Journal of Evolutionary Biology, 2025, **38**, 41–49
https://doi.org/10.1093/jeb/voae127
Advance access publication 8 November 2024
Research Article

JOURNAL OF
Evolutionary Biology
eseb
European Society for Evolutionary Biology

How host-microbiome/holobiont evolution depends on whether the microbiome affects host lifespan or fecundity

Alexandra L. Brown[1], Britt Koskella[1], Mike Boots[1,2]

[1]Department of Integrative Biology, University of California, Berkeley, CA, United States
[2]Department of Ecology and Conservation, University of Exeter, Penryn, United Kingdom

Handling editor: Xiang-Yi Li Richter, Associate editor: Kavita Jain

Corresponding author: Alexandra L. Brown, Department of Integrative Biology, University of California, Berkeley, 3060 Valley Life Sciences Building, Berkeley, CA 94720, United States. Email: alexandra_brown@berkeley.edu

Abstract

There is overwhelming evidence that the microbiome can be important to host physiology and fitness. As such, there is interest in and some theoretical work on understanding when hosts and microbiomes (co)evolve so that microbes benefit hosts and hosts favour beneficial microbes. However, the outcome of evolution likely depends on how microbes benefit hosts. Here, we use adaptive dynamics to investigate how host and symbiont evolution depend on whether symbionts increase host lifespan or host reproduction in a simple model of host and symbiont dynamics. In addition, we investigate 2 ways hosts release (and transmit) symbionts: by releasing symbionts steadily during their lifetime or by releasing them at reproduction, potentially increasing symbionts' chances of infecting the host's offspring. The former is strict horizontal transmission, whereas the latter is also a form of indirect or "pseudovertical" transmission. Our first key result is that the evolution of symbionts that benefit host fecundity requires pseudovertical transmission, while the evolution of symbionts that benefit host lifespan does not. Furthermore, our second key result is that when investing in host benefits is costly to the free-living symbiont stage, intermediate levels of pseudovertical transmission are needed for selection to favour beneficial symbionts. This is true regardless of fitness effects because release at reproduction increases the free-living symbiont population, which increases competition for hosts. Consequently, hosts could evolve away from traits that favour beneficial symbionts. Generally, our work emphasizes the importance of different forms of vertical transmission and fitness benefits in host, microbiome, and holobiont evolution as highlighted by our prediction that the evolution of fecundity-increasing symbionts requires parent-to-offspring transmission.

Keywords: host, microbiome, holobiont, evolution, lifespan, fecundity, fitness effect, horizontal transmission, pseudovertical transmission

Introduction

For multicellular organisms, microbial symbionts are a fundamentally important part of life. It is very likely that all multicellular organisms have some sort of microbial population living in or on them (Rosenberg et al., 2010). These microbes form the microbiome, and the combination of the host and its microbiome is often called the holobiont (Margulis, 1991). The microbiome is known to be an important player in host physiology, with, for example, the aphid symbiont *Buchnera aphidicola* helping its host gain adequate nutrients to survive on a diet of phloem sap. Corals have photosynthetic endosymbionts (family Symbiodiniaceae) that provide them with fixed carbon that enables them to live in nutrient-poor waters, and the stress tolerance of these symbionts can determine their host's ability to survive high (and low) temperatures (Silverstein et al., 2017) and various other stressors (Baker & Cunning, 2015). However, the microbiome extends beyond specialised partners necessary for survival. Corals also have various species of bacteria, fungi, archaea, and viruses living extracellularly in coral mucus and elsewhere. This group of symbionts, while more diverse and more variable between hosts (and within the same host over time), is still important for host physiology. For example, extracellular symbionts provide disease resistance via the production of antimicrobial compounds and fix nitrogen that the coral can then use (Morrow et al., 2018; van Oppen & Blackall, 2019). Similarly, for fish, the gut microbiome is known to be important for digestion, development, and disease protection, while microbial diversity is also generally correlated with health (Legrand et al., 2020; Merrifield & Rodiles, 2015; Talwar et al., 2018).

The important physiological role of the microbiome in many organisms has led to the hypothesis that the host and microbiome together might behave as a single evolutionary evolutionary unit. This is known as hologenome theory (Zilber-Rosenberg & Rosenberg, 2008), and it proposes that the primary unit of selection is the holobiont (although later versions of the theory sometimes argue instead that the holobiont is *a* unit of selection (Theis et al., 2016)). Arguments for hologenome theory include the evolutionary importance of cooperation and mutualism (Lloyd & Wade, 2019), the holobiont's possible role as an evolutionary interactor (Roughgarden et al., 2018), and the unique physiological

Received April 10, 2024; revised August 28, 2024; accepted October 25, 2024

relationship between host and microbiome that may resemble the relation between an individual and its genes (Bordenstein & Theis, 2015). Hologenome theory is disputed for several reasons. Primarily, without vertical transmission to link host and symbiont evolutionary fates, it is unclear how they might behave as a single evolutionary unit (Douglas & Werren, 2016; Moran & Sloan, 2015). Other issues include the fact that selection to benefit hosts is not necessary to produce helpful microbes (Moran & Sloan, 2015) and that classical coevolutionary theory may work just as well to understand host–microbiome evolution (Koskella & Bergelson, 2020). Fundamentally, to be the primary unit of selection, the fate of these many briefly linked organisms has to be more important than selection on the individual.

Here, we use a mathematical model to investigate how the component of fitness (reproduction or survival) that symbionts affect influences whether symbionts can evolve to benefit their hosts and whether hosts can evolve to encourage beneficial symbionts. Such a mutual benefit is not necessarily a sign that the holobiont is behaving as a single selective unit (Koskella & Bergelson, 2020), but it seems unlikely that the holobiont could be the primary unit of selection if mutual benefit cannot evolve (at the very least because a lack of mutual benefit would dilute the nonbenefiting partner relative to the other partner). Previous work has investigated the evolution of microbial effects on hosts when holobionts experience selection such that host benefits are directly tied to microbial reproduction (although microbes grow within the host as well) (Roughgarden, 2020), including in cases where microbes experience within-host costs to benefiting their hosts (Rougharden, 2023). These models provide insight into how a correlation between host and symbiont reproduction can lead to beneficial symbiont evolution even without vertical transmission. We remove this direct correlation between host and symbiont fecundity and investigate the conditions under which beneficial microbes can evolve. (an additional difference is that in our model, microbial investment in host benefits comes at the cost of increased mortality in the free-living state, rather than at the cost of within-host growth). Other work on holobiont evolution by Sakal (2023) developed a mathematical connection between individual change, ensemble change (something a bit like the holobiont, but containing additional information), and holobiont change and showed that the distribution of holobionts in the population can be found from the individual and ensemble dynamics (although the reverse is not necessarily true). With system-specific knowledge of how to classify ensembles as holobionts, application of the Price Equation becomes possible, as does determining the relative strength of selection on the holobiont as opposed to the individual. However, with large amounts of microbes or rapid microbial change, the method is somewhat complicated to apply, as the matrices involved get larger and each microbial change can potentially count as a "generation" in the population. Our investigation of a simpler system is thus useful for narrowing down the conditions under which evolution as a holobiont is even possible, and thus it is worthwhile to investigate with methods such as Sakal's.

We model selection on hosts and symbionts to behave in ways that (potentially) benefit the other's fitness. For hosts, we model two mechanisms of possible symbiont release from the host, both of which enable the symbionts to enter the free-living population and eventually acquire another host (i.e., both function as forms of symbiont transmission). The first mechanism is the release of the symbiont during the host's lifetime, which captures the horizontal acquisition of the symbiont by a new host. The second mechanism is the release of the symbiont upon host reproduction (and death). Because we assume that host reproduction and death are spatially and temporally coupled, this second form of release captures the situation where there is both indirect "pseudovertical" transmission and regular horizontal transmission (Wilkinson, 1997). Symbionts can potentially benefit their hosts either by increasing their host's fecundity (the number of offspring the host has) or their host's survival (the ability to reach reproductive maturity). We numerically evaluate our model (numerical integration being required for some expressions) to understand when hosts and symbionts with particular mutations can invade. We demonstrate the importance of different forms of vertical transmission and the nature of fitness benefits in the evolution of hosts and their microbiomes, the holobiont.

Methods

We model a population of hosts and their sometimes free-living microbial symbionts. We investigate how hosts and symbionts evolve when the symbionts affect either their host's lifespan or fecundity and how hosts adjust the release (transmission) of their symbionts as a result. We first describe the population dynamic of the model (host and symbiont life cycle shown in Figure 1).

The model

Hosted symbionts grow logistically with an intrinsic growth rate g and a carrying capacity k_S. (We model growth as logistic because the capacity of individual host to host symbionts is likely bounded by host space or host nutrient supply, and logistic growth has been used previously to model microbiome dynamics, for example, by Hara and Iwasa (2019) to model the intestinal microbiome.) Symbionts are also released from the host at a constant, per-capita rate x, similar to the loss of coral microbiome symbionts due to mucus sloughing off, as modelled by Mao-Jones et al. (2010). Released symbionts join the free-living symbiont population, discussed below. The hosted symbiont population then changes at the rate

$$\frac{ds(t)}{dt} = gs(t)(1-s(t)) - xs(t), \qquad (1)$$

where $s(t)$ is measured in units of k_S.

Starting from an initial symbiont population s_0, we can solve the above equation to get the total symbionts within a living host at time t since its birth

$$s(t) = \begin{cases} \dfrac{(g-x)s_0}{(g(1-s_0)-x)e^{(x-g)t} + gs_0}, & g \neq x, \\ \dfrac{s_0}{1+s_0 tx}, & g = x. \end{cases} \qquad (2)$$

We assume that all symbiont types within a host have the same growth and release rates (i.e., within-host growth and release rates are host traits, determined by the physical space and nutrients the host provides and the host's biological processes that cause symbionts to be "sloughed-off" or otherwise lost) and that all symbionts inhibit each other's growth equally (as they are essentially identical apart from a single mutation and so take up the same amount of space and resources). Of course, individual symbionts will reproduce and exit the host

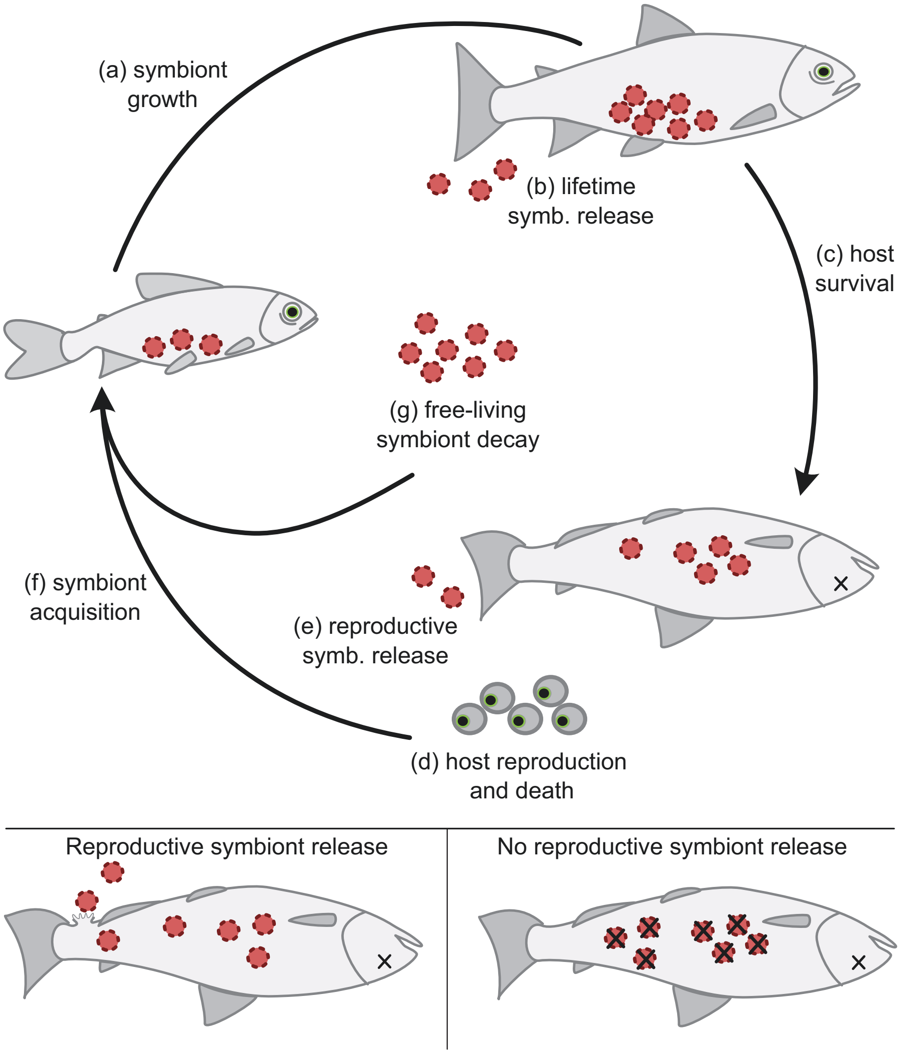

Figure 1. Upper panel: model diagram. (a) Symbionts grow logistically within their hosts. (b) Living hosts steadily release a small fraction of their symbionts, which enter the free-living population. (c) Host survival depends on the number of symbionts and the benefits they provide (lifespan case only). (d) Hosts that survive to reproductive age reproduce and then immediately die. (Fecundity case only: the number of offspring depends on hosts' symbionts.) (e) Upon reproduction/death, hosts may release a fraction of their symbionts in the vicinity of their offspring. (f) Newborn hosts acquire symbionts from the surrounding area, including those released by their parents and other free-living symbionts that happen to be nearby. (g) Any symbionts that remain enter the free-living state, where they do not grow but experience a constant mortality rate. Lower panel: reproductive release of symbionts. Hosts that release their symbionts into the environment allow those symbionts to survive in the free-living state. Symbionts that are not released die with their host.

stochastically; however, for large symbiont populations, such as we assume, the central limit theorem will ensure that the aggregate population behaves according to Equation (2). (For rare mutant symbionts, the effect of variation in the experiences of individual symbionts can be analysed through the lens of genetic drift, once the expected invasion fitness is found.) If there are multiple symbiont types, the assumptions of equal growth, expulsion, and inhibition of each other's growth, mean that the growth rate of symbiont type i is

$$\frac{ds_i(t)}{dt} = gs_i(t)(1 - s(t)) - xs_i(t), \tag{3}$$

(Note that the $1 - s(t)$ term represents growth inhibition by all symbionts within a host, not just those of type i.) As all symbionts have the same per-capita growth rate, $g(1-s(t))-x$, their proportions within the host remain constant over time (this can be seen by taking the derivative of the ratio of $s_i(t)$ to $s(t)$). The size of subpopulation s_i at time t can then be written as its initial proportion of the population times the total symbiont population size at time t

$$s_i(t) = \frac{s_i(0)}{\sum_j s_j(0)} s(t). \tag{4}$$

Hosts may experience differential survival depending on their symbionts (Figure 1C). We assume hosts experience some baseline rate of premature death p_0 (where "premature" death means death before reaching their maximum lifespan). This death rate is decreased by the survival benefit from symbionts $ps(t)$ (if there are multiple symbiont types, p is a vector of benefits). In other words, the host population decreases at the per-capita rate $p_0 - ps(t)$.

$$\frac{dh}{dt} = -h(t)(p_0 + ps(t)). \tag{5}$$

In order to find the fraction of hosts still alive at time t, we solve the above differential equation for $h(t)$, using the fact that the initial fraction of hosts alive is 1 (see below description of host reproduction). The fraction of hosts alive at time t is then

$$h(t) = \exp\left[\int_0^t (ps(\tau) - p_0)d\tau\right]. \tag{6}$$

Any hosts that survive to the end of their lifespan, $t = \ell$, reproduce (Figure 1D). Symbionts can benefit their hosts by increasing their fecundity by an amount $bs(\ell)$ above the baseline (b_0), so that host i's fecundity is proportional to

$$b_i = b_0 + bs(\ell). \tag{7}$$

We assume that hosts produce a large number of offspring so that after reproduction the population is always filled to carrying capacity, k_H. The fraction of hosts in the new generation who are offspring of parental type i is determined by the parents' abundance in the population and fecundity; that is, it is proportional to $h_i(\ell)b_i$. The total host offspring of type i is thus

$$h_i' = \frac{h_i(\ell)b_i}{\sum_j h_j(\ell)b_j}, \tag{8}$$

where h_i' is measured in units of host carrying capacity, k_H.

Adult hosts die shortly after reproduction. At the point of death, some fraction y of their symbionts may be released and enter the free-living pool. (All unreleased symbionts die with their host.) Since we assume host death and reproduction happen in close proximity to each other, these newly released symbionts are concentrated around their former host's offspring. The local free-living symbionts around the newborn hosts are then a combination of the well-mixed, global free-living population (f) and the symbionts just released by their parent

$$f_{\text{local}} = h_i(\ell)ys(\ell) + f, \tag{9}$$

where f_{local} is measured in units of $k_H k_S$.

We assume newborn hosts acquire their symbionts from their local environment, with their symbiont concentrations a saturating function of their local free-living symbionts. We represent the fact that newborn hosts may be smaller or for some other reason have a lower symbiont carrying capacity than adults by setting the newborn carrying capacity k_N to a fraction of the adult carrying capacity.

$$s_i'(0) = \left(\frac{k_N}{k_S}\right) f_{i,\,\text{local}} \Big/ \left(1 + \sum_j (f_{j,\,\text{local}})\right). \tag{10}$$

We assume the free-living symbiont population returns to a well-mixed state relatively quickly after symbiont acquisition happens, or at least before the next generation of hosts is born. This is probably reasonable if free-living symbionts are aquatic and there is not a strong directional flow to the environment, or if the symbionts are terrestrial and easily dispersed by air or animal movement, and possibly also if the symbionts are motile and disperse by their own movement. During the hosts' lifetime, we also assume that the population remains well-mixed, that is, that any symbionts released during a host's lifetime are evenly distributed throughout the free-living population. The previous forces could accomplish such distribution, as could the movement of the hosts themselves. (A lack of mixing is likely to look like an increase in pseudovertical transmission if hosts also tend not to mix or random variation in the symbionts available to hosts, if not.) The free-living symbiont population decays at rate m (Figure 1G), which we can think of as representing some net negative growth rate in the free-living state (i.e., if free-living symbionts do reproduce, they die at a higher rate, so that their net growth rate is $-m$). This difference in growth rates between the free-living and hosted states represents the benefit that symbionts get from the nutrients and protection in the environment provided by the hosts.

Host and symbiont evolution

We model two possible ways symbionts might benefit their host: by increasing their host's lifespan (via the survival benefit p) or increasing its fecundity (b). We consider the two benefits separately and compare their effects. In both cases, symbionts' benefits to their hosts come at a cost to their own ability to survive in the free-living state. Hosts can evolve by increasing or decreasing their rate of symbiont release during their lifetime (x) or the fraction of their symbionts they release at reproduction (y).

To estimate the invasion fitness of mutant hosts (i.e., the initial growth rate of the mutant), we allow the resident population to equilibrate and then introduce a mutation into a single host ($1/k_H$ of the host population) at birth (Bräström et al., 2013). We calculate the invasion fitness as the growth rate of the mutant population after two host generations have passed. We wait this extra generation before measuring growth rate because mutants with different levels of symbiont release at reproduction do not exhibit their traits until the end of their lives, so any effects on fitness will only be shown in the grand offspring of the original mutant. Inclusion of effects on future generations has been done previously, for example, for finding the invasion fitness of niche construction traits (Lehmann, 2008), and we have previously used the approach of averaging over a few early generations when invasion fitness has to be numerically estimated (Brown et al., 2023).

We find the invasion fitness of mutant symbionts using a similar process, except that we must account for symbionts having a faster generation time than hosts and for mutations arising in either hosted or free-living symbionts. To do this, we estimate the expected invasion fitness by calculating the invasion fitness of a mutant that arises at ℓ evenly spaced time points during the host lifespan. We do this for both hosted and free-living symbionts and calculate the expectation using the symbiont population at each time point and in each state to determine the probability of a mutant arising in each.

$$w \approx \frac{\sum_t^\ell \left[h(t)s(t)w_{\text{hosted}}(t) + f(t)w_{\text{free}}(t)\right]}{\sum_t^\ell \left[h(t)s(t) + f(t)\right]}, \tag{11}$$

where w is the expected invasion fitness, and $w_{\text{hosted}}(t)$ and $w_{\text{free}}(t)$ are the invasion fitness starting from an initial hosted or free-living symbionts at time t, respectively. To account for mutations whose effects will only be apparent when the original mutant's host reproduces, we again calculate the mutant growth rate after two host generations have passed.

Code

Host and symbiont fitnesses were calculated numerically in Mathematica (v 13; Wolfram Research, Inc., 2021).

Code is available at https://zenodo.org/doi/10.5281/zenodo.13840902.

Results

Symbiont affects host fecundity

First, we investigate the case where a mutant symbiont increases its host's fecundity at a cost to the symbiont's own ability to survive in the free-living state. When there is no correlation between host reproduction and symbiont fitness ($y = 0$) and the symbiont pays a cost for benefiting its host, the mutant symbiont is at a disadvantage and is lost from the population. Figure 2A shows that invasion fitness of such a mutant is negative when hosts do not release any symbionts at the time of their reproduction. In this case, the mutant symbiont is not enriched in the environment near the host's newborn offspring and thus does not benefit from its host's increased reproduction. Even when there is no cost to the mutant in the free-living state, increasing its host's fecundity is selectively neutral (Figure 2A thick solid line). In other words, when no symbionts are released at reproduction, investment in host fecundity is always deleterious for symbionts (or neutral if the cost to symbionts is 0), regardless of the cost of investment.

When some correlation between host reproduction and symbiont fitness is introduced via the host releasing a fraction of its symbionts upon reproduction, the fecundity-increasing mutant symbiont can invade in some cases (Figure 2A). The mutant's invasion fitness tends to be highest when the host releases some but not all of its symbionts upon reproduction (Figures 2A and 3). This is likely because release upon host reproduction increases the free-living symbiont population, which has two potential negative effects on the mutant symbiont. First, a larger free-living symbiont population means more competition to be taken up by a newborn host and decreases the fraction of local symbionts that come from the parent host. Second, a larger free-living population increases the chances that the mutation will arise in a free-living symbiont (as opposed to a hosted symbiont) and thus must survive until it is taken up by a host to experience any benefit of its traits.

When the symbiont increases host fecundity, hosts experience selection on the symbionts they release during their lifetimes and at reproduction. Hosts are selected to decrease their symbiont release during their lifetime (x), probably because it has the immediate benefit of increasing the number of symbionts they are hosting and thus their fecundity. (The equilibrium symbiont population within a host is $K_s\left(1-x/g\right)$, where x is the symbiont release rate.) The evolutionarily stable lifetime symbiont release rate for hosts appears to be no release at all, that is, $x = 0$ (Supplementary Figure S2A and B). On the other hand, hosts are selected to increase the symbionts they release at reproduction, and only releasing all of their symbionts at reproduction seems to be evolutionarily stable (Supplementary Figure S2C and D). This makes sense because host offspring benefit from the opportunity to acquire as many symbionts as possible, while reproductive release comes at the end of host life and thus at no cost to the host parent.

Symbiont affects host lifespan

When there is no cost to symbionts increasing host lifespan, beneficial symbionts can invade even when no symbionts are released upon host reproduction (Figure 2B, thick solid line). This is also the case when the costs of symbiosis are small (Figure 2B, dashed line). However, as costs increase, beneficial mutants eventually can no longer invade unless at least some symbionts are released upon host reproduction (i.e., there must be some correlation between host reproduction and symbiont fitness), despite the fact that symbionts do not directly alter host fecundity (Figure 2B, thin solid line). When extending the host's lifespan is very costly, beneficial symbionts invade only when they benefit both from the additional time to live inside their host that they provide and also from a longer-lived host's greater chances of surviving to produce offspring. In some cases, when the costs of extending host lifespan are high, beneficial symbionts may also fail to invade at too-high levels of reproductive release, so that invasion is only possible at intermediate levels (Figure 2B, thin, solid line).

Selection on hosts appears to act in a similar direction as in the case of fecundity-increasing symbionts. Hosts are selected to decrease symbiont release during their lifetimes and increase the release of symbionts at reproduction, with

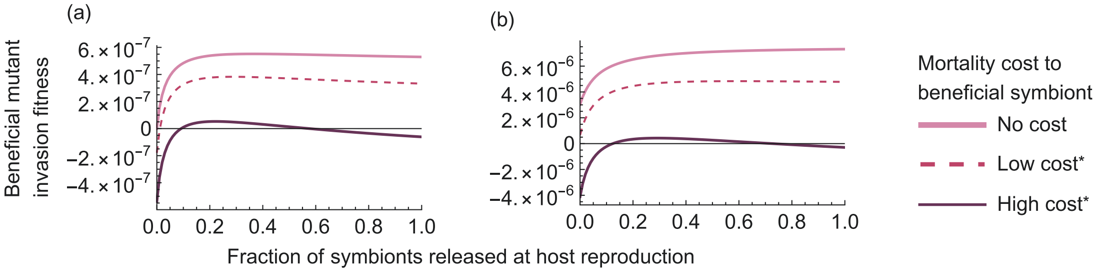

Figure 2. Invasion fitness of a mutant symbiont that increases (a) host fecundity or (b) host lifespan at the cost of increasing its own mortality in the free-living state. *Note that beneficial mutant mortality is different between panels (a) and (b). In all cases, symbionts have a baseline mortality of 0.01 per time step and experience additional mortality in proportion to the benefit they provide. (Resident in all cases provides half the benefit of the mutant and so pays half the cost.) Thick, solid line: symbionts suffer no additional mortality cost. Dashed line: symbionts suffer an additional mortality cost of (a) 1×10^{-8} (mutant) or 5×10^{-9} (resident) or (b) 1.3×10^{-7} (mutant) or 7×10^{-8}. Thin, solid line: symbionts suffer an additional mortality cost of (a) 3×10^{-8} (mutant) or 1.5×10^{-8} (resident) or (b) 4×10^{-7} (mutant) or 2×10^{-7} (resident). Other parameters: (a) fecundity benefit of resident 0.01 per k_S symbionts, fecundity benefit of mutant 0.02, release rate 0.001 per time step, no premature host death; (b) baseline rate of premature host death p_0 = 0.002, survival benefit of resident 0.001 per k_S symbionts, survival benefit of mutant 0.002, release rate 0.001 per time step. All other parameters as in Table 1. See Supplementary Table S1 for a table form of this figure.

Table 1. Model parameters.

Variable	Definition	Value
b_0	Baseline host fecundity	1
f	Free-living symbionts	Variable, units of $k_H k_S$
g	Symbiont intrinsic growth rate (within host)	0.1 per time step (t.s.)
h	Host population size	Variable, units of k_H
k_H	Host carrying capacity	100
k_N	Maximum symbionts taken up by newborn host	$0.1k_S$
k_S	Symbiont carrying capacity (within host)	10,000
ℓ	Host lifespan	100 t.s.
m	Free-living symbiont mortality rate	0.01 per t.s. or variable
p_0	Baseline host premature death rate	0 or 0.002 per t.s.
s	Number of hosted symbionts	Variable, units of k_S
s_0	Initial number of symbionts in a newborn host	Variable, units of k_S
	Host traits	
x	Symbiont release rate (living host)	
y	Fraction of symbionts released at host reproduction	
	Symbiont traits	
b	Fecundity benefit to host	
p	Survival benefit to host	

the only evolutionary stable strategies arising at no lifetime release and release of all symbionts upon reproduction.

Discussion

We have shown that whether symbionts evolve to benefit their hosts depends on both the aspect of host fitness that the symbiont affects and the level and type of symbiont release from the host. The only case where fecundity benefits can evolve is where symbiont and host fecundity are linked via the release of symbionts at host reproduction. This is true even if benefiting their hosts is not costly at all. On the other hand, symbionts that improve the host's lifespan can sometimes evolve even when there is no release at reproduction and all hosted symbionts die with their hosts. However, in order to prevent the symbiont population from going extinct, symbionts must be released during the host's lifetime if none or only a few are released at the end of the host's life. Interestingly, hosts in our model evolve to not release symbionts during their lifetimes and to release them all at reproduction. While this sometimes favours the evolution of beneficial symbionts, in some cases, when hosts benefits are particularly costly to symbionts, beneficial symbionts can only evolve at intermediate levels of symbiont release, regardless of whether they increase host fecundity or lifespan.

Although our model focuses on the roles of lifetime versus reproductive/end-of-life release of symbionts, it may apply more broadly to different mechanisms of symbiont transmission. In particular, the lifetime release of symbionts in our model is a mechanism of horizontal transmission, while release at reproduction allows both horizontal transmission and indirect vertical transmission. This is because we assume that host parents die both spatially and temporally close to their offspring, increasing the likelihood that their offspring will be able to acquire the symbionts that host parents release at reproduction. Other mechanisms of vertical or indirect vertical transmission could also potentially produce a correlation between parent and offspring symbionts, providing a link between host fertility and symbiont fitness that should allow the evolution of fecundity-increasing symbionts. (Lifespan-increasing symbionts would also benefit from this link.)

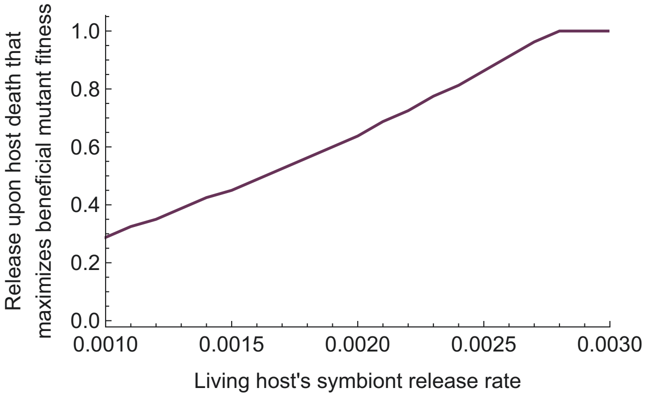

Figure 3. Fraction of symbionts released upon host reproduction that maximises a beneficial mutant's invasion fitness. Intermediate fractions are favoured, with the optimal fraction increasing with the rate of symbiont release by living hosts. Parameters: Free-living resident and mutant symbionts have a per capita mortality of $0.01 + 5 \times 10^{-9}$ and $0.01 + 1 \times 10^{-8}$ per time step, respectively. Resident symbionts provide a fecundity benefit of 0.01 per k_S symbionts, mutants a benefit of 0.02. No premature host death ($p_0 = 0$). Other parameters are as in Table 1. Invasion fitness of the mutant is shown in Supplementary Figure S1. See Supplementary Table S2 for a table form of this figure.

Interestingly, when the costs of symbiosis are high, we sometimes see that only intermediate levels of reproductive release favour the evolution of beneficial symbionts. This may have something to do with the mechanics of symbiont release at reproduction. Not all symbionts released by the parent host upon its reproduction are taken up by its offspring. Some join the free-living symbiont population. Over time, a high level of symbiont release upon reproduction can increase the size of the free-living symbiont population. This can actually have a negative effect on new mutant symbionts that arise if these benefit their host at cost to their free-living survival. This is for two reasons: first, if the free-living population is larger, then a mutation is more likely to arise in a free-living symbiont. Since beneficial mutants have a lower chance of survival in the free-living state, the expected fitness of a mutant that arises, averaged across all the symbionts that might mutate, tends to be lower with a larger free-living population. Second, free-living symbionts compete with symbionts released at reproduction to be taken up by newborn hosts. Even if a beneficial mutation is lucky enough to arise within a hosted symbiont, competition for its host's offspring is stiffer, and thus the chance that symbiont will get to experience any benefits of its "helpful" phenotype is lower. This is particularly the case when the symbiont benefits host fecundity and must remain with its host lineage over generations to benefit at all from helping its host.

Despite the benefits to hosts of exhibiting moderate levels of reproductive release when the above is true, hosts appear to always evolve to release all of their symbionts upon reproduction. As we assumed all symbionts were somewhat beneficial, pseudovertical transmission is immediately beneficial to a particular host, as it ensures its offspring have access to as many symbionts as possible. Similarly, hosts evolve to limit the release of symbionts during their lifetimes because lifetime release decreases their internal symbiont populations and thus slightly decreases the benefits the host receives. Possibly if some symbionts were harmful, release would evolve differently, or if the lifetime and reproductive release of symbionts were correlated, for example, if both happened via a similar mechanism. However, in our current model, we see that hosts evolve to favour their short-term benefit at the expense, in some cases, of encouraging the evolution of more beneficial symbionts by exhibiting intermediate levels of reproductive release. Whether this ends up disfavouring beneficial symbiont evolution or not depends on the exact parameters of the situation, but it is interesting that despite directly benefiting from their microbiomes, hosts in our model are sometimes "bad" members of the holobiont, because they do not evolve in a way that maximises the benefit to hosts and symbionts.

Our model suggests that some level of indirect pseudovertical transmission is required for fecundity-increasing symbionts to evolve. This contrasts with work by Roughgarden (2020), where horizontal transmission alone allows the proliferation of fecundity-increasing symbionts. The difference is that in that model symbionts directly benefit from increasing their host's fecundity by also increasing their own fecundity—holobionts reproduce together before being separated for horizontal transmission, so that the more offspring hosts have, the more holobionts are produced, and thus the more symbionts are produced. Similarly, work on symbioses and nonsymbiotic mutualisms shows that when symbionts/mutualistic partners benefit from increasing host fecundity, such fecundity benefits can also evolve in the absence of vertical transmission. For example, in plant-pollinator mutualisms, pollinators benefit from increasing host fecundity by receiving nectar (Soberon & Martinez del Rio, 1981). Furthermore, the mutualism-producing mechanisms of partner choice (Bull & Rice, 1991) and sanctions (West et al., 2002), provide direct benefits/consequences to beneficial/unhelpful symbionts, allowing hosts to link fecundity with symbiont fitness.

Without a direct link between host fecundity and symbiont fecundity or an indirect link such as pseudovertical transmission, our model suggests that symbionts that increase host fecundity are unlikely to evolve. This contrasts with symbionts that affect host lifespan, where increased host persistence also means increased opportunities for symbionts to multiply within the host and eventually be released to find new hosts. In this way, lifespan-increase symbionts are engaging in something like byproduct reciprocity (Connor, 1995; Sachs et al., 2004), where one partner invests in another to increase byproducts produced by the other (in our model, a longer-lasting place to live). Our model suggests that for horizontal transmission alone to favour the evolution of a beneficial microbiome, symbionts must either increase host lifespan or experience some direct benefits to helping their hosts. Thus, if there are cases where hologenome theory holds in the absence of vertical transmission or other direct symbiont benefits, they probably involve lifespan-increasing symbionts.

While our model likely applies to more general mechanisms for symbiont transmission that correspond to the same transmission modes, the particular benefit of intermediate levels of symbiont transmission for the evolution may be due to the fact that our mechanism of pseudovertical transmission also increases the free-living symbiont population. Furthermore, this mechanism is not applicable to all systems, including those where offspring are produced spatially and temporally distantly from their parent's deaths (e.g., iteroparous organisms whose offspring disperse, such as the seeds of perennial plants) and those where no or few symbionts are released upon host reproduction and death. However, the mechanism in our model captures directly natural symbioses, such as between the symbiont *Nephromyces* and its sea squirt host. *Nephromyces* is thought to be transmitted primarily horizontally upon its host's death (Saffo & Davis, 1982).

Our results provide some predictions that might be possible to compare with experimental or natural populations. In particular, it would be interesting to see if existing or experimentally evolved microbial symbioses show the same correlation between fecundity benefits and vertical/pseudovertical transmission. Experimental systems in which pseudovertical transmission is created by spatial structure would be particularly apt here, as our model suggests that the evolution of fecundity-increasing microbes but not lifespan-increasing ones might increase with increasing spatial structure.

However, the fact that hosts in our model did not evolve in a way that favoured the evolution of beneficial symbionts raises some questions that extensions to the model could be used to investigate. For example, allowing diminishing returns in symbiont benefits might alter the selective pressures on hosts to increase symbiont release during their lifetime. The model could also be expanded to allow different kinds of spatial structure, which might also affect the parent–offspring correlation and thus the benefits to hosts of symbiont release. In addition, research to understand how correlation between different modes of symbiont release affects host evolution and whether differences in symbionts between hosts can ever alter the course of host evolution would be helpful for better understanding host-symbiont coevolution and in narrowing down the conditions that might permit holobiont evolution without vertical transmission. Finally, within our current model or, more likely, a similar one that does not require numerical integration, it would be helpful to find expressions for the boundaries of parameter space that select for and against beneficial symbionts. This would give useful information about exactly how environmental and host traits interact to set the selective landscape for symbionts.

Our model provides information about the conditions under which the evolution of the holobiont as a whole may even be possible. Our results suggest that the conditions for holobiont evolution are somewhat constrained—horizontal transmission is not enough, alone, to select for fecundity benefits, and some sort of correlation between host fecundity and symbiont fitness must also be present. The fact that hosts in our model do not evolve to favour beneficial symbionts suggests that the conditions for "holobiont-like" evolution are even further constrained, and future work is needed to identify the conditions necessary for hosts to evolve in ways that encourage the evolution of symbiont benefits.

Supplementary material

Supplementary material is available at *Journal of Evolutionary Biology* online.

Author contributions

Alexandra Brown (Conceptualization-Equal, Methodology-Equal, Software-Equal, Writing—original draft-Equal, Writing—review & editing-Equal), Britt Koskella (Conceptualization-Equal, Supervision-Equal, Writing—review & editing-Equal), and Mike Boots (Conceptualization-Equal, Funding acquisition-Equal, Methodology-Equal, Supervision-Equal, Writing—review & editing-Equal)

Funding

This publication is funded by the Gordon and Betty Moore Foundation through grant GBMF10578 to M.B.

Conflicts of interest

None declared.

References

Baker, A. C., & Cunning, R. (2015). Coral "bleaching" as a generalized stress response to environmental disturbance. In C. M. Woodley, C. A. Downs, A. W. Bruckner, J. W. Porter, & S. B. Galloway (Eds.), *Diseases of coral* (pp. 396–409). John Wiley & Sons, Inc. https://doi.org/10.1002/9781118828502.ch30

Bordenstein, S. R., & Theis, K. R. (2015). Host biology in light of the microbiome: Ten principles of holobionts and hologenomes. *PLOS Biology*, *13*(8), e1002226. https://doi.org/10.1371/journal.pbio.1002226

Brännström, Å., Johansson, J., & von Festenberg, N. (2013). The hitchhiker's guide to adaptive dynamics. *Games*, *4*(3), 304–328. https://doi.org/10.3390/g4030304

Brown, A. L., Casarez, G. A., & Moeller, H. V. (2023). Acquired phototrophy as an evolutionary path to mixotrophy. *The American Naturalist*, *202*(4), 458–470. https://doi.org/10.1086/725918

Bull, J., & Rice, W. (1991). Distinguishing mechanisms for the evolution of co-operation. *Journal of Theoretical Biology*, *149*(1), 63–74. https://doi.org/10.1016/S0022-5193(05)80072-4

Connor, R. C. (1995). The benefits of mutualism: A conceptual framework. *Biological Reviews*, *70*(3), 427–457. https://doi.org/10.1111/j.1469-185X.1995.tb01196.x

Douglas, A. E., & Werren, J. H. (2016). Holes in the hologenome: Why host-microbe symbioses are not holobionts. *mBio*, *7*(2), e02099-15. https://doi.org/10.1128/mBio.02099-15

Hara, A., & Iwasa, Y. (2019). Coupled dynamics of intestinal microbiome and immune system—A mathematical study. *Journal of Theoretical Biology*, *464*, 9–20. https://doi.org/10.1016/j.jtbi.2018.12.021

Koskella, B., & Bergelson, J. (2020). The study of host–microbiome (co)evolution across levels of selection. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *375*(1808), 20190604. https://doi.org/10.1098/rstb.2019.0604

Legrand, T. P., Wynne, J. W., Weyrich, L. S., & Oxley, A. P. (2020). A microbial sea of possibilities: Current knowledge and prospects for an improved understanding of the fish microbiome. *Reviews in Aquaculture*, *12*(2), 1101–1134. https://doi.org/10.1111/raq.12375

Lehmann, L. (2008). The adaptive dynamics of niche constructing traits in spatially subdivided populations: Evolving posthumous extended phenotypes. *Evolution*, *62*(3), 549–566. https://doi.org/10.1111/j.1558-5646.2007.00291.x

Lloyd, E. A., & Wade, M. J. (2019). Criteria for holobionts from community genetics. *Biological Theory*, *14*(3), 151–170. https://doi.org/10.1007/s13752-019-00322-w

Mao-Jones, J., Ritchie, K. B., Jones, L. E., & Ellner, S. P. (2010). How microbial community composition regulates coral disease development. *PLoS Biology*, *8*(3), e1000345. https://doi.org/10.1371/journal.pbio.1000345

Margulis, L. (1991). Symbiogenesis and symbionticism. In L. Margulis & R. Fester (Eds.), *Symbiosis as a source of evolutionary innovation: Speciation and morphogenesis* (pp. 1–14). MIT Press.

Merrifield, D. L., & Rodiles, A. (2015). The fish microbiome and its interactions with mucosal tissues. In B. H. Beck, & E. Peatman (Eds.), *Mucosal health in aquaculture* (pp. 273–295). Academic Press. https://doi.org/10.1016/B978-0-12-417186-2.00010-8

Moran, N. A., & Sloan, D. B. (2015). The hologenome concept: Helpful or hollow? *PLOS Biology*, *13*(12), e1002311. https://doi.org/10.1371/journal.pbio.1002311

Morrow, K. M., Muller, E., & Lesser, M. P. (2018). How does the coral microbiome cause, respond to, or modulate the bleaching process? In M. J. H. van Oppen, & J. M. Lough (Eds.), *Coral bleaching: Patterns, processes, causes and consequences* (pp. 153–188, Vol. 233). Springer International Publishing. https://doi.org/10.1007/978-3-319-75393-5_7

Rosenberg, E., Sharon, G., Atad, I., & Zilber-Rosenberg, I. (2010). The evolution of animals and plants via symbiosis with microorganisms. *Environmental Microbiology Reports*, *2*(4), 500–506. https://doi.org/10.1111/j.1758-2229.2010.00177.x

Roughgarden, J. (2020). Holobiont evolution: Mathematical model with vertical vs. horizontal microbiome transmission. *Philosophy, Theory, and Practice in Biology*, *12*(2). https://doi.org/10.3998/ptpbio.16039257.0012.002

Roughgarden, J. (2023). Holobiont evolution: Population theory for the hologenome. *The American Naturalist*, *201*(6), 763–788. https://doi.org/10.1086/723782

Roughgarden, J., Gilbert, S. F., Rosenberg, E., Zilber-Rosenberg, I., & Lloyd, E. A. (2018). Holobionts as units of selection and a model of their population dynamics and evolution. *Biological Theory*, *13*(1), 44–65. https://doi.org/10.1007/s13752-017-0287-1

Sachs, J. L., Mueller, U. G., Wilcox, T. P., & Bull, J. J. (2004). The evolution of cooperation. *The Quarterly Review of Biology*, *79*(2), 135–160. https://doi.org/10.1086/383541

Saffo, M. B., & Davis, W. L. (1982). Modes of infection of the ascidian *Molgula manhattensis* by its endosymbiont *Nephromyces* Giard. *The Biological Bulletin*, *162*(1), 105–112. https://doi.org/10.2307/1540974

Sakal, T. (2023). *A mathematical theory of multilevel selection and holobiont evolution* (Publication No. 30694206) [Doctoral dissertation, University of California, Santa Barbara]. eScholarship. https://escholarship.org/uc/item/45w1f70z

Silverstein, R. N., Cunning, R., & Baker, A. C. (2017). Tenacious D: *Symbiodinium* in clade D remain in reef corals at both high and low temperature extremes despite impairment. *The Journal of Experimental Biology*, *220*(7), 1192–1196. https://doi.org/10.1242/jeb.148239

Soberon, J. M., & Martinez del Rio, C. (1981). The dynamics of a plant-pollinator interaction. *Journal of Theoretical Biology*, *91*(2), 363–378. https://doi.org/10.1016/0022-5193(81)\protect\penalty-\@M90238-1

Talwar, C., Nagar, S., Lal, R., & Negi, R. K. (2018). Fish gut microbiome: Current approaches and future perspectives. *Indian Journal of Microbiology*, *58*(4), 397–414. https://doi.org/10.1007/s12088-018-0760-y

Theis, K. R., Dheilly, N. M., Klassen, J. L., Brucker, R. M., Baines, J. F., Bosch, T. C. G., Cryan, J. F., Gilbert, S. F., Goodnight, C. J., Lloyd, E. A., Sapp, J., Vandenkoornhuyse, P., Zilber-Rosenberg, I., Rosenberg, E., & Bordenstein, S. R. (2016). Getting the hologenome concept right: An eco-evolutionary framework for hosts and their microbiomes. *mSystems*, *1*(2), e00028–16. https://doi.org/10.1128/mSystems.00028-16 van Oppen, M. J. H., & Blackall, L. L. (2019). Coral microbiome dynamics, functions and design in a changing world. *Nature Reviews Microbiology*, *17*(9), 557–567. https://doi.org/10.1038/s41579-019-0223-4

West, S. A., Kiers, E. T., Pen, I., & Denison, R. F. (2002). Sanctions and mutualism stability: When should less beneficial mutualists be tolerated? *Journal of Evolutionary Biology*, *15*(5), 830–837. https://doi.org/10.1046/j.1420-9101.2002.00441.x

Wilkinson, D. M. (1997). The role of seed dispersal in the evolution of mycorrhizae. *Oikos*, *78*(2), 394–396. https://doi.org/10.2307/3546308

Wolfram Research, Inc. (2021). *Mathematica, version 13.0*. Wolfram Research, Inc.

Zilber-Rosenberg, I., & Rosenberg, E. (2008). Role of microorganisms in the evolution of animals and plants: The hologenome theory of evolution. *FEMS Microbiology Reviews*, *32*(5), 723–735. https://doi.org/10.1111/j.1574-6976.2008.00123.x