Holobiont Evolution:

Model with Vertical *vs.* Horizontal Microbiome Transmission with Remarks on Lineal *vs.* Collective Inheritance

Joan Roughgarden[1,2,*]

Draft, July 5, 2019

1. Department of Biology, Stanford University, Stanford CA 94305, USA;

2. Hawaii Institute of Marine Biology, University of Hawaii, Kaneohe HI 96744, USA.

∗ Corresponding author; e-mail: joan.roughgarden@stanford.edu.

Keywords: holobiont, hologenome, hologenotype, holobiont selection, microbiome, symbiosis, evolution, model, coral

Abstract

A holobiont is a composite organism consisting of a host together with its microbiome, such as a coral with its zooxanthellae. Some investigators contend that selection operates on entire holobionts and view the microbiome's genes as extending the host's nuclear genome to jointly comprise a hologenome. Holobiont selection then operates on entire hologenomes by analogy to how ordinary natural selection operates on genes in a Mendelian population. Other investigators argue that vertical transmission of microbiomes is uncommon. Therefore, holobiont selection cannot be evolutionarily important because the microbiome is an acquired condition rather than an inherited trait. The conceptual disagreement between these positions invites a simple mathematical model to see how holobiont selection might operate and to assess the plausibility of holobiont selection as an evolutionary force.

Here I present two variants of such a model. In one variant, juvenile hosts obtain microbiomes from their parents (vertical transmission). In the other variant, microbiomes of juvenile hosts are assembled from source pools containing the combined microbiomes of all parents (horizontal transmission). According to both variants, holobiont selection indeed causes evolutionary change in holobiont traits. Therefore, holobiont selection is plausibly an important evolutionary force with either mode of microbiome transmission.

Furthermore, a new concept of inheritance emerges from the modeling: collective inheritance whereby juveniles inherit a sample of the collected genomes from all parents as contrasted with traditional lineal inheritance whereby juveniles inherit the genomes only from their own parents. This new concept of collective inheritance may apply to the evolution of soil and other microbes that feature large amounts of horizontal gene transfer and may underlie cultural evolution wherein each generation inherits a sample of knowledge from the collected knowledge of the preceding generation.

Introduction

This paper develops a simple conceptual mathematical model for the evolution of holobionts. Its purpose is to help clarify how holobiont selection may cause evolutionary change in the traits of holobionts.

A *holobiont* is a composite organism consisting of a host together with its microbiome (Margulis 1991). A *microbiome* is an "ecological community of commensal, symbiotic, and pathogenic microorganisms" that shares the "body space" of a host (Lederberg & McCray 2001). The *hologenome* is the union of all the host genes with all the genes in its microbiome (Zilber-Rosenberg and Rosenberg 2008). To these definitions I add the following: a *hologenotype* is the configuration of the hologenome in an individual holobiont, and *holobiont selection* is the differential reproduction and survival of holobionts depending on their hologenotypes.

Many researchers have suggested that a holobiont is a unit of selection in the sense that selection operates on the combination of the host together with its microbiome as a unit (Woese 2004, Rosenberg, Koren, Reshef, Efrony, & Zilber-Rosenberg 2007, Fraune & Bosch 2010, Guerrero, Margulis & Berlanga 2013, Gilbert, Sapp & Tauber 2012, McFall-Ngai, Hadfield, Bosch, Carey, Domazet-Loo, Douglas, Dubilier et a. 2013, Rosenberg & Zilber-Rosenberg 2014. Bordenstein & Theis 2015, Lloyd 2017, Rosenberg & Zilber-Rosenberg 2018). According to this perspective, the union of a host's genes together with the genes from all the microbes living in it comprises an extended genotype, called here the hologenotype. Selection on holobionts then changes hologenotype frequencies in a population of holobionts with a process envisioned as similar to how classic natural selection on individuals changes genotype frequencies in a population of individuals. Proponents of holobiont selection have documented astonishingly intricate and intimate integration between hosts and their microbiomes in physiology, anatomy, and development and argue that this integration itself is evidence of the power of holobiont selection to produce host/microbiome coadaptation.

Other researchers are unconvinced that holobiont selection actually accomplishes the evolu-

tionary results claimed for it (Booth 2014, Moran & Sloan 2015, Douglas & Werren 2016, Hester, Barott, Nulton, Vermeij, & Rohwer 2015, Skillings 2016, Chiu & Eberl 2016, Queller & Strassmann 2016, Doolittle & 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, the microbiome in a host is an acquired trait. Because the microbiome is not inherited, holobionts presumably cannot evolve through holobiont selection. The skeptics acknowledge the existence of host/microbiome integration, but deny that holobiont selection is necessary to account for the evolution of this integration. They note further that microbiomes are not necessarily helpful to the host, but can contain pathogens that hurt the host. They argue that a balanced view of the host/microbiome relation would include more mention of deleterious microbes to supplement the mutualistic microbes emphasized by the holobiont selection proponents.

The response of holobiont selection proponents to the objections raised by the skeptics is to demonstrate that vertical transmission is actually more common that the skeptics claim. The microbiome does not need to be contained in the gametes of the parental host to be considered as being transmitted vertically. The parental host's microbiome may colonize (or infect) the juveniles during birth, for example during passage through the birth canal in mammals, or from contact with nest material in birds, and so forth. Although the skeptics may underestimate the amount of vertical transmission of microbiomes, this response by the proponents still remains unsatisfying. For example, in a few species of corals the zooxanthellae are transmitted vertically in the coral's gametes (Hirose & Hidaka 2006). But the vast majority of zooxanthellae are acquired from the open sea water surrounding the coral (Babcock, Bull, Harrison et al. 1986, Trench 1993). So, the matter of limited vertical transmission remains a difficult point of holobiont selection proponents.

On the other hand, the holobiont selection skeptics face the reality that extensive and intricate host/microbiome integration has indeed evolved somehow. The skeptics have to argue that classic coevolutionary selection operating on separate interacting host and microbiome genotypes can produce the integration. Although coevolutionary selection does indeed lead to co-

adaptations between macro-organisms as in the plant-pollinator and damselfish-sea anemone associations and has also led to the evolution of reduced virulence by pathogens to their hosts, the degree of integration observed between host and microbiome is claimed by holobiont proponents to be of an entirely different scale than that seen in familiar examples of coevolutionary coadaptation. So, the skeptics have to argue that given enough time, classic coevolutionary selection will still eventually yield the degree of host/microbiome integration highlighted by the proponents. If so, the concepts of a holobiont, hologenome, hologenotype and holobiont selection have no empirical reference and can be seen as superfluous terms.

So, at this time, two quite reasonable alternative positions occur with respect to holobiont selection. The proponents face the difficulty of dealing with the largely horizontal mode of microbiome transmission. The skeptics face the difficulty that classic coevolutionary selection is not known to produce the deeply integrated coadaptation that occurs in the host/microbiome relation.

In this context, a simple conceptual model might be helpful in clarifying how holobiont evolution can be attained with holobiont selection. This article specifically addresses the question of microbiome transmission. It analyzes whether holobiont evolution *via* holobiont selection is plausible depending on the mode of microbiome transmission. This study responds to the demand from medical and conservation constituencies for understanding the microbiome/host relationship (Turnbaugh, Ley, Hamady, Fraser-Liggett, Knight & Gordon 2007, Dubilier, McFall-Ngai & Zhao 2015, OSTP 2016).

The models introduced here are intentionally simplistic. Many review articles document an extraordinary complexity of microbiomes across diverse systems in mammals, soils, and oceans of concern to medicine, agriculture and conservation. The modeling task is to distill this detail into a minimum set of assumptions relevant to conceptual clarity, not to empirical accuracy. For example, two versions of model are introduced. In one, almost all of the microbiome is transmitted vertically with but a trickle of horizontal mixing; in the other all the microbiome is transmitted horizontally without any vertical transmission. In reality of course, both modes

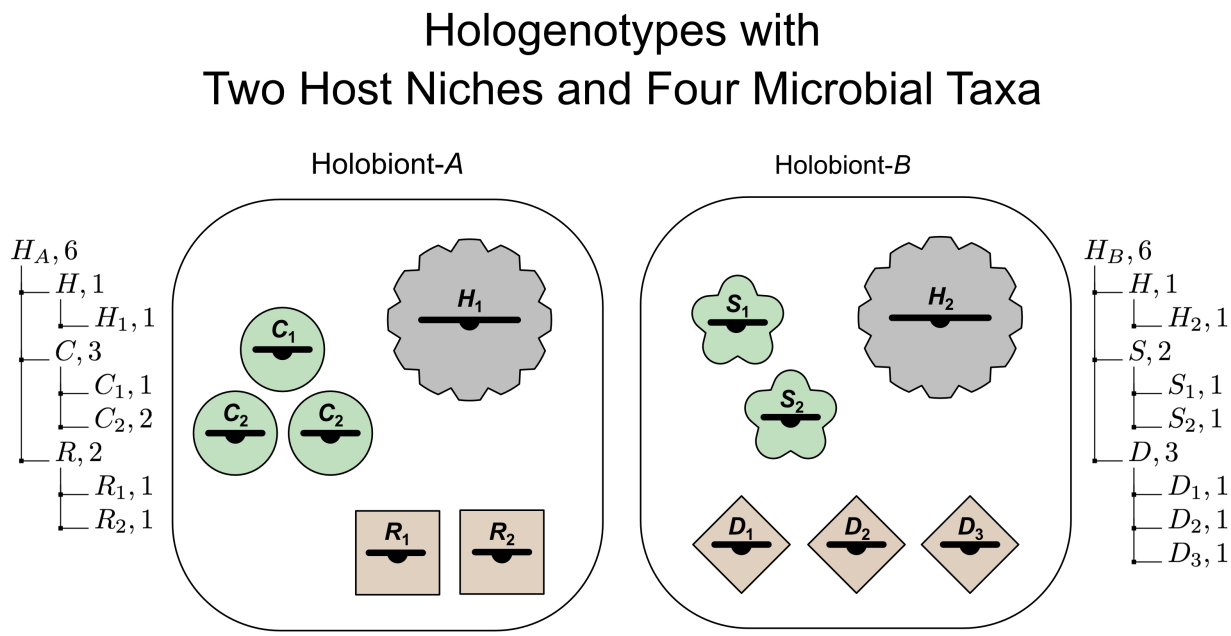
occur simultaneously. That empirical truth is not relevant here, however. If holobiont selection is effective with near total vertical transmission and also with total horizontal transmission, then it will also be effective if both modes of transmission occur simultaneously. It is sufficient for conceptual clarity to show that holobiont selection works in the two extreme cases, and to leave formulation of a model where some fraction of the transmission is vertical and the remaining fraction horizontal to the future should the need for a more elaborate model arise. I hope the reader will appreciate that simple models such as these are always vulnerable to attack for leaving out some cherished detail, and will grant me license to concentrate on assumptions that seem most relevant to bringing conceptual clarity to the holobiont/hologenome dispute. The models in this article are minimum models for concepts, not minimum models for empirical systems.

The Hologenome

Because the hologenome consists of the genes from all the microbes in the microbiome plus the host's nuclear genes, the mere description of the hologenome might seem daunting. The approach introduced here is to represent the hologenome as a hierarchy.

Figure 1 diagrams two instances of a conceptual holobiont, *A* and *B*. A representative host is depicted as a rectangle with rounded corners. A host's genome contains nuclear genes depicted in the gray gear box. Holobiont-*A* has allele H_1 and Holobiont-*B* has H_2 at a locus of interest in its nucleus. Each host has two niches for microbes depicted as green or brown that can represent physical locations within the host or functional roles (Burke, Steinberg, Rusch, Kjellberg & Thomas 2011). Two taxa of greens, circle and star, and two taxa of browns, rectangle and diamond, are available for each niche. The abundance of these taxa in each niche can vary among holobionts—Holobiont-*A* has three circles and Holobiont-*B* two stars. The allelic composition can vary among microbes—the circles have one C_1 allele and two C_2 alleles in Holobiont-*A*.

The hologenotype of an individual holobiont may be represented as a hierarchical data structure (list of lists) as depicted in Figure 1. The outer list indicates the number of genomes in the



$$\mathbf{H}_A = \{H_A, 6, \{\{H, 1, \{\{H_1, 1\}\}\}, \{C, 3, \{\{C_1, 1\}, \{C_2, 2\}\}\}, \{R, 2, \{\{R_1, 1\}, \{R_2, 1\}\}\}\}\}$$

$$\mathbf{H}_B = \{H_B, 6, \{\{H, 1, \{\{H_2, 1\}\}\}, \{S, 2, \{\{S_1, 1\}, \{S_2, 1\}\}\}, \{D, 3, \{\{D_1, 1\}, \{D_2, 1\}, \{D_3, 1\}\}\}\}\}$$

Figure 1: *Examples of hologenotypes from two hosts assuming two microbe niches (green and brown) and two possible microbe taxa per niche (circles and stars, rectangles and diamonds) together with allelic genetic variation within the microbe taxa. The hologenotype may be represented as a list of lists, as illustrated at bottom of figure. The right and left sides of the figure illustrate the genetic hierarchy for the hologenotypes.* $\boldsymbol{H}_{\mathrm{A}}$*, for example, reads as consisting of six individual genomes, one genome being the host nucleus containing one* H_1 *allele, three genomes from circle microbes containing among them one* C_1 *allele and two* C_2 *alleles, and two genomes from rectangle microbes containing among them one* R_1 *allele and one* R_2 *allele.*

holobiont. (The host nucleus is counted as a taxon containing one genome.) Nested within this are lists for the abundance and identity of each genome. And nested within this are lists for the number and identity of each allele.

In the hologenotype description the number of microbes is normalized to a standard host

cell. For example, if Holobiont-A as a whole is comprised of 1000 host cells, 3000 green circle microbes and 2000 brown rectangle microbes, then its hologenotype description, $\mathbf{H}_A$, records 1 host nucleus, 3 green circles, and 2 brown rectangles as shown in Figure 1.

A complete model of holobiont evolution predicts the trajectory through time of the hologenotype numbers for a holobiont population.

To compare the evolutionary outcomes of vertical and horizontal transmission, the special case of hologenotypic variation in microbe number, assuming one host niche, one microbial taxon, and no allelic variation in host or microbe genomes is sufficient. The microbe may be either a pathogen or a mutualist.

Vertical transmission

Three processes occur as sequential stages within each host generation, as shown in Figures 2 and 3 (Roughgarden 2017, Roughgarden, Gilbert, Rosenberg, Zilber-Rosenberg and Lloyd 2018). The host's generation time is the macro time step. Within each macro time step, micro time steps reflect the kinetics of microbe exchange and microbe generation time. In detail:

Model

Microbial Mixing

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. Hence, holobionts that had few microbes to begin with receive a net increase in microbe number and holobionts with many microbes to begin with incur a net decrease.

A small m is consistent with limited microbe interchange across hosts. The holobiont population needs limited horizontal mixing even though parents primarily transmit microbes vertically to their offspring. Otherwise, if m is 0, then holobiont selection reduces to clone selection on the

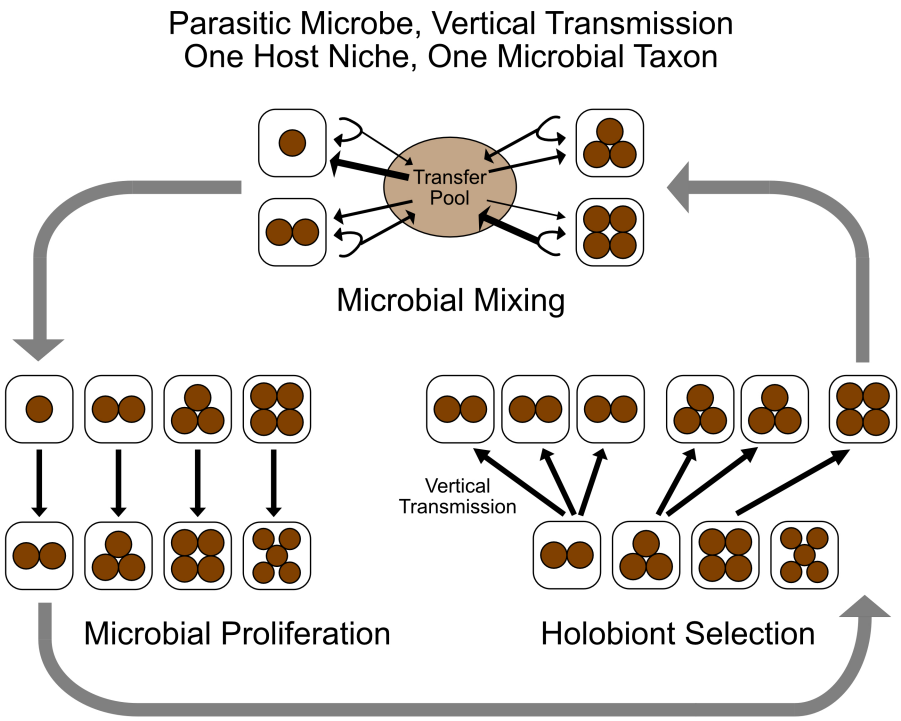


Figure 2: *Sequence of processes with vertical transmission of parasitic microbiome.*

hosts whereby the host clone with lowest number of parasites or the largest number of mutualists becomes fixed. Furthermore, if m is 0, then empty hosts cannot be colonized.

Microbial Proliferation

The microbes within each holobiont increase according to a density-dependent ecological population model. A single microbe strain obeys the logistic equation and multiple competing strains follow the Lotka-Volterra competition equations.

Holobiont Selection

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 present in its

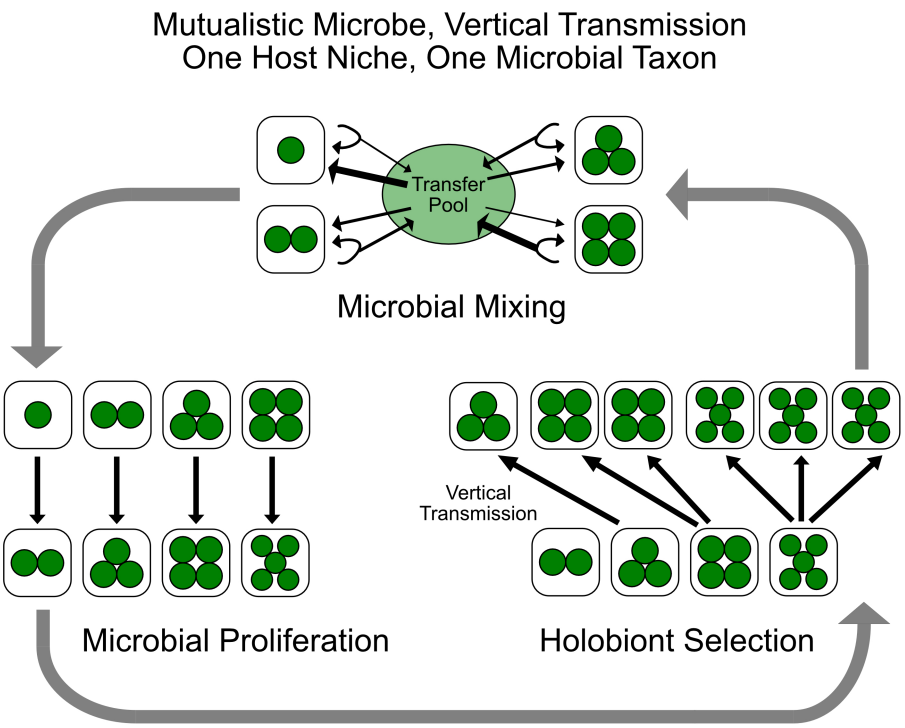


Figure 3: *Sequence of processes with vertical transmission of mutualistic microbiome.*

parent. In the case of microbial parasites in Figure 2, the holobionts with the lowest number of microbes leave the largest number of progeny and conversely for microbial mutualists in Figure 3. Holobiont reproduction and survival is independent of the density of holobionts.

Iteration of the macro time step with these three stages generates a trajectory of holobiont abundance and the hologenotype frequencies through time.

The equations for the model and instructions for how to download the model from the web appear in the mathematical appendix.

Results

Figures 4 and 5 show screen shots of the histogram through time of the hologenotype frequencies for host interactions with parasitic and mutualistic microbes. In both figures iteration of macro time steps leads from an initial uniform distribution to a stationary distribution of hologenotype

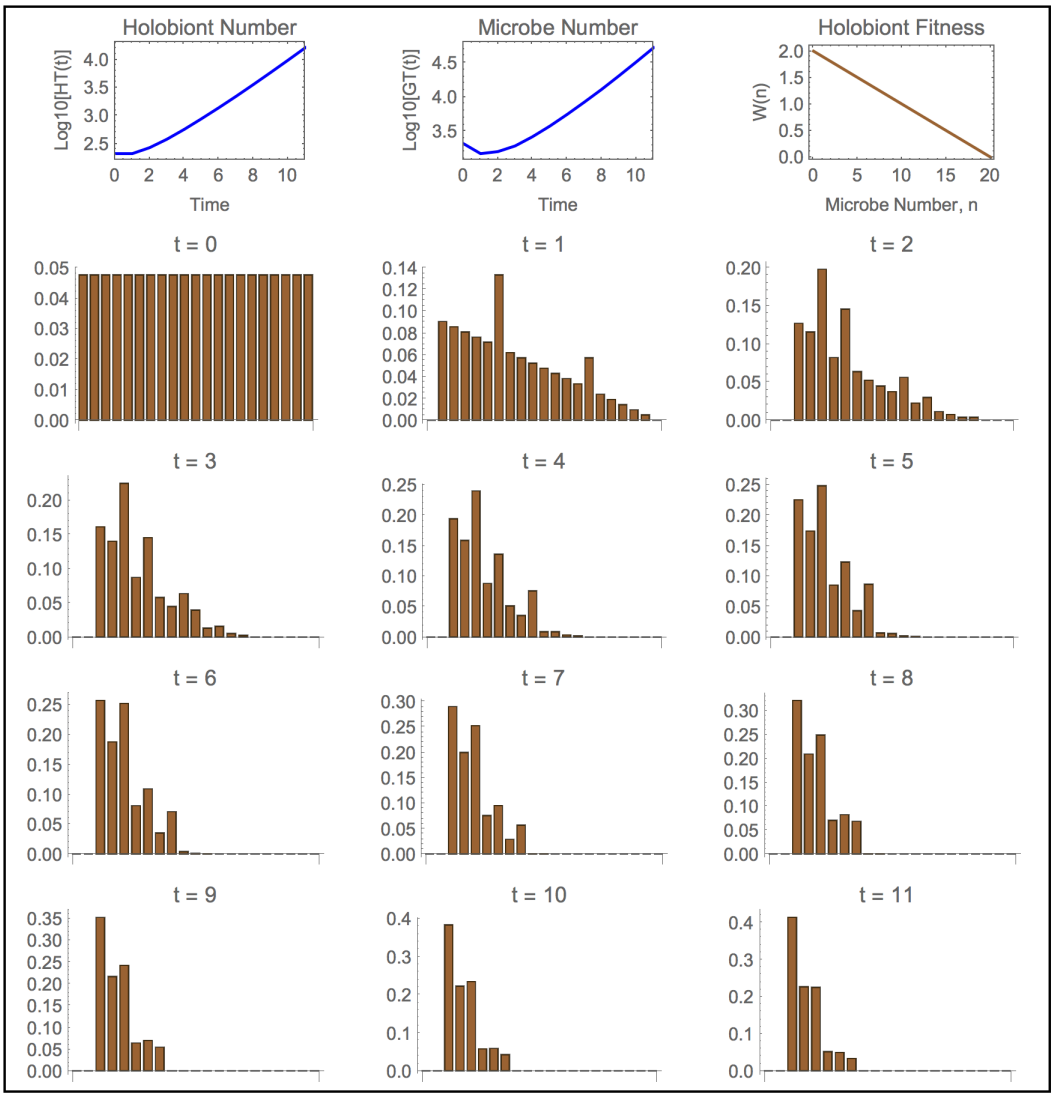


Figure 4: *Results with vertical transmission of parasitic microbiome. Top left and middle graphs show log of total holobiont and microbe numbers through time and top right graph shows holobiont fitness as function of number of microbes in host. Histograms show distribution of hologenotype frequencies through time. The horizontal axis in the histograms is number of microbes per host and vertical axis is fraction of holobiont population.*

frequencies at which holobiont selection balances microbial mixing.

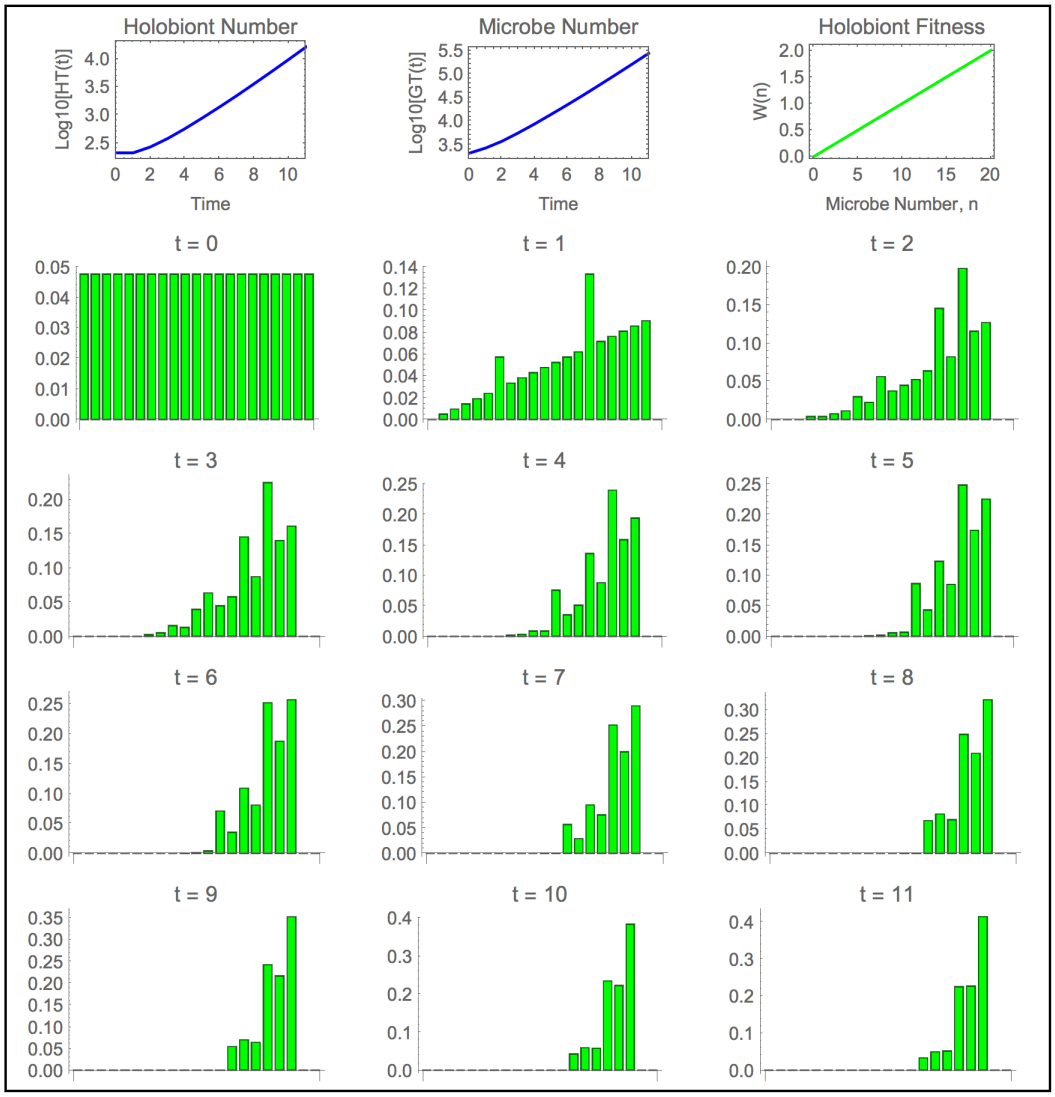


Figure 5: *Results with vertical transmission of mutualistic microbiome.*

In Figure 4 holobiont selection leads to a preponderance of holobionts with fewer parasites than initially. In Figure 5 holobiont selection leads to a preponderance of holobionts with more mutualists than initially. In the model's picture display (not shown), the holobionts with a parasitic microbiome change through time from 50% brown to almost no color and holobionts with a mutualistic microbiome change through time from 50% green to nearly solid green.

The stationary distribution of hologenotype frequencies partly depends on the initial condition. An historical accident affecting the initial condition may influence the outcome of holobiont selection in this scheme of microbial mixing.

Microbial mixing homogenizes the hologenome and retards the progress of holobiont selection, reinforcing an intuition that effective holobiont selection requires faithful vertical transmission of microbes with limited horizontal mixing.

For parasites, microbial proliferation acts in opposition to holobiont selection. By increasing the microbe's intrinsic rate of increase and/or the number of micro time steps the virulence of the parasite is increased. This increased virulence can overtake holobiont selection, leading to holobiont extinction.

These findings support comparable conclusions about the evolution of gut microbiota via host epithelial selection (Schluter & Foster 2012).

In reality, much of the microbiome does not colonize vertically. Thus, another variant of the model is needed.

One possible modification to the model is to allow the mixing probability, m, to equal 1, implying that every microbe leaves its host to enter the 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, sampling during microbe transmission can be introduced to the model. This sampling produces hologenotypic variation across holobionts and leads to the following formulation.

Horizontal transmission

Model

Three processes occur as sequential stages within each host generation, as shown in the top panels of Figures 6 and 7. In detail:

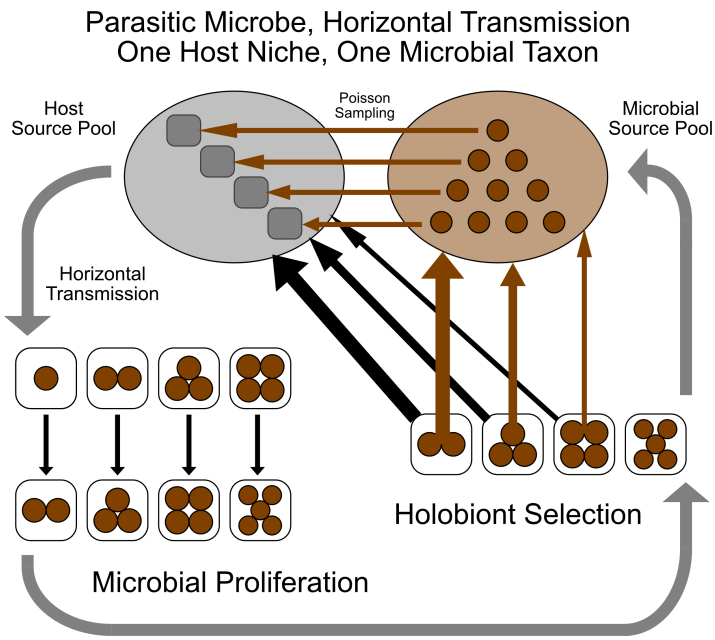


Figure 6: *Sequence of processes with horizontal transmission of parasitic microbiome.*

Microbial transmission

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.

Microbial Proliferation

The microbes within each holobiont increase according to density-dependent ecological population models, as before.

Holobiont Selection

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.

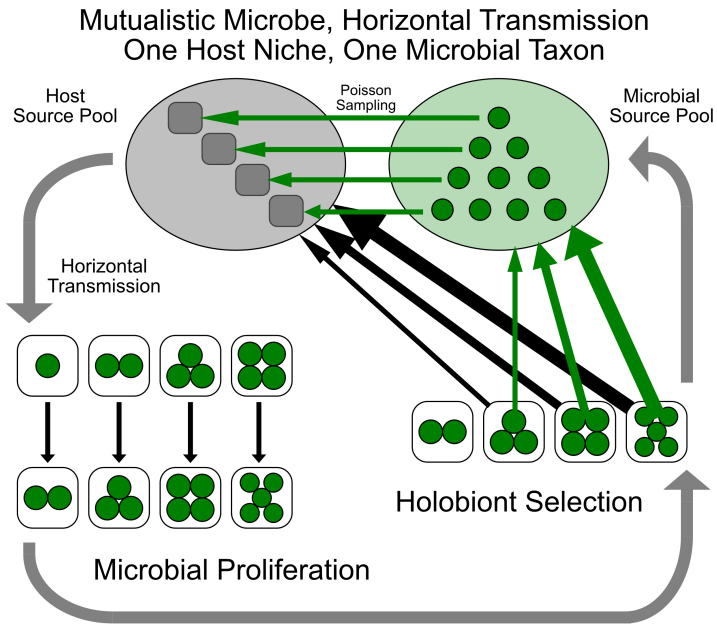


Figure 7: *Sequence of processes with horizontal transmission of mutualistic microbiome.*

For microbial parasites holobionts with the lowest number of microbes leave the largest number of progeny and conversely for the microbial mutualists. Holobiont reproduction and survival is independent of the density of holobionts.

Results

Figures 8 and 9 show screen shots of the histogram through time of the hologenotype frequencies where the microbes are parasitic or mutualistic. Overall, the results are qualitatively similar in this model variant to those in the vertical transmission variant. Holobiont selection against parasites lowers average parasite microbe number in holobionts over generations relative to the initial condition and holobiont selection for mutualists increases average microbe number in holobionts over generations relative to the initial condition.

With horizontal transmission, a stationary distribution is attained when the holobiont selection balances the variability caused by the Poisson sampling of the source pools during transmis-

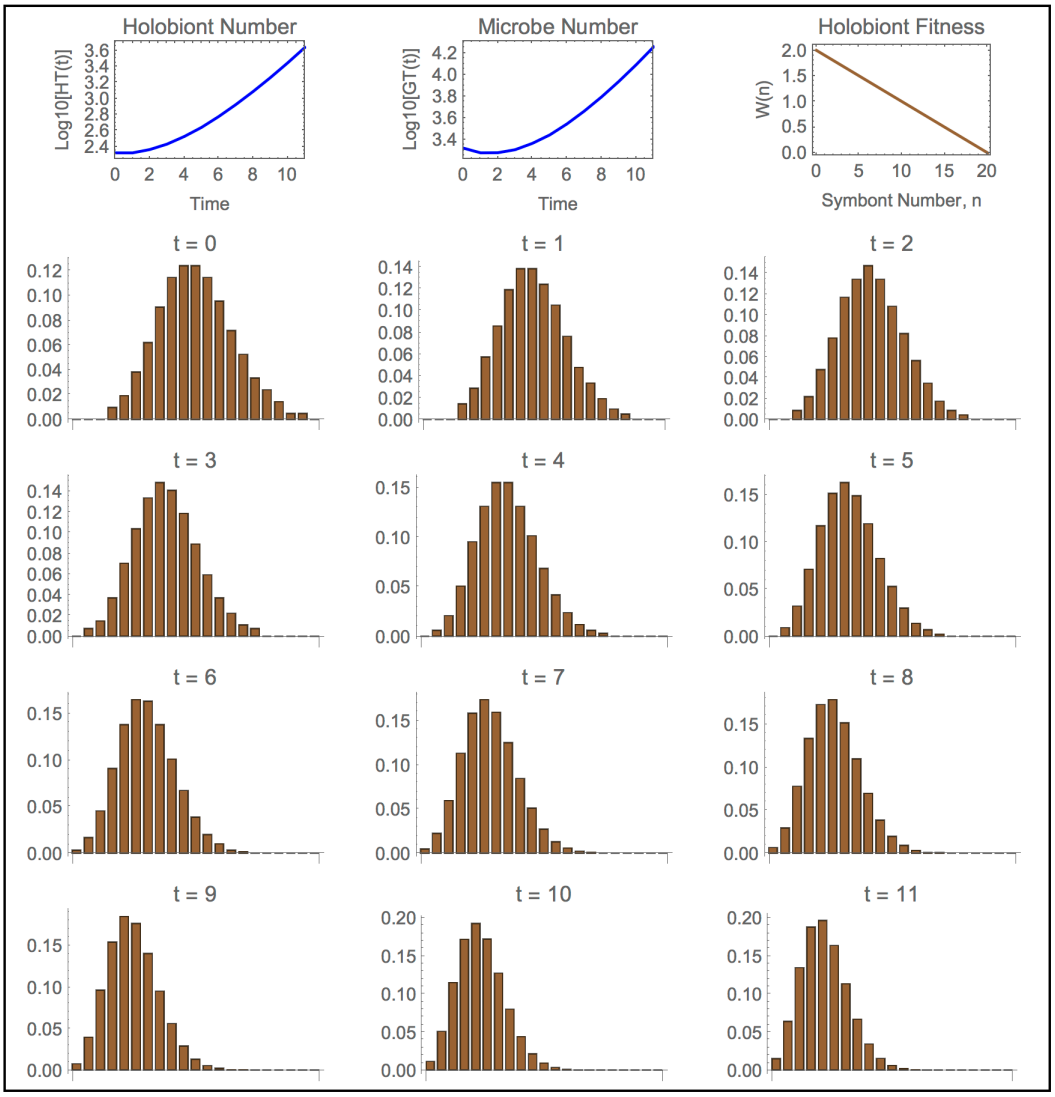


Figure 8: *Results with horizontal transmission of parasitic microbiome.*

sion. (Whether this stationary distribution depends on initial conditions has not been analyzed.) Here too the virulence of the parasites can overcome the holobiont selection if the parasite intrinsic rate of increase is high enough and/or the number of micro time steps large enough.

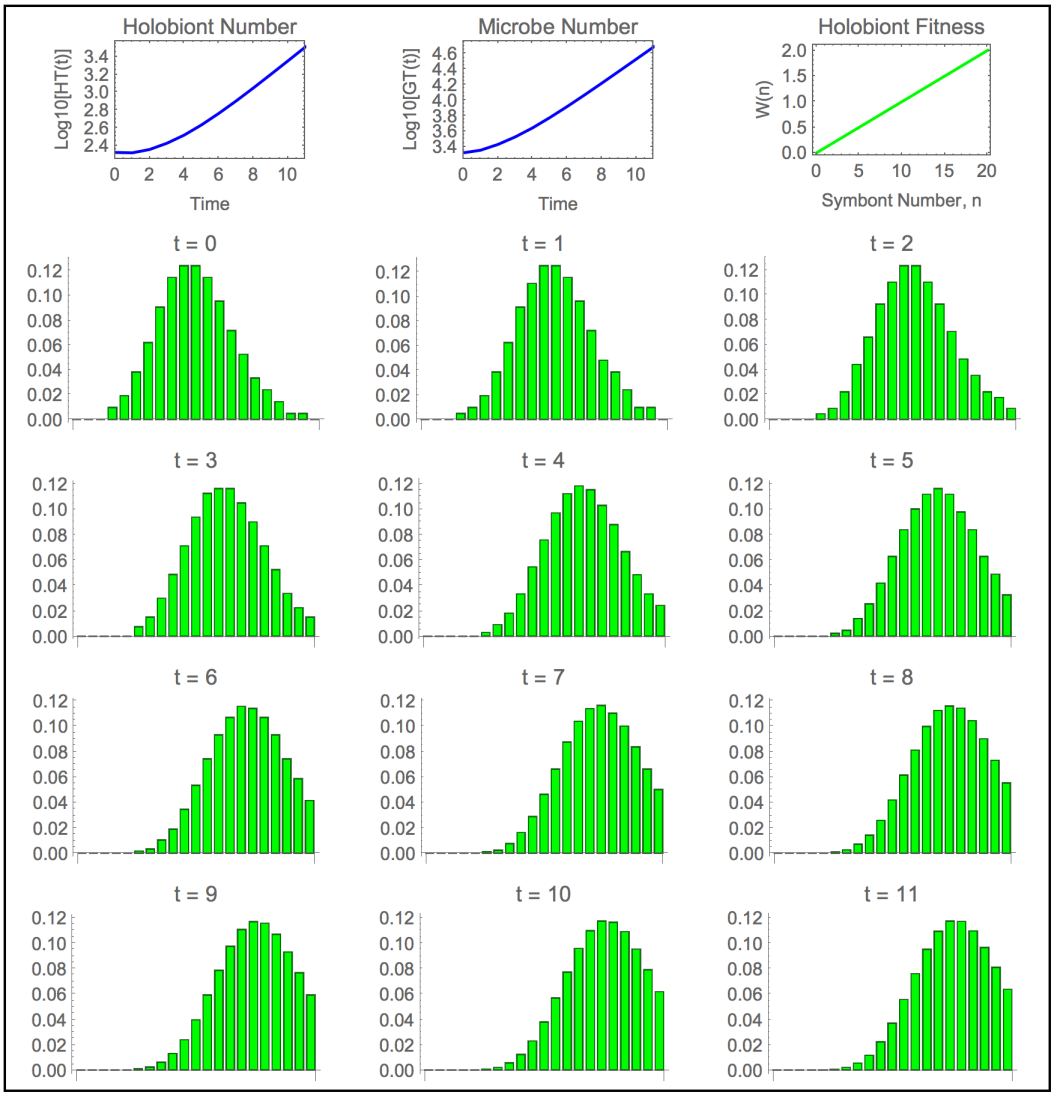


Figure 9: *Results with horizontal transmission of mutualistic microbiome.*

Discussion

The model here shows that holobiont selection causes the evolution of hosts with a reduced number of parasites and an increased number of mutualists, assuming either vertical or horizontal microbiome transmission. This outcome is to be expected given that reducing parasite load and

increasing mutualists are adaptive. But *how* this expected outcome is attained is what makes holobiont selection distinctive.

A classical route to lowered parasites and increased mutualists is *via* host control. For example, if the host has a locus in its nuclear genome with two alleles, say P_1 and P_2, such that P_1 produces an antibiotic, then ordinary natural selection will fix the P_1 allele in the host's nuclear gene pool, leading to a reduction in the parasite load. Similarly, if the host has a locus with two alleles, M_1 and M_2, such that M_1 produces a chemical that facilitates the mutualistic microbes, then ordinary natural selection will fix the M_1 allele leading to an increase of mutualists.

Contrast with holobiont selection. The genetic elements of the hologenome include not only the host's nuclear genome but also the microbes themselves. The hologenome is a dynamic genome, some of whose elements have their own ecology. Holobiont selection operates on the host plus its community of microbes which feeds back to determine the community structure of the microbiome itself. The simplest ecological community is merely a single species, as illustrated in the model presented here. Selection on the holobiont feeds back to the microbiome to influence the abundance realized by its microbe populations.

The examples illustrates cases with a single microbe population, either a parasite or mutualist. If the microbiome includes two species, one being a parasite and the other a mutualist, then the outcome is simply the combination of the outcomes from single species. Compare, for example, a nuclear genome with two unlinked non-epistatic loci, P and M, as above. Selection fixes P_1 and M_1 simultaneously. Similarly, if the parasitic and mutualistic microbes do not interact, then holobiont selection produces the same outcome for both that was illustrated for each singly, namely, a simultaneous reduction in parasites and increase in mutualists. However, if the microbe species do interact with each other, then the Proliferation Stage in the models would need to incorporate a Lotka-Volterra or other population-dynamic model for the interaction instead of the logistic equations presently being employed.

Although holobiont selection leads to a reduction of parasites and an increase of mutualists with both vertical and horizontal transmission, the underlying evolutionary processes are

different between these two modes of transmission.

In vertical transmission, microbes incorporate into a host lineage along with the host's nuclear genome. Microbial mixing loosens the association between the microbes and success of their host lineage, reducing the impact of the holobiont selection.

In horizontal transmission, beneficial microbes from hosts that are successful because of having many mutualists flood the microbial source pool. Hence, the next generation's holobionts are assembled with an increase in microbe number because the ratio of microbes to hosts has increased. Conversely, the limited production of parasitic microbes from hosts that are successful because of having few parasites dilutes the microbial source pool. Hence, the next generation's holobionts are assembled with a decrease in number of microbes because the ratio of microbes to hosts has decreased. The Poisson sampling of the microbes colonizing hosts guarantees hologenotypic variation for the holobiont selection to operate on.

A difference between model variants is that horizontal mixing with vertical transmission destroys hologenotypic variation whereas the sampling process in horizontal transmission produces hologenotypic variation.

With vertical transmission, a holobiont's microbiome is inherited by lineal descent, albeit not as a Mendelian trait. In contrast, the process of microbiome assembly from the combined parental microbe production is dubbed here as "collective inheritance".

Collective inheritance might prove valuable to understanding the evolution of bacteria and other microbes that feature large amounts of horizontal gene transfer (Mao & Lu 2016). It might also aid understanding cultural inheritance wherein offspring inherit a sample of the pooled knowledge of the parental generation.

Both lineal and collective modes of inheritance, although not named as such, are included in a recent model of microbiome evolution featuring both host and microbial selection (Zeng, Wu, Sukumaran & Rodrigo 2017).

Evolution by holobiont selection with two modes of transmission is reminiscent of evolution by two modes of group selection, one based on limited migration among demes in a metapop-

ulation (Eshel 1972) and the other based on trait groups in a population with a dispersal phase (Wilson 1975).

Previous investigators have noted that holobiont evolution is, in a sense, Lamarckian because holobiont selection affects acquired rather than inherited traits (Rosenberg, Sharon & Zilber-Rosenberg 2009, Osmanovic, Kessler, Rabin & Soen 2018). With horizontal transmission especially, a holobiont's microbial abundance is acquired from environmental source pools. Still, evolution *via* holobiont selection seems best viewed as Darwinian rather than Lamarckian because holobiont selection is a selection process acting on holobionts as individuals. However, evolution *via* holobiont selection is not neoDarwinian because neoDarwinism is founded on Mendelian inheritance (Wright 1931).

The model here shares some similarity with literature on the evolution of organelles (Birky, Maruyama, & Fuerst 1983; Rand, Hane & Fry 2004; Smith 2007) and on the evolution of reduced parasite virulence (Yamamura 1993, Lipsitch, Siller, & M. Nowak 1996). However, the model here does not adopt the oppositional framework found in traditional coevolutionary theory for interacting species, nor does it require the competitive game-theoretic concept of a Nash equilibrium or evolutionarily stable strategy (ESS). The model here does not assume host and microbiome are governed by coupled differential equations defined on the same time scale. Holobiont theory is not a straight forward application of well-known tools such as multilevel/kin selection, coevolutionary theory, and virulence evolution. Holobiont theory with its central concepts of a hologenome, hologenotypes, holobiont selection and collective inheritance is a new theoretical endeavor.

The model here and its framework are extensible and scalable. Future theoretical research might investigate the comparative effectiveness of holobiont and traditional coevolutionary selection in producing host/microbiome integration, how holobiont selection modulates microbiome community structure and how species diversity is maintained in the microbiome.

Conclusion

The model here with its two variants demonstrates theoretically that holobiont selection is plausibly an important force in evolution because holobiont selection produces evolutionary change in holobionts with both vertical and horizontal microbe host transmission. Holobiont evolution can rely on collective inheritance as well as lineal inheritance.

Acknowledgments

I am deeply grateful to Erol Akçay, Scott Gilbert, Priya Iyer, Ehud Lamm, Eugene Rosenberg, Jeremy Van Cleve, Vignesh Venkateswaran, and Ilana Zilber-Rosenberg for valuable comments on the manuscript.

Appendix A: Mathematical Details

State Variable Defined

The hologenotype numbers for a holobiont population through time are recorded in the matrix, H, where $H(t, n)$ is the number of holobionts at time t containing n microbes. The top row of H records the initial condition of the holobiont population. The model computes the row at $t + 1$ given the row at t; that is, after each macro time step, the model appends a row to H. The index, t, runs from 1, which is the initial condition, to $t_M + 1$ where t_M is the number of macro time steps in the iteration. The index, n, runs from 0 to K, where K is the maximum number of microbes in a host. At the conclusion of t_M macro time steps, H has become a matrix with dimensions, $(t_M + 1) \times (K + 1)$.

The total number of holobionts through time is recorded in the vector,

$$H_T(t) = \sum_{n=0}^{K} H(t, n) \tag{A1}$$

and the total number of microbes through time is recorded in the vector,

$$G_T(t) = \sum_{n=0}^{K} n\, H(t,n) \tag{A2}$$

Model Equations—Vertical transmission

Here are the three stages within a macro time step for the vertical-transmission variant of the model.

Horizontal Microbe Transfer Across Holobionts.

The total number number of microbes that enter the transfer pool is $m\, G_T(t)$, where m is the probability a microbe leaves its host. Regardless of the host they reside in, all microbes have the same probability of entering the transfer pool. Therefore, the number of microbes who enter the transfer pool is simply m times the total number of microbes summed over all holobionts. The transfer pool is then distributed back equally across the holobiont population. The number of transfers received back from the pool per holobiont is then $m\, G_T(t)/H_T(t)$, provided $H_T(t) > 0$. Every holobiont receives this number.

Putting the arrivals and departures together, yields a formula for the new number of microbes, $n'(t,n)$ in a holobiont that formerly contained n microbes at time, t.

$$n'(t,n) = (1-m)\, n + m\, G_T(t)/H_T(t) \tag{A3}$$

This formula says the new number of microbes in the holobiont equals the number there previously minus the number that departed plus the number that arrived.

The $n'(n)$ must be an integer. To avoid considering fractional microbes, Equation (3) is rewritten as,

$$n'(t,n) = \lfloor (1-m)\, n + m\, G_T(t)/H_T(t) \rceil \tag{A4}$$

where the symbol $\lfloor x \rceil$ indicates the nearest integer to x.

The number of holobionts with n microbes after the horizontal transfer, $H'(t,n)$, then is found by accumulating over i the holobionts whose microbe composition has changed from i to n'.

$$H'(t,n) = \sum_{i=1\,|\,n=n'(t,i)}^{K} H(t,i) \tag{A5}$$

To see how this equation works, take a particular holobiont composition after horizontal transfer. Ask, for example, how many holobionts have exactly three microbes in them after the horizontal transfer has completed? $H'(t,3)$ is found by summing over H before the transfer started and using only the terms for the holobionts who wound up with 3 microbes after the transfer was over. If the summation is indexed with i, then the terms to include in the summation are those for which $3 = n'(i)$. These are the terms for holobionts who started with i microbes and wound up with 3 microbes. The same logic applies to $H'(t,n)$ for any n.

Microbe Proliferation Within Holobionts.

The next step is for the microbes to proliferate within their holobionts. If a holobiont starts with n microbes, then after one micro time step, the number of microbes in it is $F(n)$, where $F(n)$ is a demographic model for population growth.

The widely used logistic equation predicts sigmoid population growth with the population size leveling off at K, the carrying capacity. The speed of population growth initially, is r, the intrinsic rate of increase. Here, the model is written using a general $F(n)$ to indicate that any suitable demographic model may be used, not necessarily the logistic equation.

Let t_m be the number of micro time steps per macro time step. After t_m steps, the microbe population will have grown in size to

$$n''(n) = F^{t_m}(n) \tag{A6}$$

where $F^{t_m}(n)$ is the t_m^{th} iterate of $F(n)$. To avoid fractional microbes, the nearest integer is taken, yielding

$$n''(n) = \lfloor F^{t_m}(n) \rceil \tag{A7}$$

The number of holobionts with n microbes after microbe proliferation, $H''(n)$, then is found using the same logic as in the preceding stage, by accumulating over i the holobionts whose microbe composition has changed from i to n''.

$$H''(t,n) = \sum_{i=1\,|\,n=n''(i)}^{K} H'(t,i) \tag{A8}$$

The microbe proliferation takes place in the holobiont population after the horizontal transfer stage. Hence, $H'(t,n)$ appears in Equation (8).

Holobiont Selection.

Here the number of microbes within a holobiont does not change, but the number of holobionts changes. The fitness of a holobiont with n microbes, defined as the number of progeny that the holobiont produces, is $W(n)$. Therefore, the number of holobionts with n microbes after reproduction is simply

$$H(t+1,n) = W(n)\,H''(t,n) \tag{A9}$$

The holobiont selection takes place after microbe proliferation stage. Hence, $H''(t,n)$ appears in Equation (9). Fractional holobionts are not meaningful, so the preceding equation is modified to indicate that the nearest integer is intended

$$H(t+1,n) = \lfloor W(n)\,H''(t,n) \rceil \tag{A10}$$

Overall, Equation (10) predicts the hologenotype numbers at time $t+1$ based on what it was at time t.

Model Equations—Horizontal transmission

Here are the three stages within a macro time step for the horizontal-transmission variant of the model.

Horizontal transmission From Source Pools.

The macro time step begins with values for the size of the host pool, $H_T(t)$, and the microbe pool, $G_T(t)$. Then the Poisson density parameter indicating the average number of microbes per host is

$$\mu(t) = \frac{G_T(t)}{H_T(t)} \tag{A11}$$

Next, the hologenotype numbers are found from a Poisson density function, $P_\mu(n)$, normalized on the interval, $[0, K]$, multiplied by the number of hosts at time t,

$$H'(t, n) = \lfloor \frac{P_{\mu(t)}(n)}{\sum_{n=0}^{K} P_{\mu(t)}(n)} H_T(t) \rceil \tag{A12}$$

where $P_{\mu(t)}(n) = \frac{e^{-\mu(t)}\mu(t)^n}{n!}$ for $n \geq 0$. The numerator of the fraction in the equation above is the probability that an offspring of the host is colonized by n microbes given that the average number of microbes per host is $\mu(t)$ according to a Poisson probability distribution. However, the number of microbes in a host cannot exceed the host carrying capacity for microbes, K. So, the sum of probabilities of n from 0 to K is taken as the denominator in the fraction above. Thus, the overall probability of an offspring of the host being colonized by n microbes where n is between 0 and K is the fraction in the formula above. The sum of this fraction from 0 to K equals 1, as it should. This fraction then multiplies the number of hosts waiting to be colonized, $H_T(t)$, to yield the number of host offspring with n microbes at time t.

Microbe Proliferation Within Holobionts.

As before, the next step is microbe proliferation within their holobionts. $F(n)$ is a demographic model for population growth, such as the logistic equation.

After t_m steps, the microbe population grows in size to

$$n''(n) = \lfloor F^{t_m}(n) \rceil \tag{A13}$$

where $F^{t_m}(n)$ is the t_m^{th} iterate of $F(n)$.

The number of holobionts with n microbes after microbe proliferation, $H''(n)$, is found by accumulating over i the holobionts whose microbe composition has changed from i to n''.

$$H''(t,n) = \sum_{i=1\,|\,n=n''(i)}^{K} H'(t,i) \tag{A14}$$

Holobiont Selection.

The fitness of a holobiont with n microbes, defined as the number of progeny that the holobiont produces, is $W(n)$. Therefore, the number of holobionts with n microbes after reproduction is simply

$$H(t+1,n) = \lfloor W(n)\, H''(t,n) \rceil \tag{A15}$$

Prior to the next macro time step, all the microbes leave their holobionts and join a microbe source pool and the now-empty holobionts join a host source pool. Microbe retransmission and holobiont reassembly commences at the start of the next macro time step. The number of hosts and microbes in their source pools are, respectively,

$$H_T(t+1) = \sum_{n=0}^{K} H(t+1,n) \tag{A16}$$

and

$$G_T(t+1) = \sum_{n=0}^{K} n\, H(t+1,n) \tag{A17}$$

A new value of Poisson density parameter describing the average number of microbes per holobiont, $\mu(t+1)$, is then computed for use at the beginning of the next macro time step.

Download Program

Short programs 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.

To use the .nb files one must have the *Mathematica* app which is quite expensive, although available in many universities. The .nb files allow the user not only to run the programs and also to adapt and change them by modifying the *Mathematica* code.

Using the CDF files requires a free CDF player that is downloaded from Wolfram at:
https://www.wolfram.com/cdf-player/.
The player is analogous in principle to a PDF reader only here CDF files rather than PDF files are displayed. The CDF files are fully interactive and one can change the model parameters with sliders. The only limitation to using CDF files rather than the notebook files is that one cannot modify the code of a CDF file.

If one is uncertain about whether to download the CDF player, one can use the CDF files directly online with a web browser. The vertical transmission version of the model is found at:
https://www.wolframcloud.com/objects/joan.roughgarden/Published/HolobiontDynamicsVertical-05.cdf
and the horizontal version at:
https://www.wolframcloud.com/objects/joan.roughgarden/Published/HolobiontDynamicsHorizontal-05.cdf.
The CDF files run much faster when using the CDF player on a local computer, but clicking on these links provides a preview of how the programs work prior to deciding whether to download the player.

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