Holobiont Evolution: Population Genetic Theory for the Hologenome

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Abstract

This paper develops a mathematical theory for holobiont evolution that parallels the population-genetic theory of classical evolutionary biology. It presents theory for hologenomes having two haploid microbial strains and two diploid host alleles. The theory reveals how selection on holobionts causes the joint evolution of microbial and host components of the hologenome. The paper shows how holobiont selection differs from coevolutionary selection, how holobiont selection is effective with both vertical and horizontal microbiome transmission, how inheritance can be a collective process rather than solely a lineal process, how random microbial colonization of the microbiome is the counterpart to random union of gametes for nuclear genes, how the evolutionary success of a microbial strain depends on within-host K-selection and between-host r-selection leading to an expression for the multilevel fitness per holobiont, how the hologenome is analogous to a two-loci genetic system, how holobiont selection maximizes equality of marginal fitnesses and not overall mean fitness, how the concept of an evolutionarily stable strategy (ESS) can be extended to hologenomes (HSS) leading to the evolution of cooperative microbe-host coadaptation, how the HSS can be invaded by an altruistic microbe resulting in a microbial polymorphism that expresses a balance of within-holobiont selfishness and between-holobiont altruism, how holobiont selection with horizontal transmission comprises a new third type of multilevel selection, and overall, how the concept of the hologenome with holobiont selection can be made conceptually and mathematically rigorous.

1 Introduction

This paper develops a mathematical theory for holobiont evolution that parallels the population-genetic theory of classical evolutionary biology. All organisms host a variety of microbes, their "microbiome". The combination of a host with its microbiome has been termed a "holobiont" (Margulis 1991). Studies reveal astonishing coadaptation between microbiomes and their hosts extending to development, metabolism, physiology and behavior (Gilbert, Sapp and Tauber 2012, McFall-Ngai, Hadfield, Bosch, Carey, Domazet-Loo, Douglas, Dubilier *et al.* 2013, Bordenstein and Theis 2015). Some evolutionary process must have produced this coadaptation.

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Because the phenotype of a holobiont results from the expression of the genes in the host's nucleus plus the genes in the microbes within the host, the union of the host genes with all the microbial genes comprises an extended genome that has been termed a "hologenome" (Zilber-Rosenberg and Rosenberg 2008). The configuration of the hologenome in a particular host has been called its "hologenotype"—it is a hierarchical data structure (Roughgarden 2020). Selection on holobionts with their varying hologenotypes is envisioned as analogous to classical natural selection on individuals with their varying nuclear genotypes. This holobiont selection might be the evolutionary process that accounts for microbiome-host coadaptation.

Classical population-genetic theory supplies an equation (or equations) that comprise a dynamical system, that is, equations that map nuclear gene or genotype frequencies at time t to the gene or genotype frequencies at time $t+1$, or symbolically $p_t \rightarrow p_{t+1}$ where p denotes a vector of gene or genotype frequencies. Therefore, a theory of holobiont evolution should do the same, that is, the theory should supply equations that map the hologenotype frequencies at time t to the hologenotype frequencies at time $t+1$, or symbolically, $H_t \rightarrow H_{t+1}$ where H denotes a vector of the frequencies of the hologenotypes in the holobiont population.

Holobiont selection is distinct from coevolutionary selection as diagrammed in Figure 1. With coevolutionary selection, distinct gene pools exist for the host and the microbes. For example, in a typical coevolutionary setup for two species, the fitnesses influencing a gene frequency in say, species-1 are a function of the abundances and gene frequencies in both species-1 and species-2, and conversely for the fitnesses in species-2 (Dieckmann and Law 1996; Carmona, Fitzpatrick and Johnson 2015). This setup leads to two coupled equations for changes in the gene pools of the two species, or symbolically, simultaneous mappings of $\{p(t) \rightarrow p(t+1), q(t) \rightarrow q(t+1)\}$ where p and q are gene frequencies in species-1 and species-2, respectively. The gene pools of the microbes and host are not conceptually merged and remain separate. In coevolutionary selection, the evolutionary interests of the host and the microbiome are not aligned, nor are the selection events in the species synchronized.

Holobiont selection operates on a single hologenome that itself is conceptually merged from the genomes of the host and its resident microbes. A theory of holobiont selection maps the state of a single "hologene pool" from t to $t+1$. In holobiont selection, the evolutionary interests of the microbes and host are aligned. Also, for holobionts the selection events on host and microbiome may be synchronized in the sense that the death or reproduction of a holobiont may imply the death or reproduction simultaneously of both host and microbiome.

Two species undergoing to coevolutionary selection play an evolutionary game against each other, and the coevolutionary outcome is a Nash competitive equilibrium (Brown and Vincent 1987). A coevolutionary perspective on the host-microbiome relationship has led to the claim that "the host and each individual microbial strain are distinct entities with potentially divergent

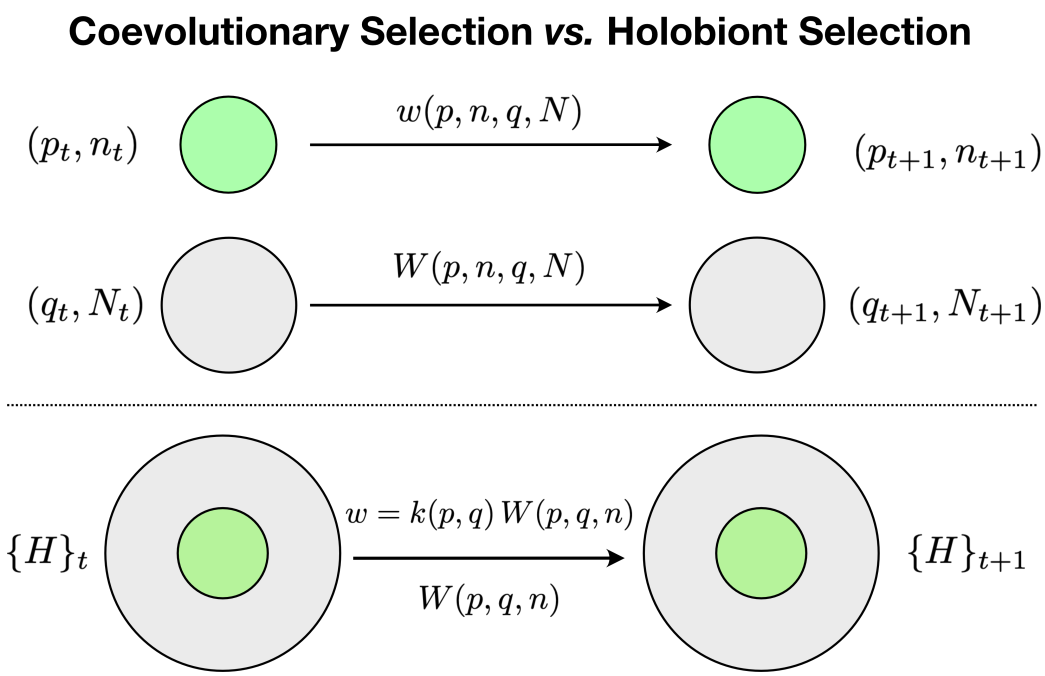

Figure 1: *Holobiont selection contrasted with coevolutionary selection. Microbes are indicated in green and hosts in gray. Coevolutionary selection is diagrammed at the top, and holobiont selection at the bottom. In coevolutionary selection, the microbe gene frequency, p, and population size, n, change from times, t to $t+1$ according to the fitness, w, which is a function of both the gene frequency and population size of the microbes themselves as well as those of the host. The host gene frequency, q, and population size, N, change from times, t to $t+1$ according to the fitness, W, which is a function of both the gene frequency and population size of the hosts themselves as well as those of the microbes. In holobiont selection, the microbe gene frequency, p, and population size, n, change from times, t to $t+1$ according to the fitness, w, which is the product of the microbe fitness within the hosts times the fitness of the host that contains them. Thus, the evolutionary interests of the microbes are partially aligned with those of the host. The host gene frequency, q changes from times, t to $t+1$ according to the fitness, W, which is a function of the gene frequency of the hosts themselves as well as the gene frequency and population size of the microbes. In the formulation here, holobiont selection does not consider that W may depend on host population size.*

selective pressures" and further, that host control is needed to constrain the microbiome, to keep the microbiome on a "leash" (Foster, Schluter, Coyte, and Rakoff-Nahoum 2017, 43).

In contrast, holobiont selection acting on the combination of a host with its microbes leads to a different evolutionary outcome because the equations for holobiont selection are different from those of coevolutionary selection. In particular, the alignment of interests between the microbes and the hosts containing them during holobiont selection presumably allows for the evolution

of more cooperation and synergism between microbes and host than is possible with coevolutionary selection. Although the evolution of reduced virulence for myxomatosis in Australian rabbits (Fenner 1953) has conventionally been assumed to result from coevolutionary selection on pathogen and host, this coadaptation might actually result from holobiont selection.

2 Vertical *vs.* Horizontal Microbe Transmission

A preliminary issue to be settled is whether holobiont evolution is even possible. Most of the reservations about the idea of holobiont selection revolve around the rarity of vertical microbiome transmission.

Many researchers have suggested that selection operates on the combination of the host together with its microbiome as a unit (Woese 2004, Rosenberg, Koren, Reshef, Efrony, and Zilber-Rosenberg 2007, Fraune and Bosch 2010, Guerrero, Margulis and Berlanga 2013, Gilbert, Sapp and Tauber 2012, McFall-Ngai, Hadfield, Bosch, Carey, Domazet-Loo, Douglas, Dubilier et al. 2013, Rosenberg and Zilber-Rosenberg 2014, Bordenstein and Theis 2015, Lloyd 2017, Rosenberg and Zilber-Rosenberg 2018, and others).

Alternatively, some researchers are unconvinced that holobiont selection actually accomplishes the evolutionary results claimed for it (Booth 2014, Moran and Sloan 2015, Douglas and Werren 2016, Hester, Barott, Nulton, Vermeij, and Rohwer 2015, Skillings 2016, Chiu and Eberl 2016, Queller and Strassmann 2016, Doolittle and Booth 2017). They observe that vertical transmission of microbiomes from parental hosts to juvenile hosts is uncommon. Instead, juvenile hosts usually acquire microbiomes from the surrounding environment leading to horizontal transmission. Hence, a holobiont's microbiome is an acquired trait. Because the microbiome is acquired, and not directly inherited, holobiont selection presumably cannot cause holobiont evolution.

A dynamical model was developed for both vertical and horizontal microbiome transmission to allow a comparison between the outcomes of holobiont selection with these two modes of transmission (Roughgarden 2020)[2]. The hologenotypic variation was solely in microbe number. The microbe was either a pathogen or a mutualist.

The model variants with vertical and horizontal transmission both feature these three processes as sequential stages: microbial mixing $\rightarrow$ within-host microbial proliferation $\rightarrow$ holobiont selection. One go-around of the life cycle is a macro time step corresponding to the host generation time. Microbial proliferation within a host occurs in micro time steps corresponding to

[2]Short programs to iterate both the vertical and horizontal transmission lifecycles written in *Mathematica* are available for download at: https://github.com/JoanKauai/Holobiont-Dynamics-Evolution. The programs are supplied as both *Mathematica* Notebooks with an .nb extension and as Computable Document Format (CDF) files with an .cdf extension. A five-minute video demonstrating the software is available at https://youtu.be/19w7ZDkRe18.

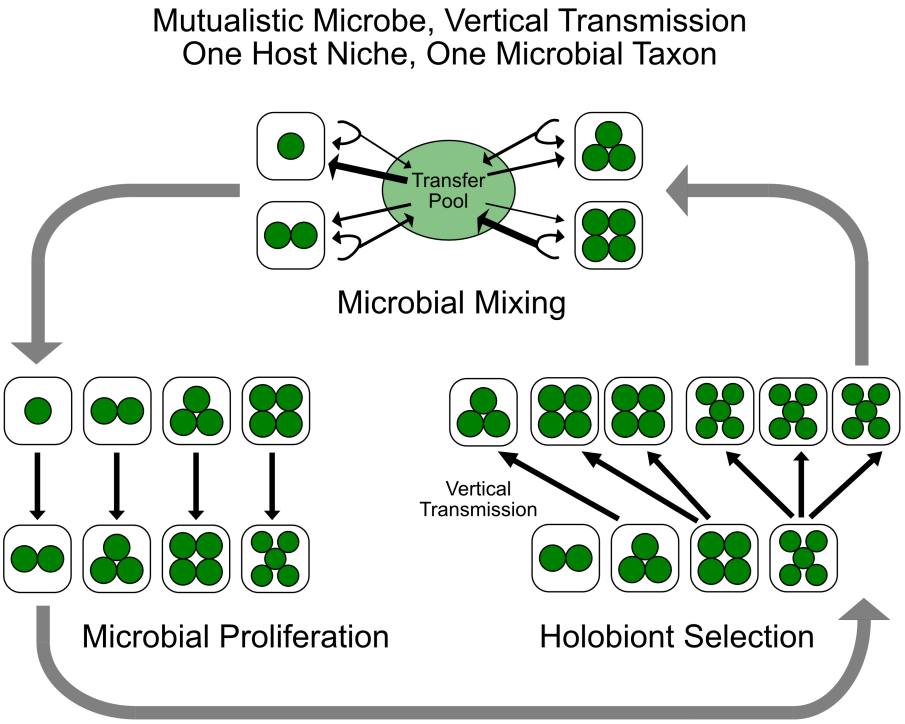

Figure 2: *Life-cycle diagram for vertical transmission assuming a mutualistic microbe. Three processes occur as sequential stages within each host generation. The host's generation time is the macro time step. The macro time step begins in the upper right of the diagram,* i.e. *after the holobiont selection stage and before the microbial mixing stage. The macro time step begins with microbial mixing, as shown at the top of the diagram. Each microbe has a small probability,* m*, of exiting its holobiont and entering a temporary "transfer pool." Then the total transfer pool is divided equally across the holobionts. Each holobiont receives the same number of transfers. After microbial mixing, the microbes proliferate within the holobionts, as shown on the left side of the diagram. The microbes within each holobiont increase according to a density-dependent ecological population model such as the logistic equation. Next the holobionts incur holobiont selection, as shown on the right side of the diagram. Each holobiont reproduces as a whole. The number of progeny a holobiont produces depends on the number of microbes in it. Parental holobionts transmit their microbes to their juveniles such that the number of microbes in each juvenile equals the number of microbes in its parent. With microbial mutualists (shown), the holobionts with the highest number of microbes leave the largest number of progeny and conversely for microbial parasites (not shown). Holobiont reproduction and survival are independent of the density of holobionts. The macro time step ends after holobiont selection, whereupon another macro time step begins at the microbial mixing stage. Iteration of the macro time step with these three stages generates a trajectory of holobiont abundance and hologenotype frequencies through time.*

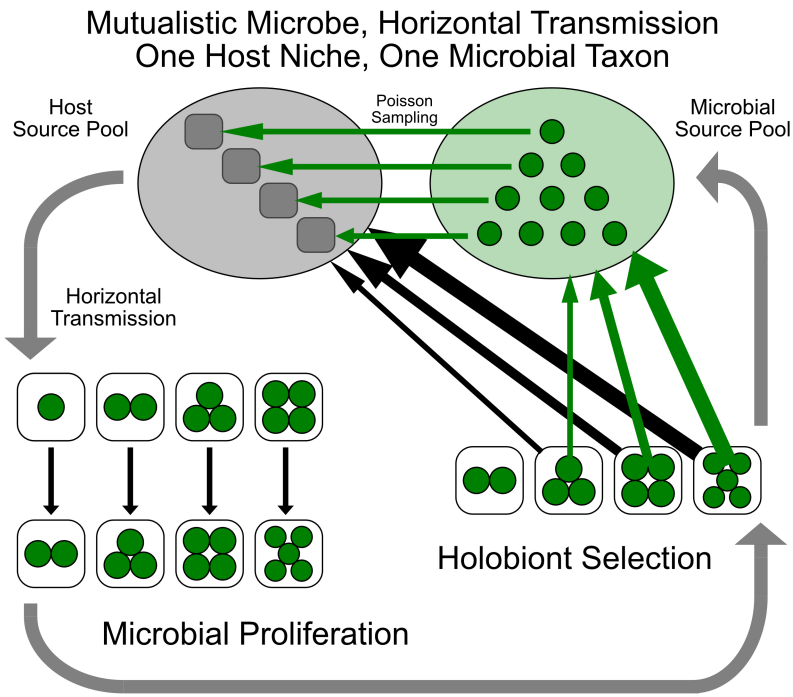

Figure 3: *Life-cycle diagram for horizontal transmission assuming a mutualistic microbe. Each generation begins with a normalized Poisson distribution of microbes across holobionts based on a density parameter equal to the number of microbes in its source pool divided by the number of juvenile hosts in its source pool. Next the microbes within each holobiont increase according to density-dependent ecological population models, as before. Finally, each holobiont reproduces as a whole. The number of progeny a holobiont produces depends on the number of microbes in it. Upon reproduction all the microbes produced from the holobionts enter a common microbe source pool and all the juvenile hosts join a common host source pool. For microbial mutualists (shown), holobionts with the highest number of microbes leave the largest number of progeny and conversely for the microbial parasites (not shown).*

the microbial generation time. The two variants of the model differ in how the microbial mixing process is visualized and how the microbial inheritance occurs.

The model variant for the life cycle with vertical transmission is shown in Figure 2. This variant of the model also allows for limited horizontal microbial exchange using the parameter m that indicates the fraction of the microbiome that is exchanged with other hosts. This number is assumed to be small.

The model variant for the life cycle with horizontal transmission is shown in Figure 3. A model for horizontal transmission could have been derived from the vertical-transmission version by allowing the mixing probability, m, to equal 1, implying that every microbe leaves its host to enter a transfer pool only to be redistributed in equal measure back into now empty hosts.

However, that would instantaneously stock all the hosts with identical microbiomes, denying holobiont selection any hologenotypic variation to operate on. Instead, a sampling process during microbe transmission was introduced.

This model has shown that directional holobiont selection causes the evolution of hosts with a reduced number of parasites and an increased number of mutualists, both for vertical and for horizontal microbiome transmission. In particular, horizontal transmission does not prevent holobiont selection from being effective—it is effective with both horizontal transmission and vertical transmission.

Moreover, 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). But the vast majority of zooxanthellae are acquired from the open sea water surrounding the coral (Babcock, Bull, Harrison, Heyward, Oliver, Wallace, and Willis 1986, Trench 1993). Even terrestrial groups may offer less vertical transmission than might be expected. In a live-bearing cockroach, researchers found only one component of the microbiome to be transmitted vertically, the rest horizontally (Jennings 2019). Therefore, in the theoretical development that follows, horizontal transmission of the microbiome will be assumed, and further development of the vertical transmission case is left to the future should the need arise.

Horizontal transmission enjoys a kind of inheritance of its own, "collective inheritance," as distinct from the "lineal inheritance" of a vertically transmitted microbiome, as illustrated in Figure 4. In collective inheritance each generation's hologenome is assembled from the collective parental hologenomes that have passed through the filter of holobiont selection. In lineal inheritance each generation's hologenome is obtained directly from the individual parental hologenomes that have passed the filter of holobiont selection. Either way, the next generation is enriched with the microbes from the selected parents. Collective inheritance is perfectly Darwinian in the sense that it supports evolutionary descent through modification, just as lineal inheritance does. However, collective inheritance is not consistent with Neo-Darwinism which relies on Mendelian inheritance (Wright 1931).

Skepticism concerning the reality of holobiont selection 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) that fitness is heritable. The third requirement is stated too narrowly. The offspring fitness must correlate with the fitness of the *selected parents*. That is, the offspring must resemble the selected parents regardless of whether those parents are their immediate parents—this is sufficient for evolution by natural selection to proceed.

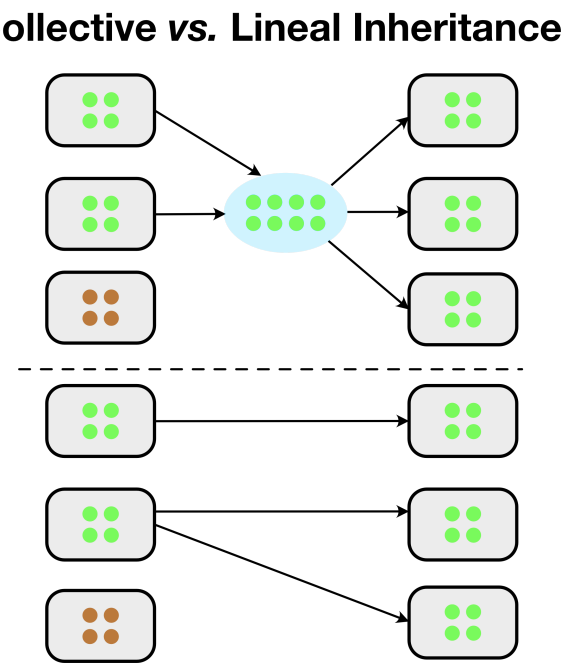

Figure 4: *Top panel illustrates collective inheritance as occurs with horizontal microbe transmission and bottom panel illustrates lineal inheritance as occurs with vertical microbe transmission. In both cases the green microbes are selected and the brown ones are not. With collective inheritance the 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.*

Because horizontal microbiome transmission has been shown to pose no challenge to considering holobiont selection as a force in holobiont evolution, the stage is now set to extend holobiont theory to include multiple microbial strains within the microbiome and multiple alleles within the host nucleus. This paper presents the new research in stages: holobionts with two haploid microbial strains, holobionts with two diploid host alleles, and holobionts having both two haploid microbial strains and two diploid host alleles combined. The mathematical theory here shows how holobiont selection causes the joint evolution of both the microbial and host components of the hologenome and reveals the distribution of microbiome configurations across hosts.

3 New Assumptions

Two new assumptions are now introduced to permit the theoretical development to move beyond computer iteration to mathematical (algebraic) analysis.

First, the generation times of the microbes are assumed to be fast relative to the generation time of the hosts, fast enough that the microbiomes within the hosts come to population dynamic equilibrium within each macro time step. Because the generation times of microbes are usually on the order of minutes to days, whereas the generation times of hosts are on the order of years to decades, this assumption seems generally reasonable.

Second, for horizontal microbiome transmission, the microbial source pool is assumed to be dilute. To see why, suppose the source pool contains two strains of microbes. If the source pool is dense, then juvenile hosts will acquire microbes from both strains. Because of the microbe sampling process, the juvenile hosts will generally have differing initial numbers of each strain, but the point is, all the juvenile hosts will acquire at least some propagules from every strain in the source pool. In contrast, if the source pool is dilute, then some juveniles will acquire propagules from say, only strain-1, others from only strain-2, and others from both strains.

With a dense source pool, even though the initial numbers of each microbial strain might differ across juvenile hosts, this difference becomes erased as the microbes in each host approach the same population dynamic equilibrium. In contrast, with a dilute source pool, the microbiomes within each host come to different population dynamic equilibria reflecting the differing strain composition that each host begins with. Thus, microbe colonization from a dense source pool leads to the loss of hologenotypic variation among hosts whereas microbe colonization from a dilute source pool preserves the hologenotypic variation. Because holobiont selection relies on hologenotypic variation among hosts, the source pool must be dilute enough to guarantee that juvenile hosts acquire different sets of species from the microbe source pool. In the following analysis, the microbiomes are assembled through binomial sampling of the microbes in the dilute microbial source pool.

4 Two Horizontally Transmitted Haploid Microbial Strains

4.1 Model

The life cycle of a holobiont with a horizontally transmitted microbiome consisting of two microbial strains in diagrammed in Figure 5. The model's detailed derivation is recorded in Appendix 1. Given that the microbe pool in the water column surrounding the empty hosts is dilute, two microbe propagules are assumed to colonize each host at random according to a binomial distribution.

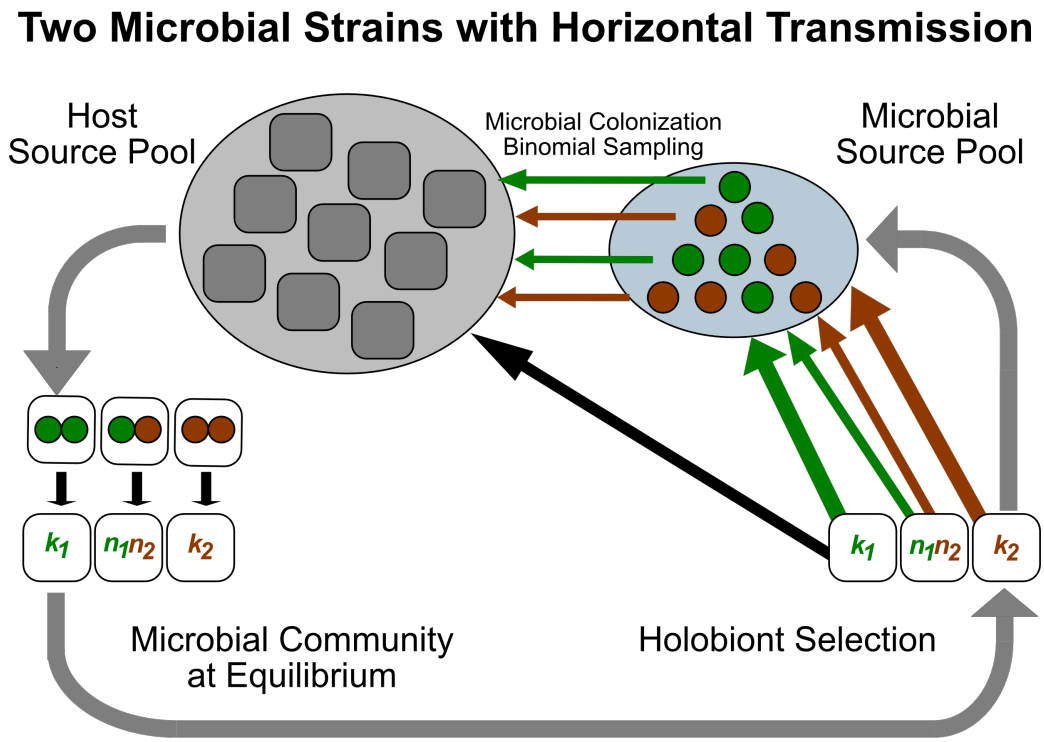

Figure 5: *Holobiont life cycle where microbiome consists of one or of two microbial strains (green and/or brown circle). In each generation the microbiomes for the juvenile hosts are assembled by sampling from the microbial source pool. The microbial source pool is dilute—hologenotypes are assembled via binomial sampling of microbial strains. Generation time of microbes is short relative to host—microbiome comes to community equilibrium within each host generation.*

A macro time step begins with the state variables $H(t)$, $G_1(t)$ 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 is $G(t) \equiv G_1(t) + G_2(t)$. The frequencies of strain-1 and strain-2 in the microbial pool at time t are $p_1(t) \equiv G_1(t)/G(t)$ and $p_2(t) \equiv G_2(t)/G(t)$ where $p_1(t) + p_2(t) \equiv 1$.

The equilibrium abundances in a dual-strain microbiome consisting of strain-1 and strain-2 are n_1 and n_2. The equilibrium abundances of pure strain microbiomes consisting of only strain-1 or strain-2 are k_1 and k_2. The holobiont fitness depend on the hologenotype, i.e., on the number of each strain of microbes in it. To simplify notation put $(W(k_1, 0), W(n_1, n_2), W(0, k_2))$ as (W_1, W_{12}, W_2),

As shown in Appendix 1, the total number of empty juvenile hosts at $t+1$ works out to be

$$\begin{aligned} H(t+1) &= p_1(t)\,\overline{W_1}(t) + p_2(t)\,\overline{W_2}(t) \\ &= \overline{\overline{W}}(t)\,H(t) \end{aligned}$$

where the holobiont mean fitness, $\overline{\overline{W}}(t)$, and the marginal host fitnesses, $\overline{W_1}(t)$ and $\overline{W_2}(t)$, at time t are

$$\begin{aligned}
\overline{W_1}(t) &\equiv p_1(t)\, W_1 + p_2(t) W_{12} \\
\overline{W_2}(t) &\equiv p_1(t) W_{12} + p_2(t)\, W_2 \\
\overline{\overline{W}}(t) &\equiv p_1(t)^2\, W_1 + 2\, p_1(t)\, p_2(t)\, W_{12} + p_2(t)^2\, W_2
\end{aligned}$$

An overline over the W indicates an average taken over the microbiome compositions, ie, with respect to p_1 and p_2. Two overlines indicate two successive averages with respect to p_1 and p_2.

Turning to the microbes, the products of microbe population sizes and holobiont fitnesses emerge as combined measures of multilevel microbe fitness per holobiont that include both success within the holobiont and overall holobiont success. Specifically, $w_{1,1}$ 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. The notation convention is that upper case W's refer to holobiont fitnesses and lower case w's refer to microbe fitnesses. These multilevel microbe fitnesses per holobiont are

$$\begin{aligned}
w_{1,1} &\equiv k_1\, W_1 \\
w_{1,12} &\equiv n_1\, W_{12} \\
w_{2,12} &\equiv n_2\, W_{12} \\
w_{2,2} &\equiv k_2\, W_2
\end{aligned}$$

The expressions for the multilevel microbe fitnesses suggest that microbes "view" their holobiont as a vehicle to increase their productivity. Because the multilevel microbe fitness is a product of microbe abundance within the holobiont times the fitness of the holobiont as a whole, the microbes increase in the hologene pool through a combination of K-selection within the holobionts and r-selection between holobionts. These expressions show that the interests of the microbes partially align with those of their hosts.

As shown in Appendix 1, the total number of microbes in the microbe pool at time $t+1$ is

$$\begin{aligned}
G(t+1) &= \left(p_1(t)\, \overline{w_1}(t) + p_2(t)\, \overline{w_2}(t)\right)\, H(t) \\
&= \overline{\overline{w}}(t)\, H(t)
\end{aligned}$$

where the multilevel microbiome mean fitness per holobiont, $\overline{\overline{w}}(t)$, and multilevel marginal mean fitnesses per holobiont, $\overline{w_1}(t)$ and $\overline{w_2}(t)$, are

$$\begin{aligned}
\overline{w_1}(t) &\equiv p_1(t)\, w_{1,1} + p_2(t)\, 2\, w_{1,12} \\
\overline{w_2}(t) &\equiv p_1(t)\, 2\, w_{2,12} + p_2(t)\, w_{2,2} \\
\overline{\overline{w}}(t) &\equiv p_1(t)\, \overline{w_1}(t) + p_2(t)\, \overline{w_2}(t)
\end{aligned}$$

Notice the factor of 2 in front of $w_{1,12}$ and $w_{2,12}$. This factor of 2 propagates thoughout the subsequent analysis.[3]

The number of strain-1 microbes placed into the microbe source pool at time $t+1$ from both single and dual strain microbiomes then is

$$\begin{aligned} G_1(t+1) &= \overline{w_1}(t)\, p_1(t)\, H(t) \\ G_2(t+1) &= \overline{w_2}(t)\, p_2(t)\, H(t) \end{aligned}$$

Therefore the microbe frequencies in the microbial pool at time $t+1$ are

$$\begin{aligned} p_1(t+1) &= \frac{G_1(t+1)}{G(t+1)} = \frac{\overline{w_1}(t)}{\overline{\overline{w}}(t)}\, p_1(t) \\ p_2(t+1) &= \frac{G_2(t+1)}{G(t+1)} = \frac{\overline{w_2}(t)}{\overline{\overline{w}}(t)}\, p_2(t) \end{aligned}$$

To summarize, the equations above for $H(t+1)$, $G(t+1)$, $p_1(t+1)$ and $p_2(t+1)$ together constitute a dynamical system for holobiont evolution with two microbe strains assuming (a) horizontal microbe transmission, (b) dilute microbe source pools and (c) macro time steps much longer than micro time steps. These equations correspond to those from classical population genetics for evolution at one locus with two alleles. Those equations predict the trajectory of alleles in the gene pool whereas these equations predict the trajectory of microbial strains in the hologene pool.

4.2 Boundary Analysis—Conditions for Increase When Rare

The condition for strain-1 to increase when rare

$$p_1(t+1) > p_1(t)\big|_{p_1 \approx 0, p_2 \approx 1}$$

[3]The coefficient of 2 in front of $w_{1,12}$ and $w_{2,12}$ will seem puzzling to readers familiar with the corresponding formulas for the marginal mean fitness of alleles at a nuclear locus (e.g., see next section). The reason for the coefficient of 2 here lies in the difference between the contribution of the second propagule in a single strain microbiome compared with the contribution of the second allele in a genetic homozygote. In a nuclear homozygote, both alleles contribute equally to the gamete pool—that is, an A_1A_1 homozygote contributes twice as many A_1 alleles to the gamete pool as an A_1A_2 heterozygote. This double gametic productivity of a homozygote compared with the heterozygote introduces a coefficient of 2 to the homozygote production relative to the heterozygote production. Meanwhile, a coefficient of 2 also appears in the production of the heterozygotes but for a different reason, namely, from the binomial distribution of genotype ratios in random mating. These coefficients of 2 in front of both the homozygote and heterozygote fitnesses are then factored out leaving the familiar expression for the marginal fitness of an allele. In contrast, when an empty host is colonized by two strain-1 propagules making a single-strain microbiome, the second propagule adds nothing to the microbiome's production. The single-strain microbiome attains the same equilibrium abundance, k_1, regardless of how many strain-1 propagules it is colonized with. Thus, a single-strain microbiome has no added productivity from the second propagule it is colonized by and no coefficient of 2 exists that can be subsequently factored out along with the coefficient of 2 from the binomial distribution. The coefficient of 2 from the binomial distribution therefore is left in the expression for the marginal mean fitness of a strain.

works out to be

$$E_1 \equiv 2\,w_{1,12} - w_{2,2} > 0$$

Similarly, the condition for strain-2 to increase when rare works out to be

$$E_2 \equiv 2\,w_{2,12} - w_{1,1} > 0$$

E_1 is the excess by which a rare strain-1 microbe out-produces a common strain-2 microbe. E_2 is the excess by which a rare strain-2 microbe out-produces a common strain-1 microbe. Strain-1 increases when it is rare if it out-produces strain-2 when strain-2 is common. Similarly, strain-2 increases when it is rare if it out-produces strain-1 when strain-1 is common. If both strain-1 and strain-2 can increase when rare, then the microbiome community consists of two strains. Otherwise, if say, strain-1 can increase when rare and strain-2 cannot, then strain-1 excludes strain-2 from the microbiome. And conversely if strain-1 cannot increase when rare while strain-2 can, then strain-2 excludes strain-1 from the microbiome.

4.3 Illustration—Altruistic and Selfish Microbes

To illustrate, consider two microbes, one altruistic and one selfish. Suppose an initially rare strain-1 (green) is altruistic in the holobiome because it transfers some of its resources to the host thereby lowering its own carrying capacity while increasing the holobiont fitness. Meanwhile the selfish common strain-2 (brown) keeps its resources to itself. Thus, $W_{12} > W_2$ while $k_1 < k_2$.

Suppose further that the two microbes compete according the the Lotka-Volterra competition equations and that the reciprocal competition coefficients are equal, $a_{12} = a_{21} = a$. The equilibrium abundances of the strains then are

$$n_1 = \frac{k_1 - a\,k_2}{1 - a^2},\ n_2 = \frac{k_2 - a\,k_1}{1 - a^2}$$

Now, as a concrete example, let, $a \to 1/2$, $k_1 \to 300$, $k_2 \to 400$, $W_1 \to 3$, $W_{12} \to 2$, $W_2 \to 1$. Then the condition for strain-1 to increase when rare works out to be

$$E_1 \equiv 2\,w_{1,12} - w_{2,2} = 400/3 > 0$$

Thus, strain-1 which has lowered its carrying capacity, k, from 400 to 300 but increased the fitness of a holobiont it resides in from 1 to 2, increases when rare against the selfish strain.

But after increasing when rare does this altruistic strain-1 proceed to exclude the selfish strain-2? To find out check the condition for increase when rare for strain-2, which works out to be

$$E_2 \equiv 2\,w_{2,12} - w_{1,1} = 1300/3 > 0$$

This condition is positive too, showing that altruistic strain-1 does not exclude the selfish strain-2. Therefore, both strain-1 and strain-2 coexist in this numerical example.

4.4 Microbiome Polymorphism

Seeing that strain-1 and strain-2 coexist in the numerical example above, the next question is how do they coexist? Do they come to come an equilibrium polymorphism, or do they oscillate in a limit cycle? Solving for the equilibrium polymorphism between the two strains, and analyzing the stability of that equilibrium will answer this question.

Putting $p_1(t+1) = p_1(t) \equiv \hat{p}$ and solving for the equilibrium frequency of strain-1 yields

$$\hat{p} = \frac{E_1}{E_1 + E_2}$$

If both strains increase when rare, then p_1 is in $(0, 1)$ as it should be. The value of the equilibrium hologenotype frequency reflects the relative speeds at which the strains can increase when rare. If strain-1 can charge ahead at gang-buster speed whereas strain-2 can barely squeeze in, then the equilibrium consists mostly of strain-1 with just a bit of strain-2.

Returning to the numerical example of altruistic and selfish microbes mentioned above, the equilibrium frequency of strain-1 works out to be

$$\hat{p} = \frac{400/3}{400/3 + 1300/3} = \frac{4}{17}$$

Therefore, the full picture of the holobiont population along with its microbiomes at equilibrium is: $(4/17) = 0.235$ of the microbial pool consists of the altruistic strain-1 microbes and $(13/17) = 0.764$ of the microbial pool consists of the selfish strain-2 microbes; after microbiome assembly, $(4/17)^2 = 0.055$ of the holobionts contain solely strain-1 microbes, $2 \times (4/17) \times (13/17) = 0.359$ of the holobionts contain both microbes and $(13/17)^2 = 0.584$ of the holobionts contain only strain-2 microbes; the holobionts with only strain-1 contain 300 microbes, holobionts with both strains each contain $(400/3) = 133$ strain-1 microbes and $(1000/3) = 333$ strain-2 microbes, and holobionts with only strain-2 contain 400 microbes in their microbiomes.

Figure 6 (Left) presents a plot of trajectories for the frequency of strain-1 through time from several initial conditions obtained by numerically iterating equation the equation for $p_1(t+1)$. The green line is the predicted equilibrium frequency. The trajectories all converge to the equilibrium.

4.5 Stability and The Direction of Microbiome Evolution

The observation that trajectories of $p_1(t)$ converge numerically to an equilibrium as shown in Figure 6 (Left) suggests that the polymorphism frequency for strain-1, p_1 is a stable polymorphism.

To analyze the stability of this equilibrium, write the dynamics as difference equation for

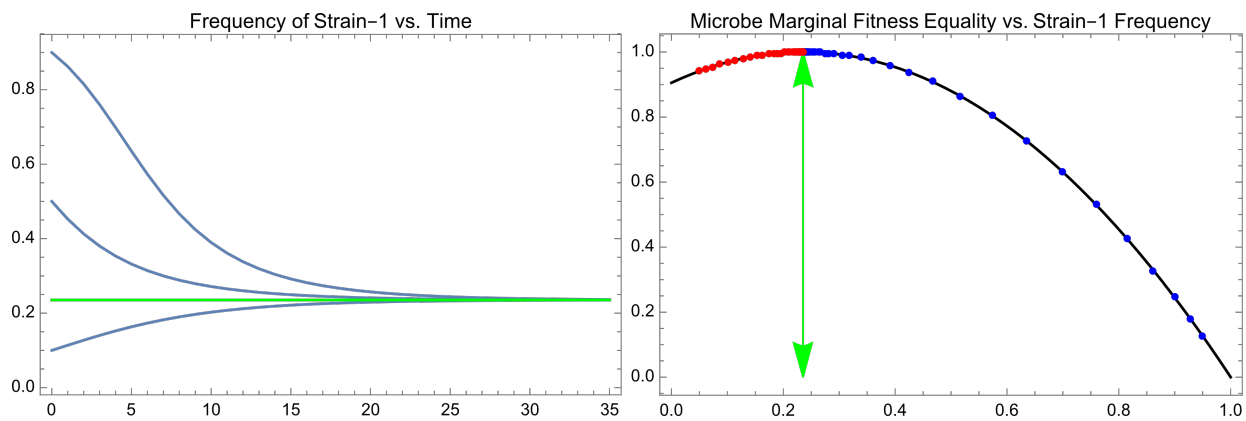

Figure 6: *Left: Frequency of the strain-1 microbe in the microbial pool vs. time, assuming strain-1 is altruistic and strain-2 is selfish. Equilibrium strain-1 frequency is horizontal line in green. Figure shows that different initial conditions all converge to the equilibrium. Right: Degree of marginal multilevel fitness equality in the microbiome vs. strain-1's frequency, assuming strain-1 is altruistic and strain-2 is selfish. Curve for degree of marginal multilevel microbial fitness equality is in black. Equilibrium strain-1 frequency is vertical arrow in green. Figure shows two different initial conditions, one (red) at* $p = 0.05$ *and another (blue) at* $p = 0.95$ *that both converge to the equilibrium at the top of the curve of fitness equality.* ($a \to 1/2$, $k_1 \to 300$, $k_2 \to 400$, $n_1 \to 400/3$, $n_2 \to 1000/3$, $W_1 \to 3$, $W_{12} \to 2$, $W_2 \to 1$)

$\Delta p_1(t) \equiv p_1(t+1) - p_1(t)$ yielding, after rearrangement,

$$\Delta p = \frac{p\,(1-p)\,(\overline{w_1} - \overline{w_2})}{\overline{\overline{w}}}$$

where to simplify notation, the subscript and time index are dropped leaving $p \equiv p_1(t)$.

Next, consider the expression,

$$-(\overline{w_1} - \overline{w_2})^2$$

which measures the degree of equality between the marginal multilevel mean fitnesses per holobiont of the two microbes. This is a quadratic function of p, a $\cap$-shaped curve whose top is at the equilibrium value of p. By taking the derivative of this function with respect to p, rearranging and substituting into the equation for Δp one obtains,

$$\Delta p = \frac{1}{2\,(E_1 + E_2)}\;\frac{p\,(1-p)}{\overline{\overline{w}}}\;\left(\frac{d\;\left(-(\overline{w_1} - \overline{w_2})^2\right)}{d\,p}\right)$$

This equation for Δp shows that holobiont selection causes p to ascend the $\cap$-shaped curve to its top where the equilibrium occurs. The expression in large parentheses is the curve's slope at p. This expression shows that if p is at a spot where the curve's slope is positive, i.e., p is to the left of the top, then holobiont selection increases p, moving p to the right and closer to the top.

Conversely, if p is at a spot where the curve's slope is negative, i.e., p is to the right of the top, then holobiont selection decreases p, moving p to the left and closer to the top. If p is exactly at the top, then Δp is zero, and equilibrium has been attained. Thus, $-(\overline{w_1} - \overline{w_2})^2$ constitutes a curve of the degree of equality between the marginal multilevel fitness per holobiont of the two strains. The result here that p ascends to the top of the curve of multilevel marginal fitness equality is the counterpart for holobiont selection to the result in classical population genetics where p climbs an "adaptive surface". Unlike classical population genetics however, the peak of the curve of marginal fitness equality per holobiont between the two microbe strains does not coincide with the peak of the overall mean multilevel microbe fitness per holobiont, $\overline{\overline{w}}(t)$, (nor with the overall mean holobiont fitness $\overline{\overline{W}}(t)$).

Although $-(\overline{w_1} - \overline{w_2})^2$ represents a curve that p ascends during holobiont evolution, this expression can be scaled and a constant added to it to produce a similar curve that is conveniently graphed between 0 and 1. So, put

$$L_G(p) \equiv \frac{-(\overline{w_1} - \overline{w_2})^2}{\max(E_1^2, E_2^2)} + 1$$

Figure 6 (Right) illustrates both $L_G(p)$ and some trajectories climbing toward its peak.

Even though the equation for Δp above shows that trajectories climb the surface of marginal multilevel mean fitness equality for the two microbes, this equation alone is not sufficient to show that the equilibrium at the top is stable. It remains theoretically conceivable that as trajectories approach the top they overshoot, and even overshoot enough to diverge away from the equilibrium. This would be realized as trajectories that constitute a multi-point limit cycle, first to the left of the equilibrium, then overshooting to the right of the equilibrium, then overshooting back to the left and so forth. This theoretical possibility can be ruled out by considering the eigenvalue of the mapping of $p(t) \to p(t+1)$ evaluated at the equilibrium p. For overshoots to occur, this quantity must be negative at the equilibrium p. And for divergent overshoots, this quantity must be strongly negative, < -1. However, this quantity works out to be positive for any p, including the equilibrium p. Therefore, overshoots do not occur, either convergent or divergent. Thus, p the top of the surface of mean fitness equality per holobiont between the two microbe strains is a globally stable equilibrium that trajectories approach smoothly.

5 Two Vertically Transmitted Diploid Host Alleles

The life cycle of a holobiont with two possible alleles in the host nucleus, A_1 and A_2, and with a single-strain horizontally transmitted microbiome is diagrammed in Figure 7.

A macro time step begins with the gamete pool. The frequencies of A_1 and A_2 in the gamete pool at time t are $q_1(t)$ and $q_2(t)$ where $q_1(t) + q_2(t) \equiv 1$. Then gametes unite at random

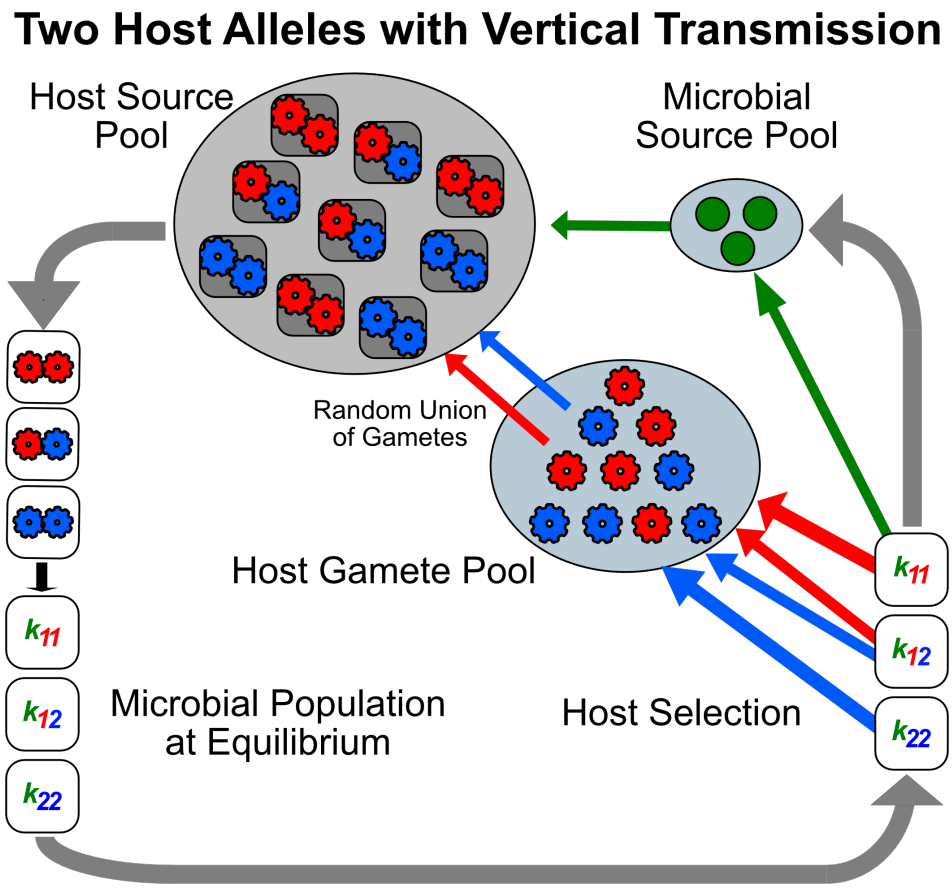

Figure 7: *Holobiont life cycle where the host nuclei contain two diploid alleles (red and/or blue cogs). In each generation the host's nuclear genotype is assembled by by random union of gamete from the preceding generation. The microbial source pool contains one microbial strain (green circles). Generation time of microbes is short relative to host—microbe population comes to equilibrium within each host generation.*

leading to a (Hardy-Weinberg) binomial distribution such that initial hologenotypes occur in the ratios: $(q_1(t)^2, 2\,q_1(t)\,q_2(t), q_2(t)^2)$. These juvenile hosts are then colonized by the single strain of microbes.

Once the juvenile hosts have been colonized, the microbes proliferate within their hosts, coming to an equilibrium microbe population size that depends on the host genotype: (k_{11}, k_{12}, k_{22}) for host nuclear genotypes (A_1A_1, A_1A_2, A_2A_2) respectively.

Next, the holobiont fitness depend on the hologenotype after microbiome proliferation, ($W(k_{11})$, $W(k_{12})$, $W(k_{22})$) which, to simplify notation, are rewritten as (W_{11}, W_{12}, W_{22}). Based on these fitnesses, the holobionts release their gametes into the gamete pool whereupon they unite at random to yield the empty juvenile hosts ready for microbe colonization. Meanwhile, the holobionts also release microbes that replenish the microbial pool.

The equation to map $q_{1,t} \to q_{1,t+1}$ is the standard equation for selection at one locus with two alleles from classical population genetics. The only new wrinkle here is that the fitness of a host depends on the population size of the microbes in it, as though the microbe population size were itself a host trait, which it effectively is because k, the host's microbe carrying capacity, is a host trait. Thus, from classical population genetics,

$$\begin{aligned} q_1(t+1) &= \frac{\widetilde{W_1}(t)}{\widetilde{\widetilde{W}}(t)}\, q_1(t) \\ q_2(t+1) &= \frac{\widetilde{W_2}(t)}{\widetilde{\widetilde{W}}(t)}\, q_2(t) \end{aligned}$$

where

$$\begin{aligned} \widetilde{W_1}(t) &\equiv q_1(t)W_{11} + q_2(t)W_{12} \\ \widetilde{W_2}(t) &\equiv q_2(t)W_{22} + q_1(t)W_{12} \\ \widetilde{\widetilde{W}}(t) &\equiv q_1(t)\widetilde{W_1}(t) + q_2(t)\widetilde{W_2}(t) \end{aligned}$$

Here, $\widetilde{W_1}(t)$ is the marginal fitness of the A_1 allele, $\widetilde{W_2}(t)$ is the marginal fitness of the A_2 allele, and $\widetilde{\widetilde{W}}(t)$ is the overall mean fitness. (The *wide tilde* above any fitness coefficient indicates an average taken over the host allele frequencies, i.e., with respect to q_1 and q_2. Contrast in the previous section where an *overline* above a fitness coefficient indicates an average taken with respect to microbiome strain frequencies, p_1 and p_2.)

The boundary analysis for this equation is well known. q_1 can increase when rare if

$$q_1(t+1) > q_1(t)\big|_{q_1 \approx 0, q_2 \approx 1}$$

which works out to be

$$F_1 \equiv W_{12} - W_{22} > 0$$

And similarly, q_2 can increase when rare if

$$F_2 \equiv W_{12} - W_{11} > 0$$

Polymorphism occurs if both alleles can increase when rare, $F_1 > 0$ and $F_2 > 0$. The polymorphism allele frequency where $q(t+1) = q(t)$ then works out to be

$$\hat{q} = \frac{F_1}{F_1 + F_2}$$

Next, develop the equation for $\Delta q \equiv q_1(t+1) - q_1(t)$ as

$$\Delta q = \frac{q(1-q)(\widetilde{W}_1 - \widetilde{W}_2)}{\widetilde{\widetilde{W}}}$$

Equilibrium results when the marginal fitness of the two alleles are equal.

To parallel the formulation in the last section, consider the expression

$$-(\widetilde{W}_1 - \widetilde{W}_2)^2$$

This expression represents the degree of equality in marginal fitness of the two alleles. It is a $\cap$-shaped quadratic function of q whose peak is at $\hat{q}$. Rewriting the equation for Δq in terms of the derivative of this expression yields

$$\Delta q = \frac{1}{2\,(F_1 + F_2)}\,\frac{q\,(1-q)}{\widetilde{\widetilde{W}}}\,\left(\frac{d\,\left(-(\widetilde{W_1} - \widetilde{W_2})^2\right)}{d\,q}\right)$$

This equation indicates that $q(t)$ climbs the curve for the degree of marginal fitness equality between the two alleles.

Scaling the expression, $-(\widetilde{W}_1 - \widetilde{W}_2)^2$, to lie between 0 and 1 yields

$$L_H(q) \equiv \frac{-(\widetilde{W}_1 - \widetilde{W}_2)^2}{\max(F_1^2, F_2^2)} + 1$$

Trajectories from holobiont selection on the host alleles climb this function representing the degree of marginal fitness equality for the two host alleles and come to equilibrium when equality is attained.

Figure 8 illustrates a numerical example with heterozygote superiority in the host alleles, $W_{11} \to 1$, $W_{12} \to 3$, $W_{22} \to 2$. The equilibrium, q, works out to be $1/3$. Figure 8 (Left) illustrates trajectories approaching the equilibrium through time from different initial conditions, and Figure 8 (Right) shows $L_h(q)$ and two trajectories climbing toward its peak.

6 Two Microbial Strains and Two Host Alleles

The life cycle of a holobiont with both two vertically transmitted alleles in the host nucleus and two horizontally transmitted microbial strains in the microbiome is diagrammed in Figure 9. This figure combines the elements both of Figure 5 for two microbes and of Figure 7 for two host alleles. The task now is to develop the equations that simultaneously map both microbe and allele frequencies at time t to their frequencies at time $t+1$.

6.1 Model

A macro time step begins with the basic state variables for the nine hologenotypes. The notation convention is that $H_{ij}(t, k, 0)$ refers to the number of holobionts at time t with k strain-1 microbes and no strain-2 microbes in the microbiome together with alleles A_i and A_j in the host nucleus,

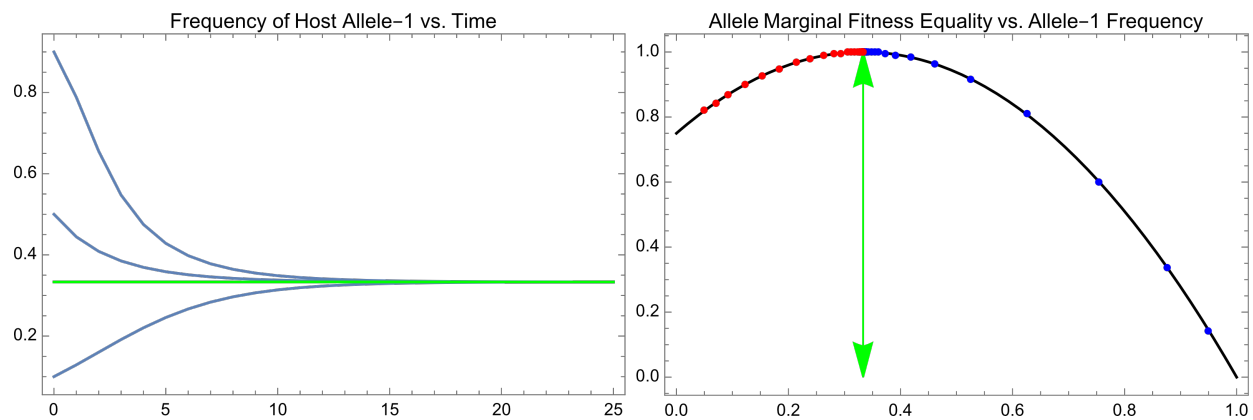

Figure 8: *Left: Frequency of the allele-1 in the host gamete pool vs. time, assuming heterozygote superiority. Equilibrium allele-1 frequency is horizontal line in green. Figure shows that different initial conditions all converge to the equilibrium. Right: Degree of allele marginal fitness equality in host vs. allele-1's frequency in the gamete pool, assuming heterozygote superiority. Curve for degree of allele marginal equality is in black. Equilibrium allele-1 frequency is vertical arrow in green. Figure shows two different initial conditions, one (red) at* $p = 0.05$ *and another (blue) at* $p = 0.95$*, whose trajectories both converge to the equilibrium at the top of the curve. (*$W_{11} \to 1$, $W_{12} \to 3$, $W_{22} \to 2$*)*

whereas $H_{ij}(t, n_1, n_2)$ refers to the number of holobionts at time t with n_1 strain-1 and n_2 strain-2 microbes in the microbiome together with alleles A_i and A_j in the host nucleus. The total at time t of the nine hologenotype numbers is $H(t)$.

Let p_1 and p_2 denote the frequencies of strain-1 and strain-2 in the microbe source pool, and let q_1 and q_2 denote the frequencies of the A_1 and A_2 alleles in the host gamete pool. The nuclei of the empty hosts are formed by random union of gametes from the host gamete pool according to the Hardy-Weinberg ratios of $(q_1^2, 2\,q_1q_2, q_2^2)$. Then these empty hosts are colonized at random through binomial sampling of the dilute microbe pool according to the ratios of $(p_1^2, 2\,p_1p_2, p_2^2)$. The model's detailed derivation is recorded in Appendix 2.

The holobiont fitnesses depend on the full hologenotype which includes both the microbiome and the host nuclear genome, eg., $W_{ij}(k_1)$ is the fitness of a single-strain holobiont with strain-1 in a host with A_iA_j alleles, and $W_{ij}(n_1, n_2)$ is the fitness for a dual-strain holobiont with n_1 strain-1 microbes and n_2 strain-2 microbes with a host genome of A_iA_j. However, to simplify notation, put $W_{ij}(k_1)$ as $W_{1,ij}$ and $W_{ij}(n_1, n_2)$ as $W_{12,ij}$ where the subscript refers to the microbiome composition followed by the nuclear genome.

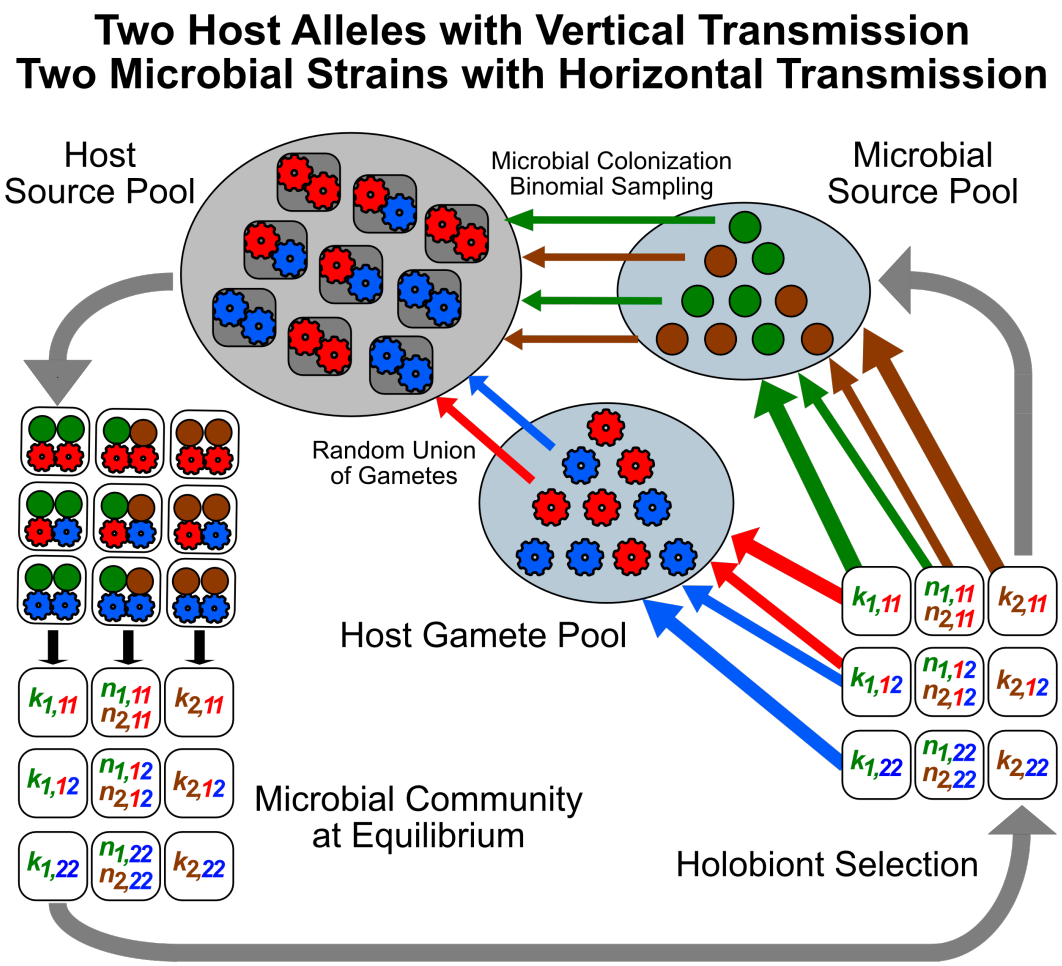

Figure 9: *Holobiont life cycle for holobiont whose microbiome consists of one or two microbial strains (green and/or brown circle) and whose nucleus consists of one allele (green or brown cog). In each generation the microbiomes for the juvenile hosts are assembled through binomial sampling from the microbial source pool. The host nuclear alleles are transmitted vertically from parent to juvenile with random union of gametes. At the holobiont selection stage, the diagram's columns indicate the strains being contributed to the microbial source pool and the diagram's rows indicate alleles being contributed to the host source pool. The microbial source pool is dilute—hologenotypes are assembled via binomial sampling of microbial strains. Generation time of microbes is short relative to host—microbiome comes to community equilibrium within each host generation. The figure illustrates how the transmission of host nuclear genes and the microbiome operate in parallel. The release of microbes to the microbe pool and the release of gametes to the gamete pool both enable genetic recombination in their respective components of the hologenome. Similarly, the statistical process of microbe colonization is the counterpart of the mating system for determining the pattern of inheritance for their respective components of the hologenome.*

The total number of empty juvenile hosts at $t+1$ works out to be

$$\begin{aligned} H(t+1) &= \left(q_1(t)^2\ \overline{W_{11}}(t) + 2q_1(t)q_2(t)\ \overline{W_{12}}(t) + q_2(t)^2\ \overline{W_{22}}(t)\right)\ H(t) \\ &= \overline{\overline{W}}(t)\, H(t) \end{aligned}$$

where the fitness for holobionts with A_1A_1, A_1A_2, and A_2A_2 nuclear genotypes averaged over their microbiomes are:

$$\begin{aligned} \overline{W_{11}}(t) &\equiv p_1(t)^2\ W_{1,11} + 2p_1(t)p_2(t)\ W_{12,11} + p_2(t)^2\ W_{2,11} \\ \overline{W_{12}}(t) &\equiv p_1(t)^2\ W_{1,12} + 2p_1(t)p_2(t)\ W_{12,12} + p_2(t)^2\ W_{2,12} \\ \overline{W_{22}}(t) &\equiv p_1(t)^2\ W_{1,22} + 2p_1(t)p_2(t)\ W_{12,22} + p_2(t)^2\ W_{2,22} \end{aligned}$$

and the marginal fitnesses for each allele are

$$\begin{aligned} \widetilde{\overline{W_1}}(t) &\equiv q_1(t)\,\overline{W_{11}}(t) + q_2(t)\overline{W_{12}}(t) \\ \widetilde{\overline{W_2}}(t) &\equiv q_1(t)\overline{W_{12}}(t) + q_2(t)\,\overline{W_{22}}(t) \end{aligned}$$

and the overall mean fitness of the holobionts is

$$\overline{\overline{W}}(t) \equiv q_1(t)\,\widetilde{\overline{W_1}}(t) + q_2(t)\,\widetilde{\overline{W_2}}(t)$$

The notation convention is that an average taken over microbe frequencies, *i.e.*, with respect to p_1 and p_2, is indicated with an overline. An average taken over allele frequencies, *i.e.*, with respect to q_1 and q_2, is indicated with a wide tilde. A wide tilde placed on top of an overline indicates an average first with respect to microbe frequencies and then with respect to allele frequencies. An overline placed over a wide tilde indicates an average first with respect to allele frequencies and then with respect to microbe frequencies. Also, to minimize the number of diacritical marks, if three marks are present, the lowest mark is understood to refer to an average over microbiome frequencies or genotype frequencies, *i.e.*, with respect to $p_1^2, 2\,p_1\,p_2, p_2^2$ and $q_1^2, 2\,q_1\,q_2, q_2^2$ rather than with respect to the strain or alleles frequencies, p_1, p_2, q_1, and q_2. (This avoids needing four diacritical marks to denote a grand mean fitness overaged over both the microbiome and the nucleus.)

Based on these definitions, the frequency of the host nuclear alleles, A_1 and A_2, respectively, in the gamete pool at time $t+1$ as a function of both the gamete pool and microbial source pool at time t works out to be

$$\begin{aligned} q_1(t+1) &= \frac{\widetilde{\overline{W_1}}(t)}{\overline{\overline{W}}(t)}\, q_1(t) \\ q_2(t+1) &= \frac{\widetilde{\overline{W_2}}(t)}{\overline{\overline{W}}(t)}\, q_2(t) \end{aligned}$$

Turning now to the microbiome, the number of strain-1 microbes in the microbial source pool at time $t+1$ works out to be

$$\begin{aligned} G_1(t+1) &= p_1(t)^2\, \widetilde{w_{1,1}}(t) + 2p_1(t)p_2(t)\, \widetilde{w_{1,12}}(t) \\ &= \overline{\widetilde{w_1}}(t)\, p_1(t)\, H(t) \end{aligned}$$

where the multilevel microbe fitnesses per holobiont are

$$\begin{aligned} w_{1,1,11} &= k_{1,11}\, W_{1,11} \\ w_{1,1,12} &= k_{1,12}\, W_{1,12} \\ w_{1,1,22} &= k_{1,22}\, W_{1,22} \\ w_{1,12,11} &= n_{1,11}\, W_{12,11} \\ w_{1,12,12} &= n_{1,12}\, W_{12,12} \\ w_{1,12,22} &= n_{1,22}\, W_{12,22} \\ w_{2,12,11} &= n_{2,11}\, W_{12,11} \\ w_{2,12,12} &= n_{2,12}\, W_{12,12} \\ w_{2,12,22} &= n_{2,22}\, W_{12,22} \\ w_{2,2,11} &= k_{2,11}\, W_{2,11} \\ w_{2,2,12} &= k_{2,12}\, W_{2,12} \\ w_{2,2,22} &= k_{2,22}\, W_{2,22} \end{aligned}$$

and where the multilevel fitnesses of a strain-1 microbe per holobiont from single and dual strain microbiomes averaged over the three nuclear genotypes are

$$\begin{aligned} \widetilde{w_{1,1}}(t) &\equiv q_1(t)^2\, w_{1,1,11} + 2q_1(t)q_2(t)\, w_{1,1,12} + q_2(t)^2\, w_{1,1,22} \\ \widetilde{w_{1,12}}(t) &\equiv q_1(t)^2\, w_{1,12,11} + 2q_1(t)q_2(t)\, w_{1,12,12} + q_2(t)^2\, w_{1,12,22} \end{aligned}$$

The notation is that $w_{1,1,11}$ indicates the multilevel fitness per holobiont of a strain-1 microbe in a single strain microbiome within an A_1A_1 host whereas $w_{1,12,12}$ indicates the multilevel fitness per holobiont of a strain-1 microbe in a dual-strain microbiome within an A_1A_2 host. Thus the subscript convention is strain identity, followed by microbiome composition, and ending with host nuclear genotype. Then strain-1's marginal multilevel fitness per holobiont averaged over the nuclear genotypes is

$$\overline{\widetilde{w_1}}(t) \equiv p_1(t)\, \widetilde{w_{1,1}}(t) + p_2(t)\, 2\, \widetilde{w_{1,12}}(t)$$

Similarly, the number of strain-2 microbes in the microbe pool at time $t+1$ is

$$\begin{aligned} G_2(t+1) &= 2p_1(t)p_2(t)\, \widetilde{w_{2,12}}(t) + p_2(t)^2\, \widetilde{w_{2,2}}(t) \\ &= \overline{\widetilde{w_2}}(t)\, p_2(t)\, H(t) \end{aligned}$$

where the multilevel fitnesses of a strain-2 microbe per holobiont from single and dual strain microbiomes averaged over the three nuclear genotypes are

$$\begin{aligned}
\widetilde{w_{2,2}}(t) &\equiv q_1(t)^2\, w_{2,2,11} + 2q_1(t)q_2(t)\, w_{2,2,12} + q_2(t)^2\, w_{2,2,22} \\
\widetilde{w_{2,12}}(t) &\equiv q_1(t)^2\, w_{2,12,11} + 2q_1(t)q_2(t)\, w_{2,12,12} + q_2(t)^2\, w_{2,12,22}
\end{aligned}$$

Then strain-1's marginal multilevel fitness per holobiont averaged over the nuclear genotypes is

$$\overline{\widetilde{w_2}}(t) \equiv p_1(t)\, 2\, \widetilde{w_{2,12}}(t) + p_2(t)\, \widetilde{w_{2,2}}(t)$$

The total number of microbes in the microbe pool at time $t+1$ then is

$$\begin{aligned}
G(t+1) &= \left(\overline{\widetilde{w_1}}(t)\, p_1(t) + \overline{\widetilde{w_2}}(t)\, p_2(t)\right)\, H(t) \\
&= \overline{\overline{\widetilde{w}}}(t)\, H(t)
\end{aligned}$$

where the microbiome multilevel mean fitness per holobiont is

$$\overline{\overline{\widetilde{w}}}(t) \equiv p_1(t)\, \overline{\widetilde{w_1}}(t) + p_2(t)\, \overline{\widetilde{w_2}}(t)$$

Therefore the microbe frequencies in the microbial pool at time $t+1$ are

$$p_1(t+1) = \frac{G_1(t+1)}{G(t+1)} = \frac{\overline{\widetilde{w_1}}(t)}{\overline{\overline{\widetilde{w}}}(t)}\, p_1(t)$$

and

$$p_2(t+1) = \frac{G_2(t+1)}{G(t+1)} = \frac{\overline{\widetilde{w_2}}(t)}{\overline{\overline{\widetilde{w}}}(t)}\, p_2(t)$$

To summarize, the equations above for $H(t+1)$, $G(t+1)$, $p_1(t+1)$, $p_2(t+1)$, $q_1(t+1)$ and $q_2(t+1)$, together constitute a dynamical system for holobiont evolution with two microbe strains and two diploid host alleles assuming (a) horizontal microbe transmission, (b) dilute microbe source pools and (c) macro time steps much longer than micro time steps. These equations correspond to those from classical population genetics for evolution at two loci with two alleles per locus. Those equations predict the trajectory in a gene pool of a two-compartment genome, one compartment per locus, whereas these equations predict the trajectory in a hologene pool of a two compartment hologenome, one compartment for the microbial genes and another compartment for the nuclear genes.

6.2 Conditions for Increase When Rare and Joint Microbiome-Host Polymorphism

Figure 10 shows the possible equilibria in the combined microbiome-nuclear gene system. The corner equilibria are shown in red and labelled A, B, C and D. The edge equilibria are shown in

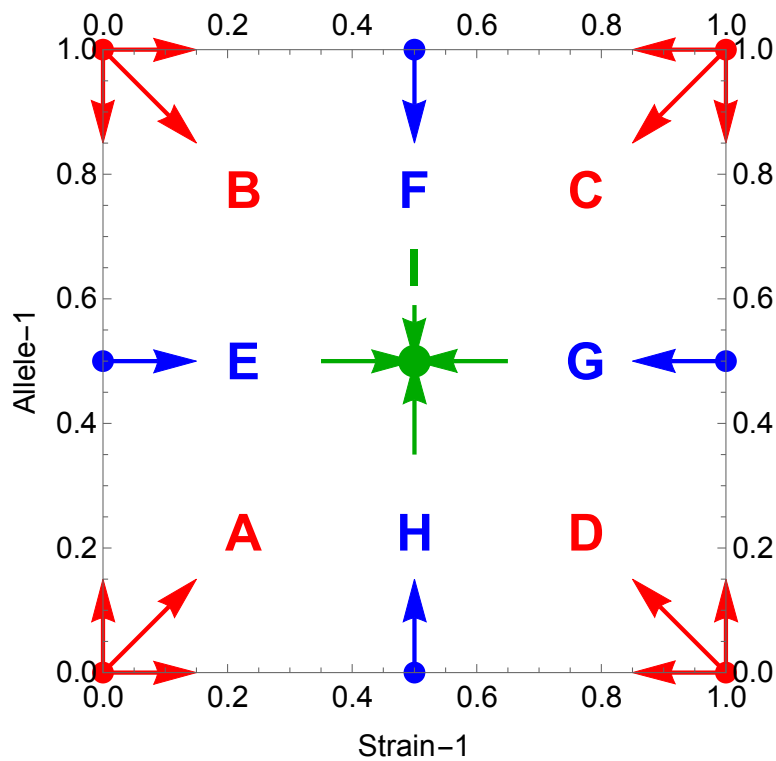

Figure 10: *Diagram of possible equilibria for holobiont evolution with two microbial strains with two host alleles. The horizontal axis is the frequency of strain-1 in the microbiome and the vertical axis is the frequency of the A_1 allele in the host nucleus. The corner equilibria are shown in red and labeled as A, B, C and D. The edge equilibria are shown in blue and labeled as E, F, G and H. The interior equilibrium is shown in green and labeled as I.*

blue and labelled E, F, G and H. The interior equilibrium is shown in green and labelled I. For the analysis, put $p_1 \to p$, $p_2 \to (1-p)$, $q_1 \to q$ and $q_2 \to (1-q)$. To understand the flow of trajectories in the p, q plane representing the combined evolution of microbial and nuclear genes, the stability of the corner equilibria are the places to investigate first.

The conditions for strain-1 and for strain-2 to increase when rare in the absence of nuclear gene variation were shown earlier. These previously derived conditions can be transferred here with the proviso that the expressions for the multilevel microbial fitnesses now depend on $q(t)$ because these fitnesses are averaged over the nuclear genotype frequencies, as indicated with the wide tilde symbol. Putting $q(t) = q$, the condition for strain-1 to increase when rare is

$$p(t+1) > p(t)\big|_{p\approx 0, q}$$

which works out to be

$$E_1(q) \equiv 2\,\widetilde{w_{1,12}} - \widetilde{w_{2,2}} > 0$$

Similarly, the condition for strain-2 to increase when rare is

$$E_2(q) \equiv 2\,\widetilde{w_{2,12}} - \widetilde{w_{1,1}} > 0$$

$E_1(q)$ is the excess by which a rare strain-1 microbe out-produces a common strain-1 microbe for a given q. $E_2(q)$ is the excess by which a rare strain-2 microbe out-produces a common strain-1 microbe for a given q.

The conditions for A_1 and A_2 to increase when rare in the absence of microbiome variation were shown earlier. These previously derived conditions can be transferred here with the proviso that the expressions for the holobiont fitnesses now depend on $p(t)$ because these fitnesses are averaged over the microbiome frequencies, as indicated with the overline symbol. Putting $p(t) = p$, the condition for A_1 to increase when rare is

$$q(t+1) > q(t)\big|_{q\approx 0,p}$$

which works out to be

$$F_1(p) \equiv \overline{W_{12}} - \overline{W_{22}} > 0$$

And similarly, A_2 can increase when rare if

$$F_2(p) \equiv \overline{W_{12}} - \overline{W_{11}} > 0$$

Applying these increase-when-rare conditions to the corner equilibria yields:

Corner A, *p*=0, *q*=0

$$\begin{aligned} E_1(0) &= 2\,\widetilde{w_{1,12}} - \widetilde{w_{2,2}} = 2\,w_{1,12,22} - w_{2,2,22} > 0 \\ F_1(0) &= \overline{W_{12}} - \overline{W_{22}} = W_{2,12} - W_{2,22} > 0 \end{aligned}$$

At this corner, all the alleles are A_2 and all the microbes are strain-2. So, the first condition means that strain-1 microbes increase if twice their multilevel fitness in a dual-strain microbiome with an A_2A_2 host exceeds the fitness of strain-2 microbes in a single strain-2 microbiome with a A_2A_2 host. The second condition means that A_1 alleles increase if their fitness in an A_1A_2 host with a single strain-2 microbiome exceeds the fitness of A_2 alleles in a A_2A_2 host with a single strain-2 microbiome. A similar interpretation applies to the other corners.

Corner B, *p*=0, *q*=1

$$\begin{aligned} E_1(1) &\equiv 2\,\widetilde{w_{1,12}} - \widetilde{w_{2,2}} = 2\,w_{1,12,11} - w_{2,2,11} > 0 \\ F_2(0) &\equiv \overline{W_{12}} - \overline{W_{11}} = W_{2,12} - W_{2,11} > 0 \end{aligned}$$

Corner C, p=1, q=1

$$\begin{aligned} E_2(1) &\equiv 2\,\widetilde{w_{2,12}} - \widetilde{w_{1,1}} = 2\,w_{2,12,11} - w_{1,1,11} > 0 \\ F_2(1) &\equiv \overline{W_{12}} - \overline{W_{11}} = W_{1,12} - W_{1,11} > 0 \end{aligned}$$

Corner D, p=1, q=0

$$\begin{aligned} E_2(0) &\equiv 2\,\widetilde{w_{2,12}} - \widetilde{w_{1,1}} = 2\,w_{2,12,22} - w_{1,1,22} > 0 \\ F_1(1) &\equiv \overline{W_{12}} - \overline{W_{22}} = W_{1,12} - W_{1,22} > 0 \end{aligned}$$

If the four corner equilibria are unstable with trajectories leading out from them into the interior of the (p, q) plane then the interior equilibrium, I, exists. This equilibrium represents a joint polymorphism for both the microbiome and host nuclear components of the hologenome. The joint equilibrium $(\hat{p}, \hat{q})$ is found from numerically solving simultaneously for p and q in

$$\begin{aligned} p &= \frac{E_1(q)}{E_1(q) + E_2(q)} \\ q &= \frac{F_1(p)}{F_1(p) + F_2(p)} \end{aligned}$$

6.3 Illustration—Joint Microbiome-Host Genetic Diversity

The full model with both two microbe strains and two host alleles may be used to investigate the maintenance of genetic diversity jointly in the microbiome and host nucleus. As a illustration, return to the previous example involving a polymorphism between two microbial strains, one altruistic and one selfish. Now, suppose strain-1 is altruistic only to one genotype of host because a host with that genotype somehow facilitates microbes of strain-1. Furthermore, suppose arbitrarily that the host genotype to which strain-1 is altruistic is the A_1A_2 heterozygote genotype. This choice is made to develop a numerical example in which both the microbiome and host components of the hologenome are likely to be polymorphic. (If say, strain-1 were altruistic to a homozygote host genotype like A_1A_1 rather than to the heterozygote A_1A_2, then the A_1 allele would fix in the host component of the hologenome, thus presenting a less interesting dynamical outcome.)

Accordingly, Table 1 presents the setup. The first column of the table details all the parameter assignments. The second column details intermediate coefficients derived from these parameters. The third column details the equilibrium outcome of holobiont selection with the equilibrium frequency of strain-1 followed by the equilibrium frequency of A_1 in the top two rows. Below these are the hologenotype frequencies, $\hat{X}_{ij,kl}$ for the hologenotype with strain-i and strain-j in the microbiome and allele A_k and A_l in the host nucleus. Figure 11 shows trajectories in the (p, q) plane that approach this equilibrium from an assortment of initial conditions.

Table 1: Numerical Example: Two Microbe Strains and Two Host Alleles

$W_{1,11} \to 2$	$n_{1,11} = 200$	$\hat{p} = 0.325939$
$W_{1,12} \to 3$	$n_{1,12} = 133.333$	$\hat{q} = 0.614992$
$W_{1,22} \to 1$	$n_{1,22} = 266.667$	
$W_{12,11} \to 1.5$	$n_{2,11} = 300$	$\hat{X}_{1,11} = 0.0401801$
$W_{12,12} \to 2.5$	$n_{2,12} = 333.333$	$\hat{X}_{12,11} = 0.509881$
$W_{12,22} \to 1$	$n_{2,22} = 266.667$	$\hat{X}_{2,11} = 0.171845$
$W_{2,11} \to 1$		$\hat{X}_{1,12} = 0.0503084$
$W_{2,12} \to 1$	$w_{1,1,11} = 700$	$\hat{X}_{12,12} = 0.638408$
$W_{2,22} \to 1$	$w_{1,1,12} = 900$	$\hat{X}_{2,12} = 0.215163$
	$w_{1,1,22} = 400$	$\hat{X}_{1,22} = 0.0157475$
$k_{1,11} \to 350$	$w_{1,12,11} = 300$	$\hat{X}_{12,22} = 0.199834$
$k_{1,12} \to 300$	$w_{1,12,12} = 333.333$	$\hat{X}_{2,22} = 0.0673501$
$k_{1,22} \to 400$	$w_{1,12,22} = 266.667$	
$k_{2,11} \to 400$	$w_{2,12,11} = 450$	
$k_{2,12} \to 400$	$w_{2,12,12} = 833.333$	
$k_{2,22} \to 400$	$w_{2,12,22} = 266.667$	
	$w_{2,2,11} = 400$	
$a_{12,11} \to 0.5$	$w_{2,2,12} = 400$	
$a_{12,12} \to 0.5$	$w_{2,2,22} = 400$	
$a_{12,22} \to 0.5$		
$a_{21,11} \to 0.5$		
$a_{21,12} \to 0.5$		
$a_{21,22} \to 0.5$		

At the joint equilibrium, the altruistic strain-1 is 33% of the microbe pool and the A_1 host allele is 61% of the host gene pool. The most common hologenotypes at equilibrium are 51% two-strain microbiomes in an A_1A_1 homozygous host, 22% single strain-2 microbiomes in an A_1A_2 heterozygous host, and 20% two-strain microbiomes in an A_2A_2 homozygous host. The microbiome in the two-strain A_1A_1 host consists of 200 strain-1 microbes and 300 strain-2 microbes. The microbiome in the single strain-2 A_1A_2 host consists of 400 strain-2 microbes. The microbiome in a two-strain A_2A_2 host consists of 267 strain-1 microbes and 267 strain-2 microbes. And similarly for the other less common hologenotypes. At equilibrium the hologenome is polymorphic for the altruistic and selfish microbial strains and for the nuclear alleles that differ in the

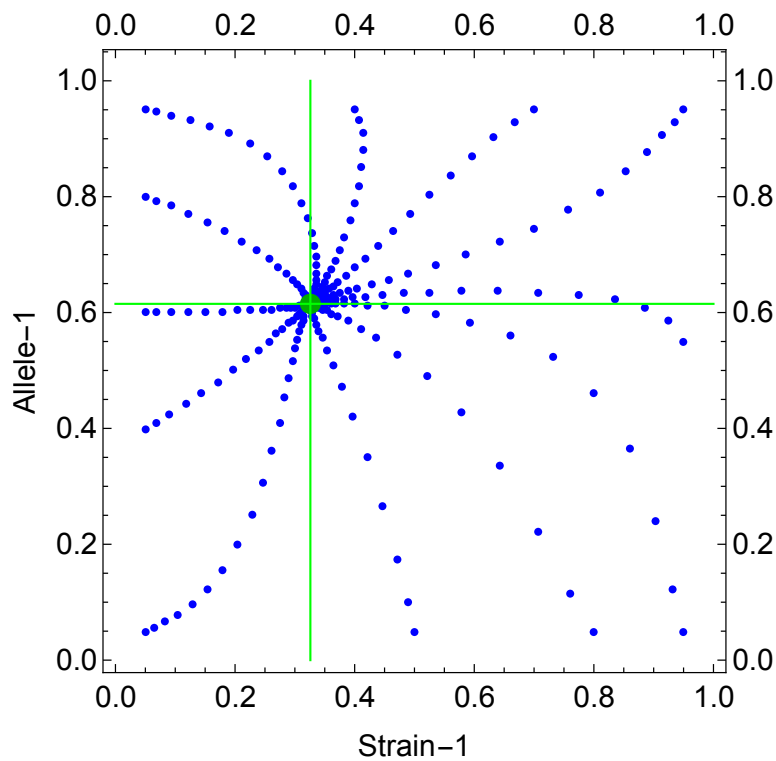

Figure 11: *Trajectories of the combined dynamics of microbe frequency and allele frequency under holobiont selection leading to a combined polymorphism representing coadaptation between the microbiome and host. Trajectories all begin at various spots near the axes and converge through time to the green dot in the interior. The equilibrium frequencies are marked with the vertical and horizontal lines in green.*

reciprocation they supply to the altruistic microbial strain.

6.4 Direction of Joint Microbiome-Host Evolution

To analyze the direction of holobiont evolution, write the dynamics as difference equations for $\Delta p_1(t) \equiv p_1(t+1) - p_1(t)$ and for $\Delta q \equiv q_1(t+1) - q_1(t)$ yielding, after rearrangement,

$$\begin{aligned} \Delta p &= \frac{p\,(1-p)\,(\overline{\widetilde{w_1}} - \overline{\widetilde{w_2}})}{\overline{\overline{\widetilde{w}}}} \\ \Delta q &= \frac{q(1-q)(\widetilde{\overline{W}}_1 - \widetilde{\overline{W}}_2)}{\widetilde{\overline{\overline{W}}}} \end{aligned}$$

where the subscript and time index are dropped leaving $p \equiv p_1(t)$ and $q \equiv q_1(t)$. At equilibrium, the marginal multilevel fitness per holobiont of both microbe strains are equal and the marginal fitness of both host alleles are equal.

In the spirit of the previous expressions for quantities maximized by holobiont selection on

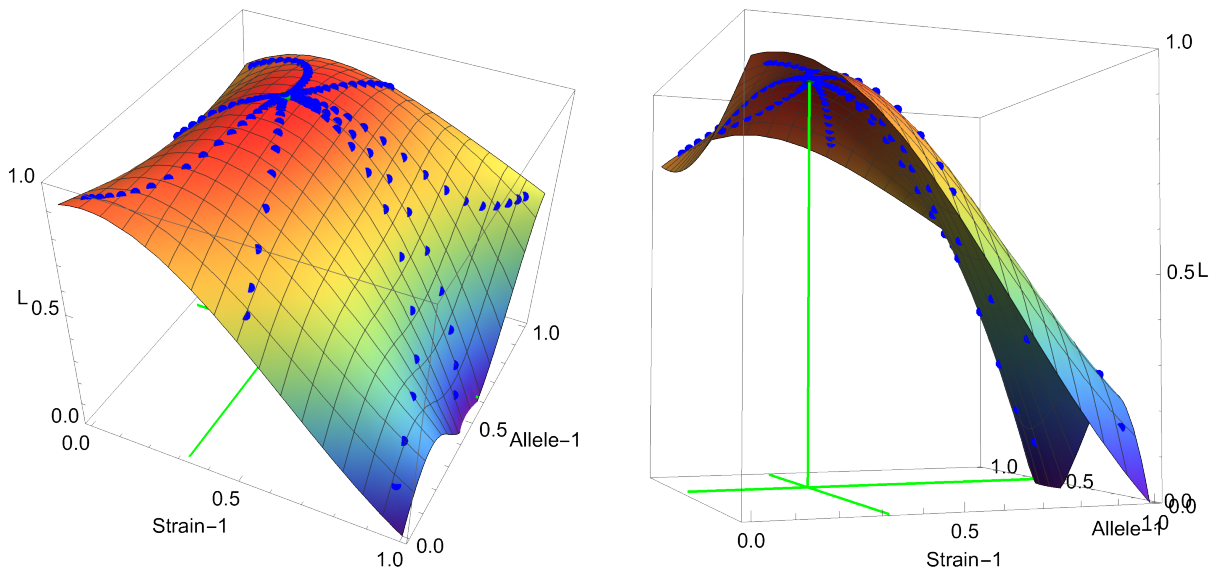

Figure 12: *The $L(p,q)$ surface representing the simultaneous degree of equality between the marginal multilevel fitness per holobiont of the microbe strains along with the degree of equality between the marginal fitness of the host alleles. The equilibrium, $(\hat{p},\hat{q})$, lies at the peak of the surface and is shown as a green dot. Trajectories of the combined dynamics of microbe frequency and allele frequency under holobiont selection are shown with blue dots from an assortment of initial conditions that start near the axes. The trajectories climb the surface and converge at the equilibrium at the top. Left, view from above the surface. Right: view from below the surface.*

a two-strain microbiome, $L_G(p)$ and on a host with two alleles $L_H(q)$, consider their product as a candidate that is maximized by holobiont selection acting simultaneously on both the microbiome and host nucleus:

$$\left(-(\overline{\widetilde{w}_1}-\overline{\widetilde{w}_2})^2\right)\left(-(\widetilde{\overline{W}}_1-\widetilde{\overline{W}}_2)^2\right)$$

This expression represents the simultaneous degree of equality between the marginal multilevel fitness per holobiont of both microbe strains along with the degree of equality between the marginal fitness of both host alleles. This expression may be scaled to lie mostly between 0 and 1 as follows:

$$\begin{aligned} L(p,q) &= \left(\frac{-(\overline{\widetilde{w}_1}-\overline{\widetilde{w}_2})^2}{\max[E_1(0)^2,E_1(1)^2,E_1(\hat{q})^2,E_2(0)^2,E_2(1)^2,E_2(\hat{q})^2]}+1\right) \\ &\times \left(\frac{-(\widetilde{\overline{W}}_1-\widetilde{\overline{W}}_2)^2}{\max[F_1(0)^2,F_1(1)^2,F_1(\hat{q})^2,F_2(0)^2,F_2(1)^2,F_2(\hat{q})^2]}+1\right) \end{aligned}$$

Figure 12 illustrates the $L(p,q)$ surface. It shows the equilibrium at the peak of this surface. The figure also shows trajectories for the simultaneous evolution of the microbiome and host nuclear components of the hologenome converging at the peak of the surface.

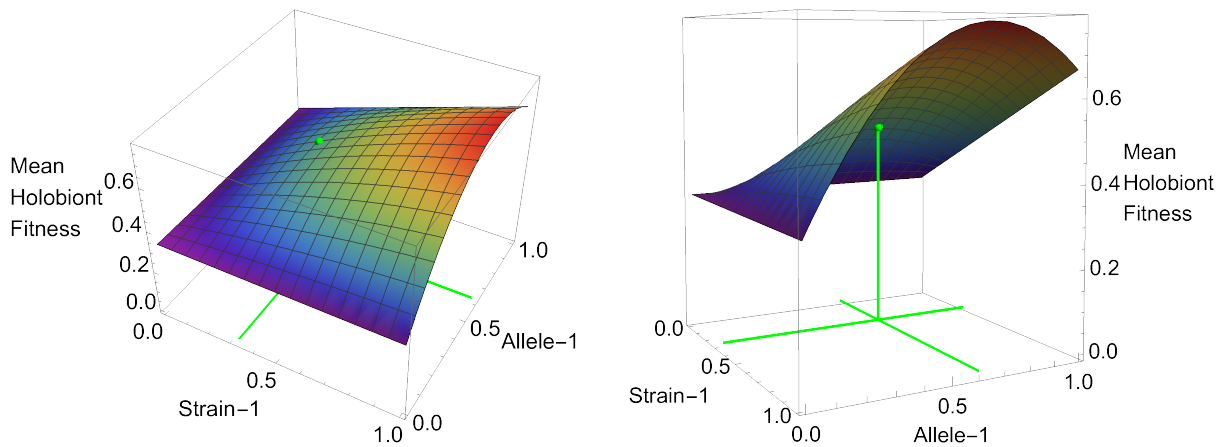

Figure 13: *The $\overline{\overline{W}}(p,q)$ surface representing overall holobiont mean fitness. The equilibrium, $(\hat{p},\hat{q})$, is shown as a green dot. Left, view from above the surface. Right: view from below the surface.*

Holobiont selection does not lead to a peak of overall adaptation for the holobiont. Figure 13 shows the surface for the overall holobiont mean fitness, $\overline{\overline{W}}(p,q)$. The equilibrium is indicated as a green dot on the surface. This dot does not lie on any peak of the surface. From the standpoint of maximal holobiont fitness, the best outcome would be for the microbiome to consist entirely of the altruistic strain-1 and for the nuclear alleles to yield the heterozygotes that benefit from strain-1 altruism. However, this outcome does not result from holobiont selection.

The difference between holobiont selection maximizing $L(p,q)$ and not $\overline{\overline{W}}(p,q)$ points out that from the standpoint of the host, a gene obtained via a microbial strain is "not as good as" the same gene present in its own nucleus. Classical natural selection with multiple genes at one locus leads to an outcome that maximizes the mean fitness. But if those same genes are embedded in microbes, then the evolutionary dynamics do not generally result in an equally adaptive outcome.

The hologenome is best compared with a multiple locus genetic system rather than a one locus multiple allele system. Classical natural selection with multiple loci generally does not result in maximizing mean fitness unless the linkage between the loci is tight (Moran 1964, Karlin 1975). The hologenome and a two-locus genetic system both contain two components. Just as selection operating on two locus genetic system does not generally maximize mean fitness, selection operating on a combined microbiome-host genetic system should not be expected to maximize mean fitness either.

On the other hand, although a microbial gene might not be "as good as" a nuclear gene for the host with respect to realizing an optimal adaptive outcome from selection, a microbial gene might be beneficial to the host for other compelling reasons. From a host's perspective, a mi-

crobial gene is a "contract gene", so to speak. A host presumably can jettison the gene if not useful, and can assimilate a replacement if needed. The facultative employment of zooxanthellae by corals under differing light and temperature conditions comes to mind. In contrast, recruiting a gene to join the nucleus represents a serious commitment by the host to pass the gene on over many generations and perhaps in perpetuity, as though granting a "genetic tenure" to the gene. The topic of hologenome architecture is a conceptual counterpart to that in classical population genetics concerning how natural selection sculpts the genetic system by modifying linkage relationships using chromosomes and modifying mutation rates using DNA repair mechanisms.

7 Strategies of Coadaptation

7.1 Definition of a Holostable Strategy (HSS)

A potential use of holobiont evolutionary theory is to account for the coadaptation between the microbiome and host that motivated the concept of a holobiont to begin with. One approach is to develop a counterpart for holobiont evolution of the evolutionarily stable strategy (EES) originally introduced by Maynard Smith (1982). A phenotypic strategy is an evolutionarily stable strategy such that, if all the members of a population adopt it, no mutant strategy can invade. This concept is realized in population-genetic terms by postulating that a strategy is associated with a corresponding gene. If the gene pool is fixed for a gene that codes for a particular strategy, and if that gene pool state is stable to invasion by any mutant gene coding for some other strategy, then that particular strategy is an ESS. This section extends the concept of an ESS to describe the evolutionarily stable configuration of host and microbiome.

Consider the hologenotype equilibrium point at which both the frequency of allele A_1 equals 1 and the frequency of strain-1 equals 1, corresponding to the corner equilibrium C in Figure 10. Next, consider a set of possible alternative alleles and a set of possible alternative microbial strains, a so-called mutant hologenotype. Then ask under what conditions this hologenotype equilibrium, C in Figure 10, is stable to invasion by any alternative nuclear allele and/or alternative microbial strain taken from the sets of possible alleles and strains—the opposite of the condition for increase when rare. That is, the $\{A_1, \text{strain-1}\}$ hologeotype is stable if the mutant hologenotypes cannot increase when rare.

If allele A_1 is fixed in the host nucleus and strain-1 is fixed in the microbiome, then alternative alleles and strains *cannot* increase when rare if

$$\begin{aligned} W_{1,11} &> W_{1,12} \\ w_{1,1,11} &> 2\,w_{2,12,11} \end{aligned}$$

for any allele, A_2 and for any strain-2 microbe. In words, a hologenotype consisting of A_1A_1

paired with the strain-1, will be called "holostable" if *both* the fitness of an A_1A_1 homozygote with a single strain-1 microbiome exceeds the fitness of an A_1A_2 heterozygote with a single strain-1 microbiome *and* the multilevel fitness per holobiont of a strain-1 microbe in a single-strain microbiome with a A_1A_1 host exceeds twice the multilevel fitness per holobiont of a strain-2 microbe in a two-strain microbiome with a A_1A_1 host. A holophenotype that leads to such a hologenotype will be called a "holostable strategy" (HSS). The HSS is stable to invasion by alternative phenotypes both ecologically and evolutionarily.

7.2 Conditions for an HSS

Consider a trait controlled by the microbe, x, and another trait controlled by the host, y, from a set of possible traits. The multilevel microbe fitness and the host fitness depend on both these traits. Write the fitnesses as functions of these traits. A focal strategy, $(\hat{x}, \hat{y})$, for a microbe and its host is an HSS if it is unbeatable by any mutant strategy, x_m, y_m, for a mutant microbe and mutant host. According to the conditions above for a non-invasible hologenotype, the conditions for the focal strategy, $(\hat{x}, \hat{y})$, to be an HSS are

$$\begin{aligned} W(\hat{x}, \hat{y}) &> W(\hat{x}, y_m) \\ w(\hat{x}, \hat{y}) &> 2\, w(x_m, \hat{y}) \end{aligned}$$

for any x_m, y_m from the set of possible traits.

To satisfy the first condition, maximize W with respect to y for any given x. The value of y that maximizes W for a particular x is the host component of the HSS at that x because any other mutant value, y_m, yields a lower W at that particular x. Hence, the focal $\hat{y}(x)$ is the host component of the HSS if it is obtained by maximizing W with respect to y for any given x,

$$\hat{y}(x) = \max_y W(x, y)$$

The second condition for an HSS is not so straight forward—it depends on the ecology of the mutant within the host. So, unpack microbial multilevel fitnesses into their components,

$$k(\hat{x}, \hat{y})\, W(\hat{x}, \hat{y}) > 2\, n(\hat{x}, \hat{y}, x_m)\, W(\hat{y}, x_m)$$

where $n(\hat{x}, \hat{y}, x_m)$ is the mutant's population size in a two-strain microbiome given that the host's trait is $\hat{y}$ and the focal microbe's trait is $\hat{x}$. The $n(\hat{x}, \hat{y}, x_m)$ stands for the mutant strain's equilibrium population size from a model of population dynamics within a two-strain microbiome. In particular, with Lotka-Volterra competition, the second condition becomes

$$k(\hat{x}, \hat{y})\, W(\hat{x}, \hat{y}) > \left(\frac{k(x_m, \hat{y}) - a\, k(\hat{x}, \hat{y})}{1 - a^2} \right) W(x_m, \hat{y})$$

for any x_m, where a is the competition coefficient (assuming for simplicity that reciprocal competition coefficients are equal and $0 \leq a \leq 1$).

The mutant strain is assumed to be identical in all respects to the focal strain except in its value of x. Therefore, the competition coefficient, a, is 1 between the mutant and the focal strain. Hence, the mutant strain cannot coexist with the focal strain if its k is less than the k of the focal strain. Therefore, the second condition for an HSS boils down to

$$k(\hat{x}, \hat{y}) > k(x_m, \hat{y})$$

The value of x that maximizes k for a particular y is the microbe component of the HSS at that y because any other value of x, say x_m, yields a lower k at that particular y. Hence, the focal $\hat{x}(y)$ is the microbe component of the HSS if it is obtained by maximizing k with respect to x for any given y.

$$\hat{x}(y) = \max_x k(x, y)$$

In summary, the focal strategy, $(\hat{x}, \hat{y})$, is an HSS if it is found by solving simultaneously from

$$\begin{aligned} \hat{y}(x) &= \max_y W(x, y) \\ \hat{x}(y) &= \max_x k(x, y) \end{aligned}$$

Whether $(\hat{x}, \hat{y})$ actually exist as a solution to these two HSS conditions depends on the tradeoffs among the host and microbe phenotypes as specified in a phenotype submodel.

This HSS represents solely self interest by both parties. The host component of the HSS maximizes the host fitness under r-selection—the host maximizes its own direct benefits and any contribution to the microbe's welfare is incidental. Conversely, the microbe component of the HSS also maximizes its own direct benefits under K-selection and any contribution to the host's welfare is incidental.

This result is surprising because the microbe's welfare is partially aligned with that of the host given that the microbe multilevel fitness is a product of its k with the host's W. However, this partial alignment does not influence the microbe's component of the HSS because the microbe's k provides a barrier to entry for a mutant strain. That is, if a strain within the host has the highest k then no mutant with a lower k can enter regardless of how much that mutant strain promotes the host's welfare and regardless of how much higher its own multilevel fitness would be enhanced as a result.

This HSS solution is a Nash equilibrium similar in principle to what could be obtained with coevolutionary selection. Because both parties in a coevolutionary model are at the same level, they typically are both jointly undergoing either r-selection or K-selection. In holobiont selection, one party is undergoing r-selection while the other is undergoing K-selection, but the solution

concept, namely the Nash equilibrium, is the same. That is, the host is playing against its microbiome and the microbiome is playing against its host similar to the parties in coevolutionary selection even though details of the model setup differ between coevolutionary and holobiont selection models.

Nonetheless, coadaptation may still result between host and microbiome even though both parties are maximizing their own direct benefits provided the within-generation reciprocation between the parties compensates for their sacrifices. This will depend on details of the phenotype submodels that describe the physiological mechanisms by which the host and microbe interact. Any coadaptation that results in this way could be considered as "by-product coadaptation" to be distinguished from "true coadaptation" that takes into account the joint welfare of one or more parties.

7.3 Invading the HSS—the Optimal Invading Strategy (OIS)

All is not lost, however. True coadaptation, rather than by-product coadaptation, can evolve if the mutant microbe has some niche differentiation from the HSS microbe. If the competition coefficient, a, is less than 1, indicating that the mutant microbe occupies a slightly different niche from the HSS microbe, then the HSS microbe can no longer serve as a barrier to entry for the mutant microbe. The difference

$$k(x_m, \hat{y}) - a\, k(\hat{x}, \hat{y})$$

indicates how much less the mutant's $k(x_m, \hat{y})$ can be relative to the HSS $k(\hat{x}, \hat{y})$ and still enter the host. This difference will be called the "altruism amplitude." If the HSS k is 100, for example, and if say, $a = 1/2$, then the mutant's k can be as low as 50 and while still allowing the mutant to enter the host. Therefore, the mutant could sacrifice up to 50 units of its k to contribute to the welfare of the host while still being able to enter the host. This will be evolutionarily profitable to the mutant microbe if the host's welfare increases enough to yield the microbe with a sufficiently higher overall multilevel fitness.

The best invader to challenge the HSS, given the competition coefficient, a, is found by reversing the second condition for an HSS. That is, let $\hat{x}, \hat{y}$ be the strategies of a particular HSS. Then a mutant microbe can invade this particular configuration if

$$k(\hat{x}, \hat{y})\, W(\hat{x}, \hat{y}) < 2 \left(\frac{k(x_m, \hat{y}) - a\, k(\hat{x}, \hat{y})}{1 - a^2} \right) W(x_m, \hat{y})$$

The best invader is the mutant, x_m that maximizes the right hand side of the inequality, i.e., maximizes

$$(k(x_m, \hat{y}) - a\, k(\hat{x}, \hat{y}))\, W(x_m, \hat{y})$$

or, rewritten as,

$$k(x_m, \hat{y})\, W(x_m, \hat{y}) - a\, k(\hat{x}, \hat{y})\, W(x_m, \hat{y})$$

The first term in the expression is the multilevel fitness of the mutant microbe. The second term is a times the multilevel fitness of the HSS microbe with which the the mutant is competing to enter the host. The best mutant microbe maximizes its own multilevel fitness while also minimizing the multilevel fitness of its HSS competitor. But in so doing, the best mutant both helps the host as part maximizing its own multilevel fitness but, depending on the value of a, also hurts the host as part of minimizing its competitor's multilevel fitness.

Denote the best invading mutant as $\widehat{x_m}$. Putting these conditions together, and assuming that the HSS strategies, $(\hat{x}, \hat{y})$, have already been determined and that a is given, then the best invading mutant as $\widehat{x_m}$ is found from

$$\widehat{x_m}(\hat{x}, \hat{y}, a) = \max_{x_m} \left(k(x_m, \hat{y})\, W(x_m, \hat{y}) - a\, k(\hat{x}, \hat{y})\, W(x_m, \hat{y}) \right)$$

The solution to this maximization is considered feasible if the inequality above is satisfied, which must be checked before the solution is accepted. $\widehat{x_m}(\hat{x}, \hat{y}, a)$ will be termed the "optimal invading strategy" (OIS). The OIS will embody true coadaptation because the microbe's optimal strategy will have incorporated some consideration of the host's welfare.

7.4 Example Phenotype Submodel

To illustrate how to develop a phenotypic submodel, consider an example concocted for an idealized coral-zooxanthellae holobiont. Let x be the "cooperative effort" by the microbe toward the host and y the "cooperative effort" by the host toward the microbe. Both x and y are in $[0, 1]$. The cooperative effort by the microbe refers to the cost of the transfer of photosynthate from the zooxanthellae to the coral, and the cooperative effort by the host refers to the cost of the infrastructure the coral provides for the zooxanthellae. Let the product, $(x\, y)$, be the "cooperative synergy" between the microbe and host. This quantity indicates how much both parties are working together to provide assistance for one another.

The cooperative synergy is taken to be the fraction of the host's base fitness, W_o, that it forgoes to provide infrastructure to the microbe and also the fraction of the microbe's base carrying capacity, k_o, that it foregoes to provide energy to the host. Accordingly,

$$\begin{aligned} W(x, y) &= (1 - x\, y)\, W_o + \ldots \\ k(x, y) &= (1 - x\, y)\, k_o + \ldots \end{aligned}$$

The costs of the cooperation from the host to the microbe and from microbe to host are only part of the picture. The other part is the benefit each receives from their cooperative synergy.

So, credit the host with the benefit that the microbe is supplying, $c_1\, x^{c_2}\, y^{c_3}\, k_o$. The coefficient, c_1, converts units of microbe carrying capacity to those of host fitness. Both x and y are raised to powers, $c_2 < 1$ and $c_3 < 1$, to describe diminishing returns to scale for the effort. Furthermore,

$c_2 < c_3$ to indicate that the effect of the host's helping the microbe must do a "round trip" to be experienced by itself, whereas the effect of the microbe's helping is only discounted once because it results from a "one-way trip".

$$W(x, y) = (1 - x\,y)W_o + c_1\,x^{c_2}\,y^{c_3}\,k_o$$

Similarly, credit the microbe with the benefit that the host is supplying to it, $c_4\,x^{c_5}\,y^{c_6}\,W_o$. The coefficient, c_4, converts the units of host fitness to those of the microbe carrying capacity and $c_5 < 1$ and $c_6 < 1$ indicate the decreasing returns to scale for the efforts. Moreover, $c_6 < c_5$ to account for the different discounting between round trip and one-way contributions of cooperative effort. Hence, the equation for the strain-1 carrying capacity in a A_1A_1 host becomes

$$k(x, y) = (1 - x\,y)k_o + c_4\,x^{c_5}\,y^{c_6}\,W_o$$

These two equations constitute the coral-zooxanthellae submodel to be analyzed. The task now is to determine the values of cooperative efforts, $\hat{x}$ and $\hat{y}$, that comprise the HSS and OIS.

7.5 Numerical Example of HSS and OIS

Table 2 offers some numerical values for parameters in the phenotype model for host-microbiome reciprocation. Table 3 lists the resulting coadaptation strategies based on these parameters. Table 4 details the microbiome polymorphism that results when the HSS holobiont configuration is invaded by the OIS microbe. Appendix 3 provides the *Mathematica* script used to compute the results for the numerical example.

Table 3 lists the Spiteful strategy first. In this strategy neither the microbe nor the host carry out any cooperative effort, so both $x = 0$ and $y = 0$. As a result, the microbe's k is only the baseline $k_o = 1000$ and the host's W is only the baseline $W_o = 1$. Spite is not stable to invasion by Cooperate—both a cooperating microbe in the microbiome can invade and a cooperating allele in the host nucleus can invade. Cooperate is an HSS because the reciprocation within a generation leads both to a higher k for the microbe and a higher W for the host. The degree of microbe cooperative effort at the HSS is $x = 0.041$ and the degree of host cooperative effort is at the HSS is $y = 0.456$. As a result of these cooperative efforts, the microbe's k increases above the baseline to $k = 1056$ and the host's W increases above the baseline to $W = 1.056$.

Table 3 lists the Altruistic strategy last. Assuming the competition coefficient, $a < 1$, the HSS cooperative strategy can be invaded by OIS altruistic strategy. In this example $a = 1/2$. In Altruism, the microbe increases its cooperative effort, x, from $x = 0.041$ to $x = 0.129$. As a result, its k drops from $k = 1056$ to $k = 1041$. This additional cooperative effort raises its multilevel fitness from $w = 1116$ to $w = 1164$. This is sufficient to allow Altruism to invade Cooperate.

Table 2: Coadaptation Example: Parameters for Host-Microbiome Reciprocation

$W_o \to 1$	$c_1 \to 1/1000$	$c_4 \to 300$
$k_o \to 1000$	$c_2 \to 3/4$	$c_5 \to 1/4$
$a \to 1/2$	$c_3 \to 1/4$	$c_6 \to 3/4$

Table 3: Coadaptation Example: HSS and OIS Solutions

Strategy	x	k	w	y	W
Spiteful	0	1000	1000	0	1
Cooperative	0.0410792	1056.25	1115.66	0.456435	1.05625
Altruistic	0.128788	1041.02	1163.77	0.456435	1.11792

Table 4: Coadaptation Example: HSS–OIS Microbiome Polymorphism

n_c	n_a	p_c	${p_c}^2$	$2\,p_c\,p_a$	${p_a}^2$
714.323	683.853	0.51182	0.26196	0.499721	0.23832

Meanwhile, the host does not change is cooperative effort, y, but receives an increase in its W from $W = 1.056$ to $W = 1.118$ through the good graces of the microbe's altruism.

The invasion of Cooperate by Altruism results in a polymorphism between the Altruistic and Cooperative microbe strains across the population of holobionts as detailed in Table 4. In a two-strain microbiome with both Cooperative and Altruistic microbes, the abundance of the Cooperative microbe, $n_c = 714$, and the abundance of the Altruistic microbe, $n_a = 684$. The abundances of microbes in single-strain Cooperator and Altruism microbiomes are their respective ks: 1056 for the Cooperator and 1041 for the Altruist. The frequency of Cooperative microbes in pool of microbes from all the holobionts combined is $p_c = 0.512$. The frequency of the Altruistic microbes is $p_a = 1 - p_c$. Then the frequency of single-strain Cooperator microbiomes across all holobionts is ${p_c}^2 = 0.262$. The frequency of two-strain microbiomes across all holobionts with both Cooperator and Altruism microbes is $(2\,p_c\,p_a) = 0.500$. The frequency of the single-strain Altruism microbiomes across all holobionts is ${p_a}^2 = 0.238$.

At the holobiont polymorphism between Cooperative and Altruistic microbiomes, the force of natural selection within the holobionts favoring a high microbe k balances the force of multilevel selection between holobionts favoring a high microbe w.

Appendix 3 provides a *Mathematica* script for computing all the quantities in the numerical example.

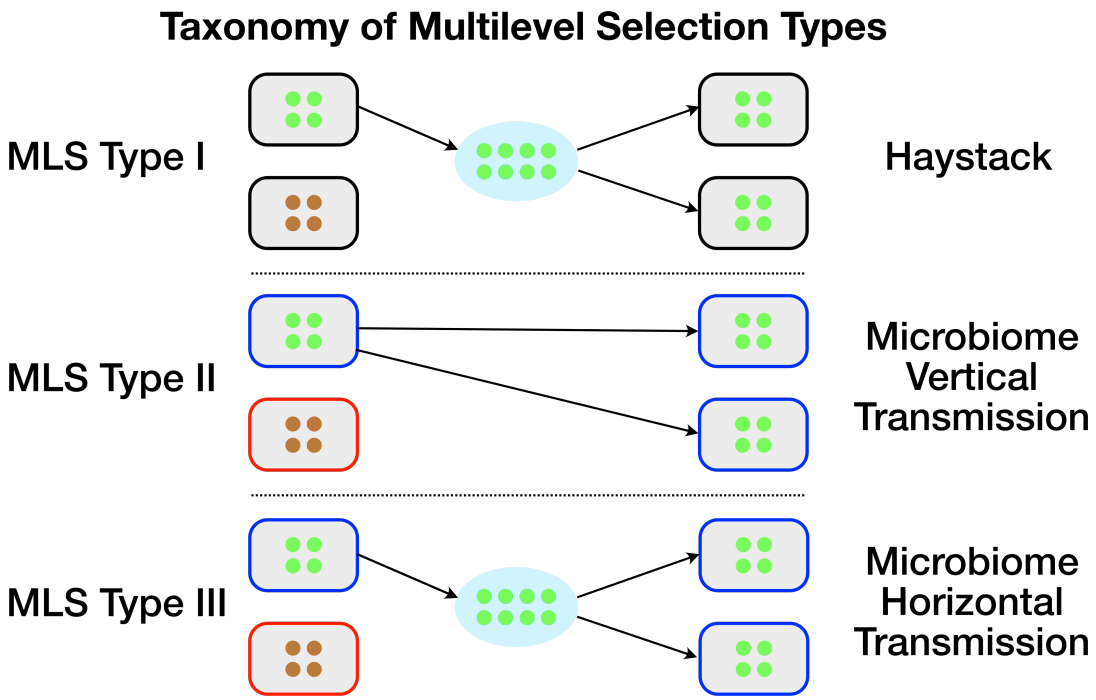

Figure 14: *Taxonomy of Multilevel Selection Types. In multilevel selection 1 (MLS1), the environment provides locations indicated as black rectangles with rounded corners. In these locations the organisms undergo natural selection favoring selfish genes, in brown. After selection, offspring from the 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 (holobiont). 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, or holobiont, is inherited as a unit from one generation to the next. MLS2 occurs during holobiont selection with vertical transmission of the microbiome. 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. MLS3 occurs during holobiont selection with horizontal transmission of the microbiome, as modeled in this paper.*

8 Discussion

Holobiont selection is relevant to a well-known distinction between types of multilevel selection, namely, multilevel selection 1 (MLS1) and multilevel selection 2 (MLS2) as diagrammed in Figure 14. (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 the recurring haystacks in farmers' fields as the collectives within which mice (particles) can interact and evolve.

A holobiont can be identified as a "collective" in Okasha's terminology. If their 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 can be regarded as an instance of MLS2. If their microbiomes are horizontally transmitted, then one might be tempted to regard holobiont selection as an instance of MLS1. However, the holobiont as a "collective" is not a recurring feature of the environment as is required by the definition of MLS1—instead it is an evolving organism itself. (Maynard Smith's haystacks do not evolve even though the mice living in them do.) Therefore, this paper considers holobiont selection with horizontal microbiome transmission to be a third type of multilevel selection, denoted here as MLS3, as diagrammed in Figure 14. MLS3 is a combination of MLS1 and MLS2.

9 Conclusions

This section records some highlights from the body of the paper and raises some additional points for discussion.

1. A hologenotype is a hierarchical data structure describing the genetic composition simultaneously in both the microbiome and the nucleus of the holobiont.

2. This paper defines holobiont selection theory as equations that map the hologenotype distribution at time t to that at time $t+1$.

3. Holobiont selection differs from coevolutionary selection. Coevolutionary selection involves separate gene pools for the interacting parties who are at the same hierarchical level. Holobiont selection involves a single hologene pool composed of microbiome and nuclear components and where the microbiome level resides within the level of the host that supplies the nucleus.

4. This paper explains why holobiont selection produces evolution with both vertical and horizontal microbial transmission, contrary to existing discussion in the literature.

5. This paper introduces the concept of collective inheritance that underlies the success of holobiont selection with horizontal microbiome transmission. Collective inheritance contrasts with the traditional linear inheritance that occurs during holobiont selection with vertical microbe transmission.

6. Holobiont selection affects both the nuclear and microbiome parts of the hologenotype in parallel. Random colonization by microbes for the microbiome portion of the hologenome is the counterpart of random union of gametes for the nuclear portion of the hologenome.

7. Holobiont selection on the microbiome portion of the hologenome with horizontal microbiome transmission depends on a new concept—the multilevel fitness per holobiont of a microbe. This multilevel fitness combines both within-host K-selection with between-host r-selection.

8. The hologenome with its two parts, the microbiome and the nucleus, is analogous to a genetic system with two loci.

9. Holobiont selection maximizes equality of marginal fitnesses, not overall mean mean fitness.

10. The concept of an evolutionarily stable strategy (ESS) can be extended to hologenomes, leading to the definition of a holostable strategy (HSS) as a holobiont configuration that cannot be invaded by alternative microbial strains and alternative nuclear alleles that code for any other holobiont configuration. In the HSS, the microbe strategy is to maximize its carrying capacity (K-selection) and the host strategy is to maximize its fitness (r-selection). Cooperative coadaptation results with suitable within-generation reciprocation of microbe-host cooperative efforts.

11. The microbiome in an HSS holobiont configuration can be invaded by a best invader microbial strain (OIS) that sacrifices some of its with-host fitness to improve the between host fitness. Invasion by the OIS microbial strain results in a microbial polymorphism that expresses a balance of within-holobiont selfishness and between-holobiont altruism.

12. Holobiont selection with vertical transmission corresponds to multilevel selection II (MLS2). Holobiont selection with horizontal transmission corresponds to a new type of multilevel selection called multilevel selection III (MLS3), which is a combination of elements from MLS1 and MLS2.

13. The concept of the hologenome together with holobiont selection can be made conceptually and mathematically rigorous.

14. Holobiont theory is useful because it considers both host and microbiome simultaneously.

15. Holobionts are widely discussed as destabilizing the concept of an individual in biology. This paper takes the position that holobionts do not destabilize the concept of individuality because the status of a holobiont as an individual derives from the status of the host. What is problematic about a coral as an individual is that a polyp is part of a colony of polyps, not that it harbors zooxanthellae.

16. What holobionts do destabilize is the concept of inheritance as necessarily a lineal process. The concept of collective inheritance challenges the notion of parental ownership of offspring and the validity of individual-based strategy arguments as criteria for evolutionary success.

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References Cited

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

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

Booth, A. 2014. "Symbiosis, Selection, and Individuality." *Biol. Philos.* 29:657–673.

Brown, J. S. and T. L. Vincent. 1987. "Coevolution as an Evolutionary Game." *Evolution* 41:66–79.

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

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

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

item Doolittle, W. F. and A. Booth. 2017. "It's the Song, Not the Singer: an Exploration of Holobiosis and Evolutionary Theory." *Biol. Philos.* 32(1):5–24.

Douglas, A. E. and J. H. Werren. 2016. "Holes in the Hologenome: Why Host-Microbe Symbioses are not Holobionts." *MBio* 7(2):e02099-15. doi:10.1128/mBio.02099-15.

Fenner, F. 1953. "Changes in the Mortality-Rate due to Myxomatosis in the Australian Wild Rabbit." *Nature* 172, 228–230 doi:10.1038/172228a0

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

Fraune, S., and T. C. G. Bosch. 2010. "Why Bacteria Matter in Animal Development and Evolution." *Bioessays* 32:571–580.

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

Guerrero, R., L. Margulis, and M. Berlanga. 2013. "Symbiogenesis: the Holobiont as a Unit of Evolution." *International Microbiology* 16:133–143.

Hester, E. R., K. L. Barott, J. Nulton, M. Vermeij, and F. Rohwer. 2015. "Stable and Sporadic Symbiotic Communities of Coral and Algal Holobionts." *ISME J.* 1–15.

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

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

Karlin, S. 1975. "General two locus selection models: some objectives, rules and interpretations."*Theoretical Population Biology* 7:364–398.

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

Lloyd, E. 2017. "Holobionts as Units of Selection: Holobionts as Interactors, Reproducers, and Manifestors of adaptation." In *Landscapes of Collectivity in the Life Sciences,* edited by S. B. Gissis, E. Lamm, and A. Shavit, 351–367. London: The MIT Press.

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

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

Maynard Smith, J. 1982. *Evolution and the Theory of Games*. Cambridge: Cambridge University Press.

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

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

Moran, P. A. P. 1964. "On the non-existence of adaptive topographies." *Annals of Human Genetics* 27:383–393.

Moran, N. A. and D. B. Sloan. 2015. "The Hologenome Concept: Helpful or Hollow?" *PLoS Biol.* 13(12):e1002311.

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

Queller, D. C. and J. E. Strassmann. 2016. "Problems of Multispecies Organisms: Endosymbionts to Holobionts." *Biol. Philos.* 31:855–873.

Rosenberg, E. and I. Zilber-Rosenberg. 2014. *The Hologenome Concept: Human, Animal and Plant Microbiota*, Springer International Publishing.

Rosenberg, E. amd I. Zilber-Rosenberg. 2018. "The Hologenome Concept of Evolution After 10 Years." *Microbiome* 6:78. https://doi.org/10.1186/s40168-018-0457-9.

Rosenberg, E., O. Koren, L. Reshef, R. Efrony, and I. Zilber-Rosenberg. 2007. "The Role of Microorganisms in Coral Health, Disease and Evolution." *Nature Reviews Microbiology*. 5(5):355–62.

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

Skillings, D. 2016. "Holobionts and the Ecology of Organisms: Multi-species Communities or Integrated Individuals?" *Bio. Philos.* 31:875–892.

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

Woese, C. R. 2004. "A New Biology for a New Century." *Microbiology and Molecular Biology Reviews*. 68(2):173–86. PMID: 15187180.

Wright, S. 1931. "Evolution in Mendelian populations." *Genetics* **16**:97–159.

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

Appendix 1: Model for Two Horizontally Transmitted Haploid Microbial Strains

The life cycle of a holobiont with a horizontally transmitted microbiome consisting of two microbial strains in diagrammed in Figure 5.

A macro time step begins with the state variables $H(t)$, $G_1(t)$ 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 is $G(t) \equiv G_1(t) + G_2(t)$. The frequencies of strain-1 and strain-2 in the microbial pool at time t are $p_1(t) \equiv G_1(t)/G(t)$ and $p_2(t) \equiv G_2(t)/G(t)$ where $p_1(t) + p_2(t) \equiv 1$.

Given that the microbe pool in the water column surrounding the empty hosts is dilute, one may posit that two microbe propagules colonize each host at random according to a binomial distribution. Thus, after the microbiome colonization phase,

$$\begin{aligned} H'(t,2,0) &= p_1(t)^2 H(t) \\ H'(t,1,1) &= 2\, p_1(t)\, p_2(t) H(t) \\ H'(t,0,2) &= p_2(t)^2 H(t) \end{aligned}$$

where $H(t, n_1, n_2)$ is the number of holobionts with a hologenotype consisting of n_1 microbes of strain-1 and n_2 microbes of strain-2 at time t.

Once the juvenile hosts have been initially populated, the microbes proliferate within their hosts, coming to an equilibrium microbiome community structure. The equilibrium abundances in a dual-strain microbiome consisting of strain-1 and strain-2 are $\widehat{n_1}$ and $\widehat{n_2}$. The equilibrium

abundances of pure strain microbiomes consisting of only strain-1 or strain-2 are k_1 and k_2. Therefore, after the microbiome proliferation phase

$$\begin{aligned} H''(t,k_1,0) &= p_1(t)^2 H(t) \\ H''(t,\widehat{n_1},\widehat{n_2}) &= 2\,p_1(t)\,p_2(t) H(t) \\ H''(t,0,k_2) &= p_2(t)^2 H(t) \end{aligned}$$

Next, the holobiont fitness depend on the hologenotype, $W(n_1,n_2)$. Accordingly, after holobiont selection, the hologenotype numbers are

$$\begin{aligned} H'''(t,k_1,0) &= W(k_1,0)\,p_1(t)^2 H(t) \\ H'''(t,\widehat{n_1},\widehat{n_2}) &= W(\widehat{n_1},\widehat{n_2})\,2\,p_1(t)\,p_2(t) H(t) \\ H'''(t,0,k_2) &= W(0,k_2)\,p_2(t)^2 H(t) \end{aligned}$$

To simplify notation put $(W(k_1,0),\ W(\widehat{n_1},\widehat{n_2}),\ W(0,k_2))$ as (W_1, W_{12}, W_2), so the the equations above are rewritten as

$$\begin{aligned} H'''(t,k_1,0) &= W_1\,p_1(t)^2 H(t) \\ H'''(t,\widehat{n_1},\widehat{n_2}) &= W_{12}\,2\,p_1(t)\,p_2(t) H(t) \\ H'''(t,0,k_2) &= W_2\,p_2(t)^2 H(t) \end{aligned}$$

These holobionts then release their microbiomes into the microbe pool and also produce empty juvenile hosts ready for the newly released microbes to colonize.

So, to obtain the state variables at time $t+1$ given the state at time t, collect the previous terms. The total number of empty juvenile hosts at $t+1$ is

$$\begin{aligned} H(t+1) &= H'''(t,k_1,0) + H'''(t,\widehat{n_1},\widehat{n_2}) + H'''(t,0,k_2) \\ &= W_1\,p_1(t)^2 H(t) + W_{12}\,2\,p_1(t)\,p_2(t) H(t) + W_2\,p_2(t)^2 H(t) \\ &= p_1(t)\,\overline{W_1}(t) + p_2(t)\,\overline{W_2}(t) \\ &= \overline{\overline{W}}(t)\,H(t) \end{aligned}$$

where the holobiont mean fitness, $\overline{\overline{W}}(t)$, and the marginal host fitnesses, $\overline{W_1}(t)$ and $\overline{W_2}(t)$, at time t are

$$\begin{aligned} \overline{W_1}(t) &\equiv p_1(t)\,W_1 + p_2(t) W_{12} \\ \overline{W_2}(t) &\equiv p_1(t) W_{12} + p_2(t)\,W_2 \\ \overline{\overline{W}}(t) &\equiv p_1(t)^2\,W_1 + 2\,p_1(t)\,p_2(t)\,W_{12} + p_2(t)^2\,W_2 \end{aligned}$$

An overline over the W indicates an average taken over the microbiome compositions, ie, with respect to p_1 and p_2. Two overlines indicate two successive averages with respect to p_1 and p_2.

To simplify notation further, put $(\widehat{n_1}, \widehat{n_2})$ as (n_1, n_2). Then the number of strain-1 microbes in the microbe pool at time $t+1$ is

$$\begin{aligned} G_1(t+1) &= k_1\, H'''(t, k_1, 0) + \widehat{n_1}\, H'''(t, \widehat{n_1}, \widehat{n_2}) \\ &= k_1\, W_1\, p_1(t)^2 H(t) + n_1\, W_{12}\, 2\, p_1(t)\, p_2(t) H(t) \end{aligned}$$

The products of microbe population sizes and holobiont fitnesses can be combined into measures of multilevel microbe fitness per holobiont that include both success within the holobiont and holobiont success itself. These multilevel microbe fitnesses per holobiont are

$$\begin{aligned} w_{1,1} &\equiv k_1\, W_1 \\ w_{1,12} &\equiv n_1\, W_{12} \\ w_{2,12} &\equiv n_2\, W_{12} \\ w_{2,2} &\equiv k_2\, W_2 \end{aligned}$$

These new coefficients refer to the multilevel success of a specific microbe 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. The notation convention is that upper case W's refer to holobiont fitnesses and lower case w's refer to microbe fitnesses. With these definitions, define strain-1's marginal multilevel fitness per holobiont as

$$\overline{w_1}(t) \equiv p_1(t)\, w_{1,1} + p_2(t)\, 2\, w_{1,12}$$

The number of strain-1 microbes placed into the microbe source pool at time $t+1$ from both single and dual strain microbiomes then is

$$G_1(t+1) = \overline{w_1}(t)\, p_1(t)\, H(t)$$

Similarly, the number of strain-2 microbes in the microbe pool at time $t+1$ is

$$\begin{aligned} G_2(t+1) &= \widehat{n_2}\, H'''(t, \widehat{n_1}, \widehat{n_2}) + k_2\, H'''(t, 0, k_2) \\ &= n_2\, W_{12}\, 2\, p_1(t)\, p_2(t) H(t) + k_2\, W(0, k_2)\, p_2(t)^2 H(t) \\ &= \overline{w_2}(t)\, p_2(t)\, H(t) \end{aligned}$$

where strain-2's marginal multilevel fitness per holobiont is

$$\overline{w_2}(t) \equiv p_1(t)\, 2\, w_{2,12} + p_2(t)\, w_{2,2}$$

The total number of microbes in the microbe pool at time $t+1$ then is

$$\begin{aligned} G(t+1) &= \left(p_1(t)\, \overline{w_1}(t) + p_2(t)\, \overline{w_2}(t)\right)\, H(t) \\ &= \overline{\overline{w}}(t)\, H(t) \end{aligned}$$

where the microbiome multilevel mean fitness per holobiont is

$$\overline{\overline{w}}(t) \equiv p_1(t)\,\overline{w_1}(t) + p_2(t)\,\overline{w_2}(t)$$

Therefore the microbe frequencies in the microbial pool at time $t+1$ are

$$p_1(t+1) = \frac{G_1(t+1)}{G(t+1)} = \frac{\overline{w_1}(t)}{\overline{\overline{w}}(t)}\,p_1(t)$$

and

$$p_2(t+1) = \frac{G_2(t+1)}{G(t+1)} = \frac{\overline{w_2}(t)}{\overline{\overline{w}}(t)}\,p_2(t)$$

To summarize, the equations above for $H(t+1)$, $G(t+1)$, $p_1(t+1)$ and $p_2(t+1)$ together constitute a dynamical system for holobiont evolution with two microbe strains assuming (a) horizontal microbe transmission, (b) dilute microbe source pools and (c) macro time steps much longer than micro time steps. These equations correspond to those from classical population genetics for evolution at one locus with two alleles. Those equations predict the trajectory of alleles in the gene pool whereas these equations predict the trajectory of microbial strains in the hologene pool.

Appendix 2: Model for Two Horizontally Transmitted Haploid Microbial Strains with Two Vertically Transmitted Diploid Host Alleles

A macro time step begins with the basic state variables for the nine hologenotypes. The notation convention is that $H_{ij}(t,k,0)$ refers to the number of holobionts at time t with k strain-1 microbes and no strain-2 microbes in the microbiome together with alleles A_i and A_j in the host nucleus, whereas $H_{ij}(t,n_1,n_2)$ refers to the number of holobionts at time t with n_1 strain-1 and n_2 strain-2 microbes in the microbiome together with alleles A_i and A_j in the host nucleus. The total at time t of the nine hologenotype numbers is $H(t)$.

Let p_1 and p_2 denote the frequencies of strain-1 and strain-2 in the microbe source pool, and let q_1 and q_2 denote the frequencies of the A_1 and A_2 alleles in the host gamete pool. The nuclei of the empty hosts are formed by random union of gametes from the host gamete pool according to the Hardy-Weinberg ratios of $(q_1^2, 2\,q_1q_2, q_2^2)$. (Hardy-Weinberg ratios would also be produced through random mating by the parental hosts.) Then these empty hosts are colonized at random through binomial sampling of the dilute microbe pool according to the ratios of $(p_1^2, 2\,p_1p_2, p_2^2)$. Random union of gametes (or random mating) followed by random colonization of microbes

leads to the following nine initial hologenotype numbers:

$$
\begin{aligned}
H'_{11}(t,2,0) &= q_1(t)^2\ p_1(t)^2\ H(t) \\
H'_{11}(t,1,1) &= q_1(t)^2\ 2p_1(t)p_2(t)\ H(t) \\
H'_{11}(t,0,2) &= q_1(t)^2\ p_2(t)^2\ H(t) \\
H'_{12}(t,2,0) &= 2q_1(t)q_2(t)\ p_1(t)^2\ H(t) \\
H'_{12}(t,1,1) &= 2q_1(t)q_2(t)\ 2p_1(t)p_2(t)\ H(t) \\
H'_{12}(t,0,2) &= 2q_1(t)q_2(t)\ p_2(t)^2\ H(t) \\
H'_{22}(t,2,0) &= q_2(t)^2\ p_1(t)^2\ H(t) \\
H'_{22}(t,1,1) &= q_2(t)^2\ 2p_1(t)p_2(t)\ H(t) \\
H'_{22}(t,0,2) &= q_2(t)^2\ p_2(t)^2\ H(t)
\end{aligned}
$$

The notation reads as follows: $H'_{11}(t,2,0)$ is the number of holobionts after random union of gametes and binomial microbe colonization with solely strain-1 as its microbiome and A_1A_1 as its nuclear genome—these holobionts have 2 microbes in them of strain-1 and 0 microbes of strain-2, and so forth for the remaining hologenotype numbers.

After the juvenile hosts have been initially populated, the microbes proliferate within their hosts and come to an equilibrium microbiome community structure. The equilibrium microbe abundances in a dual-strain microbiome consisting of strain-1 and strain-2 in a host with an A_iA_j genome are $n_{1,ij}$ and $n_{2,ij}$. The equilibrium microbe abundances of pure strain microbiomes consisting of only strain-1 or only strain-2 in a host with an A_iA_j genome are $k_{1,ij}$ and $k_{2,ij}$ respectively. Therefore, after the microbiome proliferation phase, the hologenotype numbers are:

$$
\begin{aligned}
H''_{11}(t,k_{1,11},0) &= q_1(t)^2\ p_1(t)^2\ H(t) \\
H''_{11}(t,n_{1,11},n_{2,11}) &= q_1(t)^2\ 2p_1(t)p_2(t)\ H(t) \\
H''_{11}(t,0,k_{2,11}) &= q_1(t)^2\ p_2(t)^2\ H(t) \\
H''_{12}(t,k_{1,12},0) &= 2q_1(t)q_2(t)\ p_1(t)^2\ H(t) \\
H''_{12}(t,n_{1,12},n_{2,12}) &= 2q_1(t)q_2(t)\ 2p_1(t)p_2(t)\ H(t) \\
H''_{12}(t,0,k_{2,12}) &= 2q_1(t)q_2(t)\ p_2(t)^2\ H(t) \\
H''_{22}(t,k_{1,22},0) &= q_2(t)^2\ p_1(t)^2\ H(t) \\
H''_{22}(t,n_{1,22},n_{2,22}) &= q_2(t)^2\ 2p_1(t)p_2(t)\ H(t) \\
H''_{22}(t,0,k_{2,22}) &= q_2(t)^2\ p_2(t)^2\ H(t)
\end{aligned}
$$

Next, the holobiont fitnesses depend on the full hologenotype which includes both the microbiome and the host nuclear genome, eg., $W_{ij}(k_1)$ is the fitness of a single-strain holobiont with strain-1 in a host with A_iA_j alleles, and $W_{ij}(n_1,n_2)$ is the fitness for a dual-strain holobiont with

n_1 strain-1 microbes and n_2 strain-2 microbes with a host genome of A_iA_j. However, to simplify notation, put $W_{ij}(k_1)$ as $W_{1,ij}$ and $W_{ij}(n_1, n_2)$ as $W_{12,ij}$ where the subscript refers first to the microbiome composition followed by the nuclear genome. Accordingly, after holobiont selection, the hologenotype numbers are:

$$\begin{aligned}
H'''_{11}(t, k_{1,11}, 0) &= W_{1,11}\ q_1(t)^2\ p_1(t)^2\ H(t) \\
H'''_{11}(t, n_{1,11}, n_{2,11}) &= W_{12,11}\ q_1(t)^2\ 2p_1(t)p_2(t)\ H(t) \\
H'''_{11}(t, 0, k_{2,11}) &= W_{2,11}\ q_1(t)^2\ p_2(t)^2\ H(t) \\
H'''_{12}(t, k_{1,12}, 0) &= W_{1,12}\ 2q_1(t)q_2(t)\ p_1(t)^2\ H(t) \\
H'''_{12}(t, n_{1,12}, n_{2,12}) &= W_{12,12}\ 2q_1(t)q_2(t)\ 2p_1(t)p_2(t)\ H(t) \\
H'''_{12}(t, 0, k_{2,12}) &= W_{2,12}\ 2q_1(t)q_2(t)\ p_2(t)^2\ H(t) \\
H'''_{22}(t, k_{1,22}, 0) &= W_{1,22}\ q_2(t)^2\ p_1(t)^2\ H(t) \\
H'''_{22}(t, n_{1,22}, n_{2,22}) &= W_{12,22}\ q_2(t)^2\ 2p_1(t)p_2(t)\ H(t) \\
H'''_{22}(t, 0, k_{2,22}) &= W_{2,22}\ q_2(t)^2\ p_2(t)^2\ H(t)
\end{aligned}$$

So, to obtain the state variables at time $t+1$ given the state at time t, collect the previous terms. The total number of empty juvenile hosts at $t+1$ is

$$\begin{aligned}
H(t+1) &= H'''_{11}(t, k_{1,11}, 0) + H'''_{11}(t, n_{1,11}, n_{2,11}) + H'''_{11}(t, 0, k_{2,11}) \\
&+ H'''_{12}(t, k_{1,12}, 0) + H'''_{12}(t, n_{1,12}, n_{2,12}) + H'''_{12}(t, 0, k_{2,12}) \\
&+ H'''_{22}(t, k_{1,22}, 0) + H'''_{22}(t, n_{1,22}, n_{2,22}) + H'''_{22}(t, 0, k_{2,22}) \\
&= [q_1(t)^2\ \left(p_1(t)^2\ W_{1,11} + 2p_1(t)p_2(t)\ W_{12,11} + p_2(t)^2\ W_{2,11}\right) \\
&+ 2q_1(t)q_2(t)\ \left(p_1(t)^2\ W_{1,12} + 2p_1(t)p_2(t)\ W_{12,12} + p_2(t)^2\ W_{2,12}\right) \\
&+ q_2(t)^2\ \left(p_1(t)^2\ W_{1,22} + 2p_1(t)p_2(t)\ W_{12,22} + p_2(t)^2\ W_{2,22}\right)]\ H(t) \\
&= \left(q_1(t)^2\ \overline{W_{11}}(t) + 2q_1(t)q_2(t)\ \overline{W_{12}}(t) + q_2(t)^2\ \overline{W_{22}}(t)\right)\ H(t) \\
&= \overline{\overline{W}}(t)\, H(t)
\end{aligned}$$

where the fitness for holobionts with A_1A_1, A_1A_2, and A_2A_2 nuclear genotypes averaged over their microbiomes are:

$$\begin{aligned}
\overline{W_{11}}(t) &\equiv p_1(t)^2\ W_{1,11} + 2p_1(t)p_2(t)\ W_{12,11} + p_2(t)^2\ W_{2,11} \\
\overline{W_{12}}(t) &\equiv p_1(t)^2\ W_{1,12} + 2p_1(t)p_2(t)\ W_{12,12} + p_2(t)^2\ W_{2,12} \\
\overline{W_{22}}(t) &\equiv p_1(t)^2\ W_{1,22} + 2p_1(t)p_2(t)\ W_{12,22} + p_2(t)^2\ W_{2,22}
\end{aligned}$$

and the marginal fitnesses for each allele are

$$\begin{aligned}
\widetilde{\overline{W_1}}(t) &\equiv q_1(t)\,\overline{W_{11}}(t) + q_2(t)\overline{W_{12}}(t) \\
\widetilde{\overline{W_2}}(t) &\equiv q_1(t)\overline{W_{12}}(t) + q_2(t)\,\overline{W_{22}}(t)
\end{aligned}$$

and the overall mean fitness of the holobionts is

$$\overline{\overline{\widetilde{W}}}(t) \equiv q_1(t)\,\overline{\widetilde{W_1}}(t) + q_2(t)\,\overline{\widetilde{W_2}}(t)$$

The notation convention is that an average taken over microbe frequencies, ie, with respect to p_1 and p_2, is indicated with an overline. An average taken over allele frequencies, ie, with respect to q_1 and q_2, is indicated with a wide tilde. A wide tilde placed on top of an overline indicates an average first with respect to microbe frequencies and then with respect to allele frequencies. An overline placed over a wide tilde indicates an average first with respect to allele frequencies and then with respect to microbe frequencies. Also, to minimize the number of diacritical marks placed about any quantity, the lowest mark is understood to refer to an average over microbiome frequencies and genotype frequencies, ie, with respect to $p_1^2, 2\,p_1\,p_2, p_2^2$ and $q_1^2, 2\,q_1\,q_2, q_2^2$ rather than with respect to p_1, p_2, q_1, and q_2.

Based on these definitions, the frequency of the host nuclear alleles, A_1 and A_2, respectively, in the gamete pool at time $t+1$ as a function of both the gamete pool and microbial source pool at time t is

$$\begin{aligned} q_1(t+1) &= \frac{q_1(t)\overline{W_{11}}(t) + q_2(t)\overline{W_{12}}(t)}{\overline{\overline{\widetilde{W}}}(t)}\,q_1(t) \\ q_2(t+1) &= \frac{q_2(t)\overline{W_{22}}(t) + q_1(t)\overline{W_{12}}(t)}{\overline{\overline{\widetilde{W}}}(t)}\,q_2(t) \end{aligned}$$

Turning now to the microbiome, the number of strain-1 microbes in the microbial source pool at time $t+1$ is

$$\begin{aligned} G_1(t+1) &= p_1(t)^2\left(q_1(t)^2\,k_{1,11}\,W_{1,11} + 2q_1(t)q_2(t)\,k_{1,12}\,W_{1,12} + q_2(t)^2\,k_{1,22}\,W_{1,22}\right) \\ &+ 2p_1(t)p_2(t)\left(q_1(t)^2\,n_{1,11}\,W_{12,11} + 2q_1(t)q_2(t)\,n_{1,12}\,W_{12,12} + q_2(t)^2\,n_{1,22}\,W_{12,22}\right) \\ &= p_1(t)^2\,\widetilde{w_{1,1}}(t) + 2p_1(t)p_2(t)\,\widetilde{w_{1,12}}(t) \\ &= \overline{\widetilde{w_1}}(t)\,p_1(t)\,H(t) \end{aligned}$$

where the multilevel microbe fitnesses per holobiont are

$$
\begin{aligned}
w_{1,1,11} &= k_{1,11}\, W_{1,11} \\
w_{1,1,12} &= k_{1,12}\, W_{1,12} \\
w_{1,1,22} &= k_{1,22}\, W_{1,22} \\
w_{1,12,11} &= n_{1,11}\, W_{12,11} \\
w_{1,12,12} &= n_{1,12}\, W_{12,12} \\
w_{1,12,22} &= n_{1,22}\, W_{12,22} \\
w_{2,12,11} &= n_{2,11}\, W_{12,11} \\
w_{2,12,12} &= n_{2,12}\, W_{12,12} \\
w_{2,12,22} &= n_{2,22}\, W_{12,22} \\
w_{2,2,11} &= k_{2,11}\, W_{2,11} \\
w_{2,2,12} &= k_{2,12}\, W_{2,12} \\
w_{2,2,22} &= k_{2,22}\, W_{2,22}
\end{aligned}
$$

and where the multilevel fitnesses of a strain-1 microbe per holobiont from single and dual strain microbiomes averaged over the three nuclear genotypes are

$$
\begin{aligned}
\widetilde{w_{1,1}}(t) &\equiv q_1(t)^2\, w_{1,1,11} + 2q_1(t)q_2(t)\, w_{1,1,12} + q_2(t)^2\, w_{1,1,22} \\
\widetilde{w_{1,12}}(t) &\equiv q_1(t)^2\, w_{1,12,11} + 2q_1(t)q_2(t)\, w_{1,12,12} + q_2(t)^2\, w_{1,12,22}
\end{aligned}
$$

The notation is that $w_{1,1,11}$ indicates the multilevel fitness per holobiont of a strain-1 microbe in a single strain microbiome within an A_1A_1 host whereas $w_{1,12,12}$ indicates the multilevel fitness per holobiont of a strain-1 microbe in a dual-strain microbiome within an A_1A_2 host. Thus the subscript convention is strain identity, followed by microbiome composition, and ending with host nuclear genotype. Then strain-1's marginal multilevel fitness per holobiont averaged over the nuclear genotypes is

$$\overline{\widetilde{w_1}}(t) \equiv p_1(t)\, \widetilde{w_{1,1}}(t) + p_2(t)\, 2\, \widetilde{w_{1,12}}(t)$$

Similarly, the number of strain-2 microbes in the microbe pool at time $t+1$ is

$$
\begin{aligned}
G_2(t+1) &= 2p_1(t)p_2(t)\left(q_1(t)^2 n_{2,11}\, W_{12,11} + 2q_1(t)q_2(t)\, n_{2,12}\, W_{12,12} + q_2(t)^2 n_{2,22}\, W_{12,22}\right) \\
&+ p_2(t)^2\left(q_1(t)^2\, k_{2,11}\, W_{2,11} + 2q_1(t)q_2(t)\, k_{2,12}\, W_{2,12} + q_2(t)^2\, k_{2,22}\, W_{2,22}\right) \\
&= 2p_1(t)p_2(t)\, \widetilde{w_{2,12}}(t) + p_2(t)^2\, \widetilde{w_{2,2}}(t) \\
&= \overline{\widetilde{w_2}}(t)\, p_2(t)\, H(t)
\end{aligned}
$$

where the multilevel fitnesses of a strain-2 microbe per holobiont from single and dual strain microbiomes averaged over the three nuclear genotypes are

$$\begin{aligned}
\widetilde{w_{2,2}}(t) &\equiv q_1(t)^2\, w_{2,2,11} + 2q_1(t)q_2(t)\, w_{2,2,12} + q_2(t)^2\, w_{2,2,22} \\
\widetilde{w_{2,12}}(t) &\equiv q_1(t)^2\, w_{2,12,11} + 2q_1(t)q_2(t)\, w_{2,12,12} + q_2(t)^2\, w_{2,12,22}
\end{aligned}$$

Then strain-1's marginal multilevel fitness per holobiont averaged over the nuclear genotypes is

$$\overline{\widetilde{w_2}}(t) \equiv p_1(t)\, 2\, \widetilde{w_{2,12}}(t) + p_2(t)\, \widetilde{w_{2,2}}(t)$$

The total number of microbes in the microbe pool at time $t+1$ then is

$$\begin{aligned}
G(t+1) &= \left(\overline{\widetilde{w_1}}(t)\, p_1(t) + \overline{\widetilde{w_2}}(t)\, p_2(t)\right)\, H(t) \\
&= \overline{\overline{\widetilde{w}}}(t)\, H(t)
\end{aligned}$$

where the microbiome multilevel mean fitness per holobiont is

$$\overline{\overline{\widetilde{w}}}(t) \equiv p_1(t)\, \overline{\widetilde{w_1}}(t) + p_2(t)\, \overline{\widetilde{w_2}}(t)$$

Therefore the microbe frequencies in the microbial pool at time $t+1$ are

$$p_1(t+1) = \frac{G_1(t+1)}{G(t+1)} = \frac{\overline{\widetilde{w_1}}(t)}{\overline{\overline{\widetilde{w}}}(t)}\, p_1(t)$$

and

$$p_2(t+1) = \frac{G_2(t+1)}{G(t+1)} = \frac{\overline{\widetilde{w_2}}(t)}{\overline{\overline{\widetilde{w}}}(t)}\, p_2(t)$$

To summarize, the equations above for $H(t+1)$, $G(t+1)$, $p_1(t+1)$, $p_2(t+1)$, $q_1(t+1)$ and $q_2(t+1)$, together constitute a dynamical system for holobiont evolution with two microbe strains and two diploid host alleles assuming (a) horizontal microbe transmission, (b) dilute microbe source pools and (c) macro time steps much longer than micro time steps. These equations correspond to those from classical population genetics for evolution at two loci with two alleles per locus. Those equations predict the trajectory in a gene pool of a two-compartment genome, one compartment per locus, whereas these equations predict the trajectory in a hologene pool of a two compartment hologenome, one compartment for the microbial genes and another compartment for the nuclear genes.

Appendix 3: *Mathematica* Script for Numerical Example of HSS and OIS Coadaptation

```
In[1]:= ClearAll["Global`*"]

In[2]:= (* ------------------------------------------------------------------*)

In[3]:= Wo = 1; ko = 1000;
c1 = 1/1000; c2 = 3/4; c3 = 1/4;
c4 = 300; c5 = 1/4; c6 = 3/4;
a = 1/2;

In[7]:= (* --------------- W(x,y) and yhat(x) ------------------------------- *)

In[8]:= W = (1 - x y) Wo + c1 (x^c2  y^c3) ko;

In[9]:= yhatofx = y /. Solve[D[W, y] == 0, y][[1]][[1]]
Out[9]= 1/(4 2^(2/3) x^(1/3))

In[10]:= (* --------------- k(x,y) and xhat(y) ------------------------------- *)

In[11]:= k = (1 - x y) ko + c4 (x^c5  y^c6)  Wo;

In[12]:= xhatofy = x /. Solve[D[k, x] == 0, x][[1]][[1]]
Out[12]= (3 (3/5)^(1/3))/(80 y^(1/3))

In[13]:= (* ----------------- xhat and yhat ----------------------------------*)

In[14]:= xhat = N[x /. Solve[y == yhatofx && x == xhatofy, {x, y}][[1]][[1]]]
Out[14]= 0.0410792

In[15]:= yhat = N[y /. Solve[y == yhatofx && x == xhatofy, {x, y}][[1]][[2]]]
Out[15]= 0.456435

In[16]:= N[W /. {x -> xhat, y -> yhat}]
Out[16]= 1.05625
```

```
In[17]:= N[k /. {x -> xhat, y -> yhat}]
Out[17]= 1056.25

In[18]:= (* ------------- xmhat ------------------------------------------------ *)

In[19]:= Q = (k /. {x -> xm, y -> yhat}) (W /. {x -> xm, y -> yhat}) -
   a (k /. {x -> xhat, y -> yhat}) (W /. {x -> xm, y -> yhat});

In[20]:= xmhat =  xm /. Solve[D[Q, xm] == 0, xm][[1]][[1]]
Out[20]= 0.128788

In[21]:= LHS = (k /. {x -> xhat, y -> yhat}) (W /. {x -> xhat, y -> yhat});

In[22]:= RHS = (2/(1 - a^2)) ((k /. {x -> xmhat, y -> yhat}) -
      a (k /. {x -> xhat, y -> yhat})) (W /. {x -> xmhat, y -> yhat});

In[23]:= LHS < RHS
Out[23]= True

In[24]:= xmhat > xhat
Out[24]= True

In[25]:= (* ---------- Summary of Implications --------------------------------- *)

In[26]:= (* ------ HSS Spiteful -- x = 0, y = 0 ------------------------------- *)

In[27]:= xSpite = 0;

In[28]:= ySpite = 0;

In[29]:= WSpite = W /. { x -> xSpite, y -> ySpite}
Out[29]= 1

In[30]:= kSpite = k /. {x -> xSpite, y -> ySpite}
Out[30]= 1000
```

```
In[31]:= wSpite = (k /. {x -> xSpite, y -> ySpite}) (W /. {
     x -> xSpite, y -> ySpite})
Out[31]= 1000

In[32]:= (* ------- HSS Cooperative -- x = xhat, y=yhat ---------------------- *)

In[33]:= xhat
Out[33]= 0.0410792

In[34]:= yhat
Out[34]= 0.456435

In[35]:= WCooperator = W /. {x -> xhat, y -> yhat}
Out[35]= 1.05625

In[36]:= kCooperator = k /. {x -> xhat, y -> yhat}
Out[36]= 1056.25

In[37]:= wCooperator = (k /. {x -> xhat, y -> yhat}) (W /. {x -> xhat, y -> yhat})
Out[37]= 1115.66

In[38]:= (* ------ OIS Altruistic microbe , assuming a = 1/2 ---------------- *)

In[39]:= xmhat
Out[39]= 0.128788

In[40]:= yhat
Out[40]= 0.456435

In[41]:= WAltruist = W /. {x -> xmhat, y -> yhat}
Out[41]= 1.11792

In[42]:= kAltruist = k /. {x -> xmhat, y -> yhat}
Out[42]= 1041.02
```

```
In[43]:= wAltruist = (k /. {x -> xmhat, y -> yhat}) (W /. {x -> xmhat, y -> yhat})
Out[43]= 1163.77

In[44]:= nCooperator = (kCooperator - a kAltruist)/(1 - a^2)
Out[44]= 714.323

In[45]:= nAltruist = (kAltruist - a kCooperator)/(1 - a^2)
Out[45]= 683.853

In[46]:= (* Coexistence of HSS and OIS microbes after invasion of HSS by OIS - *)
         (* strain-1 is Cooperator strain and strain-2 is Altruistic strain -- *)

In[47]:= w11 = kCooperator WCooperator
Out[47]= 1115.66

In[48]:= w112 = nCooperator WAltruist
Out[48]= 798.558

In[49]:= w212 = nAltruist WAltruist
Out[49]= 764.495

In[50]:= w22 = kAltruist WAltruist
Out[50]= 1163.77

In[51]:= E1CooperatorIncreaseWhenRare = 2 w112 - w22
Out[51]= 433.342

In[52]:= E2AltruistIncreaseWhenRare = 2 w212 - w11
Out[52]= 413.327

In[53]:= pCooperator = E1CooperatorIncreaseWhenRare/
  (E1CooperatorIncreaseWhenRare + E2AltruistIncreaseWhenRare )
Out[53]= 0.51182

In[54]:= pAltruist =  E2AltruistIncreaseWhenRare /
  (E1CooperatorIncreaseWhenRare + E2AltruistIncreaseWhenRare )
```

```
Out[54]= 0.48818

In[55]:= pCooperator^2
Out[55]= 0.26196

In[56]:= 2 pCooperator pAltruist
Out[56]= 0.499721

In[57]:= pAltruist^2
Out[57]= 0.23832

In[58]:= 2 pCooperator pAltruist + pAltruist^2
Out[58]= 0.73804
```