Holobiont Evolution: Population Theory for the Hologenome

Joan Roughgarden[1]

May 12, 2022

Abstract

This article develops mathematical theory for the population dynamics of microbiomes with their hosts and theory for holobiont 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 functional integration of microbiomes with their hosts extends to development, metabolism, physiology and behavior. The eco-evolutionary processes producing this integration are unclear.

For a microbe to join a host's microbiome, its population-dynamic parameters must sufficiently align with the host's population-dynamic parameters. Possible population-dynamic outcomes include that a microbe might be excluded from the microbiome, might initially enter the microbiome only to be shed thereafter from the holobiont, might join the microbiome and coexist with the host, or might, for a deleterious microbe, enter the microbiome culminating in its own extinction together with the host.

A horizontally transmitted microbiome constitutes a genetic system with "collective inheritance" that supports Darwinian descent with modification. The microbial source pool in the environment is the counterpart for the microbiome of the gamete pool for nuclear genes. 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 microbial genes conferring the highest holobiont fitness. The effectiveness of holobiont selection on microbial genes is limited compared to the effectiveness of natural selection on nuclear genes. In the absence of holobiont selection, a competitively inferior microbial strain may coexist with a superior strain in a colonization-extinction equilibrium. Alternatively, the presence of holobiont selection permits a polymorphism between altruistic and selfish microbial strains.

A microbe might strike an optimal fitness balance between lowering its within-host fitness while increasing holobiont fitness. However, such 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 non-helpful microbes—likely brought about by hosts making antibodies against non-helpful microbes. This discrimination constitutes 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 a ”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 being 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 processes must have produced the ubiquitous integration that occurs between microbiomes and their hosts. Furthermore, experimental studies of joint microbiome-host dynamics and evolution are increasingly appearing (*eg.* Burns *et al.* 2017, Wang *et al.* 2020, 2021).

The purpose of this paper is two-fold. The first is to understand the dynamics of coupled microbe and host populations. What conditions permit the coexistence of microbes within their hosts? The second is to determine whether a microbiome can form an extended genetic system that contributes to holobiont evolution. And in particular, a major task is to account for the formation of microbiome-host integration.

2 Preliminary Concepts

A holobiont contains two genetic systems that together comprise the hologenome—the microbial genes and the nuclear genes. This paper focuses specifically on the microbial genes because this component of holobiont inheritance is conceptually understudied relative to nuclear inheritance.

The following sections introduce special concepts relevant to theorizing about holobiont population dynamics and evolution.

2.1 Mode of Transmission

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 parental microbiome by chicks incubating in a nest. A great many such mechanisms have been described during the last two decades.

In many groups the phylogeny of the hosts parallels the composition of their microbiomes, that is, closely related hosts have more similar microbiomes than distantly related hosts (*e.g.* Moeller *et al.* 2016). This phenomenon is called "phylosymbiosis." However, as Lim and Bordenstein (2020) note, "Phylosymbiosis does not necessarily imply vertical transmission, mutualistic interactions or evolutionary splitting from a common ancestor via coevolution, cospeciation, co-diversification or cocladogenesis. Although these processes may lead to phylosymbiosis, the pattern may alternatively arise by antagonistic interactions and/or horizontal microbial transmission whereby interactions between hosts and environmental microbes establish phylosymbiosis anew each generation."

Especially in marine environments, 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). Another study with cockroaches found that the microbiome in early development was acquired vertically from the parent on egg casings but the

microbiome thereafter acquired its microbes horizontally (Renelies-Hamilton *et al.* 2021). Experiments with new-born, microbiota-free honeybees showed that horizontal transmission from hive materials and social contact with nest-mates established a typical gut composition (Powell *et al.* 2014) whereas vertical transmission from queen to workers takes place in bumblebees (Su *et al.* 2021). Thus in terrestrial environments, although vertical transmission occurs to some extent in some groups, horizontal transmission is widespread too.

At this time, many models have been developed using an assumption of vertical transmission or combined vertical/horizontal transmission (Lipsitch *et al.* 1996, Fitzpatrick 2014, 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). Generally speaking, the models tend to argue that vertical transmission promotes, and horizontal transmission degrades, the effectiveness of holobiont selection. Still, Roughgarden (2020) showed that holobiont selection is somewhat effective even with solely horizontal transmission. So, this paper differs from most of the preceding models in continuing to emphasize solely horizontal transmission. The supposition is that horizontal transmission is the generic mode of transmission and that vertical transmission can be added whenever special situations require.

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.

2.2 Collective Inheritance

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

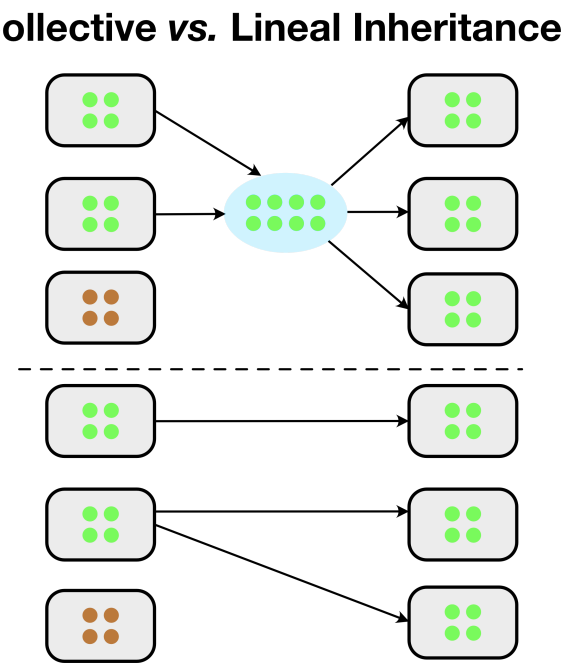

Figure 1: *Top panel illustrates collective inheritance as occurs with horizontal microbe transmission and bottom panel illustrates lineal inheritance as occurs with vertical microbe transmission. In both cases the green microbes are selected and the brown ones are not. With collective inheritance the 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.*

descent through modification, just as lineal inheritance does. However, collective inheritance is 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 including, but not limited to, their immediate parents. A further critique of the Lewontin criteria appears in Papale (2020).

2.3 Multilevel Selection

Holobiont selection is similar to concepts 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 similar to, but not the same as, MLS1 because the host is not a recurring feature of the environment—it is an evolving organism itself. Therefore, holobiont selection with horizontal microbiome transmission is similar to what must be considered as 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 density dependence within the collectives. In a holobiont, the collectives are finite in size so the number of particles that can fit in each is limited. In holobiont selection, unlike in any of the three version of MLS, the collectives have a carrying capacity for their particles. This density dependence is important in determining whether one microbe strain can competitively displace another.

Coevolution, as with flowers and their pollinators, also differs from both the MLS versions and from 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.

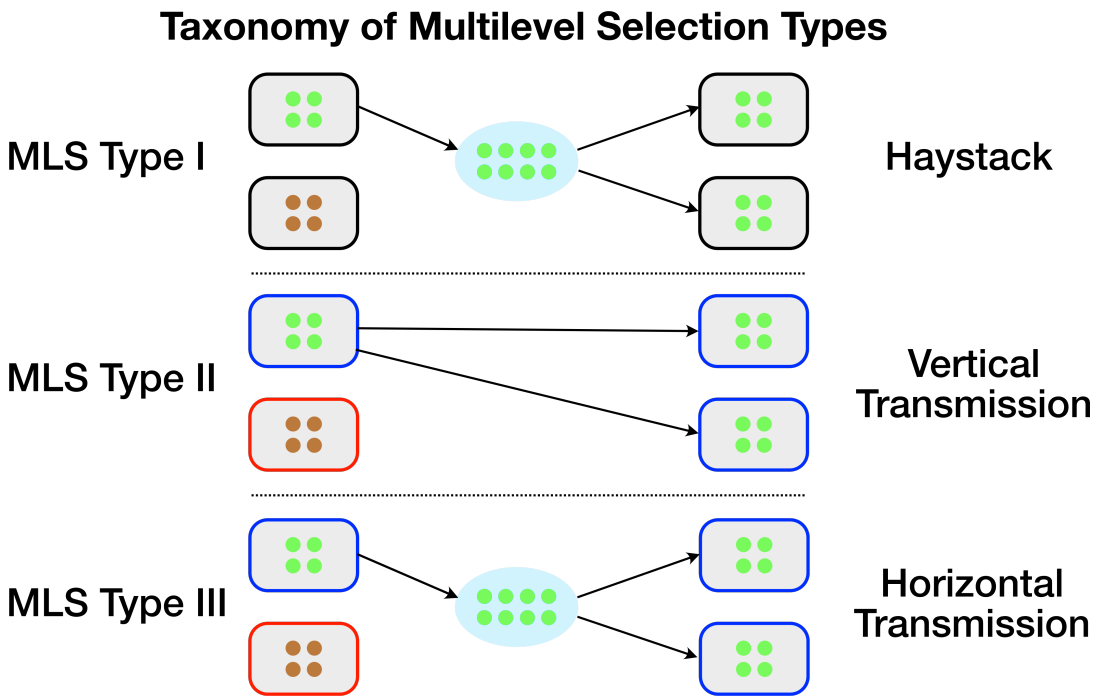

Figure 2: *Taxonomy of Multilevel Selection Types. In multilevel selection 1 (MLS1), the environment provides locations indicated as black rectangles with rounded corners. In these locations the organisms undergo natural selection favoring selfish genes, in brown. After selection, offspring from the 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 both forms of 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.*

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.

3 Host with One Microbial Strain

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.

3.1 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 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

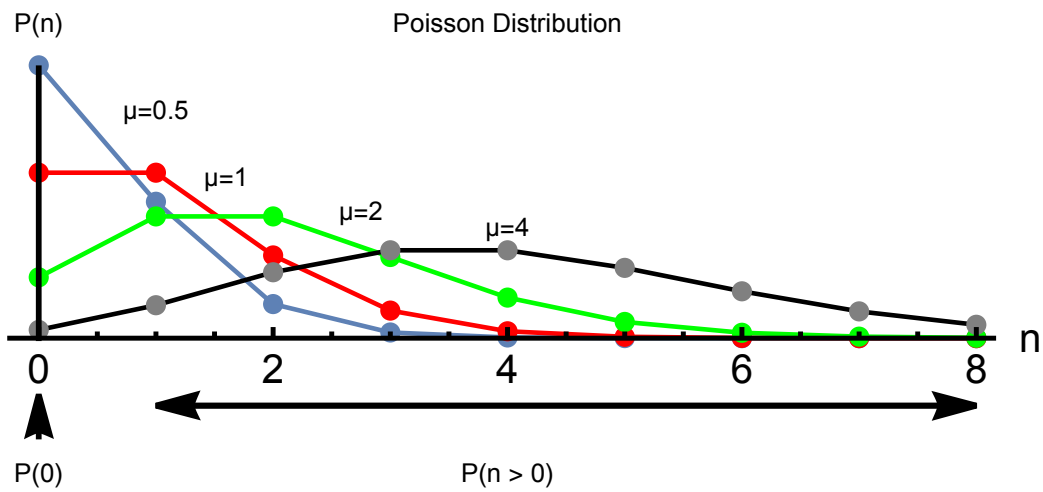

Figure 3: *Poisson Distribution for several values of μ, the Poisson density. The $P(n)$ is the probability that n microbes colonize a juvenile host. If $\mu < 1$, then the distribution peaks at $n = 0$. If $\mu > 1$, the distribution peaks at some $n > 0$. According to the formula for Poisson distribution, 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.*

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 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 col-

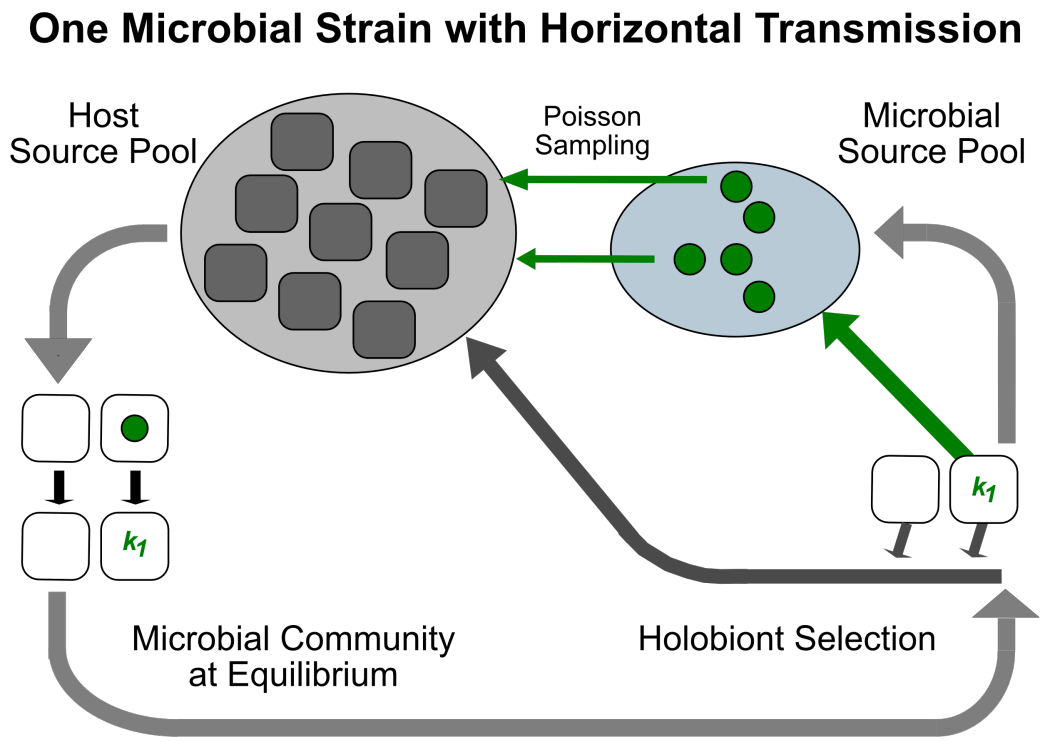

Figure 4: *Holobiont life cycle where microbiome consists of one strain (green circle). In each generation the microbiomes for the larval hosts are assembled by Poisson sampling from the microbial source pool. The microbiome comes to population-dynamic equilibrium within each host generation. Then holobiont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

umn. 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 water droplets in the air. 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 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

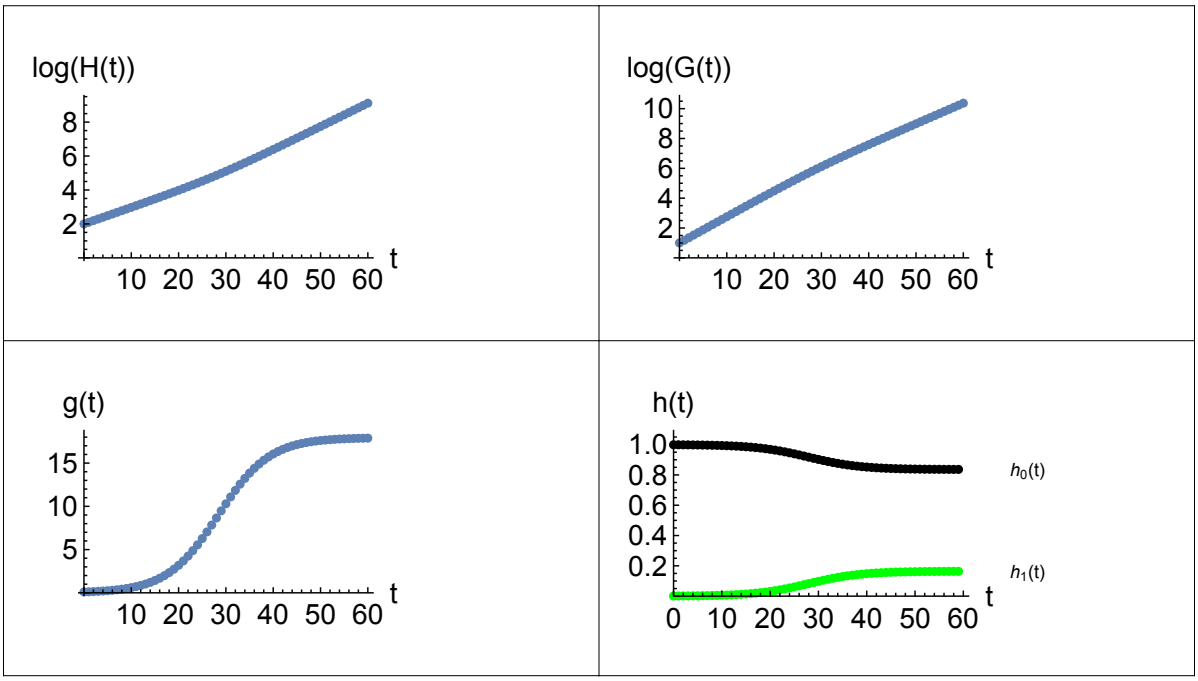

Figure 5: *Microbe-host coexistence. Top panels show trajectories of* $\log_{10} H(t)$*, and* $\log_{10} G(t)$ *through time for a beneficial microbe. The lower left panel shows,* $g(t)$*, number of microbes per host through time. The lower right panel shows the frequencies of holobionts with 0 microbes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

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 holobiont 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.

The host fitness is single-level because it depends only on microbe number and not on the fitness of the microbes themselves. 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 its other traits such as size, color etc. Instead, the microbe abundance is controlled by the *microbe's* population dynamics. Still, selection on the host does wind up indirectly controlling the microbial abundance 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. Because of population-dynamic feedback, microbe abundance becomes effectively a host trait.

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 fitness, k, and the 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 (MacArthur 1962, Roughgarden 1971).

To see what the model does, Figure 5 illustrates the output for an instance of a host with a beneficial microbe. The figure shows first, that both $H(t)$ and $G(t)$ asymptotically approach geometric growth; second, that $g(t)$ asymptotically approaches an equilibrium ratio of microbes to larval hosts in their source pools; and third, that the frequencies of holobionts with 0 microbes or k microbes asymptotically approach stable values. 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.

3.2 Conditions for Microbe-Host Coexistence

Figure 5 illustrates a typical case of microbe-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 approach 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.

3.2.1 Host with Beneficial Microbes

Consider an explicit illustration of how the condition for increase when rare interacts with the condition for coexistence. For a beneficial microbe, $W_1 > W_0 > 1$. Assume the same d, W_0 and W_1 as in Figure 5 previously, and allow the within-host microbial abundance, k, to vary. Based on the parameters in Figure 5, the condition for increase when rare requires that k must satisfy $0.01 \times k \times 2 > 1$. So, if $k > 50$, 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 $k > 62.5$, then the microbes can coexist with their hosts. In Figure 5, 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

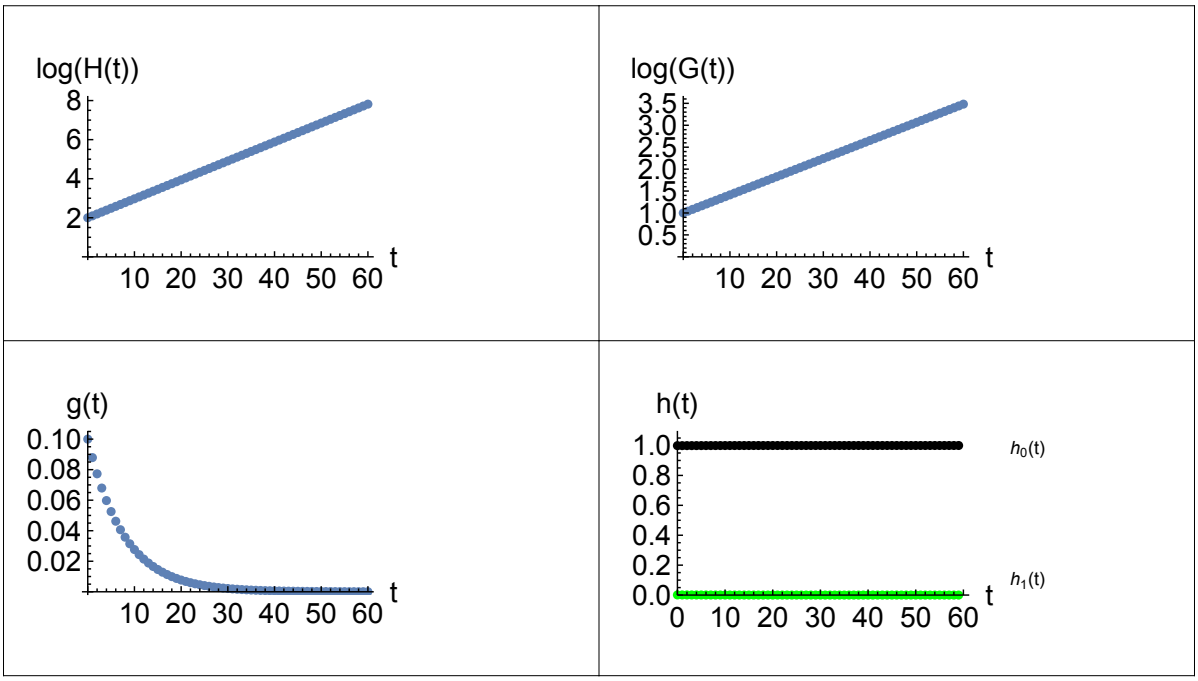

Figure 6: *Microbe shedding. Top panels show trajectories of* $\log_{10} H(t)$*, and* $\log_{10} G(t)$ *through time for a beneficial microbe. The lower left panel shows,* $g(t)$*, the number of microbes per host through time. The lower right panel shows the frequencies of holobionts with 0 microbes (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. 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 based on the parameters from Figure 5. 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-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.

The prospect of host productivity exceeding microbe productivity leading to microbe shedding seems empirically unlikely. Nonetheless, this mathematical possibility resurfaces in the analysis of further cases. The mathematical appendix details how Figure 7 is developed along with the equations to determine the conditions for microbe extinction, shedding and coexistence.

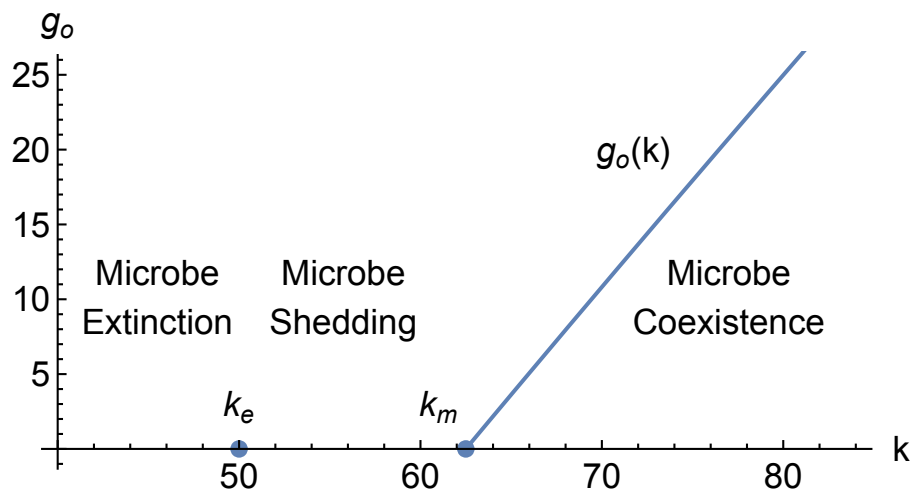

Figure 7: *Invasion-when-rare, microbial shedding, and coexistence conditions for beneficial microbe. The k_e is the minimum within-host abundance for the microbe to invade when rare, k_m is the minimum within-host abundance for the microbe and host to come to an equilibrium microbe-host ratio, and $g_o(k)$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

3.2.2 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. In this example, the fitness of a microbe containing holobionts is dropped from $W_1 = 2$ down to $W_1 = 3/4$. The parameters, d and W_0, from the preceding examples remain the same except here $k = 200$.

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 $k > 133.333$, then the microbes can increase when rare into a population of empty hosts. By the coexistence condition, k must satisfy $0.01 \times k \times (3/4)/(5/4) > 1$. So, if $k > 166.667$,

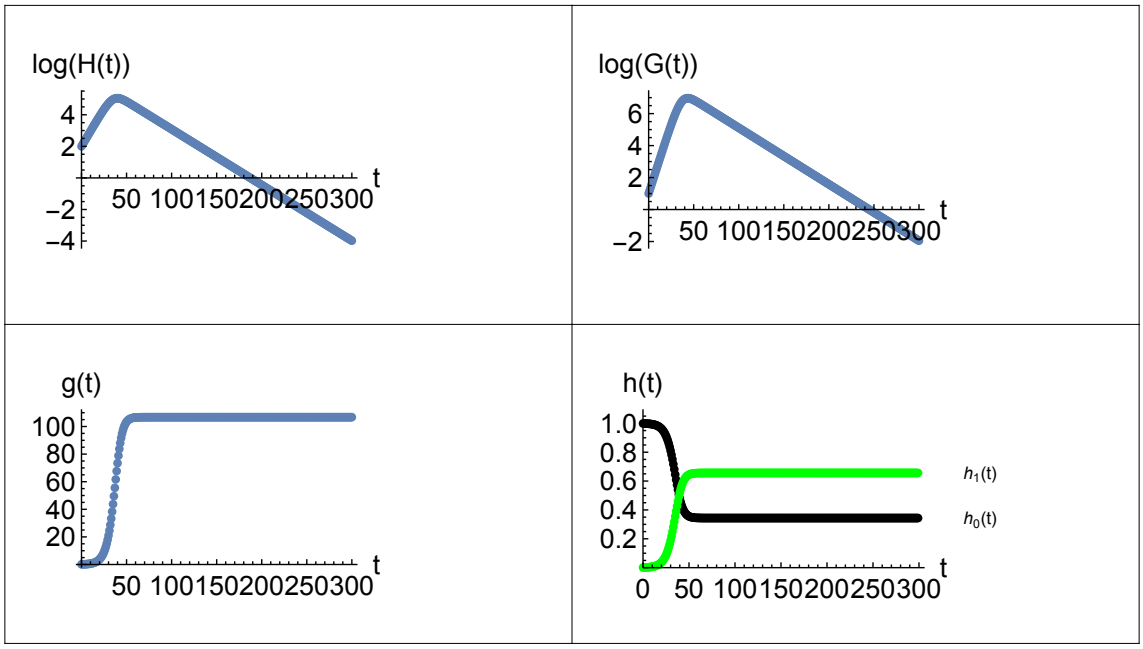

Figure 8: *Microbe-host joint extinction. Top panels show trajectories of* $\log_{10} H(t)$, *and* $\log_{10} G(t)$ *through time for a deleterious microbe. The lower left panel shows,* $g(t)$, *the number of microbes per host through time. The lower right panel shows the frequencies of holobionts with 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* $t_{\max} = 300$.

then the microbes can coexist with their hosts. In the appendix, the critical microbe-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 to intersect the horizontal line for the critical microbe-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.

This section has shown that microbe-host population dynamics leads to many different outcomes depending on microbe productivity, on host productivity with and without microbes, and on the effectiveness of microbial colonization. No outcomes involve microbe-host oscillation.

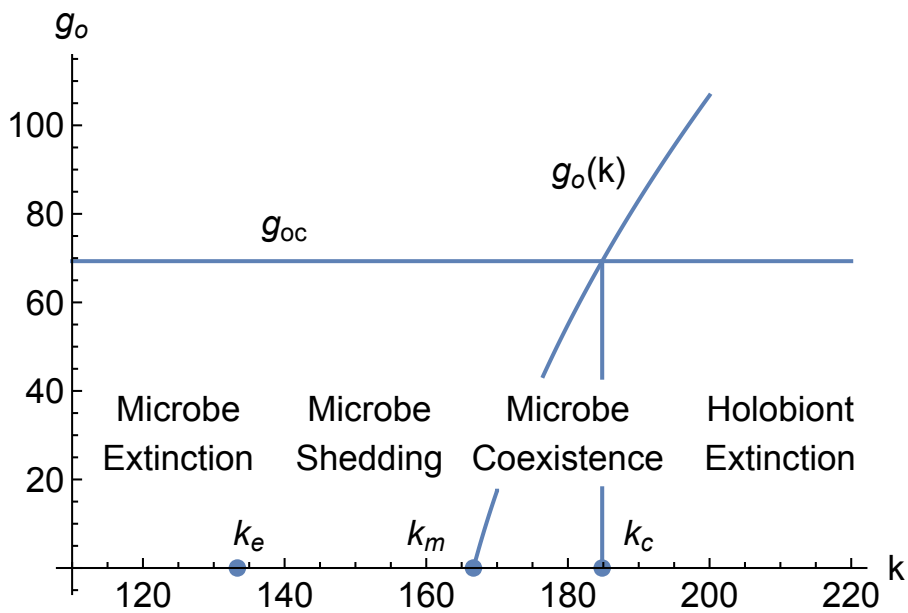

Figure 9: *Invasion-when-rare, microbial shedding, coexistence and extinction conditions for a deleterious microbe. The k_e is the minimum within-host abundance for the microbe to invade when rare, k_m is the minimum within-host abundance for the microbe and host to come to an equilibrium microbe-host 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 $k_c = 184.839$.*

4 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.

4.1 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

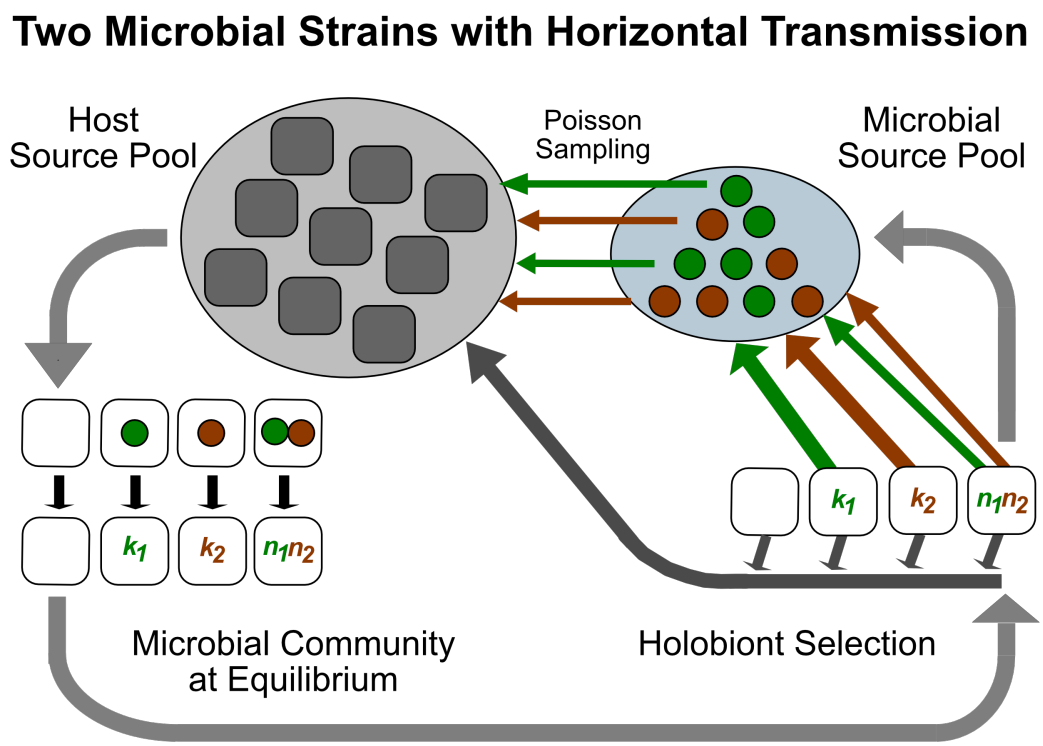

Figure 10: *Holobiont life cycle where microbiome consists of one or two microbial strains (green or brown circle). In each generation the microbiomes for the larval hosts are assembled by independent Poisson sampling of the two strains from the microbial source pool leading to four "hologenotypes". 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. The following subsections present some scenarios of holobiont selection on microbial genes that can be contrasted with corresponding scenarios of ordinary natural selection on nuclear genes.

4.2 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 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 microbial 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 plays 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.

4.3 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, $W_0 = 1$. In this situation 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 only indirectly through their effects on the phenotype. Accordingly, $k_1 = k_2 = k$, $d_1 = d_2 = d$, and $a_{12} = a_{21} = 0$.

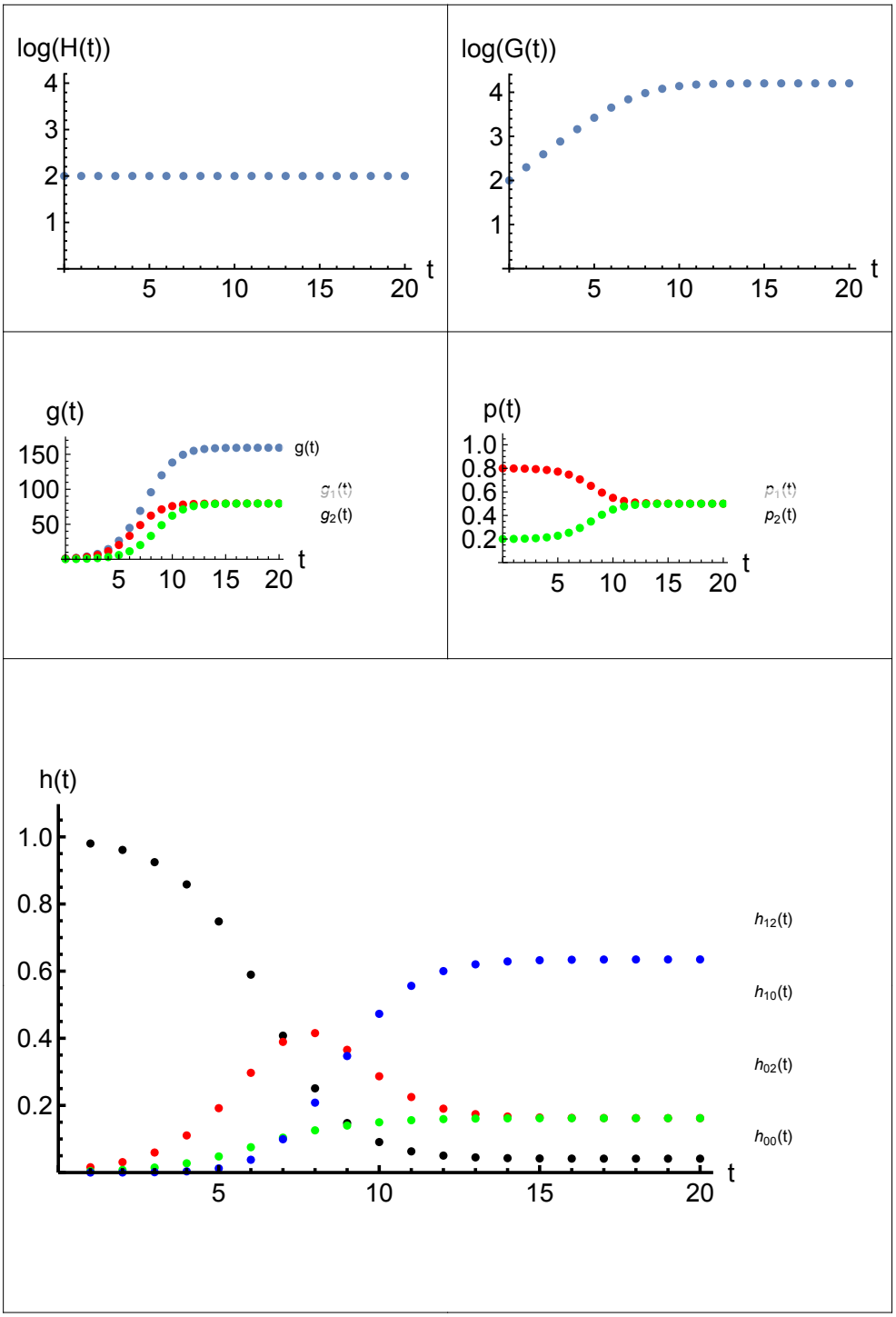

Figure 11: *Host and with two identical strains of microbes. The strains start from different initial conditions.* $d_1 = 0.02$, $d_2 = 0.02$, $k_1 = 100$, $k_2 = 100$, $a_{12} = 0$, $a_{21} = 0$, $W_0 = 1$, $W_1 = 1$, $W_2 = 1$, $W_{12} = 1$, $H_{\text{init}} = 100$, $G_{1\text{init}} = 80$, $G_{2\text{init}} = 20$, $t_{\max} = 20$.

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 colonization dynamics. The holobiont selection in favor of strain-2 pushes the frequency of strain-2 up above the center at 0.5 and draws the frequency of strain-1 down below the center of 0.5. In another example with the same parameters, 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 somewhat influence the result.

The power of the 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 selection has a much larger effect than in Figure 12.

Figure 14 shows how the equilibrium frequency of strain-1 depends on the colonization parameter. In the example, if d is greater than 0.014, polymorphism results. However, as d is increased much beyond 0.014, the equilibrium frequency of strain-1 quickly approaches 0.5, indicating that the colonization process completely overpowers the directional selection against strain-1 and favoring strain-2. Conversely, if the the colonization process is weak enough, then directional selection does fix the favored allele and eliminates the other, as in classical population genetics.

The colonization process matters because d controls whether the strains are able to express their fitness differences. If d is high, then the strains often co-occur in the same host. Figure 12 (bottom) shows that the two-strain holobiont frequency, $h_{12}(t)$ is the highest frequency. In this situation, both strains often find themselves having the same fitness, W_{12}, because they are both together in the same holobiont. Hence, neither can have an advantage over the other. Conversely, if d is low, then the strains often occupy different hosts by themselves. In Figure 13 (bottom), the strain-2 holobiont frequency, $h_{02}(t)$, is the highest frequency and $h_{12}(t)$ is very low. In this situation the fitness differences between the strains, $W_2 > W_1$, are realized and strain-2 can benefit from its advantage over strain-1. Thus, the hologenotypic variation for holobiont selection to operate on is lessened if d is high. High colonization rates homogenize the hologenotypes across the juvenile hosts. Conversely, a low d allows hologenotypic variation to form that is then acted upon by holobiont selection leading to fixation of the selectively favored microbial gene.

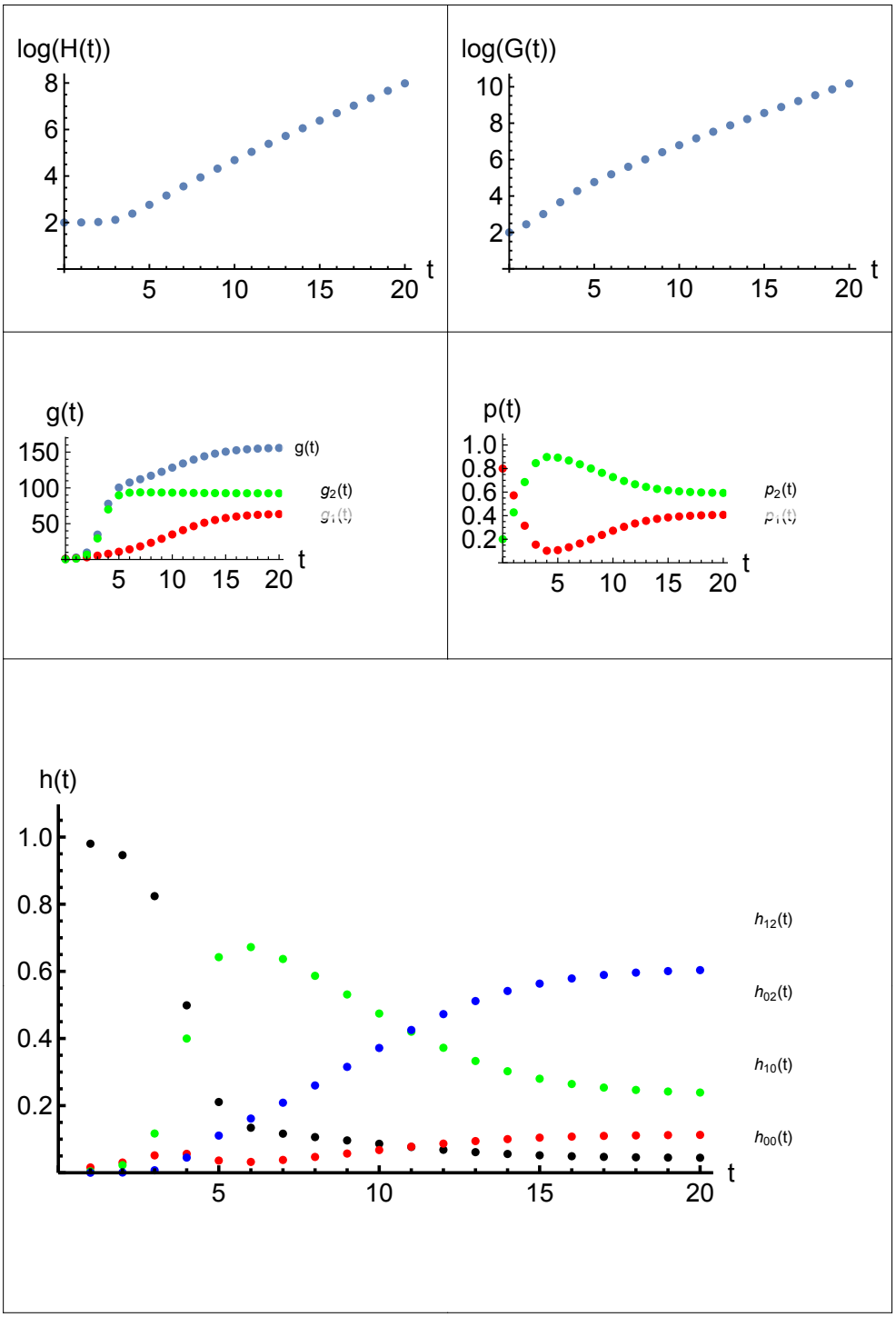

Figure 12: *Holobiont selection favoring strain-2. Here,* $W_0 = 1$, $W_1 = 1$, $W_{12} = 2$, $W_2 = 3$. *Also,* $d_1 = 0.020$, $d_2 = 0.020$, $k_1 = 100$, $k_2 = 100$, $a_{12} = 0$, $a_{21} = 0$, $H_{\text{init}} = 100$, $G_{1\text{init}} = 20$, $G_{2\text{init}} = 80$, $t_{\max} = 20$.

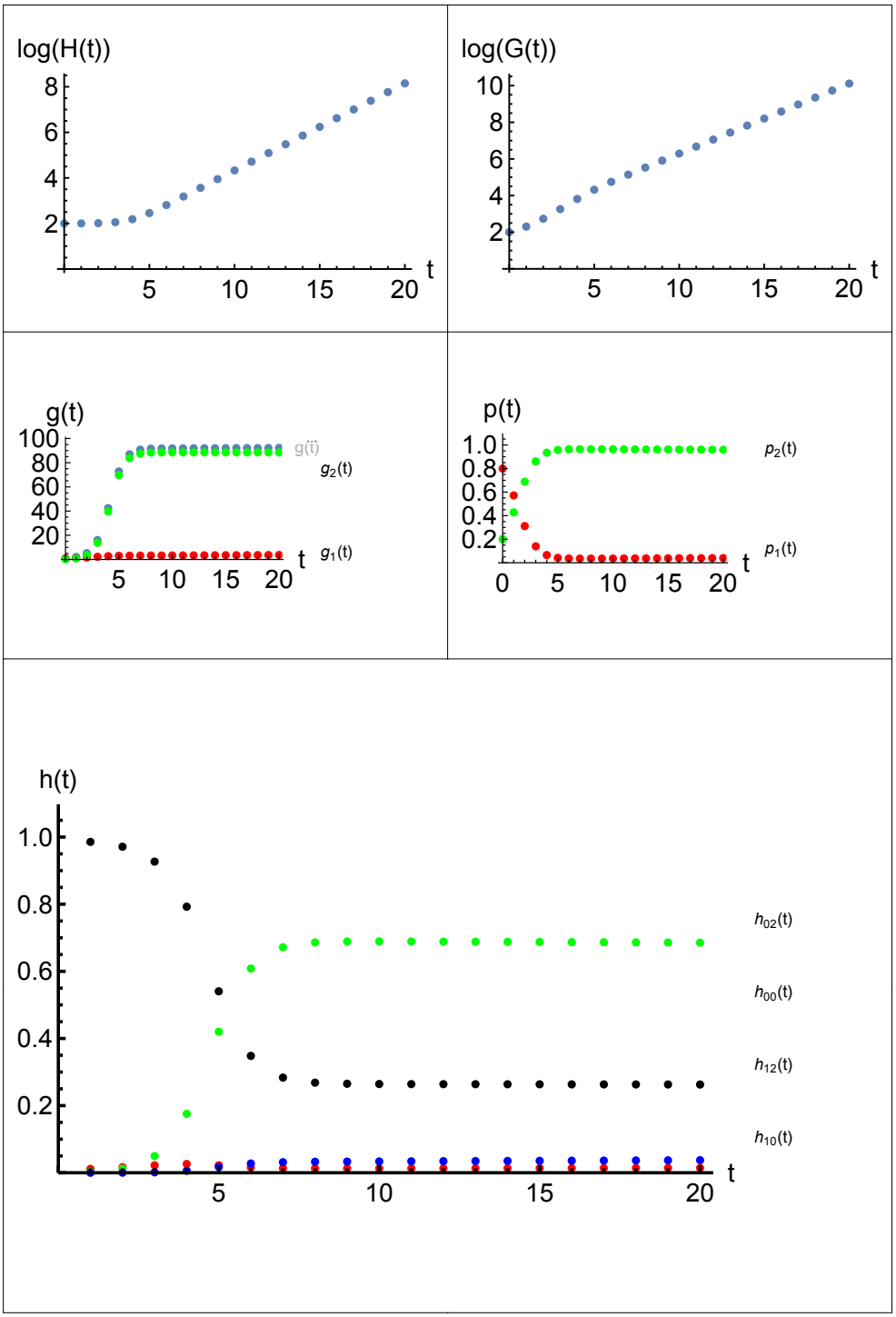

Figure 13: *Holobiont selection favoring strain-2. Here,* $W_0 = 1$, $W_1 = 1$, $W_{12} = 2$, $W_2 = 3$. *Also,* $d_1 = 0.0145$, $d_2 = 0.0145$, $k_1 = 100$, $k_2 = 100$, $a_{12} = 0$, $a_{21} = 0$, $H_{\text{init}} = 100$, $G_{1\text{init}} = 20$, $G_{2\text{init}} = 80$, $t_{\max} = 20$.

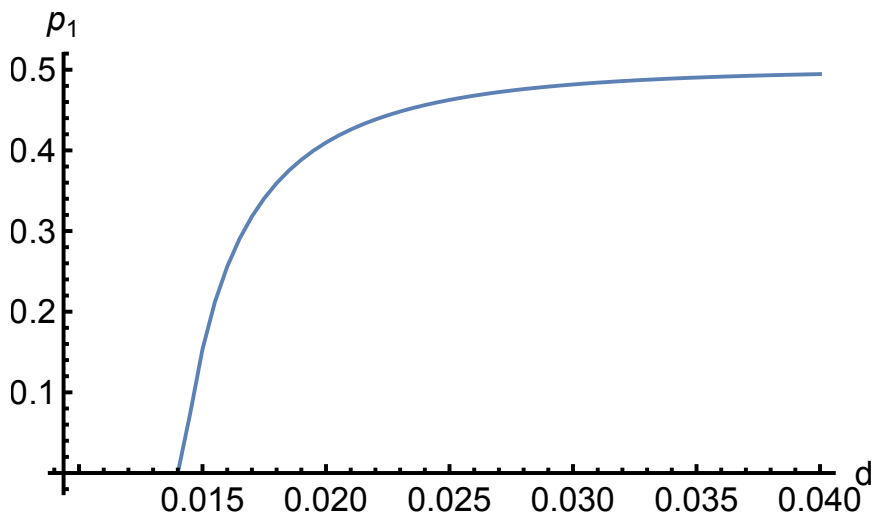

Figure 14: *Equilibrium frequency of strain-1 as a function of the colonization parameter, d. Directional holobiont selection is against strain-1 and favors strain-2. Directional holobiont selection is effective for low d but not high d. Here,* $k = 100$, $W_0 = 1$, $W_1 = 1$, $W_{12} = 2$, $W_2 = 3$

This analysis demonstrates that hologenotype frequencies show a limited response to directional holobiont selection, and the extent to which they do respond is qualified by the colonization parameter. This limited response implies that holobiont selection has a limited power to produce holobiont adaptation. However, compared with directional natural selection on nuclear genes, directional holobiont selection on microbial genes retains more genetic diversity, a property that might be beneficial in an environment that fluctuates from generation to generation.

The limited response 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.

4.4 Microbial Colonization-Extinction Coexistence

Can hologenome polymorphism can result from microbial dynamics alone without any selection at the holobiont level? The previous subsection explored the situation where the microbes did not directly interact with each other. They did affect the host fitness differently, but were otherwise identical just as two alleles in a nucleus might affect host fitness differently but not directly interact with each other. Consider now the converse situation. Suppose the two strains of microbes do interact 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.

The assumption that strain-1 out-competes strain-2 whenever the two strains are within the same host 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.

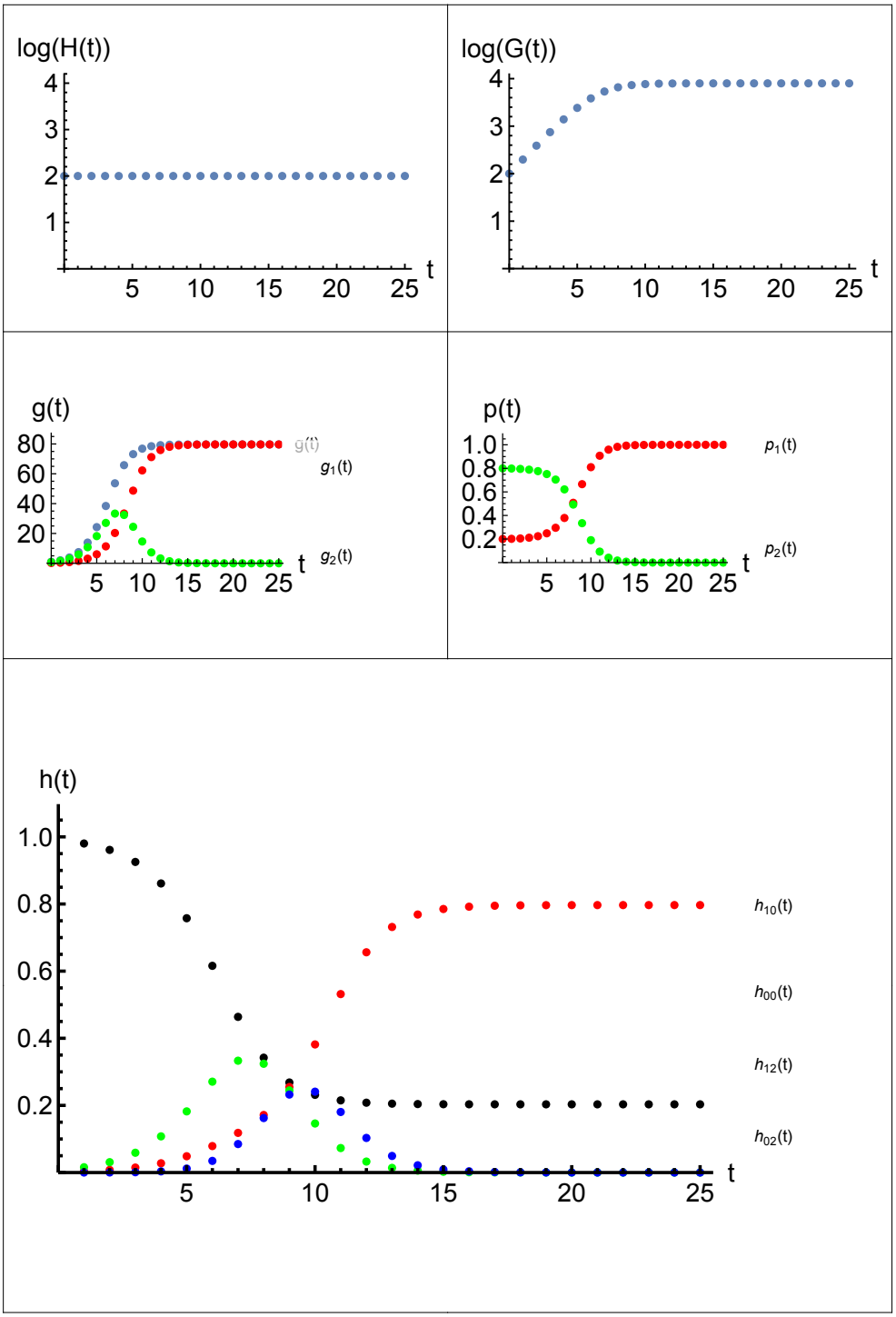

Figure 15: *Host with competitively superior strain-1 and competitively inferior strain-2. Here,* $k_2 < k_{2c} = 246$*, so strain-1 drives strain-2 to extinction. Also* $d_1 = 0.02$*,* $d_2 = 0.02$*,* $k_1 = 100$*,* $k_2 = 100$*,* $W = 1$*,* $H_{\text{init}} = 100$*,* $G_{1\text{init}} = 20$*,* $G_{2\text{init}} = 80$*,* $t_{\max} = 25$*,* $a_{12} < 1$ *and* $a_{21} > 1$*.*

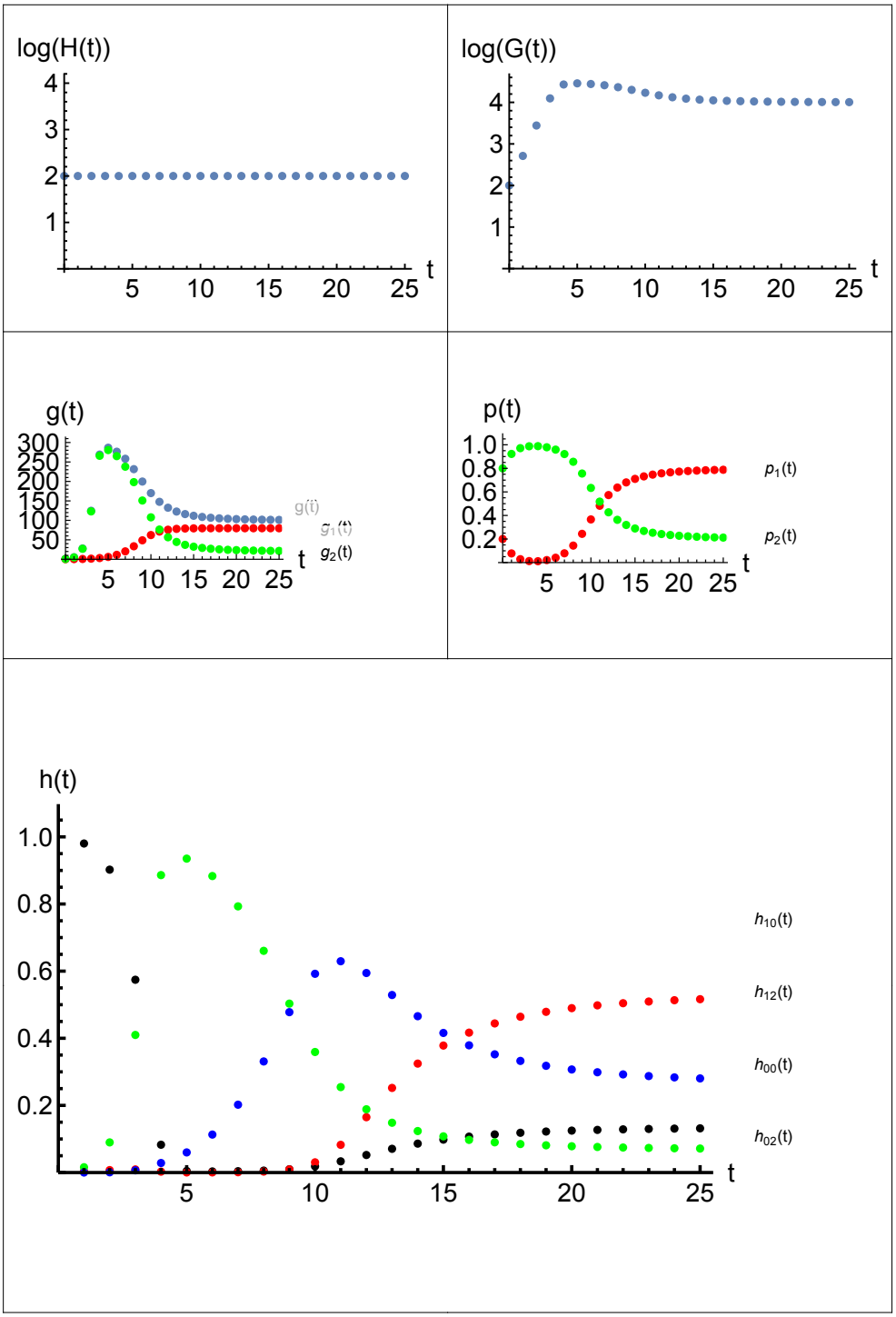

Figure 16: *Host with competitively superior strain-1 and competitively inferior strain-2. Here,* $k_2 > k_{2c} = 246$*, so strain-1 and strain-2 coexist. Also* $d_1 = 0.02$*,* $d_2 = 0.02$*,* $k_1 = 100$*,* $k_2 = 300$*,* $W = 1$*,* $H_{\text{init}} = 100$*,* $G_{1\text{init}} = 20$*,* $G_{2\text{init}} = 80$*,* $t_{\max} = 25$*,* $a_{12} < 1/3$ *and* $a_{21} > 3$*.*

4.5 Polymorphism between Altruistic and Selfish Microbes

Can the dynamics at the host level and microbe level interact?. In Subsection 4.3, the fitness differences were solely at the host level and in Subsection 4.4, 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 $W_2 > W_{12} = W_1 = W_0 = 1$.

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 for this example that if $W_2 > 1.23$, 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.

This section has presented several scenarios for how the microbiome responds to intermicrobial dynamics combined with selection on the host. The model, simple as it is, still involves ten parameters and allows a great many situations to be modeled. Other scenarios can be analyzed with this model without altering the model itself but simply by choosing different parameter values. For example, in all the scenarios presented here the host fitness in the absence of microbes, W_0, has been assumed to equal 1. The possibility that the host requires the microbes to survive might be modeled by assuming $W_0 < 1$. With this assumption, scenarios in which host and microbe coexist depending on sufficient microbe colonization abilitiy can be explored. Undoubtedly empirical situations arise to motivate still other scenarios.

What is remarkable about the hologenome is the difference for the host between the population genetics of its nuclear genes *vs.* its microbial genes. Nuclear genes are mostly inert with respect to one another. They differentially impact the host phenotype but generally do not directly impact each other even though mechanisms of intragenic conflict such as segregation distortion and meiotic drive exist (*e.g.* Sandler and Golic 1985, Taylor and Ingvarsson 2003, Lindholm *et al.* 2016). In contrast, the host's microbial genes enjoy their own community ecology spanning the host and their source pools in the environment. This, together with holobiont selection, leads overall to remarkably different evolutionary dynamics compared with the classical evolutionary dynamics of one-locus two-allele nuclear genes. Holobiont selection requires new intuition.

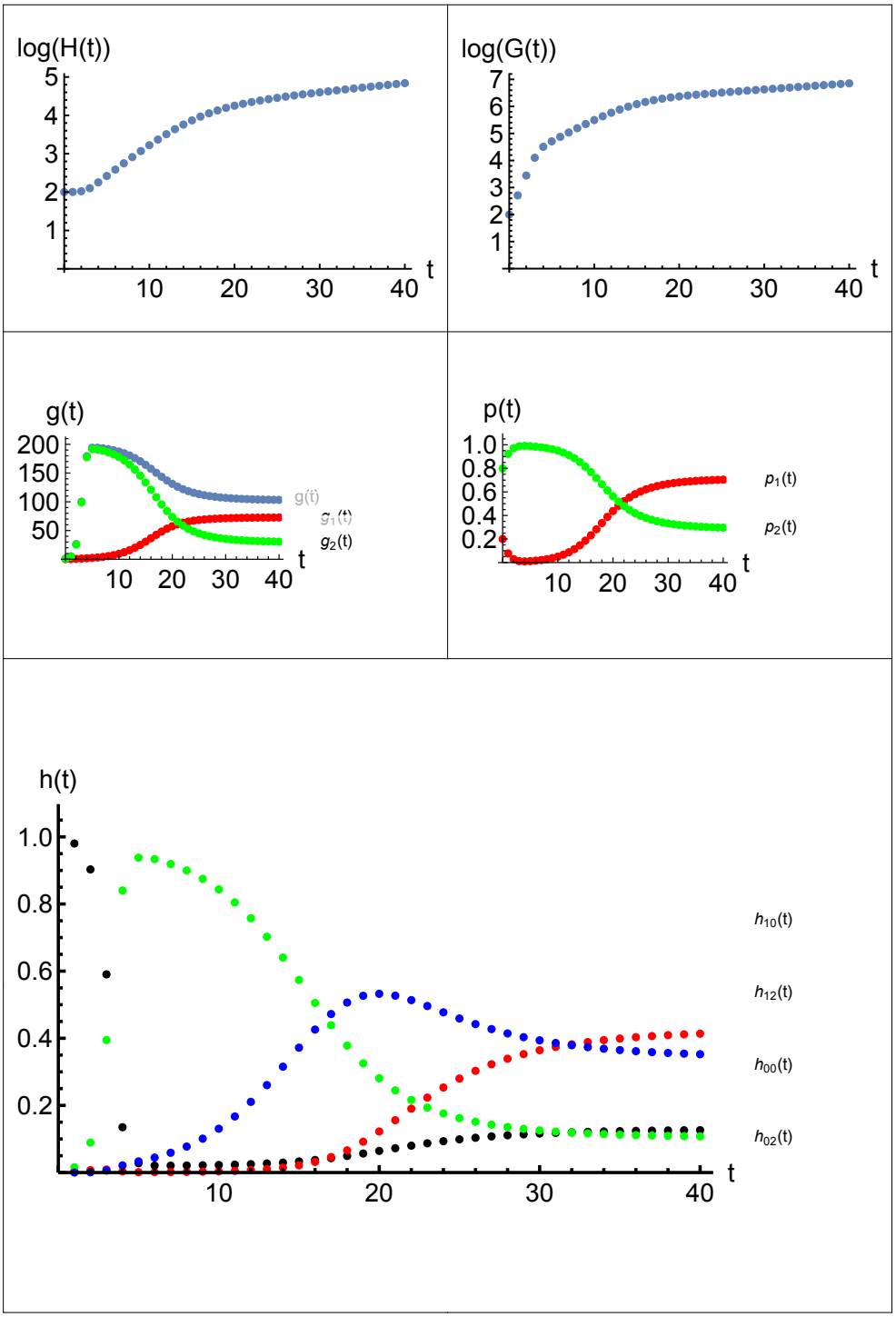

Figure 17: *Coexistence of altruistic and selfish microbes. Strain-1 is selfish and strain-2 is altruistic.* $d_1 = 0.02$, $d_2 = 0.02$, $k_1 = 100$, $k_2 = 200$, $W_0 = 1$, $W_1 = 1$, $W_{12} = 1$ *and* $W_2 = 1.5$. *Also,* $a_{12} < 1/2$ *and* $a_{21} > 2$. $H_{\text{init}} = 100$, $G_{1\text{init}} = 20$, $G_{2\text{init}} = 80$, $t_{\max} = 40$.

5 Microbiome-Host Integration

A common conjecture in discussions of holobiont evolution is that selection on the holobiont as a unit produces a mutualistic interaction between microbiome and host that is adaptive for the holobiont. The remarkable integration between microbiomes and their hosts might be interpreted as a mutualistic coadaptation that results from holobiont selection. This section investigates how microbiome-host integration might form and whether the integration is correctly viewed as a mutualism and whether the integration results from holobiont selection.

This section builds upon the preceding models of one- and two-species coupled microbe-host population dynamics. This section differs in how the coefficients are interpreted. In the preceding sections, the microbial and host fitnesses and the colonization coefficients were taken as primitive and simply stipulated for each example. Here, these parameters are interpreted in terms of underlying phenotypic models for the microbes and the host that express what a host and/or a microbe does. What a host does, or a microbe does, is called its "effort". For example, algae in the microbiome might contribute sugars to the host. The amount of sugar the algae contributes is its "effort", denoted as x, which might be expressed in units of micrograms of sugar contributed per time. This effort lowers the microbe's with-in host fitness, k, but increases the host's fitness, W. So, the population-dynamic parameters are functions of the microbe's effort, $k(x)$ and $W(x)$. Similarly, the host might expend effort, z, to inhibit the colonization of microbes by manufacturing antibodies, or might promote colonization by producing chemical attractants. The units for this effort might be the amount of ATP needed per time to produce the antibodies or attractants. The net production of this effort considers both the costs of manufacture and the benefits the antibodies or attractants confer. Thus, the host fitness is a function of both the microbes' effort and its own effort, $W(x, z)$; the microbe's within-host fitness is a function of its own effort, $k(x)$; and the microbe's colonization parameter is a function of the host's effort, $d(z)$. The question to be answered is whether holobiont selection adjusts the values of x and z to promote holobiont adaptation and maximize holobiont fitness.

5.1 Microbial Altruism *vs.* Selfishness

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. Let the microbe's effort be called its "altruistic effort". Suppose increasing 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 degree of altruism a microbe should supply the host?

To answer this question, an optimality criterion is needed. One possibility is the multilevel

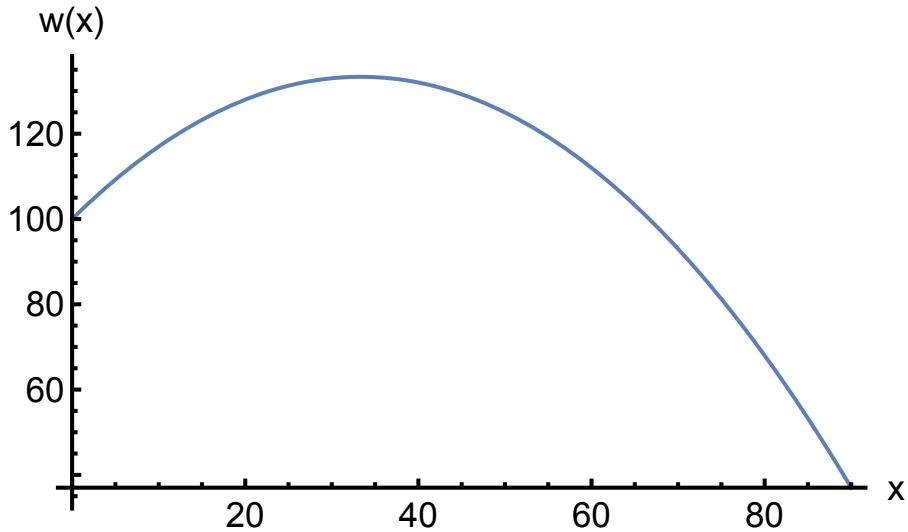

Figure 18: *Multilevel microbial fitness, w, as a function of the altruistic effort, x. The optimal effort, $\hat{x} = 33.333$. The maximal effort, $x_{max} = 90$. Here, $d = 0.1$, $k_o = 100$, $W_o = 1$, $b = 0.03$, $c = 1$.*

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 (ESS), which for nuclear genes, is a strategy that cannot be invaded by any other strategy (Maynard Smith 1974).

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 strain always displaces any established altruistic strain. Hence, the optimal degree of altruism,

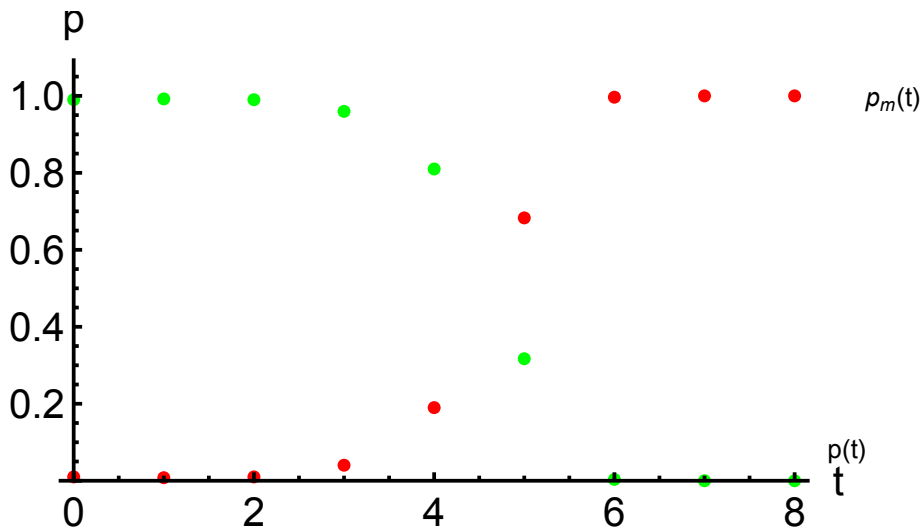

Figure 19: *Frequency of altruistic microbe, p, and selfish mutant, p_m, through time. Host fitness with altruistic microbe, $W = W_o + b\,x$. Altruistic microbe carrying capacity, $k = k_o - c\,x$, Host fitness with selfish mutant, $W_m = W_o + b\,y$, Selfish microbe carrying capacity, $k_m = k_o -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

$\hat{x}$, 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 $x = \hat{x}$.

5.2 Host Intervention

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.

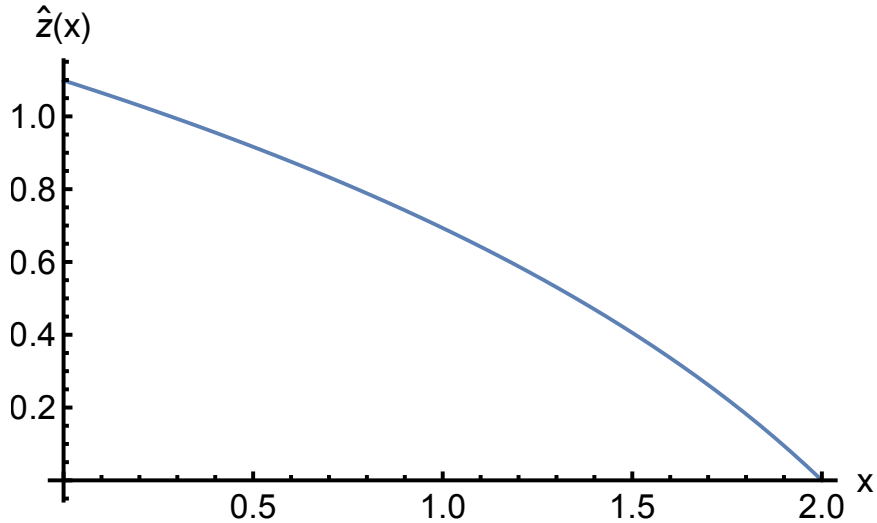

Figure 20: *Optimal antibody production, $\hat{z}(x)$, as a function of the altruism, x, being supplied by the microbe to the host. Here, $\hat{x}_{max} = 2$, $x_{max} = 3$, $\hat{z}_{max} = 1.10$, $\alpha_o = 3$, $\mu = 1$, $\beta = 1$ and $\gamma = 1$.*

The quantity of antibodies a host makes results from a balance between the direct fitness benefit and cost of making antibodies. Suppose the direct benefit to antibody production shows a decreasing return to scale and the cost of antibody production is linear. These benefit and cost functions allow an optimal antibody production to be defined. Suppose further that the benefit to the host of antibody production depends on the amount of altruism being supplied by the microbe—the more the altruism from the microbes, then the less the benefit to the host for making antibodies against the microbes. Accordingly, the optimal antibody production is a decreasing function of the resources being supplied, as shown in Figure 20 and in the mathematical appendix.

A side effect of the host's antibody production is to lower the mutant's colonization parameter. The effect of antibodies on microbial colonization is deemed a side effect because the host determines its antibody production solely from considering the *direct* fitness benefit and cost of the production regardless of how the microbes are subsequently affected.

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

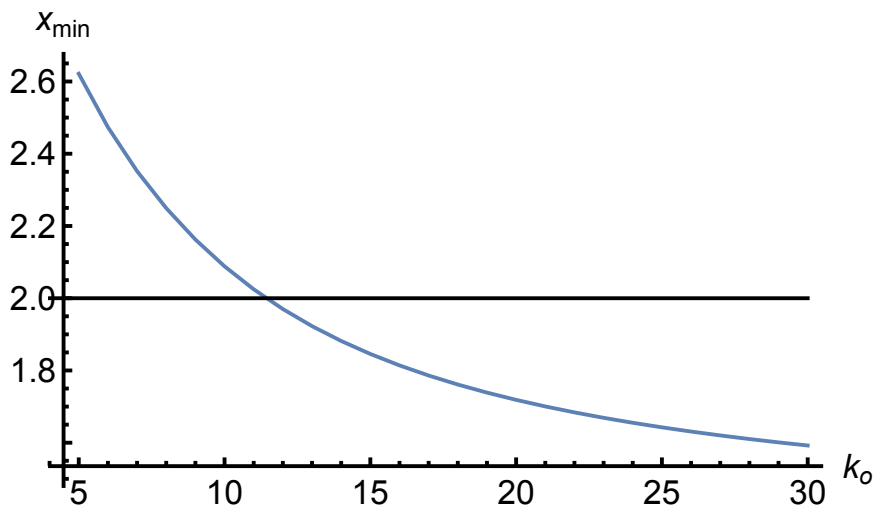

Figure 21: *Minimum altruism, $x_{min}(k_o)$, the microbe must supply to the host to increase when rare as a function of its within-host base carrying capacity, k_o. The horizontal line is $\hat{x}_{max}$, the degree of altruism that causes the host not make any antibodies at all. The intersection of 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 $W_o = 1$.*

increase the amount of altruistic effort supplied to the host.

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 of making antibodies 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. Accordingly, if a microbe's k_o is large enough that it can enter the holobiont population, the altruism it supplies must be at least $x_{min}(k_o)$. However, the microbe should not bother to supply more than what is necessary to completely shutdown the host's antibody production, $\hat{x}_{max}$. So, a microbe with base k_o needs to supply some level of altruistic effort between $x_{min}(k_o)$ and $\hat{x}_{max}$.

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, $\hat{x}_{max}$? The mathematical appendix shows that the holobionts wind up consisting of microbes that have a bit more altruism than the bare minimum together with hosts that make some level of antibodies in response. 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 end 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 limited benefit that it receives from its microbe.

This arguably imperfect outcome, which is worse than the best possible for both parties, nonetheless does represent host-microbiome integration.

5.3 Host-Orchestrated Species Sorting (HOSS)

The process whereby the host through its antibody production determines 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 production which strains in the microbial source pool can colonize.

Metaphorically, the holobiont is a seller's market for the host. The host sets up the price schedule—for each level of resource the microbes supply there is a corresponding level of antibodies produced, irrespective of any impact on the microbes. In contrast, the microbes must pay to play. They must provide enough resources to obtain a colonization parameter that permits successful colonization. From the microbes' point of view, the situation is take-it-or-leave-it—provide enough resources to colonize and you're in, otherwise you're out. And even after paying the price for successful colonization, a microbial strain faces competition from other strains who may have paid less and still successfully colonized.

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. This 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.

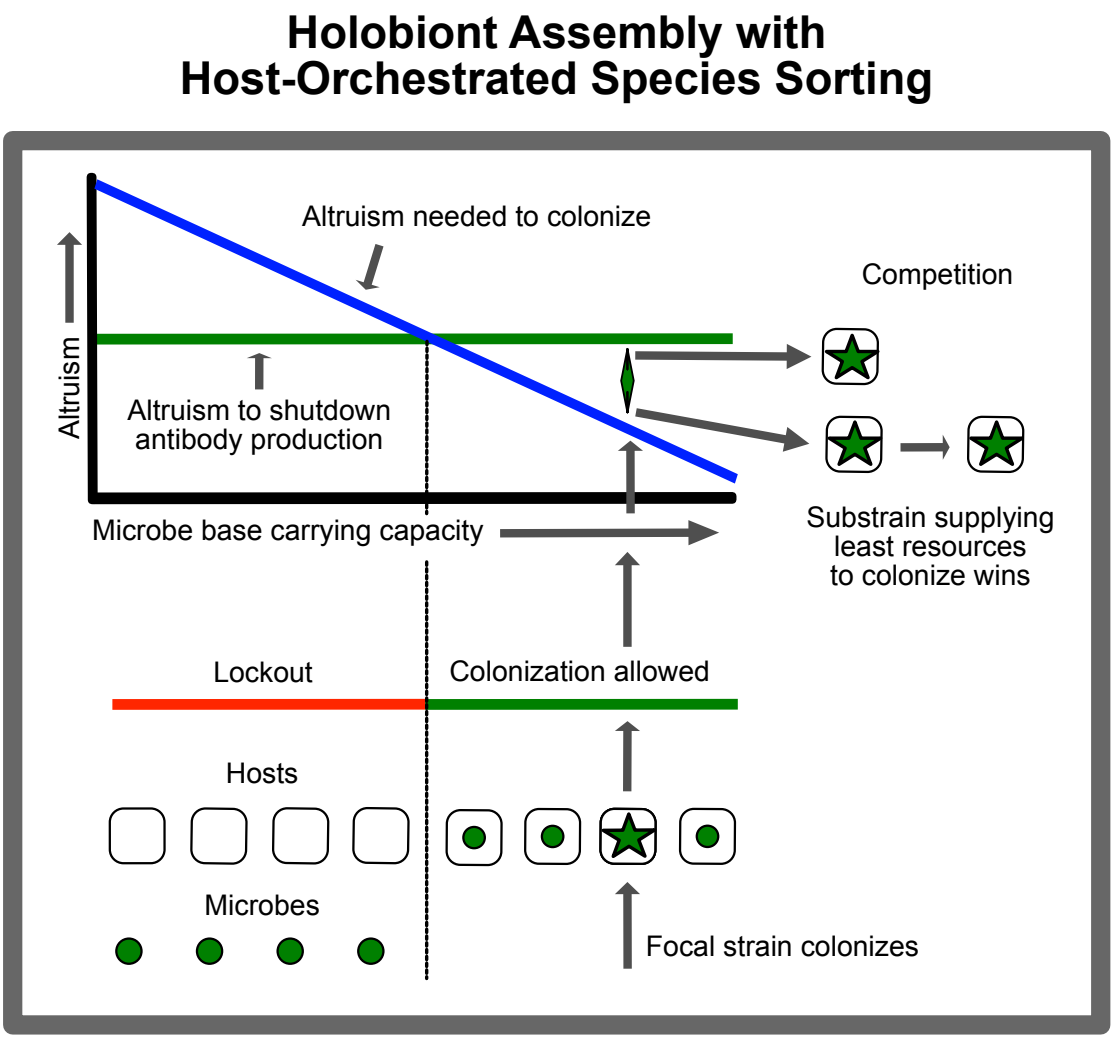

Figure 22: *Holobiont assembly with host-orchestrated species sorting (HOSS). At top left, descending blue line indicates minimum altruism microbe must supply to colonize as function of the microbe's base carrying capacity. Horizontal green line indicates amount of altruism that shuts down host antibody production. Microbes required to supply more altruism than needed to shutdown antibody production are locked out. Demarcation between rejected and eligible microbes occurs 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.*

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 proportion 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".

6 Discussion

This article has developed mathematical theory for the population dynamics, evolution and assembly of holobionts.

Several population-dynamic outcomes for microbes and hosts have been found, including that a microbe might be excluded from the microbiome, a microbe might initially enter the microbiome only to be shed thereafter from the holobiont, a microbe might join the microbiome and coexist with the host, or a deleterious microbe might enter the microbiome culminating in its own extinction together with the host.

This article has also developed the idea that a horizontally transmitted microbiome constitutes a genetic system with "collective inheritance". This form of inheritance supports Darwinian descent with modification but is inconsistent with neoDarwinism. With this form of inheritance, the microbial source pool in the environment is the counterpart for the microbiome of the gamete pool for nuclear genes. 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 microbial genes conferring the highest holobiont fitness. The effectiveness of holobiont selection on microbial genes is limited compared to the effectiveness of natural selection on nuclear genes.

The article has further shown that in the absence of holobiont selection, a competitively inferior microbial strain may coexist with a superior strain in a colonization-extinction equilibrium. Alternatively, the presence of holobiont selection permits a polymorphism between altruistic and selfish microbial strains.

The article has explored how microbiome-host functional integration might form and whether the integration is correctly viewed as a mutualism. The article shows that although a microbe might strike an optimal balance between lowering its within-host fitness while increasing holobiont fitness it is replaced by otherwise identical microbes that contribute nothing to holobiont fitness. This replacement can be reversed by hosts that discriminate against colonization by

non-helpful microbes—likely brought about by hosts making antibodies against non-helpful microbes. This discrimination constitutes microbial species sorting orchestrated by the host immune system.

Holobiont assembly with host-orchestrated species sorting (HOSS) brought about by the host immune system followed by microbial competition extends existing discussion about how the immune system determines biological individuality (Pradeu 2010, 2012, 2016, Gilbert and Tauber 2016). Pradeu (2016) writes "The immune system constitutes a discrimination mechanism, accepting some entities in the organism and rejecting others, thus participating decisively in the delineation of the organism's boundaries" (p. 803). Gilbert and Tauber (2016) write, the "immune system [is] the mediator of both defensive and assimilative environmental intercourse, where a balance of immune rejection and tolerance governs the complex interactions of the organism's ecological relationships" (p. 839). This article shows that this discrimination by the immune system results in a composite entity, the holobiont, that possesses a degree of integrated function involving both the microbes and host, even though such integration is generally less than optimal.

This article shows that microbiome-host functional integration is not the result of mutualistic coevolution because holobiont evolution is a multilevel process whereas coevolution is a single-level process. Moreover, the host-microbiome association is not mutualistic because each party is indifferent to the other's welfare and neither evolves reciprocally mutual altruism. Also, microbiome-host functional integration does not result from any of the commonly defined versions of multilevel selection because the groups have finite size implying the existence of density dependence in the within-group dynamics even in the absence of between-group density dependence. Finally, microbiome-host functional integration is not brought about solely by holobiont selection itself because the effectiveness of holobiont selection on microbial genes is limited.

Instead, holobiont evolution and assembly is a unique and simultaneous combination of both evolutionary and ecological processes. At the upper level, hosts evolve their antibody responses by ordinary natural selection on host nuclear genes. At the lower level, microbes colonize hosts in an ecological community-assembly process. The processes at the two levels are coupled because the host's antibody responses affect microbial community assembly, sorting the available microbes into those that can and those that cannot enter the holobiont. Competition between microbes that have entered the holobiont reduces the within-host microbial species diversity leaving only those that supply the least possible resources to the host consistent with being able to colonize successfully.

7 Acknowledgments

The author thanks Seth Bordenstein, Ford Doolittle, Scott Gilbert and members of the Stanford University biology department's microbiome reading group for helpful comments on the manuscript. The author also 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. 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.

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