Holobiont Evolution: Population Theory for the Hologenome

Joan Roughgarden[1]

April 7, 2022

Abstract

This article develops mathematical theory for the population dynamics of microbiomes with their hosts and theory for microbiome evolution caused by holobiont selection.

A holobiont is the union of a host with its microbiome. Holobiont selection is natural selection on the combined host and microbiome as a unit. The hologenome is the union of genes in the microbiome with genes in the host nucleus. The deep integration within many holobionts of microbiomes with their hosts extends to development, metabolism, physiology and behavior. The kind of eco-evolutionary processes producing this integration is unclear.

For a microbe to join a host's microbiome, its population-dynamic parameters must sufficiently align with the host's population-dynamic parameters. Accordingly, a microbe might be excluded from the microbiome, might enter the microbiome in a way leading to its subsequent gradual shedding from the holobiont, might join the microbiome and coexist with the host, or might, for a deleterious microbe, enter the microbiome culminating in the extinction of both it and the host.

A horizontally transmitted microbiome constitutes a genetic system that features "collective inheritance" capable of underwriting Darwinian descent with modification. The microbial source pool in the environment is the counterpart for the microbiome of the gamete pool for the nuclear genome. Poisson sampling of the microbial source pool is the counterpart of binomial sampling of the gamete pool. However, holobiont selection on the microbiome does not lead to a counterpart of the Hardy-Weinberg Law, a concept of selective neutrality, or directional selection that produces fixation of the microbial gene with the highest fitness. The effectiveness of holobiont selection on microbial genes is limited compared to the effectiveness of natural selection on nuclear genes. Nonetheless, holobiont selection can affect the composition of the microbiome by permitting the coexistence of a competitively inferior microbial strain with a superior strain in a colonization-extinction equilibrium and by permitting a polymorphism between altruistic and selfish microbial strains.

An altruistic microbe might strike an optimal balance between lowering within-host fitness to increase holobiont fitness. However, altruistic microbes are replaced by otherwise identical microbes that contribute nothing to holobiont fitness. This replacement can be reversed by hosts that discriminate against colonization by selfish microbes—likely brought about by hosts making antibodies against selfish microbes. This discrimination leads to a process of microbial species sorting orchestrated by the host immune system. Holobiont assembly with host-orchestrated species sorting (HOSS) followed by microbial competition, rather than co-evolution or multi-level selection, is the likely cause of host-microbiome integration.

[1]Hawaii Institute of Marine Biology, University of Hawaii and Department of Biology, Stanford University

1 Introduction

The combination of a host with its microbiome has been termed a ”holobiont” (Margulis 1991). A “microbiome” is an “ecological community of commensal, symbiotic, and pathogenic microorganisms” that shares the “body space” of a host (Lederberg and McCray 2001). The union of the host nuclear genes with the genes from all the microbes in its microbiome is a ”hologenome” (Zilber-Rosenberg and Rosenberg 2008). The configuration of the hologenome in a particular holobiont is its ”hologenotype” (Roughgarden 2020). “Holobiont selection” is natural selection on holobionts wherein a holobiont’s survival and/or fecundity depends on the phenotype brought about by its hologenotype. In other words, in holobiont selection, a holobiont’s fitness depends on the composition of both its microbiome and the host nuclear genes.

To a first approximation, the nuclear genome in a species has a set number of loci distributed across a set number of chromosomes together with a set number of alleles at each locus determined by the ploidy. In contrast, the hologenome is relatively extensible in the sense that the number of different strains of microbes in a host and their abundances can vary among individuals and through time. *A priori*, it is far from clear how microbial genes could contribute to holobiont evolution because hosts typically obtain some or all of their microbes from source pools in the environment. The holobiont traits brought about by microbial genes might be thought of as acquired rather than inherited and thus not eligible for evolutionary change by natural selection.

Yet, studies reveal astonishing integration between microbiomes and their hosts extending to development, metabolism, physiology and behavior (Gilbert *et al.* 2012, McFall-Ngai *et al.* 2013, Bordenstein and Theis 2015, Theis *et al.* 2016). A Google watch on the term “holobiont” reveals that new peer-reviewed papers documenting integrated microbiome-host interactions appear every day for groups ranging from sponges, corals, various other marine invertebrates, as well as terrestrial invertebrates and vertebrates. The scope of the microbiome-host interaction is enormous. Some population process must have produced the ubiquitous integration that occurs between microbiomes and their hosts. How to explain this integration is one of the principal objectives of this paper.

This paper develops mathematical theory for how, and to what extent, a host’s microbiome can form a genetic system that contributes to the joint evolution of microbiome and host. A holobiont contains two genetic systems that together comprise the hologenome—the microbial genes and the nuclear genes. This paper focuses specifically on the microbiome as a genetic system because this component of holobiont inheritance is more conceptually problematic than nuclear inheritance.

The inheritance of the microbiome is tied to the mode of microbial transmission—horizontal or vertical. Vertical transmission of microbes invites analogy with the transmission of nuclear genes. It is facilitated by mechanisms such as the acquisition of a maternal microbiome by

embryos as they pass through the birth canal and the acquisition of a microbiome by chicks incubating in a nest. Many such mechanisms have been described. Moreover, many models have already been developed using an assumption of vertical transmission or of combined vertical/horizontal transmission (Lipsitch *et al.* 1996, Shapiro and Turner 2014, Foster *et al.* 2017, Hurst 2017, Roughgarden 2017, Zeng *et al.* 2017, Osmanovic *et al.* 2018, Roughgarden *et al.* 2018, van Vilet and Doebeli 2019, Roughgarden 2020, Bruijning *et al.* 2021, Obeng *et al.* 2021).

Still, horizontal transmission appears to be the generic mode of microbe transmission, with vertical transmission being a special case. For example, in a few species of corals the zooxanthellae are transmitted vertically in the coral's gametes (Hirose and Hidaka 2006) and in a few brooding species, planulae larvae are directly released from the coral polyps, possibly also allowing for vertical transmission of zooxanthellae and other microbes acquired during the brooding (Atoda 1947, Harrison and Wallace 1990, Prasetia *et al.* 2017). But the vast majority of zooxanthellae are acquired from the open sea water surrounding the coral (Babcock *et al.* 1986, Trench 1993). Even terrestrial groups may offer less vertical transmission than might be expected. In a live-bearing cockroach, researchers found only one component of the microbiome to be transmitted vertically, the rest horizontally (Jennings 2019). Therefore, in the theoretical development that follows, horizontal transmission of the microbiome will be assumed, with further development of the vertical transmission case left to the future should the need arise.

Although this paper's focus on horizontal transmission is motivated by the biology of holobionts, the reader is invited to consider the topic of holobiont evolution more abstractly. Fancifully, imagine the Perseverance Rover presently exploring the surface of Mars actually finds life. Presumably extra-terrestrial life, like that on earth, evolves. But there is no reason to suppose that the inheritance system for life on Mars is Mendelian. Instead, it might consist of a system of gene-containing particles that are contributed to a source pool and then reacquired each generation, not unlike our earthly microbiomes. Evolution does not require Mendelian inheritance *per se*. It is conceptually interesting to explore the variety of inheritance systems that can support Darwinian evolution—a theory that was formulated before Mendel and is logically independent of it.

Horizontal transmission brings about a kind of inheritance of its own, "collective inheritance," as distinct from the "lineal inheritance" of a vertically transmitted microbiome, as illustrated in Figure 1. In collective inheritance, each offspring's microbiome is assembled from the collected microbiomes of parents who have favored by holobiont selection. In lineal inheritance, each offspring's microbiome is obtained directly from their own parents who have been favored by holobiont selection. Either way, the next generation is enriched with the microbes from the selected parents. Collective inheritance is perfectly Darwinian in the sense that it supports evolutionary descent through modification, just as lineal inheritance does. However, collective inheritance is

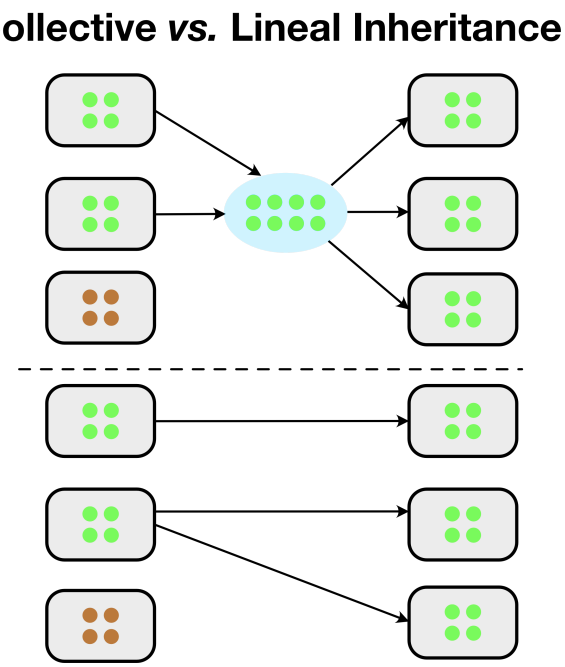

Figure 1: *Top panel illustrates collective inheritance as occurs with horizontal microbe transmission and bottom panel illustrates lineal inheritance as occurs with vertical microbe transmission. In both cases the green microbes are selected and the brown ones are not. With collective inheritance the selected parents contribute their microbes to a collective from which the microbes are distributed into the juvenile hosts. With lineal inheritance the juvenile hosts receive their microbes directly from their own selected parents. Either way, the next generation consists of hosts whose microbiomes reflect the selection process from the preceding generation. Collective inheritance and lineal inheritance both underwrite Darwinian evolutionary descent through modification. However, only lineal descent is consistent with Neo-Darwinism.*

not consistent with Neo-Darwinism which relies on Mendelian inheritance (Wright 1931).

Skepticism concerning the possibility of evolution *via* holobiont selection with horizontally transmitted microbiomes flows from a mistaken assumption that the mode of inheritance must be lineal for evolution by natural selection to proceed. For example, Lewontin (1970) famously wrote that evolution by natural selection requires three conditions: (1) phenotypic variation, (2) differential fitness, and (3) heritable fitness. The third requirement is stated too narrowly. The third requirement should state that the offspring fitness must correlate with the fitness of the *selected* parents. That is, the offspring must resemble the selected parents regardless of whether the parents they resemble are their own immediate parents.

Holobiont selection might be considered a form of multilevel selection. However, it does not accord with a well-known distinction between multilevel selection 1 (MLS1) and multilevel

selection 2 (MLS2) as diagrammed in Figure 2. (Mayo and Gilinsky 1987, Damuth and Heisler 1988, Okasha 2006).

According to Okasha (2006, 58), for MLS2 "it is essential that the collectives reproduce in the ordinary sense, that is, they 'make more' collectives; otherwise, determining parent-offspring relations at the collective level will not exist." In contrast, "the role of the collectives in MLS1 is to generate a population structure for the particles, which affects their fitnesses. For MLS1 to produce sustained evolutionary consequences, collectives must 'reappear' regularly down the generations, but there is no reason why the collectives themselves must stand in parent-offspring relations." A "collective" in MLS1 is a recurring feature in the environment, possibly inanimate, that confines organisms into a social group. Indeed, Maynard Smith (1964) developed a model for MLS1 that referred to recurring haystacks in farmers' fields as collectives within which mice (particles) can interact and evolve.

A holobiont is similar to a "collective" in Okasha's terminology. If the microbiomes are vertically transmitted, then parent-offspring relations exist among the holobionts of successive generations as required by the definition of MLS2. Therefore, holobiont selection with a vertically transmitted microbiome is similar to MLS2. However, if the microbiomes are horizontally transmitted, holobiont selection is not similar to MLS1 because the host is not a recurring feature of the environment but is an evolving organism itself. Therefore, holobiont selection with horizontal microbiome transmission is similar to a third type of multilevel selection, MLS3, as diagrammed in Figure 2. MLS3 is a combination of MLS1 and MLS2. Still, holobiont selection with either vertical or horizontal transmission is not *exactly* the same as either MLS2 or MLS3 respectively, because the definitions of MLS do not consider the possibility of density dependence within the collectives. In a holobiont, the collectives are finite in size so that the number of particles can fit in each is limited. Hence, in holobiont selection, unlike in MLS, the collectives have a carrying capacity for their particles. The density dependence is important during the analysis of conditions for one microbe strain to competitively displace another.

Coevolution, as with flowers and their pollinators, differs from both MLS and holobiont selection. In coevolution the fitnesses in species depend on the gene frequencies and population sizes of other species at the same organizational level. (Roughgarden 1983, Dieckmann and Law 1996; Carmona *et. al.* 2015). The coupled equations for coevolution differ from coupled equations for a host with its microbes because host and microbes occupy different organizational levels.

This research contribution is organized as a single narrative together with a large mathematical appendix provided as supplementary material. The mathematical appendix is self-contained and can be read by itself. The narrative—which is this article, offers a verbal account of what is shown in the appendix. This two-part organization is intended to make the research accessible to both mathematical and non-mathematical readers.

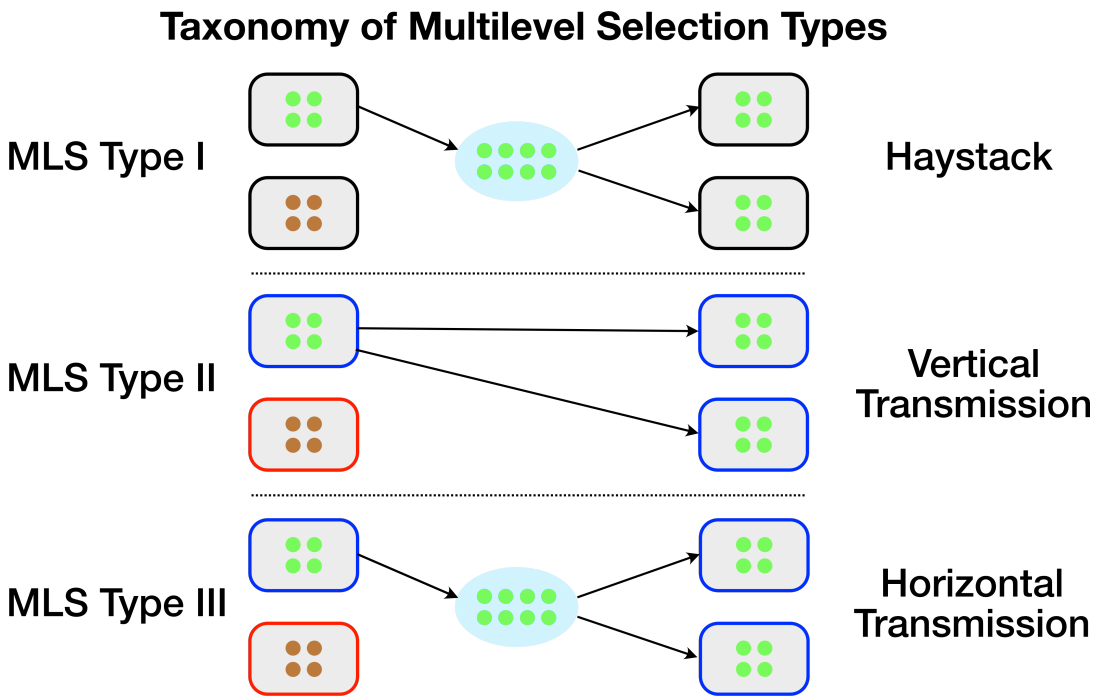

Figure 2: *Taxonomy of Multilevel Selection Types. In multilevel selection 1 (MLS1), the environment provides locations indicated as black rectangles with rounded corners. In these locations the organisms undergo natural selection favoring selfish genes, in brown. After selection, offspring from the locations are released into a common pool which are then redistributed to the locations for the next generation. The locations with many altruistic genes, in green, deliver more offspring into the common pool than locations dominated by selfish genes. If the between-location productivity advantage of altruistic genes exceeds their within-location disadvantage to selfish genes, then altruistic genes evolve overall. Thus, the organisms evolve but the locations themselves do not because they are physical features of the environment. (Maynard Smith in 1964 referred to such locations as haystacks.) In multilevel selection 2 (MLS2), small organisms (microbes) are contained in larger organisms (hosts), resulting in a symbiotic pair of two species. While in the host, the microbes undergo natural selection favoring brown selfish microbes. However, hosts containing green altruistic microbes, shown as blue rectangles with rounded corners, survive and reproduce with their microbes better than hosts with brown selfish genes, shown as orange rectangles with rounded corners. The symbiotic pair is inherited as a unit from one generation to the next. Multilevel selection 3 (MLS3) is a combination of MLS1 and MLS2. MLS3 shares with MLS1 that microbes are released into a common pool from which they are redistributed into hosts for the next generation. MLS3 shares with MLS2 that hosts themselves evolve along with the microbes. Holobiont selection with vertical or horizontal transmission is similar to MLS2 or MLS3 respectively. However, holobiont selection differs from MLS by including density dependence within the hosts, leading to each host having a carrying capacity for the particles within it. The within-host density dependence is important when analyzing the conditions for one microbial strain to competitively displace another.*

2 Host with One Microbial Strain

Coexistence between a microbe and host is not a given. The existence of a holobiont presupposes a population-dynamic compatibility between the microbes and host. This section investigates the population dynamics of a host with a single-species microbiome. It discusses conditions for the coexistence of the host with its microbe. This population-dynamic analysis is first step prior to analyzing the evolution that results when the microbiome consists of two (or more) microbial strains.

2.1 Model

The model assumes that larval hosts are colonized at random by microbes according to a Poisson distribution, an assumption introduced in Roughgarden (2020). The Poisson distribution is widely used for random phenomena as disparate as the number of ticks per second by a Geiger counter, the number of blood cells in each well of a microscope slide, and even the number of phage in bacteria (Ellis and M. Delbrück 1939). The assumption of Poisson colonization implies that some larval hosts remain uncolonized by any microbes while others are colonized by one or more microbes.

The generation time for the microbes within a host is here assumed to be short relative to the generation time of the host. As a result, the microbes come to population-dynamic equilibrium within the host during each host generation. This assumption in turn implies that the initial condition for larval hosts colonized by one or more microbes is erased. That is, the number of microbes in a mature host is either 0, for larval hosts not colonized by any microbes, or k for all larval hosts colonized by one or more microbes because the microbes in all the colonized hosts come to the same within-host equilibrium abundance, k.

The assumption of a short microbial to host generation time allows results to be derived mathematically, unlike Roughgarden (2020). The earlier model relied solely on computer iteration of the equations describing how the holobiont population changes through time. Here, the outcome of the microbe-host interaction is derived analytically and then confirmed with computer iteration. The use of mathematical analysis allows a wider parameter space and more types of outcomes to be appreciated that is possible with computer iteration alone.

The Poisson distribution has one parameter, sometimes called the "Poisson density", often denoted as μ. Figure 3 illustrates the Poisson Distribution for several values of μ. If μ is low, then many larval hosts are left uncolonized whereas if μ is large almost all larvae are colonized.

The μ depends on the ratio of microbes in the microbial source pool to the number of larval hosts in the host source pool. Clearly, if there are few microbes per larval host, then μ is low and few hosts are colonized, and conversely if there are many microbes per host, then many hosts are

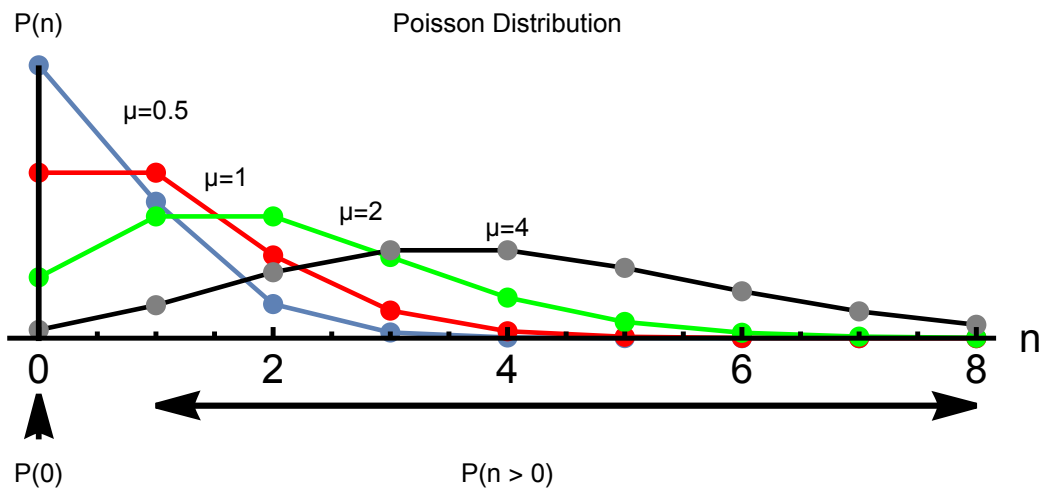

Figure 3: *Poisson Distribution for several values of μ, the Poisson density. The $P(n)$ is the probability that n microbes colonize a juvenile host. If $\mu < 1$, then the distribution peaks at $n = 0$. If $\mu > 1$, the distribution peaks at some $n > 0$. According to the formula for Poisson distribution, the probability that a host is not colonized by any microbes, $P(0)$, is $e^{-\mu}$. Therefore, the probability that a host is colonized by one or more hosts, $P(n > 0)$, is $1 - e^{-\mu}$. Microbial populations in the hosts colonized by one or more hosts all come to equilibrium at the same abundance, k, regardless of how many microbes each was colonized to begin with.*

colonized. The μ changes through time as the ratio of microbes to larval hosts change. Let $H(t)$ be the number of larvae in the host source pool and $G(t)$ the number of microbes in the microbial source pool at time t. The Poisson density parameter, $\mu(t)$, is then assumed to equal $d \times g(t)$, where $g(t)$ is the ratio of microbes to hosts in their source pools, $G(t)/H(t)$, and d is an important parameter, usually small, between 0 and 1. The earlier model did not use the parameter d, which in effect was assumed to equal 1. Here, the d assumes considerable importance.

A low d indicates a dilute microbial source pool relative to the host source pool, and a relatively high d indicates a dense microbial source pool relative to the host source pool. The d might arise from physical mixing processes in the water column that contains the microbial and host source pools. For example, a low d might arise if the host source pool is concentrated near one spot on the benthos while the microbial source pool is broadly distributed in the water column. The d might also be interpreted as a coefficient of transmission such as found in models of disease dynamics in epidemiology. For example, a low d might describe a microbe transmitted only through physical contact and a high d might describe a microbe transmitted through aerosols. Furthermore, the d might be interpreted as a recruitment coefficient such as found in marine-biology models whereby a high d indicates a high recruitment rate by microbes to empty

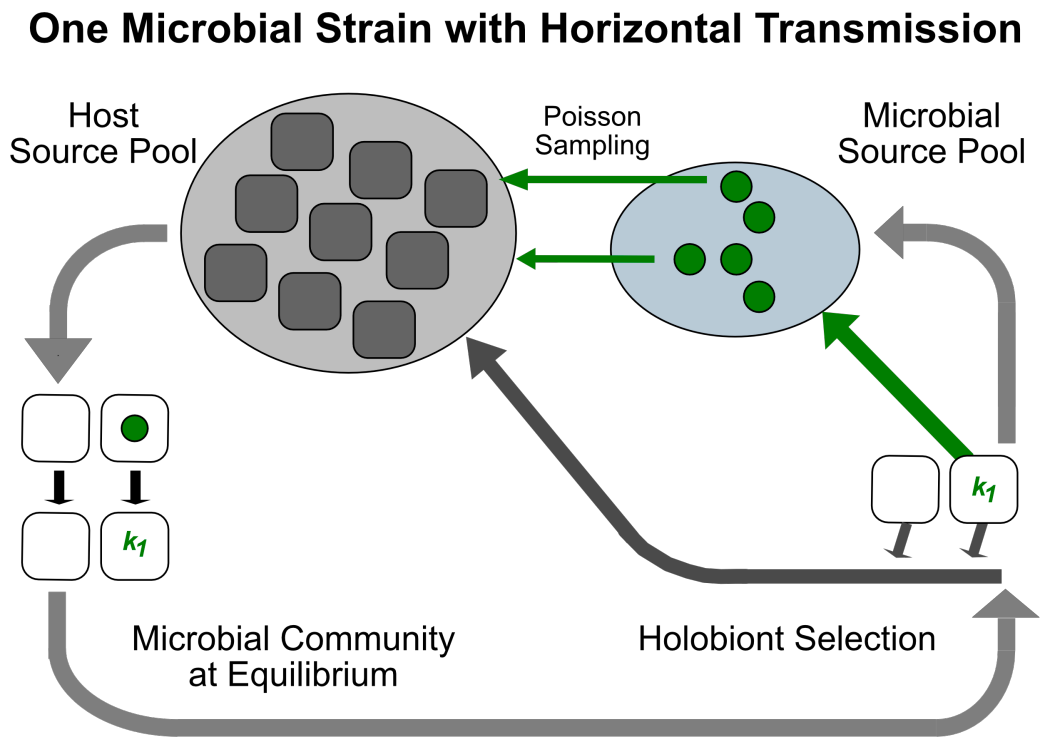

Figure 4: *Holobiont life cycle where microbiome consists of one strain (green circle). In each generation the microbiomes for the larval hosts are assembled by Poisson sampling from the microbial source pool. The microbiome comes to community equilibrium within each host generation. Then holobiont selection occurs whereby the holobionts contribute the next generation's larval hosts to the larval source pool and the next generation's microbes to the microbial source pool.*

hosts. The d may also be a host trait indicating the degree to which the host accepts or rejects the microbes, perhaps mediated through the host's immune system. Accordingly, a low d indicating a microbe strain rejected by the host and a high d a microbial strain whose colonization is allowed by the host. Depending on context, d may be called the dilution factor, the transmission parameter, or the colonization parameter.

The life cycle of a holobiont with a microbiome consisting of one horizontally transmitted microbial strain is diagrammed in Figure 4. The life cycle begins with $H(t)$ and $G(t)$ for the host and microbial source pools. Then the larval hosts are colonized by microbes according to a Poisson distribution as just discussed. After colonization, microbes proliferate within the juvenile hosts and come to an equilibrium abundance. Next, the holobiont fitness depends on whether the microbe abundance is 0 or k. Let W_0 be the holobiont fitness with an empty microbiome and W_1 be the holobiont fitness with a strain-1 microbial abundance of k. The W is interchangeably referred to as the holobiont fitness or as the host fitness depending on context. Finally, each holo-

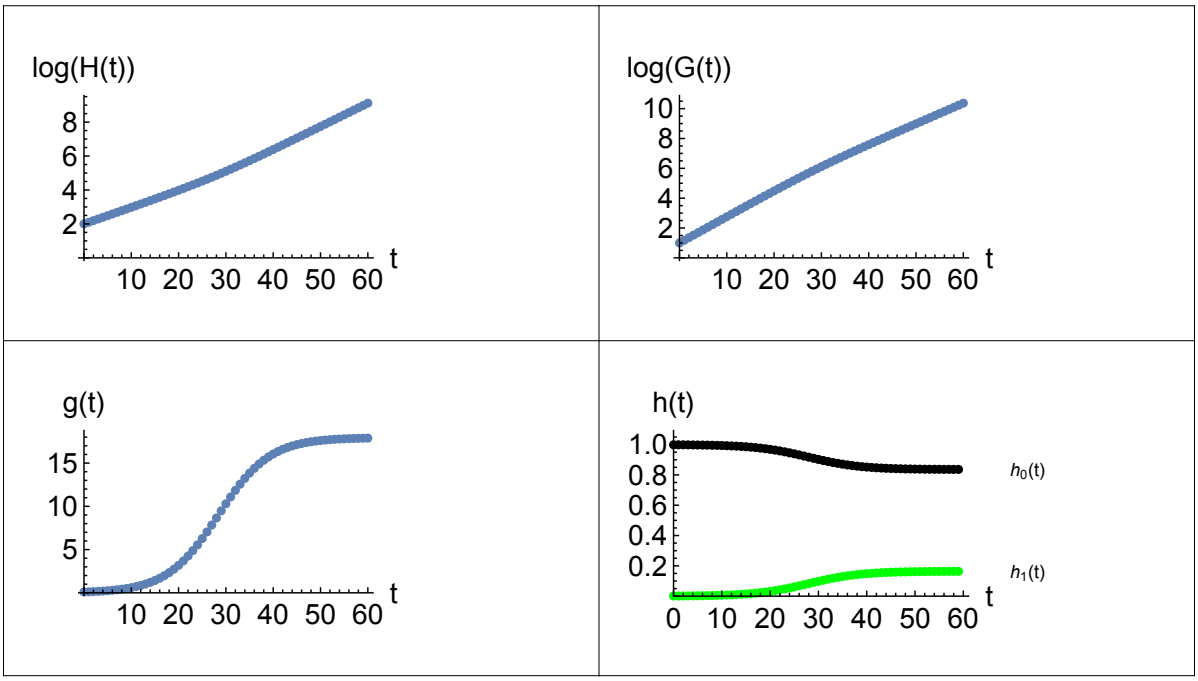

Figure 5: *Microbe-host coexistence. Top panels show trajectories of* $\log_{10} H(t)$*, and* $\log_{10} G(t)$ *through time for a beneficial microbe. The lower left panel shows,* $g(t)$*, number of microbes per host through time. The lower right panel shows the frequencies of holobionts with 0 microbes (top) and with k microbes (bottom) through time. Here,* $d = 0.01$*,* $W_0 = 5/4$*,* $W_1 = 2$*,* $k = 75$*,* $H(0) = 100$*,* $G(0) = 10$*, and* $t_{\max} = 60$*.*

biont contributes larval hosts to the host source pool based on W_0 or W_1 depending on whether the holobiont has microbes or not. That is, each holobiont without any microbes contributes W_0 larvae to the new host source pool and each holobiont with k microbes contributes W_1 larvae to the new host source pool. Furthermore, the holobionts with microbes in them also contribute to the microbial source pool based on both k *and* on W_1. That is, each holobiont with k microbes in it contributes $k \times W_1$ to the microbial source pool. The next go-around of the life cycle begins with these reconstituted larval host and microbial source pools.

Overall, the model contains only four parameters: k, which is the equilibrium number of microbes in the microbe-containing hosts; d, which is the colonization parameter; W_0, which is the fitness of holobionts without microbes; and W_1, which is the fitness of holobionts with k microbes in each.

For holobionts, host and microbial fitnesses have unusual properties. The host fitness, W, is a function of the *number* of microbes in it, either 0 or k. The microbe abundance is a *trait* from the standpoint of the host. But the microbe abundance is not a host trait in the usual sense because the host does not contain a gene for the microbe abundance as it does for other host traits such

as size, color etc. Instead, the microbe abundance is a variable controlled by the *microbe's* population dynamics. Still, selection on the host does wind up indirectly controlling the microbial abundance within it as though its microbial abundance were an ordinary trait because of "population dynamic feedback." If the microbe is beneficial, then the selection favoring hosts with large numbers of microbes increases the number of microbes in the microbial pool that in turn leads to a higher number of microbes colonizing the larval hosts in the next generation. Conversely, if the microbe is deleterious, the selection favoring hosts with low numbers of microbes decreases their number in the microbial pool, in turn leading to a lower number colonizing the next generation's host larvae. However, despite this population-dynamic feedback, selection on the host is single-level selection because the host fitness does not depend on microbe fitness, only microbe number.

In contrast, the microbe fitness *is* multilevel because a microbe's success at supplying the microbial pool for the next generation depends on *both* its within-host fitness, k, and the between-host fitness, W_1. To combine both these levels into one expression, the "microbe multilevel fitness per holobiont" for strain-1, w_1, is defined as $k \times W_1$. (Upper case pertains to hosts and lower case to microbes.) In ecological terminology, the microbes are undergoing "K-selection" within their hosts and "r-selection" between hosts.

To see what the model does, Figure 5 illustrates the output for an instance of a host with a beneficial microbe, $W_1 > W_0 > 1$. In this illustration, the parameters are arbitrarily set to $d = 0.01$, $W_0 = 5/4$, $W_1 = 2$, and $k = 75$. The figure shows first, that both $H(t)$ and $G(t)$ asymptotically approach geometric growth with a geometric growth factor each time step that computes to 1.373; second, that $g(t)$ asymptotically approaches an equilibrium indicating that 17.918 microbes exist in the microbial source pool for each empty host in the host source pool; and third, that the frequencies of holobionts with 0 microbes or k microbes asymptotically approach 0.836 and 0.164 respectively. The asymptotic Poisson density parameter, μ_o, is 0.179. Therefore, the average number of colonizing microbes per host, averaged over both the empty and non-empty hosts is itself 0.179. The average number of colonizing microbes in the non-empty hosts is 1.092. The hosts and their microbes asymptotically approach geometric growth because the host population dynamics has no density dependence. The only density dependence in the model is for the within-host microbial dynamics, not the between-host dynamics.

The mathematical appendix supplies equations that predict the asymptotic population growth rate for host and microbe, the equilibrium ratio of microbes to hosts in their source pools, and the asymptotic frequencies of the holobionts with 0 and k microbes.

2.2 Conditions for Microbe-Host Coexistence

Figure 5 illustrates a typical case of micro-host coexistence. However, this outcome is only one of several possibilities. To determine when a case such as Figure 5 occurs, the conditions for microbe-host coexistence must be determined.

Typically in models of population dynamics, the possibility of coexistence between two species is ascertained by seeing if each species can increase when it is rare while the other species is common. If both species can increase when rare while the other is common, one typically concludes that the species coexist. However, microbiome-host coexistence is unusual in that this familiar criterion does not hold.

The condition for the host to increase when the microbe is rare is simply $W_0 > 1$, *i.e.*, each host has to do better than simply replace itself in the absence of microbes—this is assumed to be true *apriori* because otherwise there would be no hosts to begin with.

In the mathematical appendix, the condition for the microbe to increase when rare into a population of empty hosts shown to be $d \times k \times W_1 > 1$. This condition is plausible because it means that a microbe must do better than to replace itself, taking into account both its within-host and between-host fitness as well as the colonization parameter. This condition is necessary for microbe-host coexistence, but surprisingly, this condition is not sufficient for microbe-host coexistence.

The appendix also shows that the condition for a microbe-host equilibrium ratio to exist is $d \times k \times W_1/W_0 > 1$. This expression is nearly the same as the condition for increase when rare from the preceding paragraph, except here W_1 is divided by W_0. Thus, whether the microbe can enter the holobiont population to begin with depends only on its own properties, d, k, and W_1. In contrast, whether microbes can coexist with the host *after* entry depends on the success of the hosts they are inhabiting *relative* to the success of the hosts they are not inhabiting. If the host without any microbes in it is able to grow by itself, $W_0 > 1$, then the condition for coexistence is more stringent than the condition for increase when rare because $W_1/W_0 < W_1$, which makes the condition for the microbe-host equilibrium ratio harder to satisfy than the condition for invasion when rare.

2.2.1 Host with Beneficial Microbes

Consider an explicit illustration of how the condition for increase when rare interacts with the condition for coexistence. As in Figure 5 previously, again assume $d = 0.01$, $W_0 = 5/4$, and $W_1 = 2$. Now focus on the within-host microbial abundance, k. By the condition for increase when rare, k must satisfy $0.01 \times k \times 2 > 1$. So, if $k > 1/(0.01 \times 2) = 50$, then the microbes can increase when rare into a population of empty hosts. However, by the coexistence condition, k must satisfy $0.01 \times k \times 2/(5/4) > 1$. So, if $k > 1/(0.01 \times 2/(5/4) = 62.5$, then the microbes can

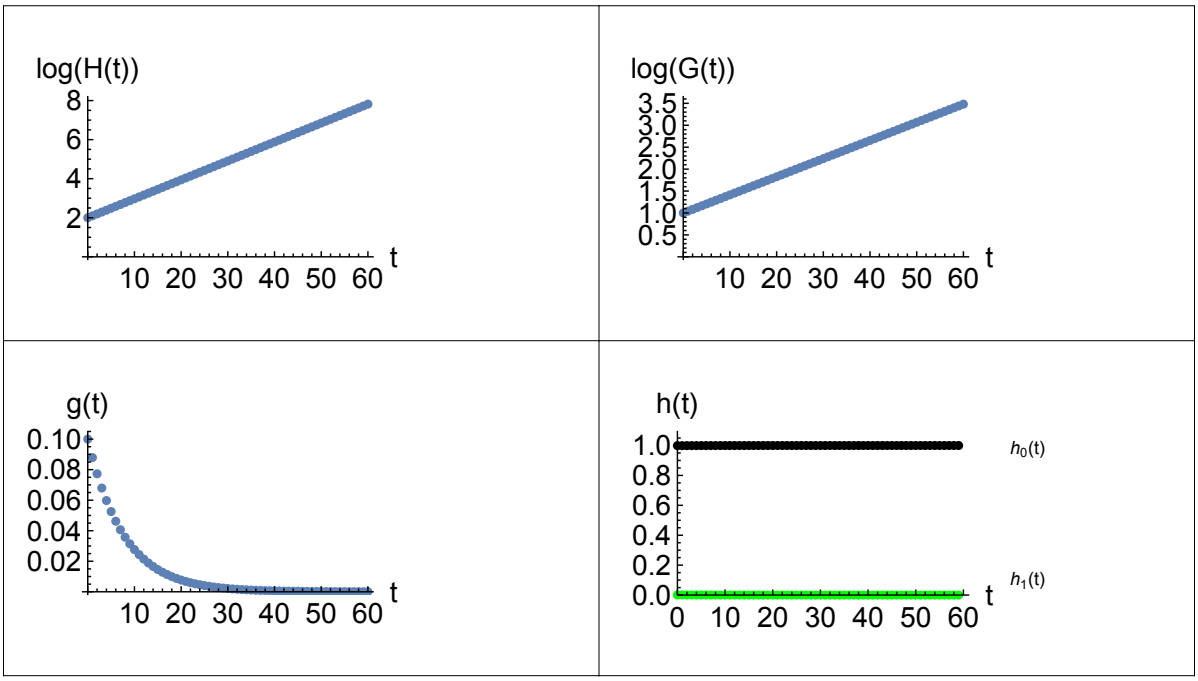

Figure 6: *Microbe shedding. Top panels show trajectories of* $\log_{10} H(t)$*, and* $\log_{10} G(t)$ *through time for a beneficial microbe. The lower left panel shows,* $g(t)$*, the number of microbes per host through time. The lower right panel shows the frequencies of holobionts with 0 microbes (top) and with k microbes (bottom) through time. Here,* $d = 0.01$*,* $W_0 = 5/4$*,* $W_1 = 2$*,* $k = 55$*,* $H(0) = 100$*,* $G(0) = 10$*, and* $t_{\max} = 60$*.*

coexist with their hosts. Recall in Figure 5 that k is 75—larger than both the minimum necessary to increase when rare (50) and the condition for coexistence (62.5). Thus, Figure 5, illustrated successful microbe-host coexistence.

But what if k is between 50 and 62.5? Then the microbes can increase when rare but cannot coexist with their hosts. In this situation, microbe production does not keep up with host production. Although the microbes are not excluded from the holobiont population and continue to maintain a presence in the microbial source pool, the fraction of holobionts that are colonized by microbes drops to zero because the quantity of empty hosts increases much faster than the quantity of microbes available to colonize them. This situation is termed "microbial shedding". Figure 6 illustrates microbe shedding for $k = 55$, a number between 50 and 62.5.

Figure 7 summarizes the possibilities. If $k < 50$, then the microbes cannot enter the holobiont population. If k is between 50 and 62.5, then microbe shedding occurs. If $k > 62.5$, then microbes and hosts coexist. The figure also shows the curve relating the equilibrium microbe to host ratio to k. The curve equals zero when $k = 62.5$ and increases as k increases because increasing the number of microbes in each colonized host in turn increases the number of microbes released into the microbial source pool.

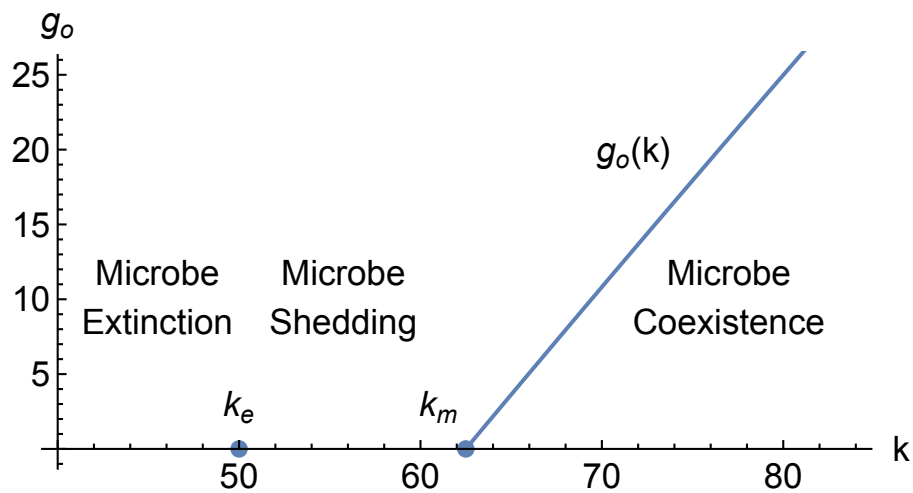

Figure 7: *Invasion-when-rare, microbial shedding, and coexistence conditions for beneficial microbe. The k_e is the minimum within-host abundance for the microbe to invade when rare, k_m is the minimum within-host abundance for the microbe and host to come to an equilibrium microbe-host ratio, and $g_o(k)$ is the equilibrium microbe-host ratio as a function of k. Here, $d = 0.01$, $W_0 = 5/4$, $W_1 = 2$, $k_e = 50$, $k_m = 62.5$.*

2.2.2 Host with Deleterious Microbes

If the microbes are deleterious, then $W_0 > 1 > W_1$. Suppose that deleterious microbes are introduced to a holobiont population. Initially, the holobiont population can grow because most holobionts lack any deleterious microbes and their fitness without microbes, W_0, is > 1 by assumption. But as the microbes increase, the average growth rate of the holobiont population declines as more holobionts harbor the deleterious microbes. If the microbes increase to the point where the average holobiont fitness drops below 1, then the holobiont population declines. This situation is one where the microbe and host coexist in the sense that the condition for a microbe-host equilibrium ratio to exist is satisfied, but the microbe to host ratio is large enough to drive the average holobiont fitness below 1. In this situation, the microbe enters the host population, then both microbe and host coexist in a stable ratio, and finally both decline together to extinction. This is illustrated in Figure 8.

As an example, suppose the fitness of a microbe containing holobionts is dropped from $W_1 = 2$ down to $W_1 = 3/4$. The other parameters from the preceding examples remain the same, $d = 0.01$ and $W_0 = 5/4$. Figure 9 summarizes the possibilities for microbe-host coexistence with this deleterious microbe for various values of k. By the condition for increase when rare, k must satisfy $0.01 \times k \times 3/4 > 1$. So, if $k > 1/(0.01 \times (3/4)) = 133.333$, then the microbes can increase when rare into a population of empty hosts. By the coexistence condition, k must satisfy

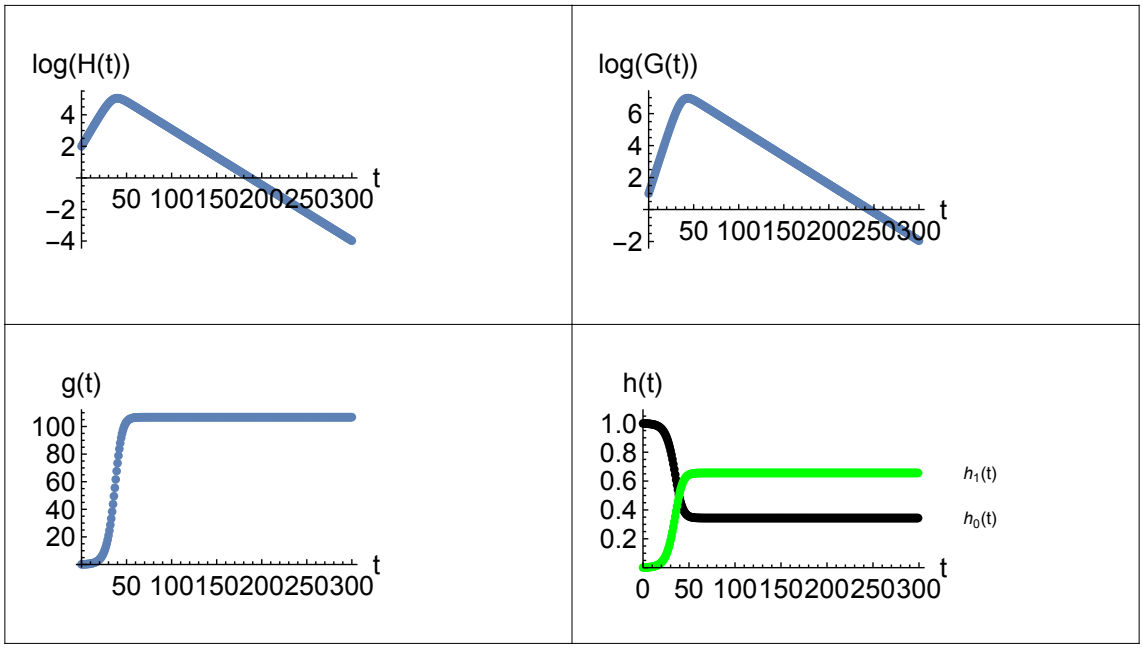

Figure 8: *Microbe-host joint extinction. Top panels show trajectories of* $\log_{10} H(t)$, *and* $\log_{10} G(t)$ *through time for a deleterious microbe. The lower left panel shows,* $g(t)$, *the number of microbes per host through time. The lower right panel shows the frequencies of holobionts with 0 microbes (top) and with* k *microbes (bottom) through time. Here,* $d = 0.01$, $W_0 = 5/4$, $W_1 = 3/4$, $k = 200$, $H(0) = 100$, $G(0) = 10$, *and* $t_{\max} = 300$.

$0.01 \times k \times (3/4)/(5/4) > 1$. So, if $k > 1/(0.01 \times (3/4)/(5/4)) = 166.667$, then the microbes can coexist with their hosts. In the appendix, the critical microbe to host ratio that makes the average holobiont fitness equal 1 is found numerically to equal 69.315. The microbe host ratio increases with k and is shown in Figure 9 as a curved line starting at 166.67 that rises intersecting the horizontal line for the critical microbe to host ratio. The intersection point is found numerically to occur at $k = 184.839$. In summary, if k is below 133.333, the deleterious microbes cannot enter the population; if k is between 133.333 and 166.667, the deleterious microbes can enter but their reproduction cannot keep up with that of the host, resulting in microbe shedding; if k is between 166.667 and 184.839, then the deleterious microbes and host coexist and both increase through time; and finally, if k is greater than 184.839, then the deleterious microbes and host coexist but both decrease through time resulting in their joint extinction. In the illustration of Figure 8, k is 200, which is larger than 184.839, and so joint microbe-host extinction results in accord with Figure 9.

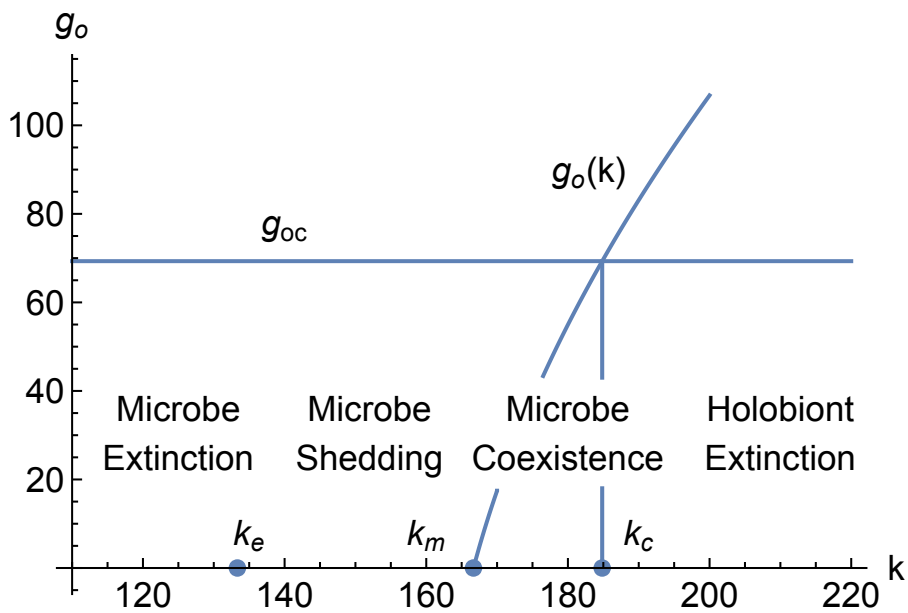

Figure 9: *Invasion-when-rare, microbial shedding, coexistence and extinction conditions for a deleterious microbe. The k_e is the minimum within-host abundance for the microbe to invade when rare, k_m is the minimum within-host abundance for the microbe and host to come to an equilibrium microbe-host ratio, k_c is the maximum within-host abundance for the microbe and host to avoid joint extinction, g_{oc} is the critical value of g_o for which the average holobiont fitness is 1, and $g_o(k)$ is the equilibrium microbe-host ratio as a function of k. Here, $d = 0.01$, $W_0 = 5/4$, $W_1 = 3/4$, $k_e = 133.333$, $k_m = 166.667$, $g_{oc} = 69.315$, and $k_c = 184.839$.*

3 Host with Two Microbial Strains

This section extends the model of a host with one microbial strain to include two strains. The presence of two strains provides hologenotypic variation among the holobionts upon which holobiont selection can operate.

3.1 Model

To set the stage, recall the setup for one locus with two alleles from textbook population genetics. The alleles are A_1 and A_2 with frequencies in the gamete pool of p_1 and $(1 - p_1)$, respectively. The three zygotic genotypes, A_1A_1, A_1A_2, and A_2A_2, are formed from binomial sampling of the gamete pool leading to the famous Hardy-Weinberg ratios of p_1^2, $2\,p_1\,(1 - p_1)$ and $(1 - p_1)^2$, respectively. That is, the A_1A_1 genotype is formed by drawing one A_1 gamete from the gamete pool followed by another A_1; the A_1A_2 genotype is formed by drawing an A_1 followed by an A_2 or the reverse order, leading to the factor of 2; and the A_2A_2 genotype is formed by drawing two

A_2 gametes in succession. In keeping with this paper's terminology, one might say instead that the A_1 and A_2 alleles "colonize" the empty zygotes from the gametic source pool according to binomial sampling. The "copy number" of the A_1 allele is 2 in a A_1A_1 homozygote and is 1 in a A_1A_2 heterozygote, and similarly for the copy number of A_2 in the A_2A_2 homozygote is 2 and is 1 in the A_1A_2 heterozygote.

Here, the microbes of each strain supply genes for the holobiont and comprise its hologenome. The abundance of a microbial strain is the copy number of the gene from that strain. The four hologenotypes consist of {0,0}, $\{k_1, 0\}$, $\{0, k_2\}$, and $\{n_1, n_2\}$ where k_1 and k_2 are the within-host equilibrium abundances of single-strain microbiomes consisting of strain-1 and of strain-2 respectively, and n_1 and n_2 are the within-host equilibrium abundances in a two-strain microbiome obtained from a within-host population-dynamic model such as the Lotka-Volterra competition equations.

The ratio of these four hologenotypes after colonization results from independent Poisson sampling of each microbial strain from the microbial source pool. Let the Poisson probability of a host being colonized by one or more microbes of strain-1 as P_1 and for strain-2 as P_2. Accordingly, the probability of empty hosts not being colonized by any microbes of strain-1 is $(1 - P_1)$ and the probability of not being colonized by any microbes of strain-2 is $(1 - P_2)$. Then the Poisson ratios for the four hologenotypes, {0,0}, $\{k_1, 0\}$, $\{0, k_2\}$, and $\{n_1, n_2\}$, are $(1 - P_1)\,(1 - P_2)$, $P_1\,(1 - P_2)$, $(1 - P_1)\,P_2$ and $P_1\,P_2$, respectively. In words, the {0,0} hologenotype frequency refers to larval hosts not colonized by either strain-1 or strain-2, the $\{k_1, 0\}$ hologenotype frequency refers to larval hosts colonized by strain-1 and not strain-2, the $\{0, k_2\}$ hologenotype frequency refers to larval hosts not colonized by strain-1 and colonized by strain-2, and the $\{n_1, n_2\}$ hologenotype frequency refers to larval hosts colonized by both strain-1 and strain-2.

The life cycle of a holobiont with a microbiome consisting of two horizontally transmitted microbial strains is diagrammed in Figure 10. The life cycle begins with the three state variables for the source pools, $H(t)$, $G_1(t)$ and $G_2(t)$ where $H(t)$ is number of empty hosts in the host pool at time t, and $G_1(t)$ and $G_2(t)$) are the number of microbes of strain-1 and strain-2 in the microbial pool at time t. The total number of microbes in the microbial source pool at time t, $G(t)$, is $G_1(t) + G_2(t)$. The ratio of strain-1 microbes to hosts in their source pools, $g_1(t)$, is $G_1(t)/H(t)$, and similarly $g_2(t)$ is $G_2(t)/H(t)$. The ratio of both microbe strains combined to hosts in their source pools, $g(t)$, is $G(t)/H(t)$. The frequency of strain-1 in the microbial pool at time t, $p_1(t)$ is $G_1(t)/G(t)$ and for strain-2, $p_2(t)$ is $G_2(t)/G(t)$. The Poisson density parameters for strain-1 at time t, $\mu_1(t)$, is $d_1 \times g_1(t)$ and for strain-2, $\mu_2(t)$ is $d_2 \times g_2(t)$, where d_1 is the dilution factor for strain-1 and d_2 is the dilution factor for strain-2.

The two strains independently colonize the empty hosts according to a Poisson distribution as just discussed. Once the juvenile hosts have been initially populated, the microbes proliferate

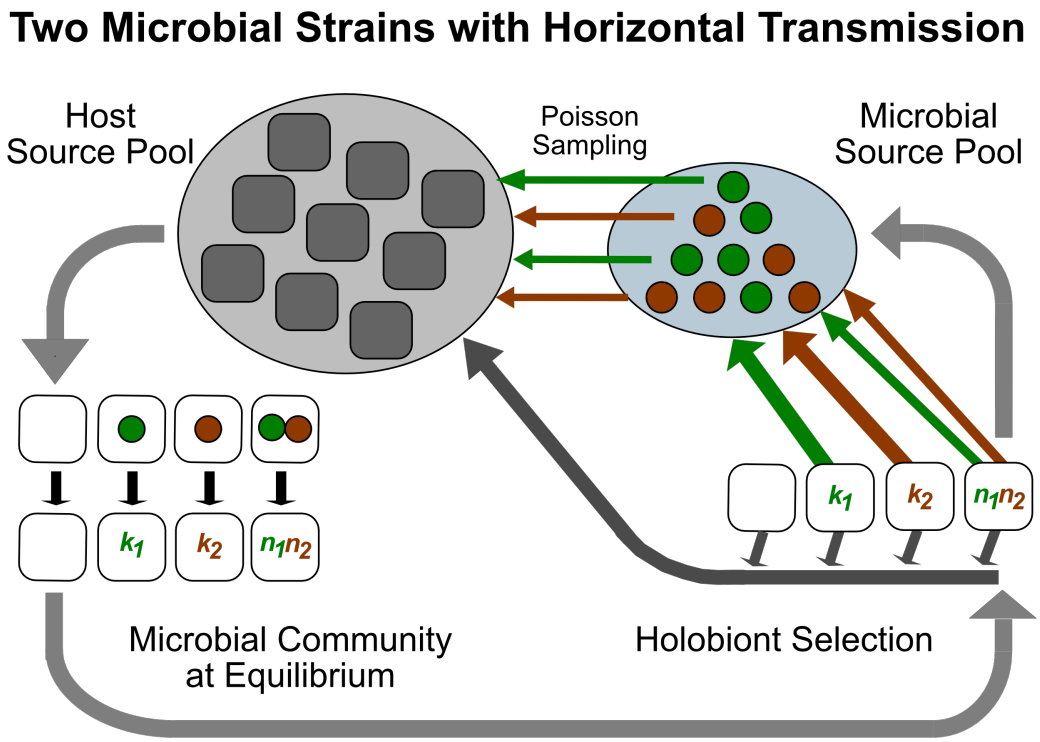

Figure 10: *Holobiont life cycle where microbiome consists of one or two microbial strains (green or brown circle). In each generation the microbiomes for the larval hosts are assembled by independent Poisson sampling of the two strains from the microbial source pool leading to four "hologenotypes". The microbiome comes to community equilibrium within each host generation. Then holobiont selection occurs whereby the holobionts contribute the next generation's larval hosts to the larval source pool and the next generation's microbes of both strains to the microbial source pool.*

within their hosts, coming to an equilibrium microbiome community. The process of attaining the population-dynamic equilibrium within the hosts erases the initial conditions with which the hosts were colonized. Hence, the equilibrium abundances of single-strain microbiomes consisting of only strain-1 or strain-2 are k_1 and k_2 respectively, regardless of the number of colonizing microbes. Similarly, the equilibrium abundances in a dual-strain microbiome consisting of strain-1 and strain-2 are n_1 and n_2 regardless of the number of colonizing microbes. The n_1 and n_2 are the equilibrium within-host microbe population sizes from a species-interaction model such as the Lotka-Volterra (LV) competition equations or other appropriate species-interaction model.

Next, the holobiont fitness depend on the hologenotype. Let the holobiont fitness of the hologenotypes, $(0,0)$, $(k_1,0)$, $(0,k_2)$ and (n_1,n_2) be W_0, W_1, W_2 and W_{12}, respectively. The holobionts release their empty juvenile hosts into the host source pool based on these fitnesses.

Turning now to the microbes, as before, the products of within-host microbe population sizes

and the holobiont fitnesses can be combined into measures of multilevel microbe fitness per holobiont. These measures represent simultaneous success in both within-holobiont K-selection and between-holobiont r-selection. The multilevel microbe fitnesses per holobiont are: $w_{1,1}$ is $k_1 \times W_1$, $w_{1,12}$ is $n_1 \times W_{12}$, $w_{2,12}$ is $n_2 \times W_{12}$ and $w_{2,2}$ is $k_2 \times W_2$. These coefficients refer to the multilevel success of a specific microbial strain within a specific microbiome. Thus, $w_{1,1}$ is the multilevel fitness per holobiont of a strain-1 microbe in a single-strain microbiome consisting only of strain-1, $w_{1,12}$ is the multilevel fitness per holobiont of a strain-1 microbe in a dual-strain microbiome, and so forth. Each single-strain holobiont with k_1 microbes in it contributes $k_1 \times W_1$ microbes of strain-1 to the microbial source pool. Each dual-strain holobiont contributes $n_1 \times W_{12}$ microbes of strain-1 and $n_2 \times W_{12}$ microbes of strain-2 to the microbial source pool, and so forth. The next go-around of the life cycle begins with these reconstituted larval host and microbial source pools.

Overall, the model now contains ten parameters: k_1 and k_2, which are the equilibrium number of microbes in the single-strain hosts hosts; n_1 and n_2 which are the equilibrium number of microbes of each strain in the two-strain hosts; d_1 and d_2, which are the colonization parameters for each strain; W_0, which is the fitness of holobionts without microbes; W_1, which is the fitness of single-strain holobionts with k_1 strain-1 microbes in each; W_2, which is the fitness of single-strain holobionts with k_2 strain-2 microbes in each; and W_{12}, which is the fitness of dual-strain holobionts with n_1 strain-1 microbes and n_2 strain-2 microbes in each. The n_1 and n_2 parameters might themselves be derived from an underlying within-host species-interaction model such as the LV competition equations. In this case, the LV reciprocal competition coefficients, denoted as a_{12} and a_{21}, together with k_1 and k_2 are sufficient to determine n_1 and n_2.

The model's predictions are best viewed in relation to specific situations. It is impractical to give an exhaustive list of all possible outcomes as was done for the host-single strain model of the previous section.

3.2 No Selective Neutrality

A fundamental feature of classical population genetics is the possibility of selective neutrality provided the mating system consists of random union of gametes (or random mating). Recall, for example, the classic model for selection on a diploid locus with two alleles, A_1 and A_2. If the fitnesses for the three genotypes, A_1A_1, A_1A_2 and A_2A_2 are equal, then any initial allele frequency persists unchanged. That is, the random union of gametes process does not itself alter the allele frequencies—only "forces" of evolution such as natural selection, drift, mutation and so forth can cause allele frequency change. This result is the familiar Hardy-Weinberg Law.

The process whereby empty hosts in the host source pool are colonized by microbes from the microbial source pool is for the hologenome the conceptual analogue of the mating system for

the nuclear genome. The colonization process produces a random assortment of microbe strains in the hosts according to Poisson sampling analogous to how the mating system produces a random assortment of alleles in the nucleus according to Binomial sampling. However, the Poisson colonization process is *not* neutral and it produces changes in the frequency of the microbial strains by itself even if the microbe strains are functionally identical. There is no analogue of the Hardy-Weinberg Law for the hologenome.

Figure 11 illustrates this situation. The properties of both strains are identical. However, the initial frequency of strain-1 is 0.8 and of strain-2 is 0.2. After 20 generations, both strain frequencies converge to 0.5. Thus, the colonization process itself influences the strain frequencies. Therefore, the effect of any within-holobiont K-selection between the microbial strains and of any between-holobiont r-selection on the entire holobionts play out upon the non-level stage supplied by the colonization process. The Poisson colonization process supplies a strong pull to the center, and the effect of any selection will be combined with this central pull to yield a net result.

3.3 Limited Response to Directional Selection

Textbook population genetics reveals the effectiveness of natural selection in bringing about evolution with nuclear genes. For example, with one diploid locus and two alleles, directional selection results in fixation of the favored allele and elimination of the alternative allele. Now suppose that the same two alleles from the nucleus are instead found in two strains of the microbiome—microbes are the vehicles delivering genes to the host instead of gametes to the nucleus. If so, is there any analogue to the classical outcome of directional selection for microbial genes compared to nuclear genes?

Specifically, if two nuclear alleles lead to the nuclear genotype fitnesses, $W_{11} = 1$, $W_{12} = 2$, and $W_{22} = 3$, then the A_2 allele becomes fixed in the gene pool and A_1 is eliminated. Now suppose instead that the same alleles are lodged in microbial strains leading to the hologenotype fitnesses, $W_1 = 1$, $W_{12} = 2$, and $W_2 = 3$. Also, $W_0 = 1$. This situation can be explored by supposing the two strains of microbes are identical and transparent to each other but differ only in their impact on holobiont fitness. This setup is is similar to two alleles on homologous chromosomes that do not directly interact with each other but do so indirectly through their effects on the phenotype. Accordingly, $k_1 = k_2 = k$, $d_1 = d_2 = d$, and $a_{12} = a_{21} = 0$.

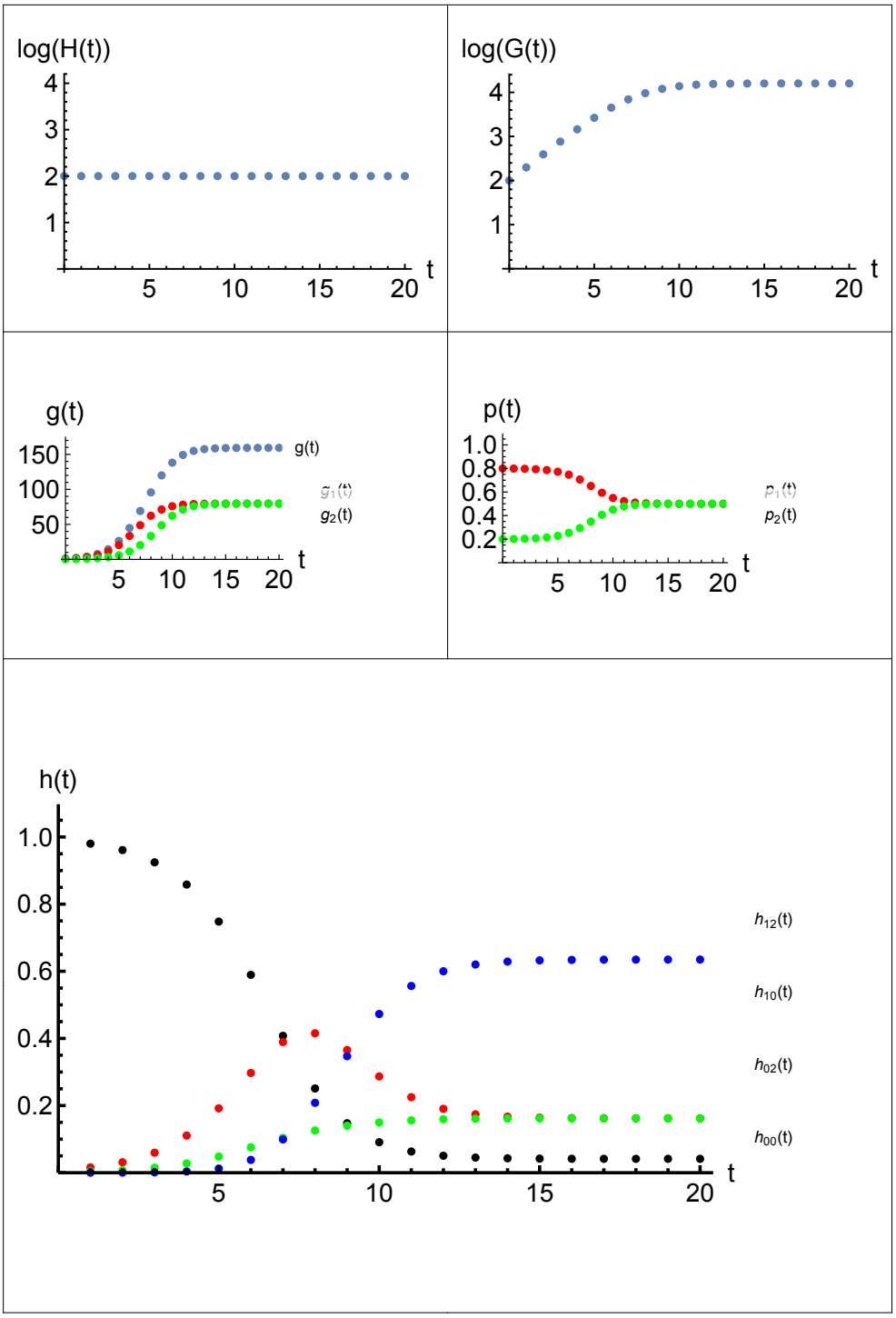

Figure 11: *Host and with two identical strains of microbes. The strains start from different initial conditions.* $d_1 = 0.02$, $d_2 = 0.02$, $k_1 = 100$, $k_2 = 100$, $a_{12} = 0$, $a_{21} = 0$, $W_0 = 1$, $W_1 = 1$, $W_2 = 1$, $W_{12} = 1$, $H_{\text{init}} = 100$, $G_{1\text{init}} = 80$, $G_{2\text{init}} = 20$, $t_{\max} = 20$. *Asymptotically,* $R_H = 1.00$, $R_G = 1.00$, $g = 159.36$, $g_1 = 79.68$, $g_2 = 79.68$, $p_1 = 0.5$, $p_2 = 0.5$, $h_{00} = 0.04$, $h_{10} = 0.16$, $h_{02} = 0.16$, *and* $h_{12} = 0.63$.

As Figure 12 shows, the result of directional holobiont selection in this instance is polymorphism, not fixation of strain-2. Holobiont selection does *not* eliminate strain-1 even though it is inferior to strain-2. In the figure, the equilibrium strain frequencies are $p_1 = 0.410$ and $p_2 = 0.590$. The equilibrium reveals the strong pull to the center caused by the Poisson colonization dynamics. The selection in favor of strain-2 draws the frequency of strain-2 somewhat above the center at 0.5 and pushes the frequency of strain-1 somewhat below the center of 0.5. In another example with the same d, k, a_{12} and a_{21}, not illustrated, the strength of selection favoring strain-2 over strain-1 is increased to $W_1 = 1$, $W_{12} = 4$, and $W_2 = 6$ with $W_0 = 1$. As a consequence, the equilibrium shifts slightly farther away from the center to $p_1 = 0.396$ and $p_2 = 0.604$, showing that increasing the strength of selection does move the net result a bit further away from the center.

The mathematical appendix shows that in the case of directional selection, if both strains can exist by themselves together with the host, then both strains can increase when rare. However, the condition to increase when rare is necessary, not sufficient, to conclude that a polymorphism occurs because one of the strains might be shed even though it can increase when rare.

The power of the Poisson colonization process to override the holobiont selection is controlled by the colonization parameter, d. If d is lowered, then the directional selection is able to drive the frequency of the inferior strain-1 lower. In Figure 12, $d = 0.020$. In contrast, Figure 13 uses $d = 0.0145$. In this figure, the equilibrium strain frequencies now are $p_1 = 0.040$ and $p_2 = 0.960$, showing that directional holobiont selection has a much larger effect than previously.

Figure 14 shows the dependency of the equilibrium frequency of strain-1 on the colonization parameter, d, The d must be greater than 0.010 for strain-1 to increase when rare. According to the figure, d must be greater than about 0.014 for strain-1 to coexist with strain-2 and the host. So, if d is between 0.010 and 0.014, strain-1 is shed from the holobiont population even through it can increase when rare. And if d is greater than 0.014, then polymorphism results. However, as the figure illustrates, as d is increased much beyond the value of 0.014, the equilibrium frequency of strain-1 quickly approaches 0.5, indicating that the Poisson colonization process is completely overpowering the directional holobiont selection against strain-1 and favoring strain-2.

Thus, hologenotype frequencies show a limited response to directional holobiont selection, and the extent to which they do respond is qualified by the colonization parameter. Holobiont selection does not cause fixation of the favored allele because the Poisson sampling provides a refuge for the unfavored allele, preventing its exclusion.

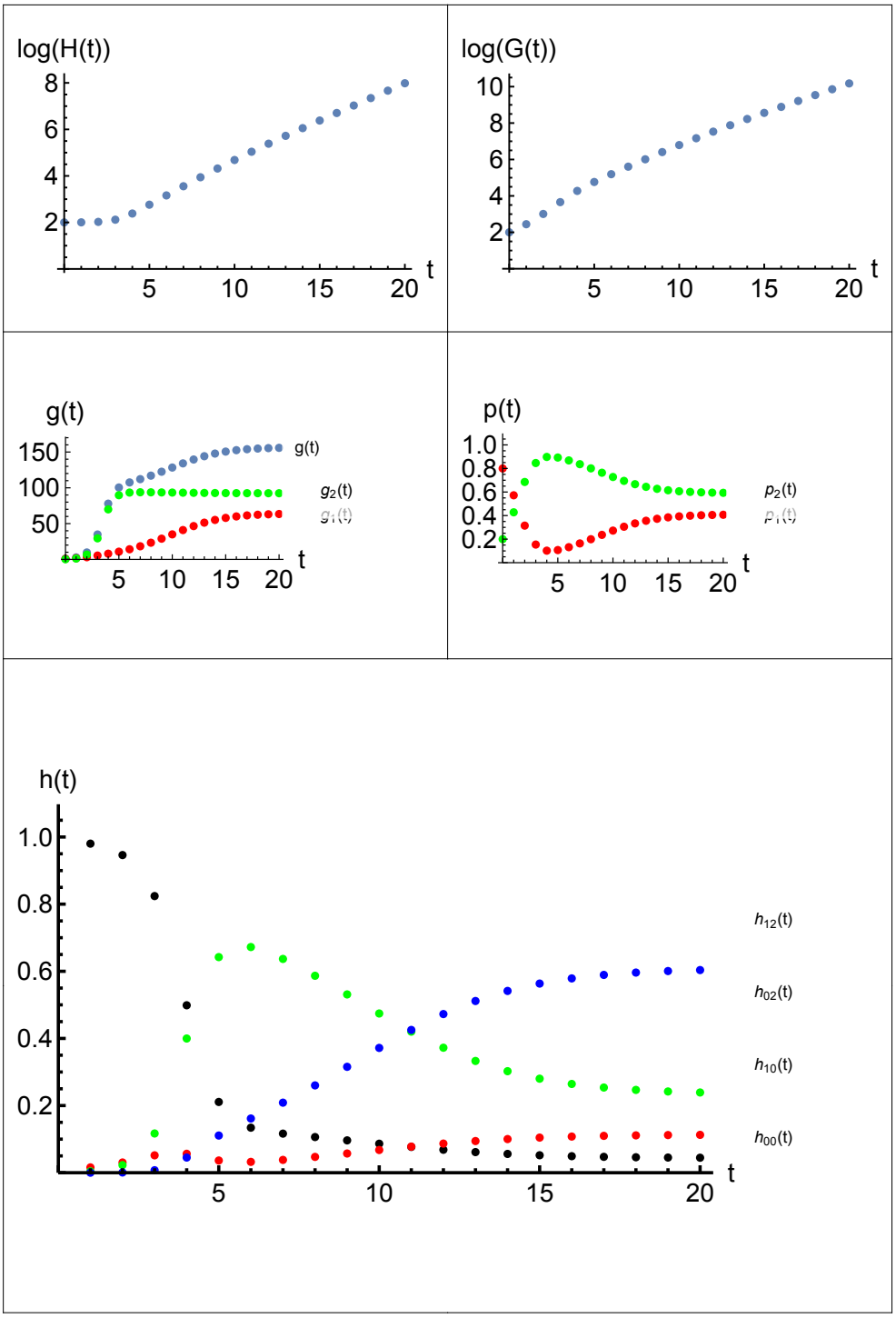

Figure 12: *Holobiont selection favoring strain-2. Here,* $W_0 = 1$, $W_1 = 1$, $W_{12} = 2$, $W_2 = 3$. *Also,* $d_1 = 0.020$, $d_2 = 0.020$, $k_1 = 100$, $k_2 = 100$, $a_{12} = 0$, $a_{21} = 0$, $H_{\text{init}} = 100$, $G_{1\text{init}} = 20$, $G_{2\text{init}} = 80$, $t_{\max} = 20$. *Asymptotically,* $R_H = 2.08$, $R_G = 2.08$, $g = 155.86$, $g_1 = 63.41$, $g_2 = 92.44$, $p_1 = 0.407$, $p_2 = 0.593$, $h_{00} = 0.045$, $h_{10} = 0.113$, $h_{02} = 0.239$, *and* $h_{12} = 0.604$.

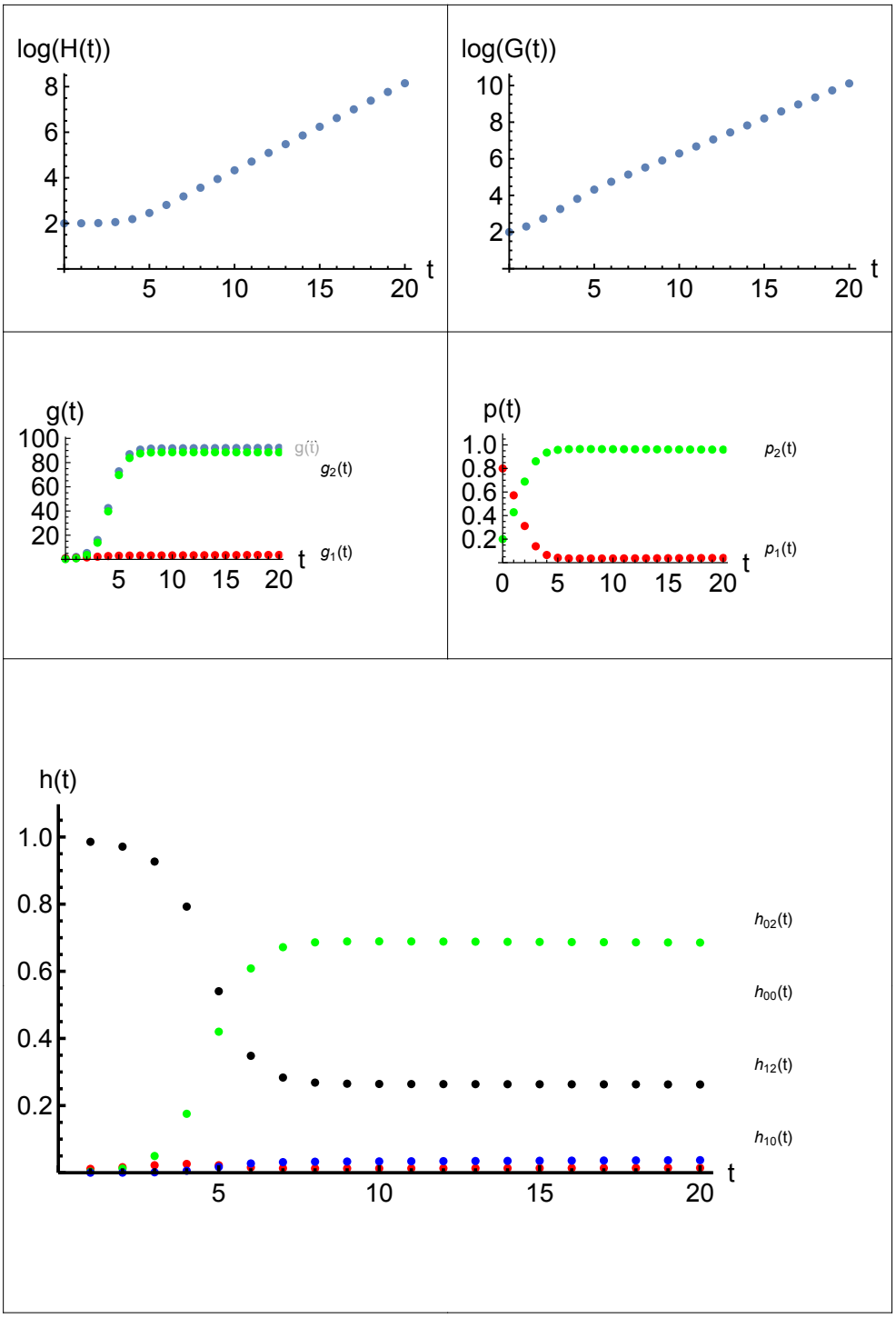

Figure 13: *Holobiont selection favoring strain-2. Here,* $W_0 = 1$, $W_1 = 1$, $W_{12} = 2$, $W_2 = 3$. *Also,* $d_1 = 0.0145$, $d_2 = 0.0145$, $k_1 = 100$, $k_2 = 100$, $a_{12} = 0$, $a_{21} = 0$, $H_{\text{init}} = 100$, $G_{1\text{init}} = 20$, $G_{2\text{init}} = 80$, $t_{\max} = 20$. *Asymptotically,* $R_H = 2.409$, $R_G = 2.409$, $g = 92.191$, $g_1 = 3.700$, $g_2 = 88.491$, $p_1 = 0.040$, $p_2 = 0.960$, $h_{00} = 0.263$, $h_{10} = 0.014$, $h_{02} = 685$, *and* $h_{12} = 0.037$.

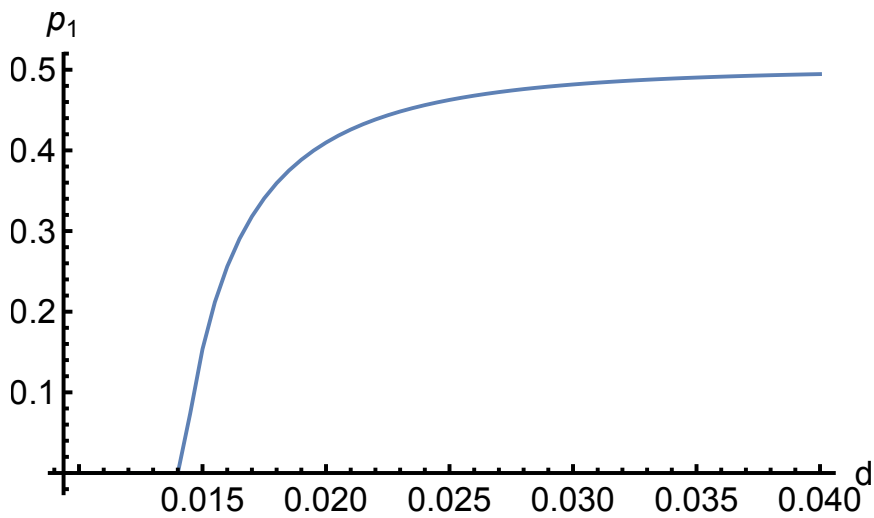

Figure 14: *Equilibrium frequency of strain-1 as a function of the colonization parameter, d. Directional holobiont selection is against strain-1 and favors strain-2. Directional holobiont selection is effective for low d but not high d. Here,* $k = 100$, $W_0 = 1$, $W_1 = 1$, $W_{12} = 2$, $W_2 = 3$

The limited response of the hologenome to holobiont selection has precedence in the classical population genetics of multiple locus genetic systems (Moran 1964, Karlin 1975). There, the evolutionary outcome depends on combining the dynamics of selection with the dynamics of recombination. Here, the evolutionary outcome depends on combining the dynamics of selection with the dynamics of colonization. Evolutionary biology over the years has relied on the canonical one-locus-two-allele setup as a metaphor in which selection completely determines the outcome. It is more realistic, both in classical population genetics and here as well, to regard evolutionary outcomes as resulting from mixing multiple processes, only one of which is natural selection.

The mathematical appendix further explores the situations where the two-strain holobiont is superior or inferior to either of the single-strain holobionts—these correspond to the other standard one-locus-two-allele cases in textbook population genetics.

3.4 Microbial Colonization-Extinction Coexistence

Hologenome polymorphism can result from microbial dynamics alone without any selection at the holobiont level. To illustrate, suppose the two strains of microbes compete with each other within the host but each strain has the same effect on the host. From the host's point of view, the strains are identical, but from the microbes' point of view, one strain is superior to the other—say, strain-1 out-competes strain-2 whenever the two strains are within the same host. This assumption requires strong competitive asymmetry which, with Lotka-Volterra competition,

implies that $a_{12} < k_1/k_2$ and $a_{21} > k_2/k_1$. Can strain-2 persist in the holobiont population despite losing in competition to strain-1 whenever both occur together?

To model this case, let $W_0 = W_1 = W_2 = W_{12} = W$. The appendix shows that a critical k_2, called k_{2c}, exists such that if $k_2 < k_{2c}$ then the competitively superior strain-1 microbe excludes the competitively inferior strain-2 microbe from the holobiont population. In contrast, if $k_2 > k_{2c}$, then strain-1 cannot exclude strain-2 from the holobiont population even though it can exclude strain-2 whenever both colonize the same host. In this case, the two strains coexist in a colonization-extinction equilibrium in the holobiont population.

The reason that the strains can coexist if $k_2 > k_{2c}$ despite the competitive asymmetry is that the colonization process provides some empty hosts that end up being colonized only by strain-2, thereby providing a refuge for the competitively inferior strain from the competitively superior strain. However, the production of strain-2 microbes in these refuge hosts must be high enough to compensate for their inability to produce anything in those hosts where strain-1 is also present. This result is an instance of a meta-population model for patch dynamics featuring a colonization-extinction equilibrium (Levins and Culver 1971, Amarasekare and Possingham 2001).

Figures 15 and 16 illustrate the the dynamics of the host with a two-strain microbiome, assuming that strain-1 competitively excludes strain-2 whenever both colonize the same host. For the parameters of the illustration, the critical value, k_{2c} works out to be 246. In Figure 15, k_2 is less than k_{2c}, and strain-1 drives strain-2 to extinction in the holobiont population. In this case, strain-2 does not produce enough where it is alone in a host to compensate for the loss of production from hosts that both strains colonized. In Figure 16, k_2 is greater than k_{2c}, and strain-1 and strain-2 coexist in the holobiont population. Here, strain-2 does produce enough in hosts where it is alone to compensate for the loss of production in hosts where both strains colonize.

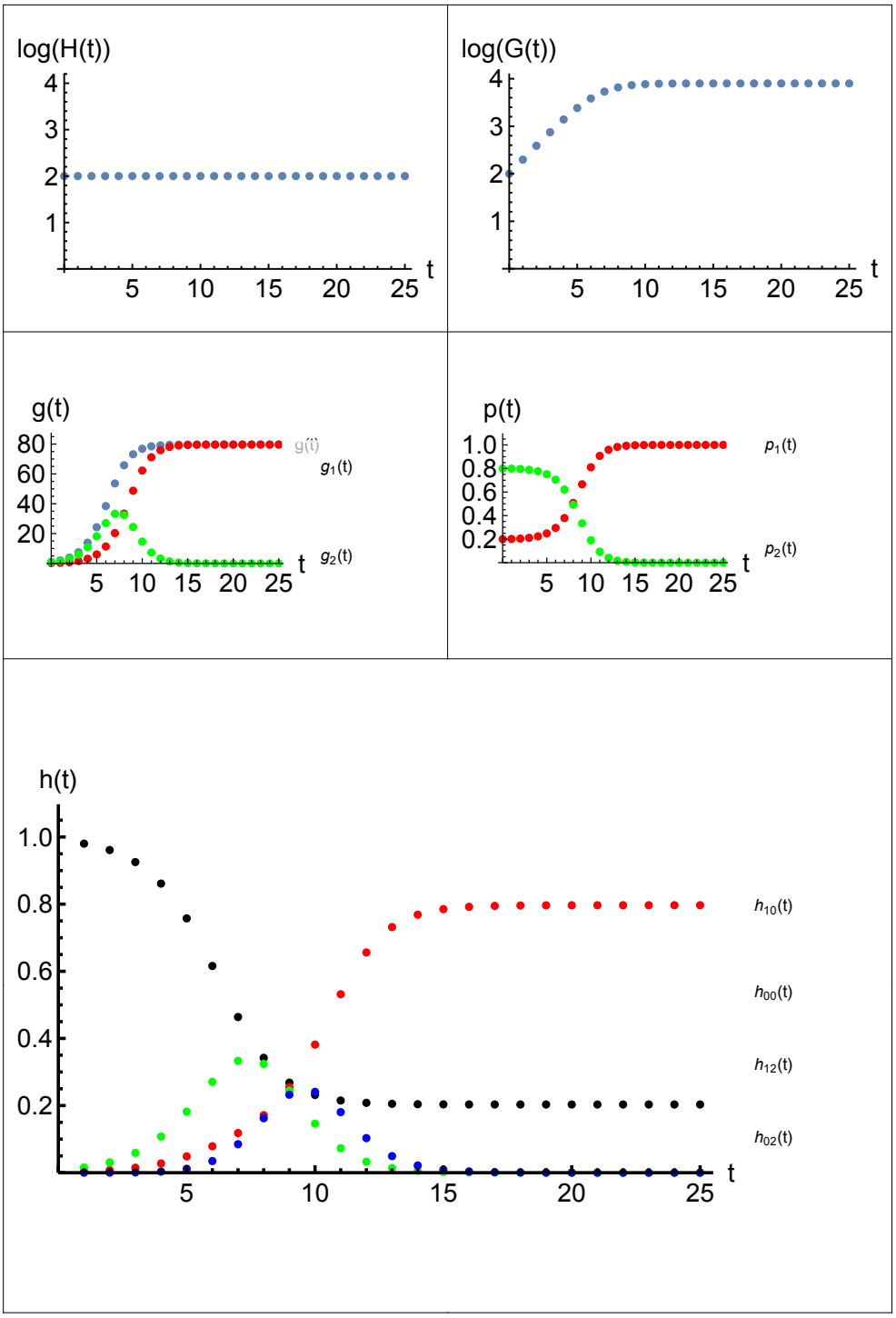

Figure 15: *Host with competitively superior strain-1 and competitively inferior strain-2. Here,* $k_2 < k_{2c} = 246$*, so strain-1 drives strain-2 to extinction. Also* $d_1 = 0.02$*,* $d_2 = 0.02$*,* $k_1 = 100$*,* $k_2 = 100$*,* $W = 1$*,* $H_{\text{init}} = 100$*,* $G_{1\text{init}} = 20$*,* $G_{2\text{init}} = 80$*,* $t_{\max} = 25$*,* $a_{12} < 1$ *and* $a_{21} > 1$*. Asymptotically,* $R_H = 1.00$*,* $R_G = 1.00$*,* $g = 79.68$*,* $g_1 = 79.68$*,* $g_2 = 0$*,* $p_1 = 1$*,* $p_2 = 0$*,* $h_{00} = 0.20$*,* $h_{10} = 0.80$*,* $h_{02} = 0$*, and* $h_{12} = 0$*.*

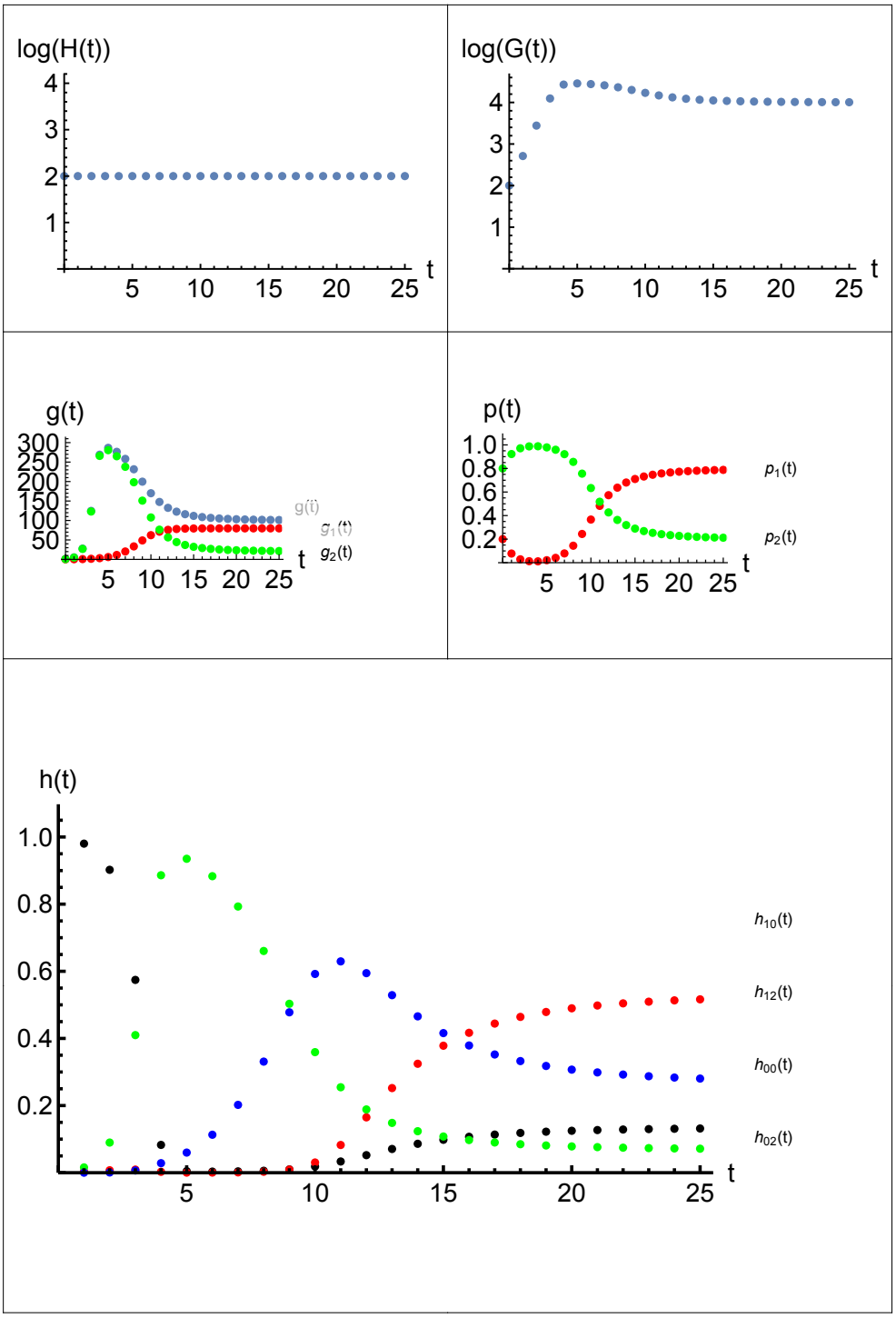

Figure 16: *Host with competitively superior strain-1 and competitively inferior strain-2. Here,* $k_2 > k_{2c} = 246$*, so strain-1 and strain-2 coexist. Also* $d_1 = 0.02$*,* $d_2 = 0.02$*,* $k_1 = 100$*,* $k_2 = 300$*,* $W = 1$*,* $H_{\text{init}} = 100$*,* $G_{1\text{init}} = 20$*,* $G_{2\text{init}} = 80$*,* $t_{\max} = 25$*,* $a_{12} < 1/3$ *and* $a_{21} > 3$*. Asymptotically,* $R_H = 1.00$*,* $R_G = 1.00$*,* $g = 101.13$*,* $g_1 = 79.68$*,* $g_2 = 21.45$*,* $p_1 = 0.79$*,* $p_2 = 0.21$*,* $h_{00} = 0.13$*,* $h_{10} = 0.52$*,* $h_{02} = 0.07$*, and* $h_{12} = 0.28$*.*

3.5 Polymorphism between Altruistic and Selfish Microbes

The dynamics at the host level and microbe level can interact. In Section 3.3, the fitness differences were solely at the host level and in Section 3.4, the fitness differences were solely at the microbe level. Here there two levels are combined.

To consider an example where microbial dynamics and holobiont selection jointly occur, suppose that strain-1 always excludes strain-2 in a host where both are present, as previously shown in Figs. 15 and 16, but now also allow directional holobiont selection in favor of strain-2. That is, strain-2 (the altruistic microbe) sacrifices its competitive ability with respect to strain-1 (the selfish microbe), but receives a higher fitness at the holobiont level. Can holobiont selection favoring strain-2 keep it from going extinct? Specifically, if $k_2 < k_{2c}$ such that strain-2 is excluded in the absence of holobiont selection, can directional holobiont selection in favor of strain-2 retain it in the holobiont population? The directional selection favoring strain-2 is implemented by assuming $W_2 > W_{12} = W_1 = W_0$.

The mathematical appendix offers a case based on Figure 15 in which strain-1 excludes strain-2 because $k_2 < k_{2c}$. In the appendix it is shown in this example that if $W_2 \approx 1.23$, then strain-2 is rescued by holobiont selection because of its positive contribution to host fitness. The net result is a polymorphism between strain-2 and strain-1. Figure 17 provides an example where W_2 is 1.5, which is larger than 1.23, the minimum necessary to rescue strain-2.

3.6 Microbiome-Host Integration

This section revisits a principal motivation for this paper's research—what accounts for the remarkable integration between microbiomes and their hosts as noted in the paper's Introduction?

One might suppose that multilevel selection would lead to an optimal tradeoff by a microbe between its within-host carrying capacity and the between-host fitness of the holobiont. The optimal tradeoff is expressed in terms of a phenotypic variable, x, that is a measure of the "altruistic effort" by the microbe. The x measures how much of a microbe's within-host carrying capacity it foregoes to improve the holobiont fitness. The effort could refer to the amount of some expensive metabolite that the microbe transfers to the host. What amount of this metabolite should the microbe transfer?

For example, suppose that increasing altruistic effort, x, lowers a microbe's k according to a linear formula. Similarly, suppose that increasing the altruistic effort raises the host's W according to a linear formula. What is the optimal x?

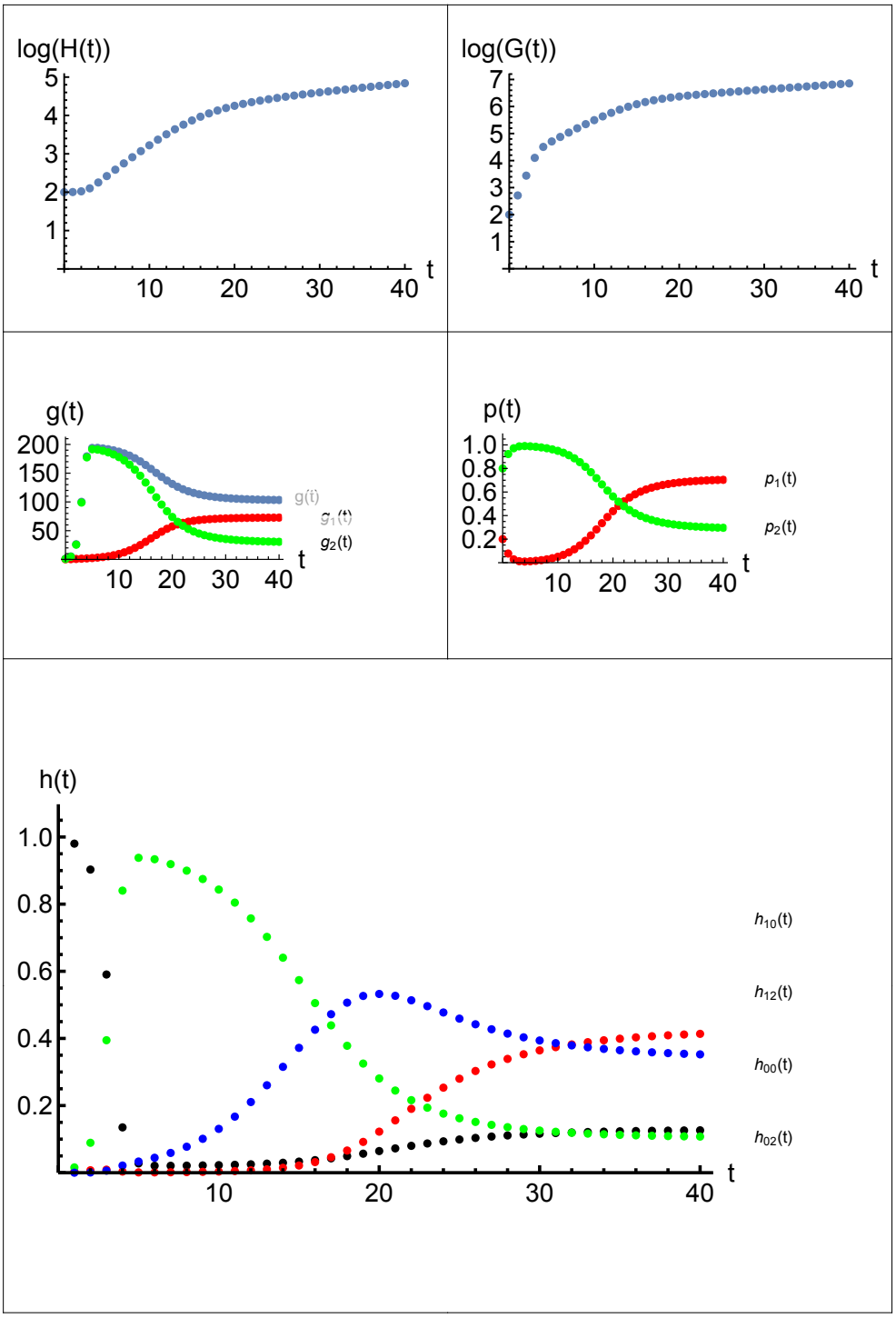

Figure 17: *Coexistence of altruistic and selfish microbes. Strain-1 is selfish and strain-2 is altruistic.* $d_1 = 0.02$, $d_2 = 0.02$, $k_1 = 100$, $k_2 = 200$, $W_0 = 1$, $W_1 = 1$, $W_{12} = 1$ *and* $W_2 = 1.5$. *Also,* $a_{12} < 1/2$ *and* $a_{21} > 2$. $H_{\text{init}} = 100$, $G_{1\text{init}} = 20$, $G_{2\text{init}} = 80$, $t_{\max} = 40$. *Asymptotically,* $R_{oH} \approx 1.05$, $R_{oG} \approx 1.05$, $g_o = 103.33$, $g_{1o} = 72.70$, $g_{2o} = 30.63$, $p_{1o} = 0.704$, $p_{2o} = 0.296$, $h_{00o} = 0.126$, $h_{10o} = 0.414$, $h_{02o} = 0.108$, *and* $h_{12o} = 0.352$.

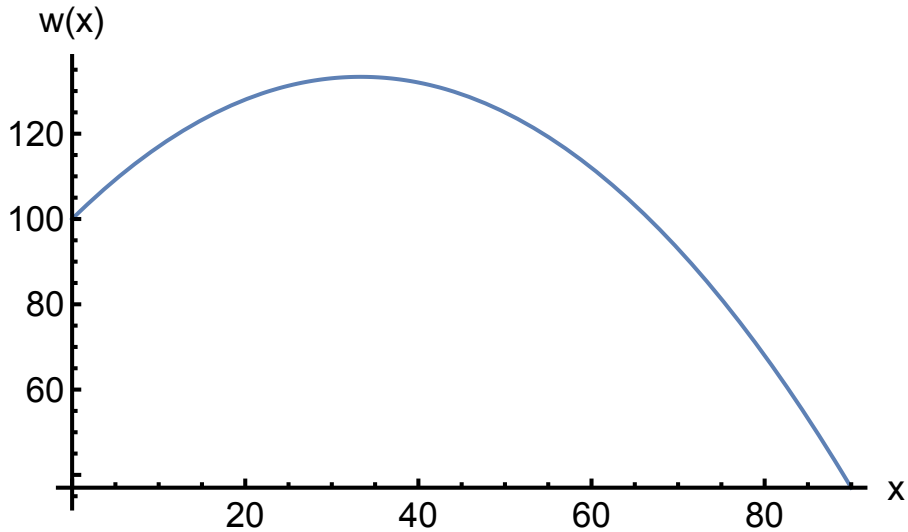

Figure 18: *Multilevel microbial fitness, w, as a function of the altruistic effort, x. The optimal effort, $\hat{x} = 33.333$. The maximal effort, $x_{max} = 90$. Here, $d = 0.1$, $k_o = 100$, $W_o = 1$, $b = 0.03$, $c = 1$.*

To answer this question, an optimality criterion is needed. One possibility is the multilevel microbial fitness, w. Because $k(x)$ and $W(x)$ are linear functions of x, the multilevel fitness, $w(x) = k(x) \times W(x)$, is a quadratic function of x. The formula for the optimal altruistic effort, $\hat{x}$, that maximizes $w(x)$ is noted in the mathematical appendix. An example appears in Figure 18.

Next, do microbes with the optimal effort exclude all other microbe strains with a non-optimal effort? Suppose a mutant strain arises that is identical in all respects to the optimal strain except for expending a different altruistic effort, $y \neq \hat{x}$. By this assumption, the reciprocal competition coefficients both equal 1 and the other parameters of the altruistic and mutant strains are the same. All that differs between the strains is their altruistic efforts. Can the optimal altruistic strain exclude all non-optimal altruistic strains? If it can, then $\hat{x}$ would represent a microbial counterpart to an evolutionarily stable strategy, which for nuclear genes, is a strategy that cannot be invaded by any other strategy.

Indeed, consider the more general question of whether a selfish mutant can invade *any* established altruistic strain, including an optimally altruistic strain. Let the established strain exhibit some degree of altruism, not necessarily optimal, at $x > 0$. Can an otherwise identical but completely selfish mutant with $y = 0$ invade any altruistic strain with $x > 0$?

The mathematical appendix shows that the completely selfish mutant with $y = 0$ *always* increases when rare into a holobiont population fixed for a strain supplying *any* altruistic effort, including $x = \hat{x}$. The reason is that a completely selfish mutant has a higher k than that of any altruism-providing strain because it does not incur any cost of altruism, assuming all else is equal. Therefore, when the mutant and established strain both colonize a holobiont together, the selfish

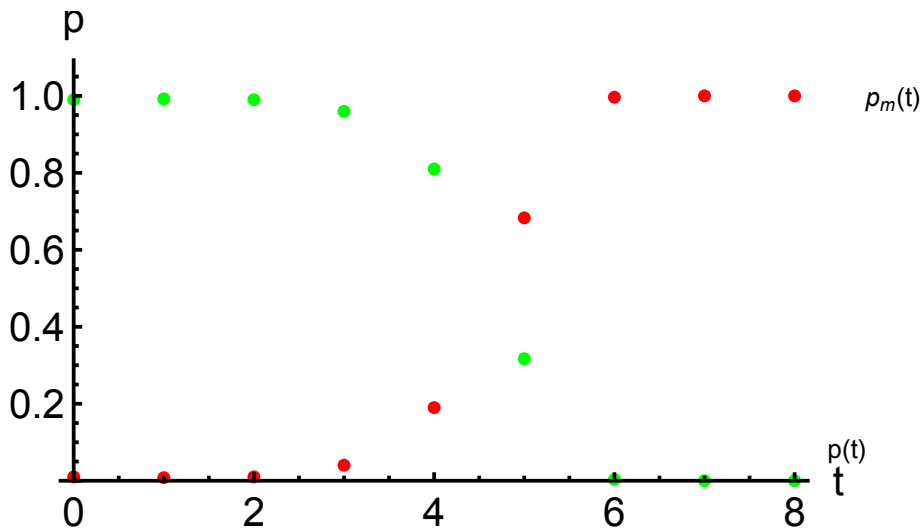

Figure 19: *Frequency of altruistic microbe, p, and selfish mutant, p_m, through time. Host fitness with altruistic microbe, $W = W_o + b\,x$. Altruistic microbe carrying capacity, $k = k_o - c\,x$, Host fitness with selfish mutant, $W_m = W_o + b\,y$, Selfish microbe carrying capacity, $k_m = k_o - c\,y$. Here, $d = 0.1$, $W_o = 1$, $k_o = 100$, $b = 0.03$, $c = 1$, $x = 33.3333$, $y = 0$, $H_{init} = 100$, $G_{a,init} = 99$, $G_{m,init} = 1, t_{max} = 8$.*

strain always displaces any established altruistic strain. Hence, the optimal degree of altruism, $\hat{x}$, or indeed any altruism at all cannot be the counterpart of an evolutionarily stable strategy because it can always be invaded by a more selfish strategy. Instead, only the completely selfish microbe itself, $x = 0$, is an evolutionarily stable strategy. Figure 19 illustrates a selfish microbe with $y = 0$ excluding an altruistic microbe with $x = \hat{x}$.

To obtain some degree of evolutionarily stable altruistic effort by the microbes to the host, the host needs to discriminate against microbial selfishness. The host can cause the mutant's colonization coefficient to be less than the altruist's colonization parameter. How, could a host intervene in the colonization parameter of a microbial strain? One way is *via* the host's immune system.

Suppose that a host initially "sees" a microbe that colonizes it as a deleterious invader. If so, it creates antibodies to the microbe which has the effect of lowering the microbe's colonization parameter. That is, if a microbe is deactivated by a host's immune system upon entry into the host, it has failed to colonize. So the question now becomes how much should the host's immune system deactivate the entering microbes as a function of how altruistic the microbes are. This situation gives rise to a "microbiome-host game" wherein the host adjusts the amount of antibody it produces depending on the resources the microbe supplies to it and the microbe adjusts the amount of resources it provides consistent with its own successful colonization.

The quantity of antibodies a host makes toward a particular microbial strain results from

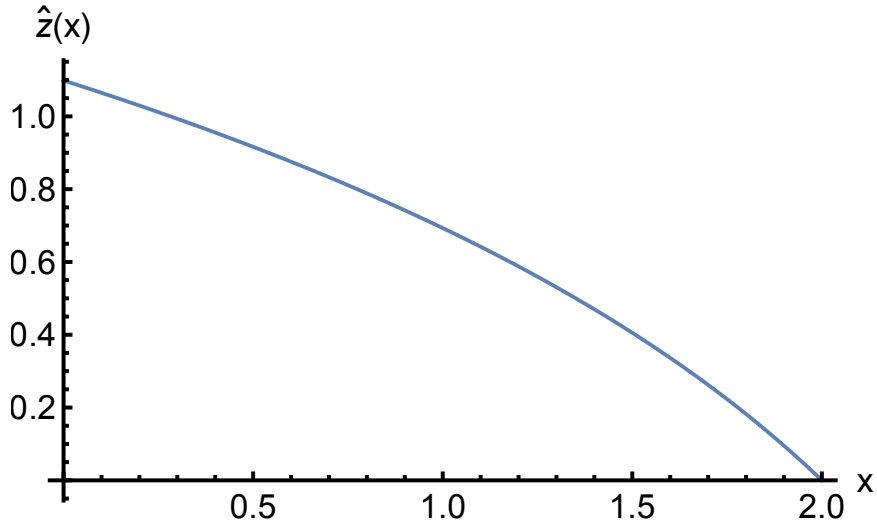

Figure 20: *Optimal antibody production, $\hat{z}(x)$, as a function of the altruism, x, being supplied by the microbe to the host. Here, $\hat{x}_{max} = 2$, $x_{max} = 3$, $\hat{z}_{max} = 1.10$, $\alpha_o = 3$, $\mu = 1$, $\beta = 1$ and $\gamma = 1$.*

a balance between the fitness benefit and the fitness cost of making antibodies for that strain. Suppose the benefit to antibody production shows a decreasing return to scale and the cost of antibody production is linear. This benefit and cost determine an host optimal antibody production for that particular strain. Suppose further that the amplitude of the antibody's benefit to the host depends on the amount of altruism being supplied by the strain. The benefit to the host of making antibodies to a microbe is lessened if the microbe is supplying some nutrients to it. Accordingly, the optimal antibody production by the host as a function of the amount of resource being supplied by the microbes is a decreasing function of the resources supplied, as shown in the mathematical appendix and in Figure 20.

A side effect of the host's antibody production is to lower the mutant's colonization parameter. Suppose increasing the antibody level lowers colonization according to a linear formula. Then the mathematical appendix shows that for each microbial base carrying capacity, k_o, there is a minimum amount of altruism, $x_{min}(k_o)$, the microbe must supply for it to increase when rare into the holobiont population, as shown in Figure 21.

The minimum degree of altruism a microbe must supply is a decreasing function of its base carrying capacity, as shown in Figure 21, because the lower k_o is, the fewer microbes it can release into the microbial source pool. Then these fewer microbes in turn need a higher colonization rate to sustain their population, and the only way to obtain a higher colonization parameter is to increase the amount of altruistic effort supplied to the host.

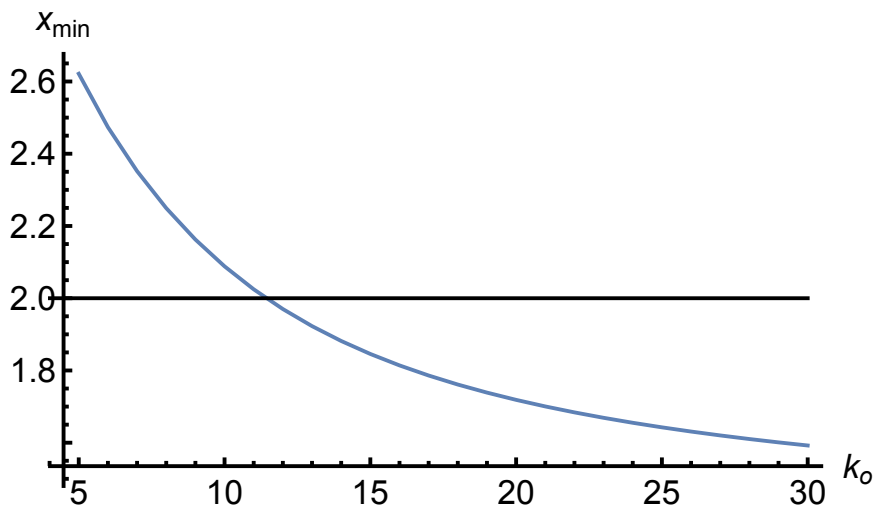

Figure 21: *Minimum altruism, $x_{min}(k_o)$, the microbe must supply to the host to increase when rare as a function of its within-host base carrying capacity, k_o. The horizontal line is $\hat{x}_{max}$, the degree of altruism that causes the host not make any antibodies at all. The intersection of the $x_{min}(k_o)$ curve with the $\hat{x}_{max}$ line occurs at $\tilde{k}_o = 11.43$. The $\tilde{k}_o$ marks the cutoff between insufficiently altruistic strains excluded from the holobiont and the altruistic strains capable of joining. Here, $\hat{x}_{max} = 2$, $\alpha_o = 3$, $\mu = 1$, $\beta = 1$, $\gamma = 1$, $d_o = 0.1$, $\nu = 0.2$, $b = 0.03$, $c = 1$, and $W_o = 1$.*

However, from the host's perspective, if the altruism supplied is greater than a certain maximum level, $\hat{x}_{max}$, it will shutdown antibody production altogether because the benefit is no longer worth the cost. So, the possibility arises that a microbe's k_o may be low enough that the altruistic effort it would need to increase when rare exceeds the level at which the host has already shut down antibody production. Increasing altruistic effort beyond $\hat{x}_{max}$ does nothing to improve colonization. If a microbe cannot make it into the holobiont population when the host is not making any antibodies at all, then it is locked out of the holobiont population. Figure 21 shows $\hat{x}_{max}$ as a horizontal line. All microbes whose baseline carrying capacity lie to the left of the intersection between the curve and the line are locked out from the holobiont population.

If a microbe's baseline carrying carrying capacity is to the right of the intersection between the curve and line in Figure 21, then the microbe is not locked out of participating in the holobiont population. However, its altruistic effort must exceed the minimum needed for the host to reduce its antibody production enough to permit sufficient colonization. So, a microbe of baseline carrying capacity, k_o, needs to supply some level of altruistic effort between $x_{min}(k_o)$ and $\hat{x}_{max}$ to enter the holobiont population.

Well, which is it? Should the microbe supply the minimum altruism needed to enter the holobiont population, $x_{min}(k_o)$, or enough altruism to shut down the host antibody production, $\hat{x}_{max}$? Increasing the altruism increases the speed at which the microbes can increase when rare—this speed combines both the within-host net carrying capacity after the cost of altruism has been subtracted and the between-host fitness after the benefit of altruism has been added. From this perspective, the microbe should supply $\hat{x}_{max}$. However, the criterion for increase when rare is necessary, but not sufficient, for microbe-host coexistence. The mathematical appendix shows that the holobionts wind up consisting of microbes that have a bit more altruism than the minimum necessary to colonize together with hosts exhibiting a correspondingly greater antibody response. The numerical examples in the mathematical appendix suggest that holobionts end up with microbes that supply about 10% more altruism than the minimum necessary to colonize. If the microbes supply exactly the minimum, they are excluded by an established strain that supplies more than the bare minimum. But a strain that supplies enough altruism to shut down host antibody production altogether is itself shed once a more selfish strain colonizes. So, the result consists of holobionts that integrate a microbial strain supplying a bit more resource to the host than the minimum necessary to colonize together with a host that makes an amount of antibody that reflects the benefit it receives from its microbe. Nonetheless, this arguably imperfect outcome, which is less than the best possible for both parties, does represent host-microbiome integration.

The process whereby the host determines through its antibody production which microbes are eligible to colonize represents a kind of species sorting that is termed here as "host-orchestrated species sorting (HOSS)". Species sorting is well known in environmental microbiology. Baas-Becking (1934, *cf.* de Witand Bouvier. 2006) famously wrote "everything is everywhere, but the environment selects." As Van der Gucht *et. al.* (2007) note, this phrase assumes that high dispersal rates for microorganisms are ubiquitous, an assumption receiving active investigation (Székely and Langenheder 2014, Wu *et. al.* 2018). Here, the idea of species sorting refers to hosts selecting through their antibody response which strains in the microbial source pool can colonize in light of the amount of resources they supply.

Metaphorically, the holobiont is a seller's market for the host which determines its antibody production in response to the resources it receives, irrespective off how the microbes might be affected whereas the microbes must pay to play by providing resources to be allowed to colonize.

The combination of host-orchestrated species sorting followed by competition among the colonizing microbes leads to a conceptual model of holobiont assembly. A diagram illustrating the process of holobiont assembly appears in Figure 22. Holobiont assembly based on host-orchestrated species sorting followed by microbial competition, rather than coevolution or multilevel selection, is the likely cause of host-microbiome integration.

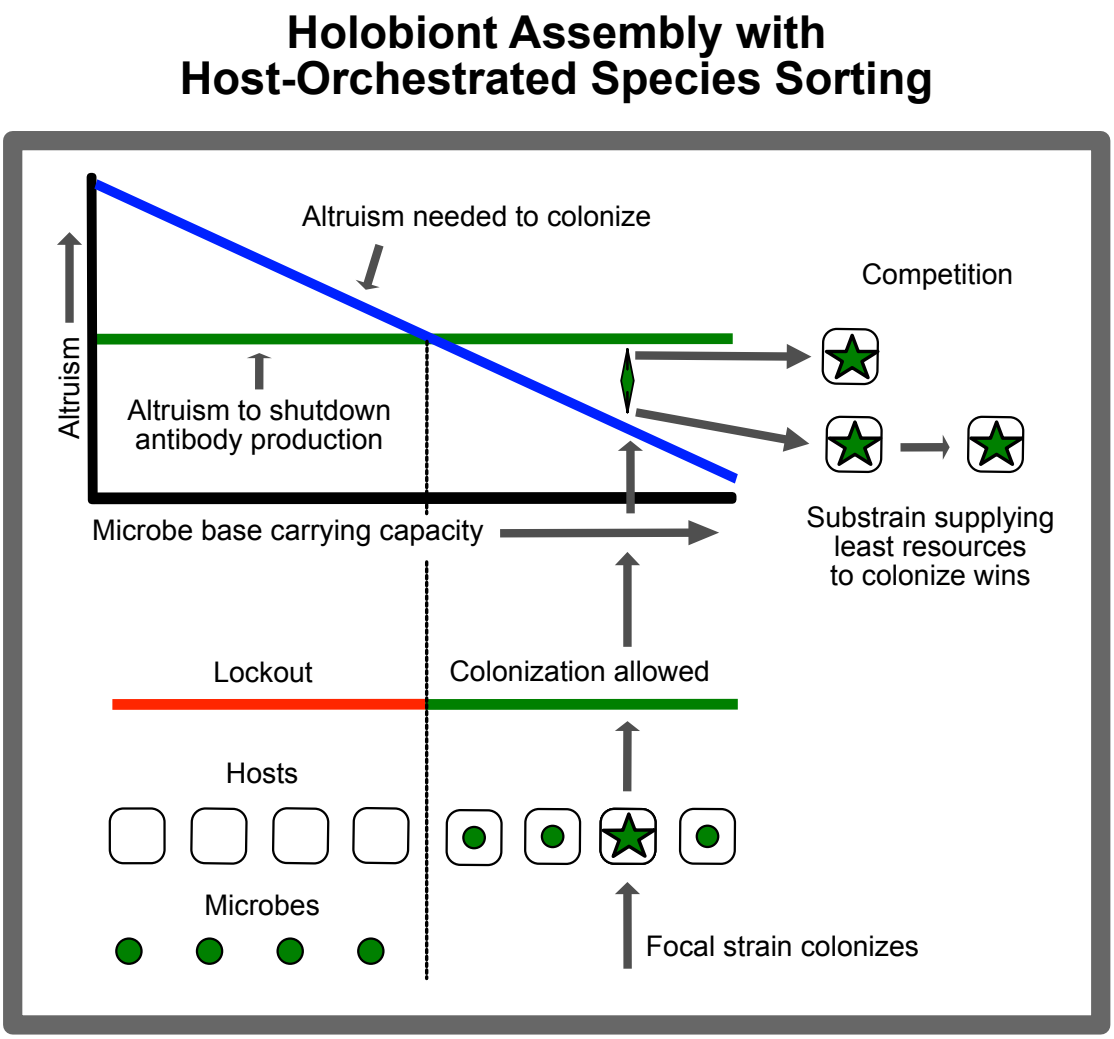

Figure 22: *Holobiont assembly with host-orchestrated species sorting (HOSS). At top left, descending blue line indicates minimum altruism microbe must supply to colonize as function of the microbe's base carrying capacity. Horizontal green line indicates amount of altruism that shuts down host antibody production. Microbes required to supply more altruism than needed to shutdown antibody production are locked out. Demarcation between rejected and eligible microbes at intersection of blue and green lines. Suite of microbes differing in base carrying capacities depicted at bottom left and center. Microbes with base carrying capacities left of demarcation are locked out. Microbes right of demarcation become incorporated into hosts. Thus, host's altruism requirement "sorts" available microbes into those that can and cannot colonize. A focal microbe is shown with a star (⋆). Its base carrying capacity makes it eligible to incorporate into hosts. Substrains differ in amount of altruism supplied. Some supply minimum to colonize—their altruism level is near the blue line. Other substrains supply more than the minimum—their altruism is near the green line. Minimum altruism substrain excludes all other substrains. Host-orchestrated species sorting followed by competition among substrains to supply least altruism consistent with colonization results in holobionts that integrate altruism-supplying microbes with with hosts that produce a corresponding level of antibodies.*

4 Discussion

Other situations could be explored with this model. For example, tradeoff assumptions could be introduced to consider costs and benefits to a host who gives up some ability to live without microbes, W_0, to increase its fitness when with microbes, W_1. Presumably the colonization parameter must be high so that a host could rely on being colonized rather than being left to die in the absence of microbes.

Also, the theory of HOSS was developed assuming the species sorting is accomplished by the host's immune system. However, the logic of HOSS remains valid for other mechanisms of host selectivity. The host could employ toxic mucus, chemicals or physical deterrents like spines—any mechanism applied in relation to the benefit that the microbes supply. Moreover, the host could even facilitate the entry of a benefit-producing microbe rather than merely oppose entry depending on the amount of benefit supplied. Host facilitation could be represented as a negative antibody, *i.e.*, a "probody". The cost-benefit equations in the microbiome-host game could then be modified accordingly.

5 Acknowledgments

The author thanks two anonymous reviewers of a previous version of this article–a version based on binomial sampling of the microbial source pool rather than the Poisson sampling here. The critiques of these reviewers led to many improvements in both analysis and exposition. This article is Contribution #1 from a project, The Theory of Holobiont Evolution, funded by the Gordon and Betty Moore Foundation through Grant GBMF10000 to the University of Hawaii.

6 References

Amarasekare, P. and H. Possingham. 2001. "Patch Dynamics and Metapopulation Theory: the Case of Successional Species." *J. Theor. Biol.* 209:333–344, doi:10.1006/jtbi.2001.2269

Atoda K. 1947. The Larva and Postlarval Development of Some Reef-building Corals. I. *Pocillopora damicornis cespitosa* (Dana). *Sci Reports Tohoku Univ*. 18: 24–47.

Babcock, R. C., G. D. Bull, P. L. Harrison, A. J. Heyward, J. K. Oliver, C. C. Wallace and B. L. Willis. 1986. "Synchronous Spawnings of 105 Scleractinian Coral Species on the Great Barrier Reef." *Marine Biology* 90:379–394.

Baas-Becking L. G. M. 1934. *Geologie of Inleidning tot de Milieukunde*. W. P. Van Stokum, The Hague, The Netherlands.

Bordenstein, S. R. and K. R. Theis. 2015. "Host Biology in Light of the Microbiome: Ten Principles of Holobionts and Hologenomes." *PLoS Biol.* 13(8):e1002226.

Bruijning, M., L. Henry, S. K. G. Forsberg, C. J. E. Metcalf, J. F. Ayroles. 2021. "Natural selection for Imprecise Vertical Transmission in Host-microbiota Systems." *Nature Ecology and Evolution.* https://doi.org/10.1038/s41559-021-01593-y

Carmona, D., C. R. Fitzpatrick, and M. T. Johnson. 2015. "Fifty years of Co-evolution and Beyond: Integrating Co-evolution from Molecules to Species." *Mol. Ecol.* 24:5315–5329.

Damuth, J. and L. Heisler. 1988. "Alternative Formulations of Multilevel Selection." *Biology and Philosophy* 3:407–430.

de Wit, R. and T. Bouvier. 2006. " 'Everything is Everywhere, *but*, the Environment Selects'; What did Baas Becking and Beijerinck Really Say?" *Environmental Microbiology* 8:755–758. doi:10.1111/j.1462-2920.2006.01017.x

Dieckmann, U., and R. Law. 1996. "The Dynamical Theory of Coevolution: a Derivation from Stochastic Ecological Processes." *J. Math. Biol.* 34:579–612.

Ellis, E., and M. Delbrück. 1939. "The Growth of Bacteriophage." *J. General Physiology* 22:365–384. doi: 10.1085/jgp.22.3.365

Foster, K. R., J. Schluter, K. Coyte, and S. Rakoff-Nahoum. 2017. "The Evolution of the Host Microbiome as an Ecosystem on a Leash". *Nature* 548:43–51.

Gilbert, S. F., J. Sapp, and A. I. Tauber. 2012. "A Symbiotic View of Life: We Have Never Been Individuals." *Q. Rev. Biol.* 87:325–341.

Harrison P. L. and C. C. Wallace 1990. Reproduction, Dispersal and Recruitment of Scleractinian Corals. In: Dubinsky Z, editor. *Ecosystems of the World*. Amsterdam: Elsevier Science; 1990. pp. 133–207.

Hirose, M. and M. Hidaka. 2006. "Early Development of Zooxanthella-containing Eggs of the Corals, *Porites cylindirica* and *Montipora digitata*: The Endodermal Localization of Zooxanthellae." *Zoological Science* **23**:873–881.

Hurst, G. D. D. 2017. "Extended Genomes: Symbiosis and Evolution." *Interface Focus* 7: 20170001. http://dx.doi.org/10.1098/rsfs.2017.0001

Jennings, E. C. 2019. "A Holobiont Characterization of Reproduction in a Live-bearing Cockroach, *Diploptera punctata*." Phd. diss. Retrieved from https://etd.ohiolink.edu.

Karlin, S. 1975. "General Two Locus Selection Models: Some Objectives, Rules and Interpretations."*Theoretical Population Biology* 7:364–398.

Lederberg J, McCray AT. 2001. "Ome Sweet Omics"—a Genealogical Treasury of Words. *Scientist*. 15:8.

Levins, R. and D. Culver. 1971. "Regional Coexistence of Species and Competition between Rare Species." *Proc. Nat. Acad. Sci. (USA)* 68:1246–1248.

Lewontin, R. C. 1970. "The Units of Selection." *Annual Review of Ecology and Systematics*, 1:1–18.

Lipsitch, M. S. Siller, and M. Nowak. 1996. "The Evolution of Virulence in Pathogens with Vertical and Horizontal Transmission." *Evolution* 50:1729–1741.

Margulis, L. 1991. "Symbiosis as a Source of Evolutionary Innovation: Speciation and Morphogenesis." In *Symbiogenesis and Symbionticism,* edited by L. Margulis and R. Fester, 1–14, Cambridge: MIT Press.

McFall-Ngai, M., M. G. Hadfield, T. C. Bosch, H. V. Carey, T. Domazet-Loo, A. E. Douglas, N. Dubilier, et al. 2013. "Animals in a Bacterial World, A New Imperative for the Life Sciences." *Proc. Natl. Acad. Sci. (USA)* 110:3229–3236.

Maynard Smith, J. 1964. "Group Selection and Kin Selection." *Nature* 201:1145–1147.

Mayo, D. and N. Gilinsky. 1987. "Models of Group Selection." *Philosophy of Science* 54:515–538.

Moran, P. A. P. 1964. "On the Non-Existence of Adaptive Topographies." *Annals of Human Genetics* 27:383–393.

Narayana, J. K., M. M. Aogááin, W. W. B. Goh, K. Xia, K. Tsaneva-Atanasova, S. H. Chotirmall. 2021. Mathematical-based Microbiome Analytics for Clinical Translation. *Computational and Structural Biotechnology Journal.* 19:6272–6281.

Obeng, N., F. Bansept, M. Sieber, A. Traulsen, and H. Schulenburg. 2021. Evolution of Microbiota-Host Associations: The Microbe's Perspective. *Trends in Microbiology* https://doi.org/10.1016/j.tim.2021.02.005

Okasha, S. 2006. *Evolution and the Levels of Selection*. Oxford UK: Oxford University Press

Osmanovic, D., D. A. Kessler, Y. Rabin, and Y. Soen. 2018. "Darwinian Selection of Host and Bacteria Supports Emergence of Lamarckian-like Adaptation of the System as a Whole." *Biology Direct*. https://doi.org/10.1186/s13062-018-0224-7.

Prasetia, R., F. Sinniger, K. Hashizume, S. Harii 2017. Reproductive Biology of the Deep Brooding Coral *Seriatopora hystrix*: Implications for Shallow Reef Recovery. *PLOS ONE* https://doi.org/10.1371/journal.pone.0177034

Roughgarden, J. 1983. "The Theory of Coevolution." In *Coevolution*, edited by D. J. Futuyma and M. Slatkin, 33–64, Sunderland MA: Sinauer.

Roughgarden, J. 2017. "Model of Holobiont Population Dynamics and Evolution: a Preliminary Sketch." In *Landscapes of Collectivity in the Life Sciences,* edited by S. B. Gissis, E. Lamm, and A. Shavit, pp. 325–350. London: The MIT Press.

Roughgarden, J., S. F. Gilbert, E. Rosenberg, I. Zilber-Rosenberg, and E. Lloyd. 2018. "Holobionts as Units of Selection and a Model of Their Population Dynamics and Evolution." *Biological Theory* 13:44–65. https://doi.org/10.1007/s13752-017-0287-1.

Roughgarden, J. 2020. "Holobiont Evolution: Mathematical Model with Vertical *vs.* Horizontal Microbiome Transmission." *Philosophy, Theory and Practice in Biology*, 12:2. http://dx.doi.org/10.3998/ptpbio.16039257.0012.002.

Shapiro, J. and P. E. Turner. 2014. The Impact of Transmission Mode on the Evolution of Benefits Provided by Microbial Symbionts. *Ecology and Evolution* 4: 3350–3361.

Székely, A. J. and S. Langenheder. 2014. "The Importance of Species Sorting Differs Between Habitat Generalists and Specialists in Bacterial Communities." *FEMS Microbiol Ecol* 87:102–112. DOI: 10.1111/1574-6941.12195

Theis K. R., N. M. Dheilly, J. L. Klassen, R. M. Brucker, J. F. Baines, T. C. G. Bosch, J. F. Cryan, S. F. Gilbert, C. J. Goodnight, E. A. Lloyd, J. Sapp, P. Vandenkoornhuyse, I. Zilber-Rosenberg, E. Rosenberg, S. R. Bordenstein. 2016. Getting the Hologenome Concept Right: an Eco-Evolutionary Framework for Hosts and Their Microbiomes. *mSystems* 1(2):e00028-16. doi: 10.1128/mSystems.00028-16.

Trench, R. K. 1993. "Microalgal-Invertebrate Symbioses: A Review." *Endocytobiosis Cell Res.* 9:135–175.

van Vilet, S. and M. Doebeli. 2019. "The Role of Multilevel Selection in Microbiome Evolution." *Proc. Nat. Acad. Sci. (USA)* www.pnas.org/cgi/doi/10.1073/pnas.1909790116.

Van der Gucht, K., K. Cottenie, K. Muylaert, N. Vloemans, S. Cousin, S. Declerck, E. Jeppesen, J-M. Conde-Porcuna, K. Schwenk, G. Zwart, H. Degans, W. Vyverman, and L. De Meester.

2007. "The Power of Species Sorting: Local Factors Drive Bacterial Community Composition Over a Wide Range of Spatial Scales." *Proc. Natl. Acad. Sci. (USA)* 104:20404–20409. doi/10.1073/pnas.0707200104

Wright, S. 1931. "Evolution in Mendelian populations." *Genetics* **16**:97–159. *The ISME Journal* 12:485–494. doi:10.1038/ismej.2017.183.

Wu, W., HP. Lu, A. Sastri, YC. Yeh, GC. Gong, WC. Chou and CH. Hsieh. 2018. "Contrasting the Relative Importance of Species Sorting and Dispersal Limitation in Shaping Marine Bacterial versus Protist Communities." *The ISME Journal* 12:485–494; doi:10.1038/ismej.2017.183

Zeng, Q., S. Wu, J. Sukumaran, and A. Rodrigo. 2017. "Models of Microbiome Evolution Incorporating Host and Microbial selection." *Microbiome* 5(1):127. doi:10.1186/s40168-017-0343-x.

Zilber-Rosenberg, I. and E. Rosenberg. 2008. "Role of Microorganisms in the Evolution of Animals and Plants: the Hologenome Theory of Evolution." *FEMS Microbiology Reviews* 32:723–735. https://doi.org/10.1111/j.1574-6976.2008.00123.x