\rho = 1$, $S = 1$), expression (3) reduces to $F > 1/\tau$, precisely the condition for symbiont persistence in an annual, unstructured host: fertility benefits must exceed the inverse of the vertical transmission rate (Gundel et al. 2008). This tells us that demographic complexity per se does not modify symbiont persistence in host populations; seed banks matter only when they provide new avenues for symbiont loss or effects on hosts. Second, if symbionts have no effect on seed survival ($S = 1$), the potential for imperfect retention ($\rho < 1$) means that the existence of a seed bank would only destabilize symbiont persistence, all else equal. However, if symbionts enhance seed survival ($S > 1$), then a seed bank may stabilize symbiont persistence but only if $S > 1/\rho$; that is, seed survival benefits must balance imperfect retention in the same way that fertility benefits must balance imperfect transmission in models lacking seed banks.

Inequality (3) indicates that host-symbiont interactions during dormant versus reproductive life stages are completely interchangeable. This is true for both the effects of symbionts on host demography (product FS) and pathways of symbionts loss (product $\tau\rho$). Obligate dormancy dictates a linear progression through the life cycle, so it makes sense that a fitness effect on one stage would have the same consequence as the same effect on the other. The interchangeability of demographic effects also makes them compensatory, as shown in F - S space (fig. 2). For example, a twofold survival cost ($S = 0.5$) can be perfectly balanced by a twofold fertility benefit ($F = 2$). Transmission and retention rates are similarly interchangeable, and the product $\tau\rho$ can modify the compensatory relationship between costs and benefits (fig. 2). As $\tau\rho$ decreases, stronger benefits in one vital rate would be needed to offset a given cost in the other vital rate.

Finally, we can observe that parameter a does not appear in inequality (3). This makes sense, since inequality (3) com-

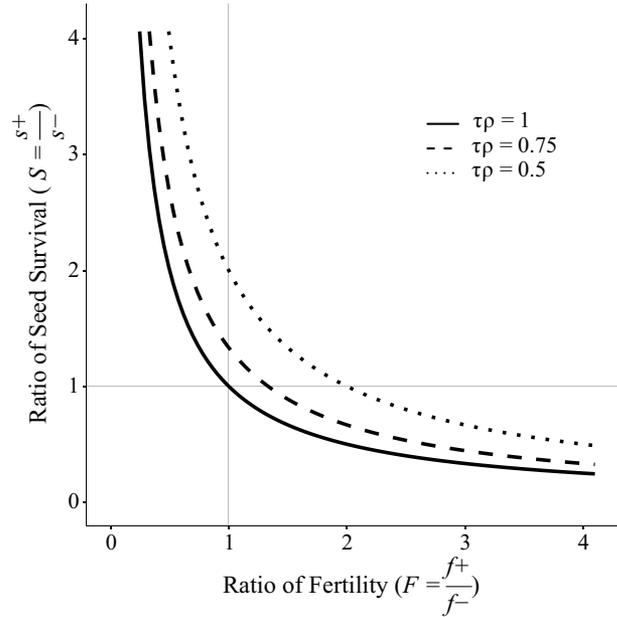


Figure 2: Persistence conditions for obligate seed-banking model (eq. [3]). Lines show isoclines that separate regions of endophyte persistence (above) or extinction (below) for different levels of symbiont loss ($\tau\rho$, product of vertical transmission and seed bank retention rates). Axes represent the effects of symbionts on host fertility ($F = f^+/f^-$) and seed survival ($S = s^+/s^-$), respectively. Gray lines at $F = 1$ and $S = 1$ represent neutral effects.

pares the production of E+ hosts by E+ hosts to production of E− hosts by E− hosts; production of E− hosts by E+ hosts contributes to neither (app. C). Thus, the fate of converted hosts has no effect on symbiont persistence, but it does affect prevalence, as we show next.

Symbiont Prevalence. The equilibrium fraction of plants that are E+ is given by

$$\hat{E}^+ = \begin{cases} \frac{FS\rho\tau - 1}{F - 1 + FS\rho\tau - F\rho\tau - a(FS\rho\tau - FS\tau - F\rho\tau + F\tau)} & \text{if } FS > \frac{1}{\tau\rho} \\ 0 & \text{otherwise} \end{cases} \quad (4)$$

This equilibrium is derived from the stable population structure; the frequency of symbiosis reaches an equilibrium, but the population continues to grow geometrically (app. C). Given that persistence condition (3) is satisfied, we can first notice that if symbionts are retained perfectly and have no effect on seed survival ($\rho = 1$, $S = 1$), symbiont prevalence reduces to $(F\tau - 1)/(F - 1)$. As above, we recover the limiting case of annual hosts that lack a storage stage (Gundel et al. 2008). When symbionts are lost during seed storage, prevalence is sensitive to the effective survival rate of con-

verted seeds, in contrast to the persistence condition. When converted hosts lose symbiont effects ($a = 0$), the nonzero prevalence in equation (4) becomes

$$\widehat{E}_+ = \frac{FS\tau\rho - 1}{F - 1 + FS\tau\rho - F\tau\rho}, \quad (5)$$

and when converted hosts instead retain the E_+ survival rate as a legacy effect ($a = 1$), it becomes

$$\widehat{E}_+ = \frac{FS\tau\rho - 1}{F - 1 + FS\tau - F\tau}. \quad (6)$$

These expressions differ only in the presence of ρ in the denominator, and they are equal when $S = 1$. As a consequence, symbiont prevalence given by equation (5) is always greater than that given by equation (6) as long as $S > 1$ and vice versa. This means that, all else equal, we should expect lower population-level symbiont prevalence when converted E_- hosts retain benefits of symbiosis than when converted hosts lose these benefits. Conversely, if symbionts impose costs on the storage stage ($S < 1$), we should expect greater prevalence when these costs are retained in converted hosts than when they are lost. These outcomes occur because imperfect retention promotes recruitment of E_- hosts. This effect is amplified when converted E_- seeds carry survival benefits of their former symbionts and dampened when converted E_- seeds carry survival costs.

The effective seed survival rate modifies how host-symbiont interactions that occur during dormant versus reproductive stages influence symbiont prevalence. First, increasing symbiont transmission (τ) and retention (ρ) always leads to greater prevalence, as expected (fig. 3A, 3B). When converted seeds take on the E_- survival rate ($a = 0$), the two pathways of symbiont loss influence endophyte prevalence in exactly the same way (overlapping lines in fig. 3A), mirroring their interchangeable effects on endophyte persistence (fig. 2). However, when converted seeds retain legacy effects ($a = 1$), imperfect transmission and retention play different roles (fig. 3B). For symbionts that are beneficial during storage ($S > 1$), losing symbionts via imperfect retention reduces prevalence more strongly than losing symbionts via imperfect vertical transmission and vice versa for symbionts that are costly during storage ($S < 1$). As above, this asymmetry arises because retention of fitness benefits or costs in converted seeds promotes or reduces, respectively, recruitment of E_- hosts into the reproductive stage.

Second, the effective survival of converted hosts modifies the relative importance of host fertility in the reproductive stage versus survival in the storage stage as determinants of symbiont prevalence. Increasing benefits to either life stage

increases prevalence (fig. 4A, 4B), as expected. Under most conditions, beneficial effects on survival promote prevalence more strongly than beneficial effects on reproduction. The asymmetry between reproduction and seed survival in these cases can be derived by comparing endophyte prevalence in the limit as F or S becomes large. If converted seeds lose the demographic effects of symbionts ($a = 0$), we find

$$\lim_{F \rightarrow \infty} \widehat{E}_+ = \frac{S\tau\rho}{S\tau\rho - \tau\rho + 1}, \quad (7)$$

$$\lim_{S \rightarrow \infty} \widehat{E}_+ = 1. \quad (8)$$

Symbionts become fixed in the population as seed survival benefits become large (eq. [8]), regardless of what happens in the reproductive stage, while benefits to reproduction cannot alone drive symbionts to fixation (eq. [7]). If converted seeds maintain legacy effects ($a = 1$), we find

$$\lim_{F \rightarrow \infty} \widehat{E}_+ = \frac{S\tau\rho}{S\tau - \tau + 1}, \quad (9)$$

$$\lim_{S \rightarrow \infty} \widehat{E}_+ = \rho. \quad (10)$$

As above, the role of the seed bank in symbiont prevalence dominates the role of the reproductive stage: seed bank effects and loss continue to limit symbiont prevalence even as fertility benefits become large (eq. [9]), while the reverse is not true (eq. [10]). However, with legacy effects, even strong seed survival benefits cannot fix symbionts in the plant population because converted E_- seeds carry those benefits and thus recruit into the population as E_- plants; failed retention in the seed bank becomes the ultimate limit on symbiont prevalence (eq. [10]). In all cases, the dominant role of the seed bank reflects the facts that prevalence is censused in reproductive plants and that the seed bank is obligate and upstream in the life cycle. Therefore, any benefits to plant fertility must be filtered through the seed bank before they can affect prevalence.

Summary of Obligate Seed Dormancy. For a host life cycle with obligate seed dormancy, the seed bank exerts an important influence on symbiont persistent and prevalence—as strong as or stronger than the reproductive stage. Obligate storage allows for demographic compensation between stages, where survival benefits in the seed bank may balance plant fertility costs or vice versa. While increasing benefits to storage and reproductive stages promotes symbiont prevalence,

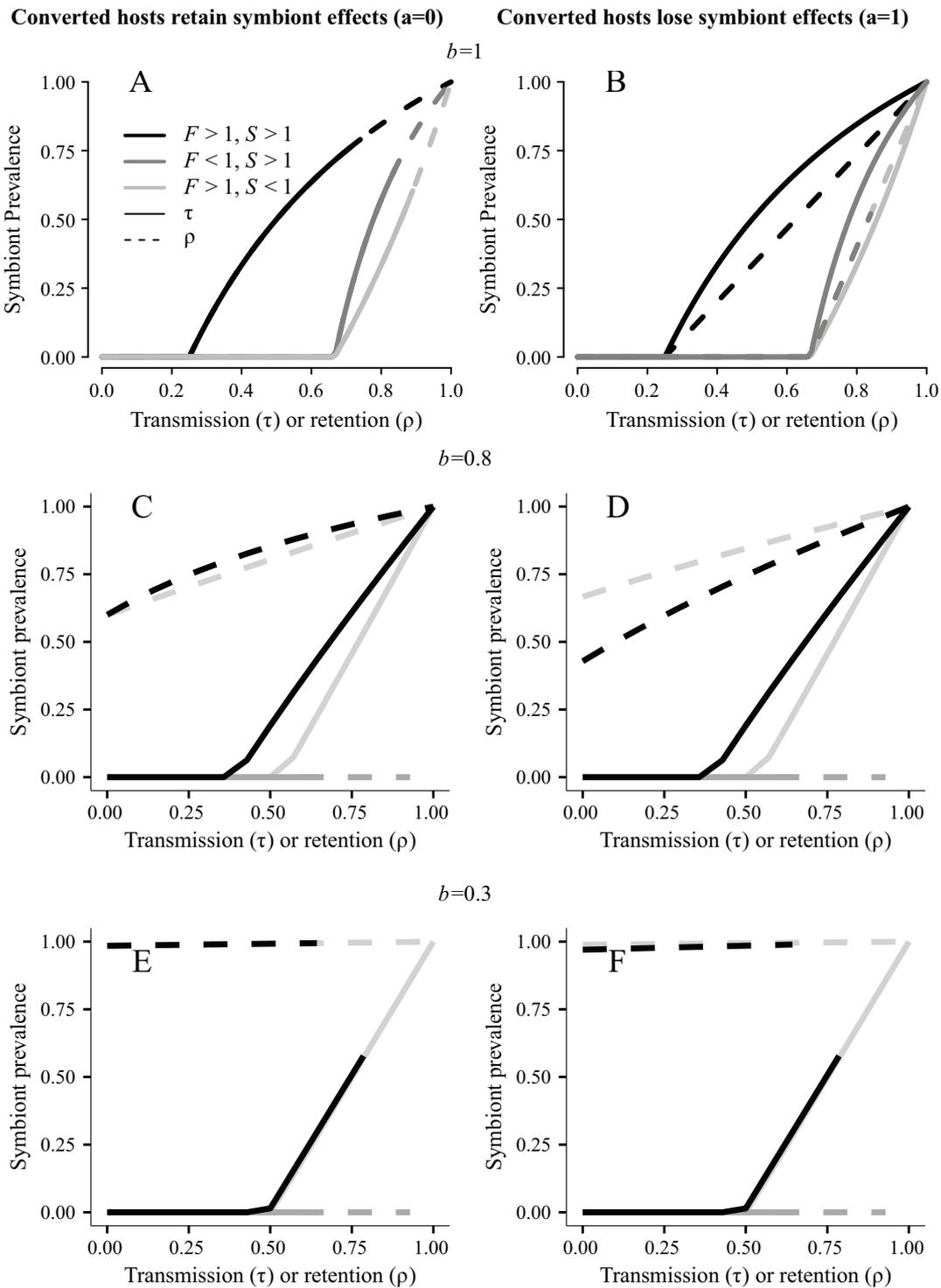


Figure 3: Equilibrium symbiont prevalence in relation to symbiont vertical transmission (τ) and retention (ρ). A, C, E, Prevalence when converted hosts lost symbiont effects ($a = 0$). B, D, F, Prevalence when converted hosts retain symbiont effects ($a = 1$). Rows correspond to the obligate seed bank model ($b = 1$; A, B) and two levels of partial seed banking ($b = 0.8$; C, D; $b = 0.3$, E, F). In each panel, either τ (solid lines) or ρ (dashed lines) varies along the X-axis; the rate that is not varying is held at τ or $\rho = 1$. Line shading represents effects of symbionts on host fecundity (F) and survival (S), which may be positive (F or $S = 2$) or negative (F or $S = 0.75$) for either or both vital rates. A and B show analytical results (eq. [4]) and C-F show results from numerical simulation. Overlap of multiple lines is represented by single lines that change shading or type.

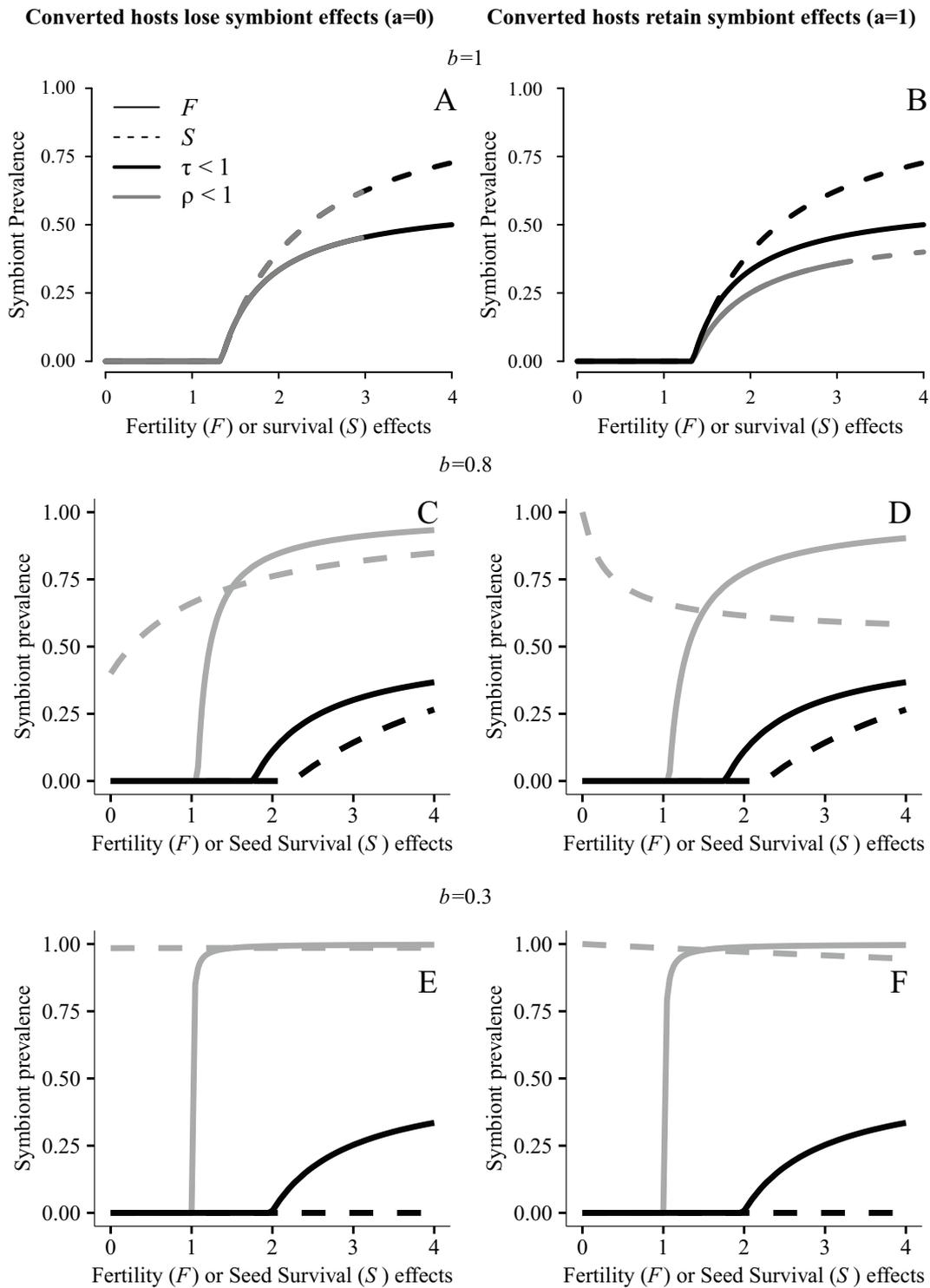


Figure 4: Equilibrium symbiont prevalence in relation to effects of symbionts on host reproduction (F) and seed bank survival (S). In each panel, either F (solid line) or S (dashed line) varies along the X -axis; the process that is not varying is held at F or $S = 1.5$. Line shading represents symbiont loss through imperfect transmission only ($\tau = 0.5$, $\rho = 1$) or imperfect retention only ($\rho = 0.5$, $\tau = 1$). Row (varying b) and column (varying a) layout as in figure 3. A and B show analytical results (eq. [4]), and C - F show results from numerical simulation. Overlap of multiple lines is represented by single lines that change shading or type.

seed survival limits prevalence more strongly because effects on host reproduction must be filtered through the seed bank. Imperfect retention of symbionts in the seed bank has similar effects on symbiont persistence and prevalence as imperfect transmission from plant to seeds. However, the two pathways of symbiont loss can have different effects on prevalence depending on whether seeds that convert to the non-symbiotic state retain legacy effects of their former symbionts. Collectively, results indicate that patterns of symbiosis in reproductive stages cannot be understood without considering processes that occur upstream in the host life cycle.

Partial Seed Dormancy

Thus far we have considered a host life cycle in which entry into the storage stage is obligate. A common and more flexible life-history strategy is one in which a fraction of offspring transitions to storage while the other fraction recruits directly into the reproductive stage. For example, many plant and arthropod hosts use partial seed or egg banking as a risk-spreading strategy in unpredictable environments (Evans and Dennehy 2005; Childs et al. 2010). Here we relax the assumption of obligate dormancy to accommodate partial propagule banking (fig. 1). The transition matrix for a partial seed bank model is

$$\mathbf{A} = \begin{bmatrix} 0 & f^-b & 0 & f^+b(1-\tau) \\ s^- & f^-(1-b) & s^-(1-\rho) & f^+(1-b)(1-\tau) \\ 0 & 0 & 0 & f^+b\tau \\ 0 & 0 & s^+\rho & f^+(1-b) \end{bmatrix}. \quad (11)$$

Parameter b represents the probability that a seed will enter the seed bank, where it remains for 1 year, versus recruit directly as a reproductive plant ($1 - b$). Note that $b = 1$ corresponds to the previous obligate seed bank model and $b = 0$ corresponds to an unstructured annual model where there is no reproductive delay. We assume that the seed-banking parameter is a species-level trait and does not differ with respect to symbiont status. We also assume that the seed bank is the only possibility for seed mortality, such that seeds that bypass the seed bank recruit perfectly to the reproductive stage (fig. 1). It may be realistic to include an additional source of mortality not associated with the seed bank (e.g., predispersal seed predation), but, to minimize the number of parameters, we proceed with the simplifying assumption. All other parameters and assumptions are carried over from model (1).

This model, while complicated by only one additional parameter over the obligate model, is mathematically less tractable; it has analytical solutions, but they do not lend

themselves to interpretation. Therefore, we assessed symbiont persistence and prevalence by numerical simulation (we verified that simulation results match analytical predictions for $b = 1$). We assessed symbiont persistence and prevalence after 1,000 years of simulated population dynamics, which was sufficient time to reach equilibria. We imposed density dependence in our simulations to keep population sizes finite, for computational convenience. The addition of density dependence does not affect equilibrium symbiont dynamics as long as E+ and E- hosts are similarly affected by density (app. B). We continue to focus on the symbiont loss rates τ and ρ and effect ratios F and S , although we found that quantitative details of some results were sensitive to the absolute values of fecundity and seed survival (lower-level parameters f and s). We present these details in appendix D and focus here on qualitative results that are robust to variation in lower-level values. All source code for our simulation work is deposited in the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.54rv6> (Bibian et al. 2016).

Partial Seed Dormancy: Symbiont Persistence. We find that flexible entry to the seed bank substantially weakens its influence on symbiont persistence. Because partial seed banking also changes the relative importance of imperfect transmission versus retention, figure 5 separates the influence of τ (A, C, E) and ρ (B, D, F). As the fraction of seeds that enter the seed bank decreases from $b = 1$, isoclines that separate regions of symbiont extinction and persistence become vertical (fig. 5, cf. rows), and isoclines for different levels of imperfect retention overlap (fig. 5B, 5D, 5F). These changes to the isoclines indicate that effects on seed survival (S) and symbiont death in the seed bank (ρ) have virtually no influence on symbiont persistence as the seed-banking probability decreases. Instead, persistence is determined entirely by the balance of symbiont effects on host fertility and loss from imperfect vertical transmission. The value of b for which seed banks no longer affect symbiont persistence is dependent on lower-level parameter values, as described in appendix D. However, under a wide range of parameter values, the rate of seed banking must be very high ($b \geq 0.9$) to strongly affect symbiont persistence. Thus, unless seed banking is obligate or nearly so, benefits to seed survival cannot compensate for fertility costs, and, conversely, costs to seed survival and failed retention in the seed bank cannot threaten symbiont persistence. As in the obligate seed dormancy model, the effective survival rate of converted seeds has no influence on symbiont persistence in the host population, since it does not affect the fitness contrast between host types.

Partial Seed Dormancy: Symbiont Prevalence. Although partial seed banking weakens the effect of host demographic

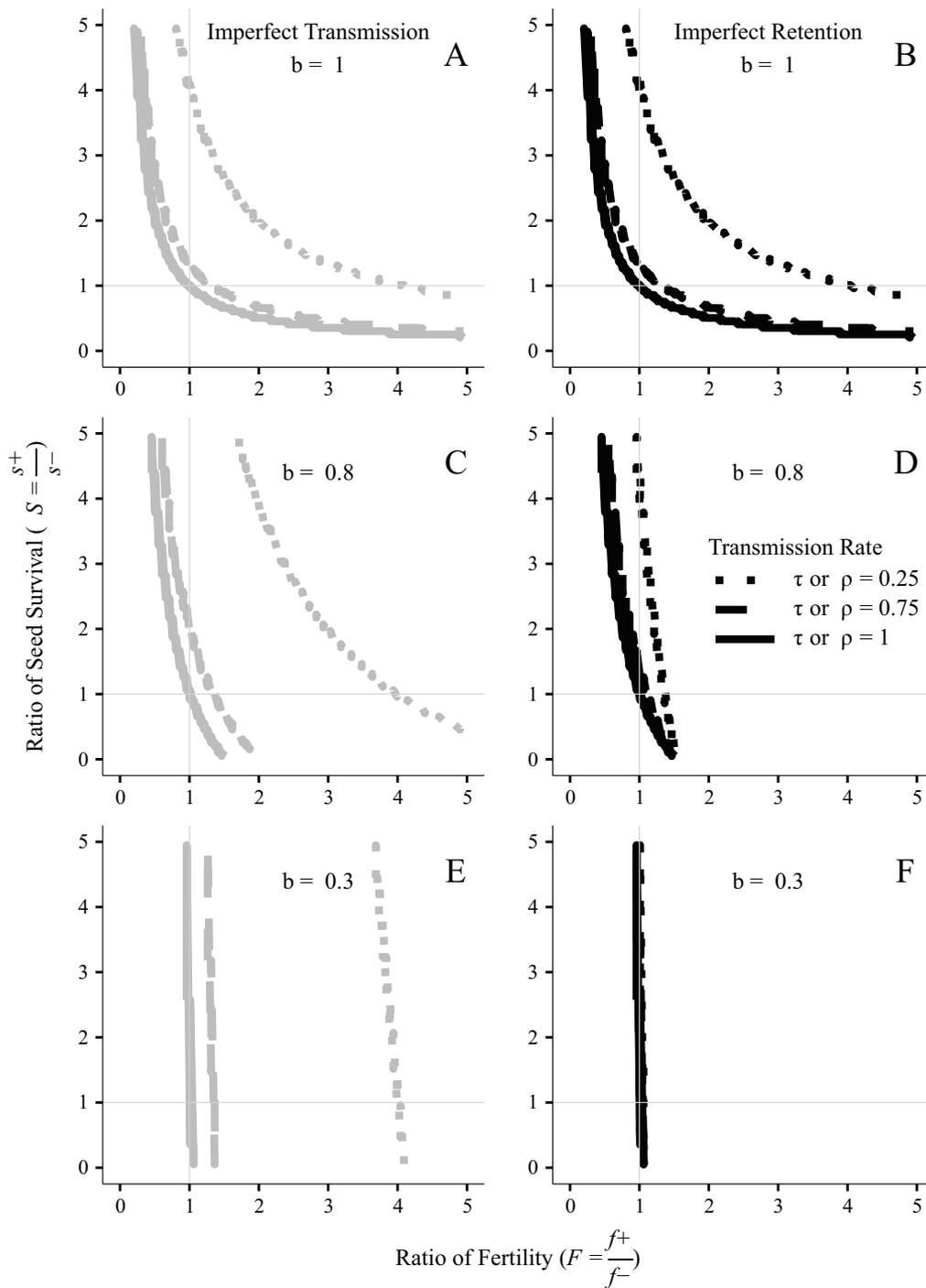


Figure 5: Symbiont persistence conditions in relation to symbiont effects on host reproduction (F) and survival (S) for varying levels of host seed banking: $b = 1$ (A, B), $b = 0.8$ (C, D), $b = 0.3$ (E, F). As in figure 2, lines separate regions of symbiont persistence and extinction, and thin gray lines indicate neutral effects. Columns show variation in vertical transmission rate τ (gray lines; A, C, E) and imperfect retention rate ρ (black lines; B, D, F). Line types represent different levels of endophyte loss (τ or $\rho = 1, 0.75, 0.25$).

storage on symbiont persistence, symbiont prevalence can remain sensitive to host-symbiont interactions that occur during dormant versus reproductive stages and in some surprising ways. As in the obligate seed bank model, population-level prevalence depends on the effective survival rate of converted seeds, as determined by parameter a .

Increasing vertical transmission (τ) or seed bank retention (ρ) of symbionts promotes their prevalence in the host population, as expected (fig. 3). As the probability of seed banking decreases from $b = 1$, symbiont prevalence becomes less sensitive to imperfect retention than it is to imperfect transmission and can remain high even when retention is very low (fig. 3C, 3D). When seed banking is infrequent ($b \leq 0.3$), prevalence is unaffected by imperfect retention and instead completely determined by the reproductive stage (fig. 3E, 3F). The effective survival rate of seeds in the seed bank modified the consequences of imperfect retention in a similar way as in the obligate storage model (fig. 3): a given reduction in retention has a greater negative effect on prevalence when converted E⁻ seeds retain survival benefits ($S > 1$) than when they do not and a weaker negative effect when converted seeds retain survival costs ($S < 1$) than when they do not. However, these effects are apparent only at moderate to high seed-banking probabilities, while prevalence remains sensitive to symbiont loss in the seed bank.

The responsiveness of symbiont prevalence to variation in demographic effects on host fertility (F) versus survival (S) is shown in figure 4. The most important and surprising result is that, under partial seed banking, increasing beneficial effects of symbionts on host survival in storage can decrease symbiont prevalence in the host population. Whether demographic benefits increase or decrease symbiont prevalence depends on the rate of seed banking and whether converted E⁻ seeds lose or keep the influence of former symbionts. When seed banking is very high, the dynamics converge on those of the obligate storage model, and beneficial effects on seed survival have strictly positive effects on prevalence, regardless of legacy effects (fig. 4A, 4B). However, when seed banking is less frequent, benefits to seed survival decrease symbiont prevalence but only if benefits are retained in converted E⁻ hosts ($a = 1$; fig. 4C, 4D). The key to understanding negative effects of benefits on prevalence under partial seed banking is that these effects occur when symbiont persistence is maintained entirely by fertility benefits (i.e., the persistence isocline is vertical; fig. 5). If the reproductive stage, alone, satisfies the persistence condition, then symbiont effects on seed survival are free to take any value; this allows for high symbiont prevalence despite costs to a partial storage stage that could not be tolerated with an obligate storage stage. Because symbiont persistence is insensitive to seed survival costs under partial seed banking, strong costs can eliminate converted E⁻ hosts that arise via imperfect retention, thus promoting the relative abun-

dance of E⁺ hosts (fig. 4D). As costs weaken and transition to increasing benefits, converted E⁻ seeds recruit at a greater rate, causing a decline in prevalence as S increases. As b becomes small, the negative effect of benefits on prevalence is diminished because opportunities for host conversion during storage become infrequent (fig. 4E, 4F).

Summary of Partial Seed Dormancy. We find that flexible entry into a storage stage of the host life cycle can substantially weaken the role of demographic storage in symbiont persistence and qualitatively alter how effects of symbionts on their host translate to population-level prevalence. The potential for demographic compensation between costs and benefits to different host life stages, as seen in the case of the obligate seed bank, disappears once passage through those stages is not strictly sequential. The insensitivity of symbiont persistence/extinction to seed bank processes allows for costs to the storage stage that increase symbiont prevalence, as long as imperfect retention generates E⁻ hosts that carry those costs via legacy effects. Thus, partial seed banking may not importantly affect whether symbionts can persist in host populations—this is dominated by interactions during the reproductive stage—but it can modify the relationship between individual-level effects and population-level prevalence in counterintuitive ways.

Discussion

Microbial symbionts are common and influential in nature. Understanding their ecological dynamics takes on urgency when considering their role in host responses to global change (Correa and Baker 2011; Kivlin et al. 2013) and their applications in pest and disease control (Zindel et al. 2011; Hoffmann et al. 2015). Previous theory for symbiont dynamics has generated the intuitive expectations that greater fitness benefits of heritable symbionts should promote their persistence and prevalence in host populations and that fitness costs are incompatible with host-symbiont mutualism. These expectations have provided a lens through which empirical patterns of symbiosis have been interpreted (Oliver et al. 2008; Semmartin et al. 2015) and debated (Faeth 2009; Rudgers et al. 2010). Our work advances this conversation by resolving connections between individual-level interactions (the effects of symbionts on hosts and pathways of loss from host lineages) and patterns of symbiont occurrence at the population level. We show that a simple but realistic modification to host-symbiont theory—the introduction of a “storage” stage of the host life cycle—can change expectations for symbiont persistence and prevalence. Ours is not the first model of heritable symbiosis to include host life cycle complexity (Ravel et al. 1997; Hancock et al. 2011), but we are the first, to our knowledge, to evaluate when and how this complexity affects symbiont dynamics, which may aid

in comparative analyses across hosts with variable life histories. Our work argues for careful consideration of population structure, including the fates of hosts that lose symbionts during life cycle transitions, a poorly understood but surprisingly consequential dimension of symbiosis dynamics. We have analyzed our model with inspiration from grass-endophyte symbioses. Here we discuss the more general implications of our results.

Our two model variants, obligate and partial seed banking, reveal when and how storage stages of the host life cycle matter for symbiont dynamics and when and how they do not. Storage stages critically affect symbiont dynamics when passage through storage is obligate. In this case, symbiont effects on and loss from the storage stage are as important as or more important than these same processes in the reproductive stage, and their interchangeability provides opportunity for compensation of costs in one stage by benefits in the other. Thus, costs of symbiosis are compatible with symbiont persistence and thus stable host-symbiont mutualism as long as costs are balanced by benefits elsewhere in the life cycle, revealing many ways in which symbionts may act as mutualists (Rudgers et al. 2012; Yule et al. 2013; Chung et al. 2015). We have emphasized seed banks as the ecological setting of demographic storage, but similar dynamics would play out in other contexts. For example, metamorphic stages of arthropod hosts represent obligate storage in that hosts must survive these demographic transitions before reaching reproductive maturity. Similarly, plant hosts must pass through seedling and juvenile stages prior to flowering. Throughout both arthropod and plant life cycles, heritable symbionts may be lost along the way (Afkhani and Rudgers 2008; Geib et al. 2009). Empirical estimates of symbiont prevalence typically come from surveys of reproductive host stages (Hilgenboecker et al. 2008; Rudgers et al. 2009; Gibert and Hazard 2013; Semmartin et al. 2015). Our results indicate that patterns of symbiosis in reproductive stages cannot be understood without considering interactions in upstream life stages, particularly when hosts have obligate, linear transitions through the life cycle.

Not all organisms do progress linearly through a life cycle, which motivated our consideration of partial seed banking. Flexible entry to the seed bank strongly dilutes its importance for symbiont persistence. This should also weaken selection for symbionts to benefit their hosts during storage, because symbiont fitness rapidly becomes insensitive to their interactions with hosts in storage as the probability of hosts entering storage decreases. Weak selection associated with partial seed banking may explain the substantial variability observed in the effects of fungal endophytes on seed bank survival and germination, including positive, neutral, and negative effects (e.g., Novas et al. 2003; Vila-Aiub et al. 2005; Gundel et al. 2006a, 2006b, 2009, 2010). While partial entry makes the storage stage unimportant

for symbiont persistence, it can continue to affect symbiont prevalence. Ironically, in some cases seed banks are able to modify symbiont prevalence precisely because they do not affect symbiont persistence; this allows for negative effects on seed stages that affect prevalence via the recruitment of converted hosts.

We have presented obligate and partial storage as alternatives, but it is likely that many host life cycles include elements of both, such as arthropods that bank eggs and also transition through prereproductive instars. Even for annual plants with a partial seed bank, as in our model, seeds that bypass the storage stage must still survive and germinate to reach the reproductive stage. To keep our theoretical framework as simple as possible, we assumed perfect recruitment of nonbanked seeds, but our model could be expanded to include additional obligate filters on recruitment to reproduction. We expect our qualitative conclusions for obligate and facultative transitions to continue to apply when both types are represented within a single life cycle, but exploring their interactions in greater detail could be a fruitful area for further work.

Formulating the model forced us to consider whether hosts that lose symbionts during development keep their demographic influence as a legacy effect. Legacy effects in symbiosis are poorly understood and may arise from several mechanisms. For example, Gundel et al. (2009) hypothesized that the concentration of endophyte mycelium in grass seeds can alter the distribution of water content in ways that affect seed survival, regardless of endophyte viability. We found in both the obligate and partial seed-banking models that, all else equal, positive legacy effects (hosts retaining benefits of former symbionts) reduce symbiont prevalence via the recruitment of converted E⁻ hosts. For an obligate storage life cycle, legacy effects cause imperfect retention to be more consequential at the population level than imperfect vertical transmission. We even find that, for partial seed banking, legacy effects can cause beneficial effects on host vital rates to reduce symbiont prevalence. We have discovered conditions under which benefits reduce prevalence, but the empirical literature leaves us ill-equipped to speculate how often these conditions may be realized. The critical next step will be to evaluate how the potential role of symbiont legacy effects plays out in nature. Our consideration of legacy effects was narrow in that converted seeds could keep an E⁺ benefit or cost through seed survival but not further into the reproductive stage. We thus assume that even strong legacy effects are short-lived relative to host development, which makes our conclusions about their effects conservative.

As in all theoretical work, results should be interpreted in light of model assumptions. We assumed that symbiont transmission is exclusively vertical. Zero horizontal transmission may be unrealistic. For example, in grass-endophyte

symbioses where transmission is predominantly vertical, low frequencies of horizontal transmission have been reported (Tintjer et al. 2008; Tadych et al. 2014). We conducted additional analyses that relaxed the assumption of strictly vertical transmission and found that our main inferences are robust to low levels of horizontal transmission ($\leq 10\%$ of E- seeds recruit as E+ plants). Horizontal transmission can rescue symbionts from extinction if their fitness effects and transmission efficiency are not adequate for persistence via vertical transmission alone, but symbiont dynamics are otherwise weakly responsive to low levels of horizontal transmission (app. A). While rare horizontal transmission may have little influence on ecological dynamics (see also Kwiatkowski and Vorburger 2012), it could modify selective pressures on symbionts and therefore alter evolutionary dynamics (Lipsitch et al. 1996). For example, horizontal transmission could weaken positive fitness feedbacks and lead to the breakdown of host-symbiont mutualism (Sachs et al. 2004; Sachs and Simms 2006).

Another important assumption of our theoretical framework relates to the role of density dependence in host population dynamics. We show in appendix B that our results apply across density-independent and density-dependent environments, as long as E- and E+ hosts are similarly affected by density dependence. However, empirical evidence suggests that symbiosis can influence host competitive ability (Faeth et al. 2004; Miller and Rudgers 2014). Our approach also assumes that all stages contribute equally to population regulation, whereas it is more likely that dormant stages contribute less if at all. These considerations would give rise to a more complex influence of density dependence than we have included.

Finally, our analysis assumed that the environment is constant. Storage stages are hypothesized to be adaptations to temporally variable environments (Evans and Dennehy 2005; Koons et al. 2008; Childs et al. 2010). While environmental constancy is an obvious starting point, it may be valuable to consider dynamics in a stochastic environment, which sets the stage for conflict between host and symbiont. For example, partial storage can be an adaptive bet-hedging strategy for hosts, but it may be maladaptive for symbionts if they lose viability during storage faster than their hosts. Symbionts may therefore be selected to accelerate host life cycles through storage stages and into reproductive maturity (splitting our banking rate b into $b^- \neq b^+$), as has been suggested by empirical studies (Faeth 2009). Temporal and spatial environmental variation may also promote host-symbiont conflict over transmission and retention strategies. In a constant environment, hosts should be selected to perfectly transmit and retain beneficial symbionts. However, if the environment fluctuates such that harboring symbionts is occasionally costly (e.g., benign years or locations in which costs of maintaining symbionts exceed their pro-

ductive benefits), then imperfect transmission and retention may be evolutionarily stable bet-hedging strategies for hosts, though this would decrease symbiont fitness. The model we have developed provides a framework to explore the adaptive dynamics of host-symbiont conflict in a stochastic setting.

Conclusion

Ecologists aim to develop general theory that distills nature to its simplest possible components; complexity should always be added with caution. We suggest that better accounting for life cycle complexity is a warranted addition to theory for host-symbiont dynamics, as is recognized in other types of interspecific interactions (Miller and Rudolf 2011). Our work is motivated by the observation that host organisms typically possess complex life cycles with opportunities for symbionts to affect and be lost from hosts in various ways over host ontogeny. We show that the addition of life cycle complexity can qualitatively change understanding of host-symbiont dynamics. We are optimistic that theoretical advances such as we have presented can facilitate empirical work by targeting key elements of host-symbiont interactions that drive their coupled dynamics. Empirical studies will be essential for understanding whether the potential consequences of host demographic storage identified by our models are realized in nature.

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Appendix A from A. J. Bibian et al., “The Role of Host Demographic Storage in the Ecological Dynamics of Heritable Symbionts” (Am. Nat., vol. 188, no. 4, p. 000)

The Effect of Horizontal Transmission on Symbiont Prevalence

We explored how the addition of low levels of horizontal symbiont transmission affect predictions for symbiont prevalence. Starting with the generalized model that includes partial seed banking (eq. [11] in the main text), the transition matrix becomes

$$\mathbf{A} = \begin{bmatrix} 0 & f^-b & 0 & f^+b(1-\tau) \\ s^-(1-h) & f^-(1-b) & s^+(1-\rho) & f^+(1-b)(1-\tau) \\ 0 & 0 & 0 & f^+b\tau \\ s^-h & 0 & s^+\rho & f^+(1-b)\tau \end{bmatrix}. \quad (\text{A1})$$

We include the possibility that E⁻ seeds recruit into the E⁺ plant population with probability h . We also explored horizontal transmission at the transition from E⁻ plants to E⁺ seeds, and results were qualitatively very similar. We assessed symbiont prevalence in the aboveground plant stage after 10,000 generations of simulated population dynamics. We explored horizontal transmission rates from 0% to 10%.

We found that a low level of horizontal transmission usually has little influence on long-run symbiont prevalence (fig. A1). Horizontal transmission has the greatest influence when symbiont prevalence at $h = 0$ is low, and it can allow the persistence of symbionts that would become extinct with vertical transmission alone (e.g., fig. A1D, thin lines). However, we have modeled the horizontal transmission probability with the constant h . It is more likely that the probability of horizontal infection is frequency dependent, increasing with greater prevalence. This makes our result an upper bound for the influence of horizontal transmission, since realized transmission rates are likely to be lower at a lower prevalence than is assumed here.

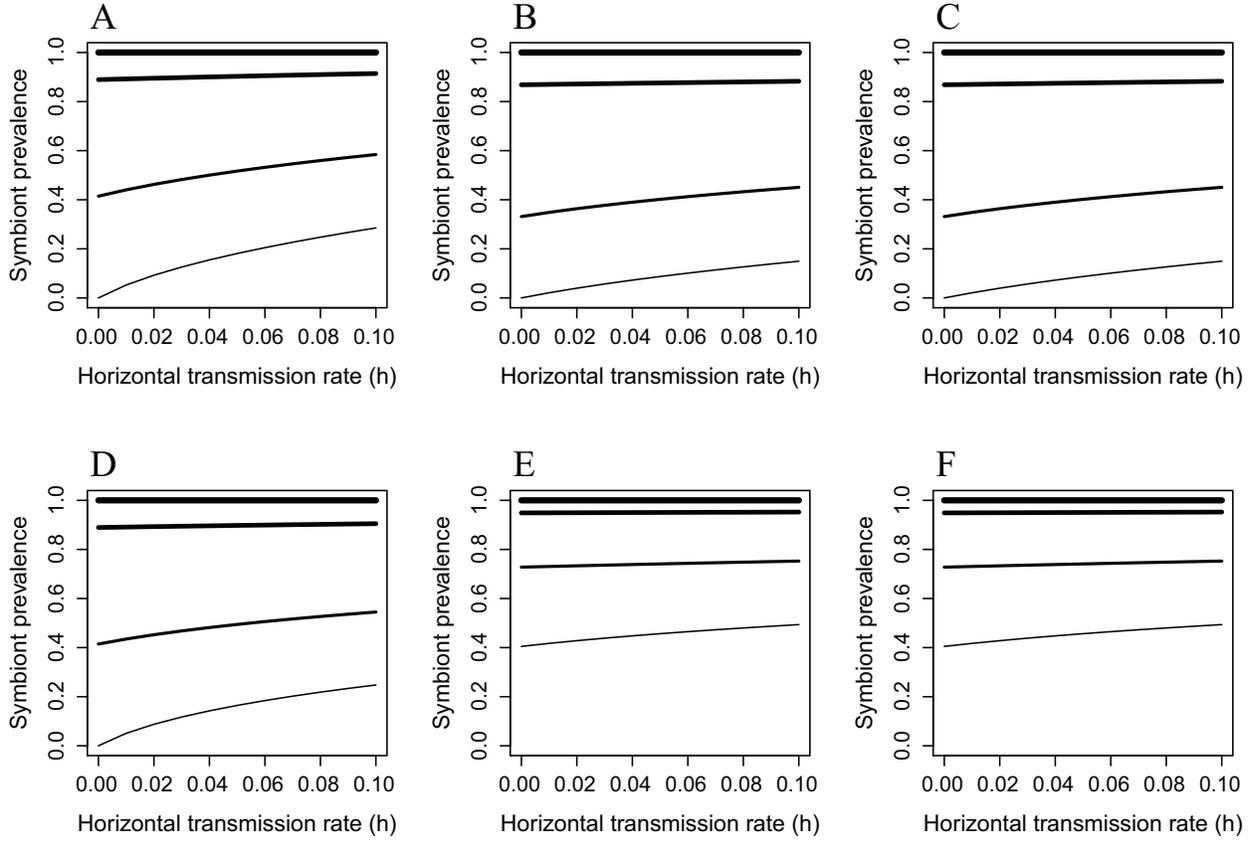


Figure A1: Symbiont prevalence in relation to the probability of horizontal transmission from E⁻ seed to E⁺ plant (eq. [A1]). Lines show different levels of vertical transmission (τ ; A–C) and retention (ρ ; D–F), from thinnest to thickest: 0.5, 0.75, 0.95, 1.0. Columns represent levels of seed banking: $b = 1$ (A, D), $b = 0.8$ (B, E), $b = 0.3$ (C, F). Host demographic parameter values for these simulations are as follows: $F = 1.5$, $S = 1.1$, $a = 0$.

Appendix B from A. J. Bibian et al., “The Role of Host Demographic Storage in the Ecological Dynamics of Heritable Symbionts” (Am. Nat., vol. 188, no. 4, p. 000)

Density Dependence in Simulations of Host-Symbiont Dynamics

In our simulations of the partial seed bank model (eq. [11] in the main text), we imposed a simple form of density dependence to maintain finite population sizes, for computational convenience. Specifically, the projection matrixes for these simulations were scaled with a negative exponential function to impose density dependence equally on all life stages. Here we show that this method for imposing density dependence has no effect on equilibrium symbiont prevalence.

For the density-dependent simulations, change in population size is governed by a time-vary transition matrix \mathbf{A}_t , according to $\mathbf{n}_{t+1} = \mathbf{A}_t \mathbf{n}_t$. At each time step, the transition matrix is given by the low-density matrix \mathbf{A}_0 , scaled by a negative exponential function of total population size such that

$$\mathbf{A}_t = \mathbf{A}_0 \times e^{-d \sum \mathbf{n}_t}. \quad (\text{B1})$$

The low-density matrix is given by equation (11) in the main text. The term $\sum \mathbf{n}_t$ is the total population size at time t , summed across all stages and endophyte states, and d controls the strength of density dependence. When $d = 0$, equation (B1) reduces to a density-independent population projection. By imposing density dependence in this way, we assume that all matrix elements are equally affected by density dependence and that all life stages and symbiont states contribute equally to density dependence.

To assess the sensitivity of our results to this method of imposing density dependence, we compared symbiont prevalence under varying values of the density-dependence parameter d (eq. [B1]). There was no difference in prevalence as we increased density dependence from $d = 0$ to $d = 0.01$, though equilibrium population size decreased as d increased (not shown), as expected. These results suggest that our method for imposing density dependence does not affect our conclusions regarding how demographic storage can alter host-symbiont dynamics.

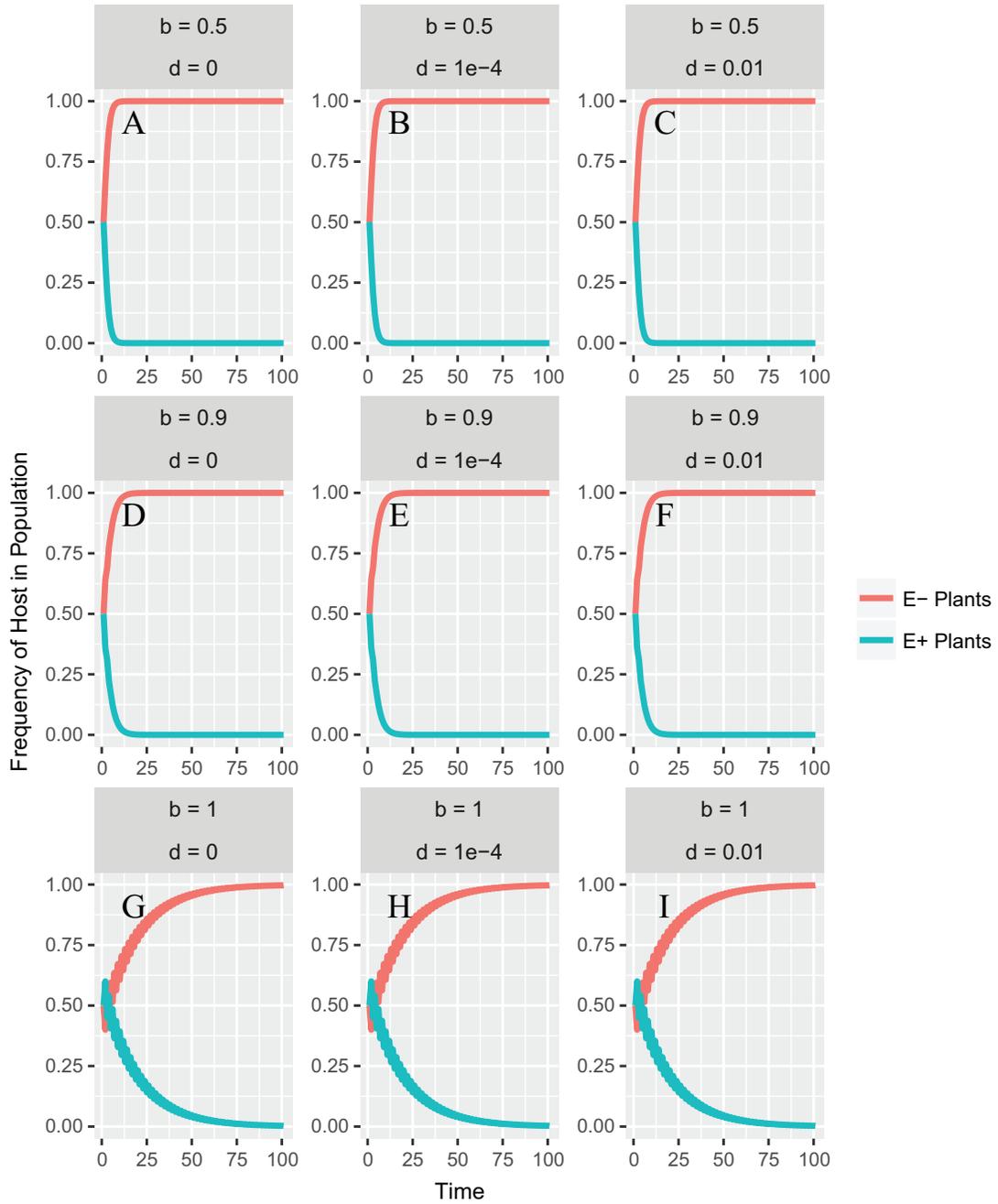


Figure B1: Symbiont prevalence in the plant stage across 100 time steps of our simulation. Rows represent different levels of seed banking: $b = 0.5$ (top), $b = 0.9$ (middle), $b = 1$ (bottom). Columns represent different levels of density dependence: $d = 0$ or density independence (left), $d = 0.0001$ (middle), and $d = 0.5$ (right). Host demographic parameters are $F = 2$, $S = 0.5$, $\tau = 1$, $\rho = 0.9$, $a = 1$.

Appendix C from A. J. Bibian et al., “The Role of Host Demographic Storage in the Ecological Dynamics of Heritable Symbionts” (Am. Nat., vol. 188, no. 4, p. 000)

Derivation of Expressions for Symbiont Persistence and Prevalence

The obligate storage model is given by $\mathbf{n}_{t+1} = \mathbf{A}\mathbf{n}_t$, where the vector \mathbf{n}_t contains the densities of E⁻ seeds, E⁻ reproductive plants, E⁺ seeds, and E⁺ reproductive plants. The matrix \mathbf{A} defines transitions between these states and is given by

$$\mathbf{A} = \begin{bmatrix} 0 & f^- & 0 & f^+(1-\tau) \\ s^- & 0 & [as^+ + (1-a)s^-](1-\rho) & 0 \\ 0 & 0 & 0 & f^+\tau \\ 0 & 0 & s^+\rho & 0 \end{bmatrix}. \quad (\text{C1})$$

Parameters are described in the main text. This matrix has four right eigenvectors, which represent the possible steady-state relative abundances of E⁻ seeds and plants and E⁺ seeds and plants. Two of these eigenvectors correspond to symbiont extinction (E⁻ seeds and plants only), and two of them correspond to symbiont persistence (all host types can occur). The leading eigenvalue of the symbiont extinction eigenvectors represents the fitness of E⁻ hosts (λ^-) and is given by

$$\lambda^- = \sqrt{f^-s^-}. \quad (\text{C2})$$

The leading eigenvalue of the symbiont persistence eigenvectors represents the fitness of E⁺ hosts (λ^+) and is given by

$$\lambda^+ = \sqrt{f^+\tau s^+\rho}. \quad (\text{C3})$$

If $\lambda^+ < \lambda^-$, then, for any initial conditions, the population will asymptotically converge on the symbiont-free stage distribution and the symbiont will become extinct. Therefore, symbiont persistence requires that

$$\sqrt{s^+\tau f^+\rho} > \sqrt{s^-f^-}. \quad (\text{C4})$$

After substituting the demographic effect ratios $F = f^+/f^-$ and $S = s^+/s^-$ and rearranging terms, we get expression (3) of the main text.

The unscaled right eigenvector associated with λ^+ is

$$\mathbf{w}^+ = \begin{bmatrix} -\frac{\rho f^-s^- - a\rho f^-s^+ - \rho\tau f^+s^+ - a f^-s^- + a f^-s^+ - \rho f^-s^- + \rho f^+s^+ + f^-s^-}{-\rho\tau f^+s^+ + f^-s^-} \\ -\frac{\sqrt{f^+\tau s^+\rho}(a\rho\tau s^- - a\rho\tau s^+ - a\tau s^- + a\tau s^+ - \rho\tau s^- + s^-)}{\tau(-\rho\tau f^+s^+ + f^-s^-)} \\ \frac{1}{\sqrt{f^+\tau s^+\rho}} \\ \frac{f^+\tau}{f^+\tau} \end{bmatrix}. \quad (\text{C5})$$

If condition (C4) is satisfied, the population will converge on this stable structure and grow geometrically at a rate given by equation (C3). We can calculate from equation (C5) the equilibrium proportion of reproductive plants that are symbiotic as

$$\widehat{E^+} = \frac{w_4^+}{w_2^+ + w_4^+} = \frac{\rho\tau f^+s^+ - f^-s^-}{a\rho\tau f^+s^- - a\rho\tau f^+s^+ - a\tau f^+s^- + a\tau f^+s^+ - \rho\tau f^+s^- + \rho\tau f^+s^+ - f^-s^- + f^+s^-}. \quad (\text{C6})$$

Substituting the effect ratios $F = f^+/f^-$ and $S = s^+/s^-$ gives the symbiont prevalence expression shown in equation (4) of the main text.

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