Holobiont Evolution:

Model with Vertical *vs.* Horizontal Microbiome Transmission

Joan Roughgarden[1,2,*]

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.

Manuscript elements: Figures 1–9, Appendix A.

Keywords: Holobiont, symbiosis, microbiome, hologenome, hologenotype, model, coral.

Manuscript type: Article.

Abstract

A holobiont is a composite organism consisting of a host together with its microbiome, such as a coral with its zooxanthellae. The intimate integration of microbiomes with their hosts implies to some that the holobiont is a unit of evolution and moreover, that selection on holobionts is an important evolutionary force. Skeptics argue that vertical transmission of microbiomes is too uncommon for holobiont selection to be evolutionarily important. What has been missing is a simple conceptual mathematical model to assess how holobiont selection may operate. 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 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 model: collective inheritance whereby juveniles inherit a sample of the collected genomes of all parents as contrasted with traditional lineal inheritance whereby juveniles inherit the genome(s) only of their own parent(s). 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 its knowledge from the collected knowledge of the preceding generation.

Introduction

This paper develops a simple conceptual mathematical model for the evolution 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 evolutionary results claimed for it (Booth 2014, Moran & Sloan 2015, Douglas & Werren 2016, Hester, Barott, Nulton, Vermeij, & Rohwer 2015, Skillings 2016, Chiu & Eberl 2016, Queller & Strass-

mann 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 in processes that amount to horizontal transmission. Hence, the microbiome in a host is effectively 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 be underestimating 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 the separate host and microbiome genotypes can produce the integration. Although coevolutionary selection does indeed lead to co-adaptations in

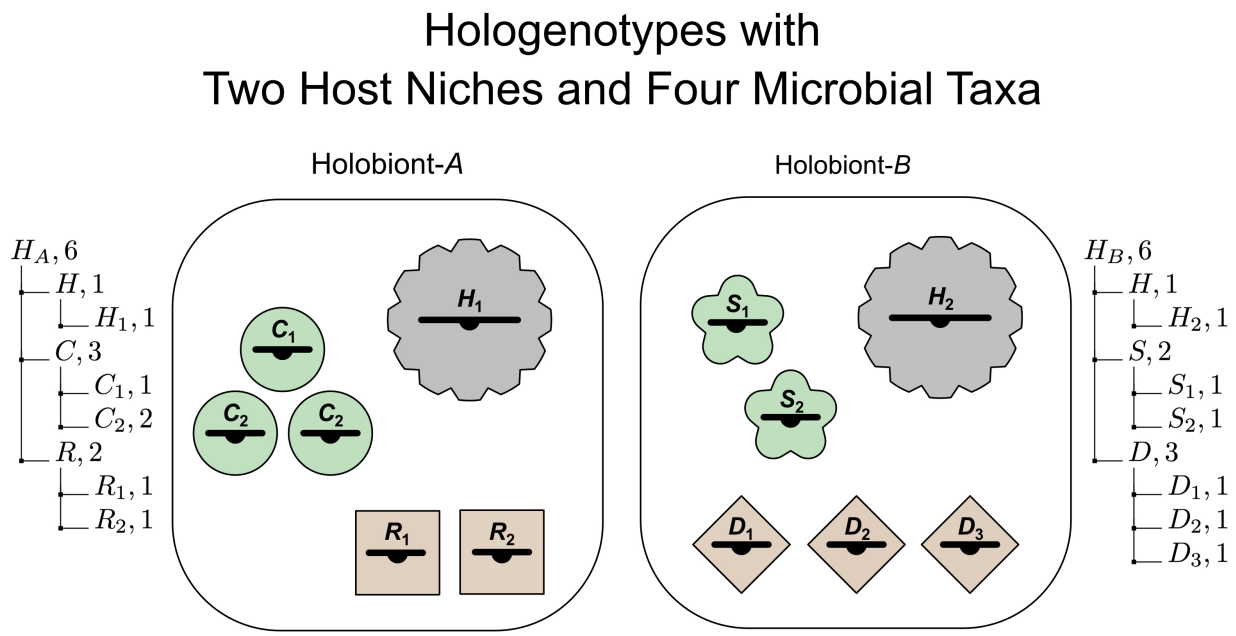
plant-pollinator and damselfish-sea anemone associations and so forth, the degree of integration between host and microbiome seems of an entirely different scale than that seen in familiar examples of coevolutionary coadaptation. The skeptics have to argue that given enough time, then coevolutionary selection will eventually yield the degree of host/microbiome integration highlighted by the proponents.

So, at this time, two quite reasonable alternative positions occur with respect to holobiont selection. The proponents face the difficulty of dealing with the mode of microbiome transmission. The skeptics face the difficulty that coevolutionary selection apparently does not lead to 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. Moreover, 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 Hologenome

The hologenome includes multiple components in 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



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

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

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. $\mathbf{H}_A$, 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.

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

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, holobiontsthat had few microbes to begin with receive a net increase in microbe number and holobionts with many microbes to begin with incur

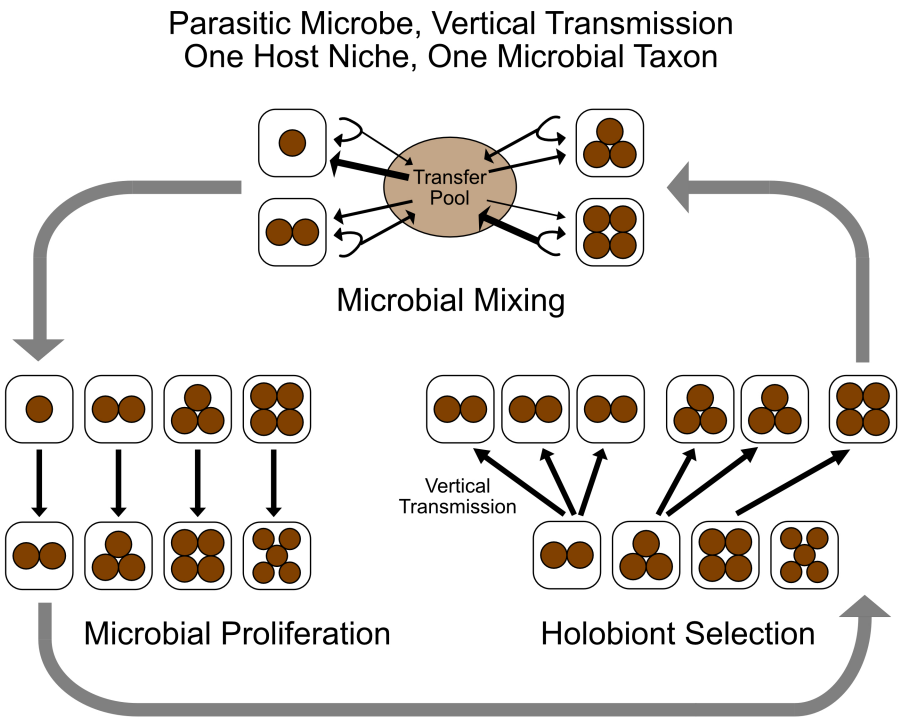


Figure 2: Sequence of processes with vertical transmission of parasitic microbiome.

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 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.

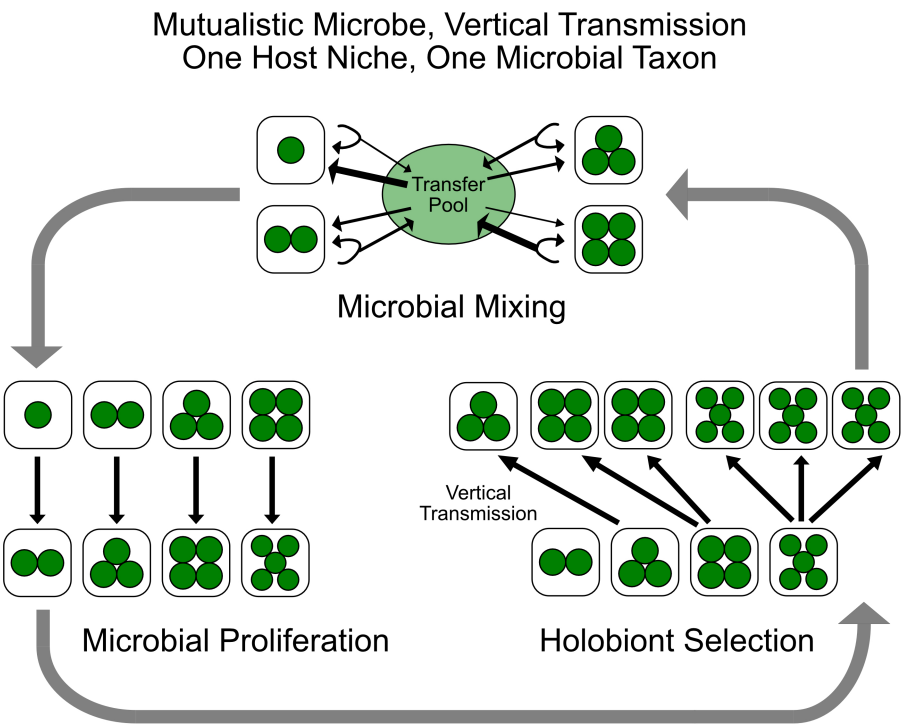


Figure 3: Sequence of processes with vertical transmission of mutualistic microbiome.

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 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 Figure 5 show screen shots of the histogram through time of the hologenotype frequencies for parasitic and mutualistic microbes. In both figures iteration of macro time steps leads from an initial uniform distribution to a stationary distribution of hologenotype frequencies at which holobiont selection balances microbial mixing.

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

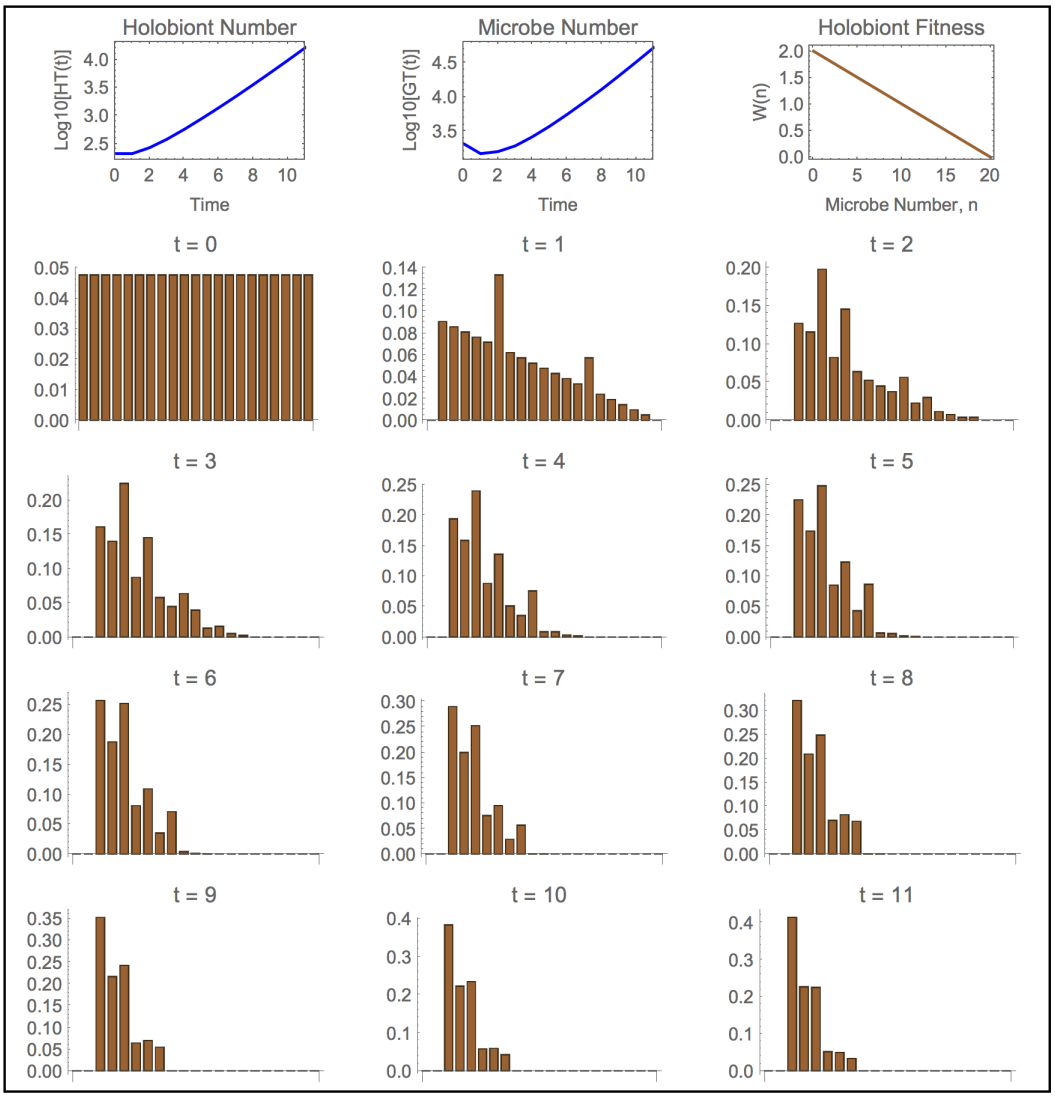


Figure 4: Results with vertical transmission of parasitic microbiome. Top left and middle graphs show log of total holobiont and microbe numbers through time and top right graph shows holobiont fitness as function of number of microbes in host. Histograms show distribution of hologenotype frequencies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

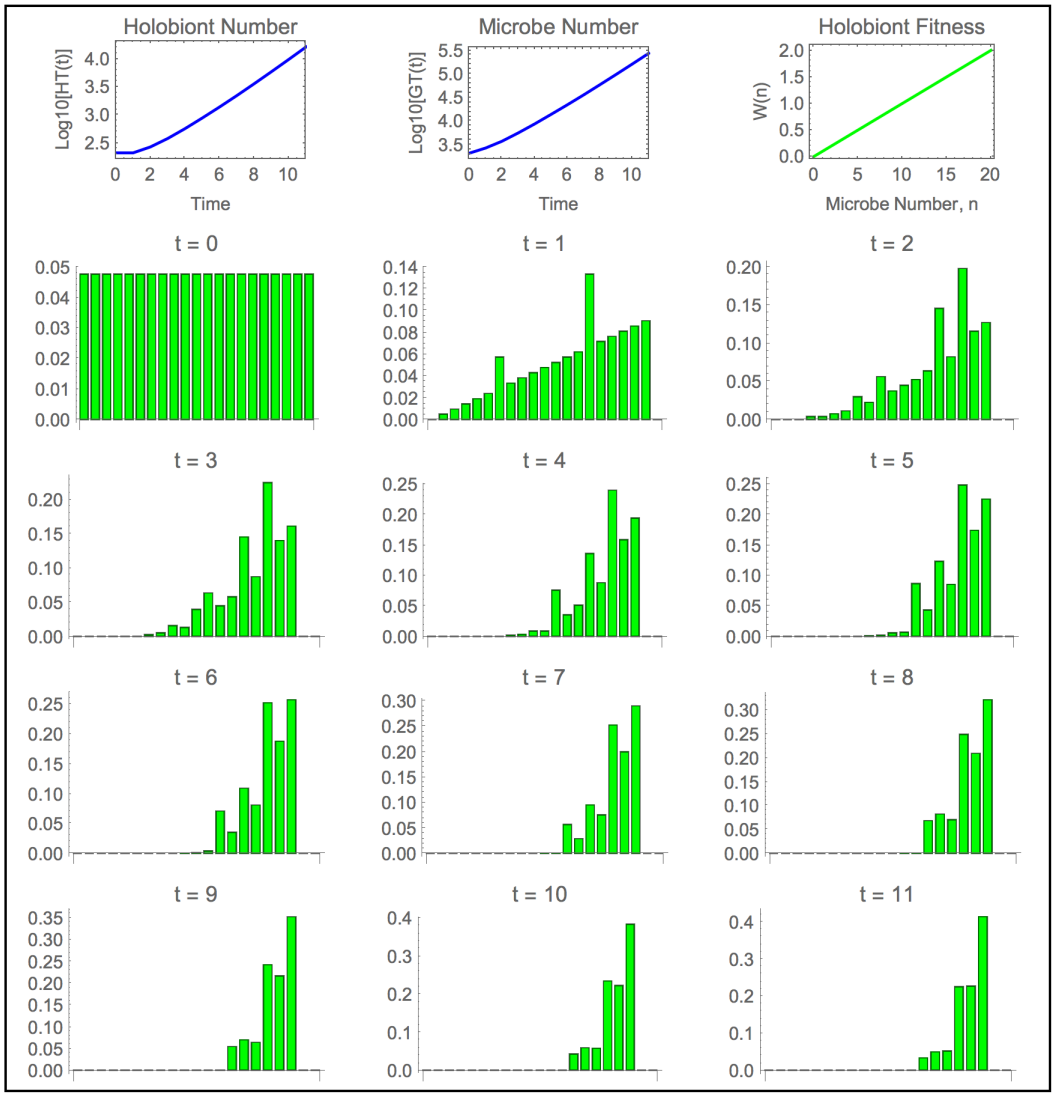


Figure 5: Results with vertical transmission of mutualistic microbiome.

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.

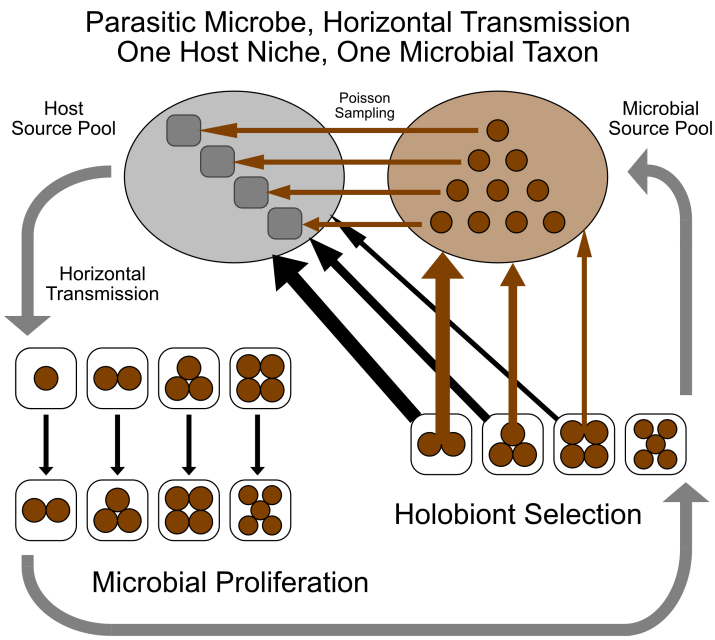


Figure 6: Sequence of processes with horizontal transmission of parasitic microbiome.

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:

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.

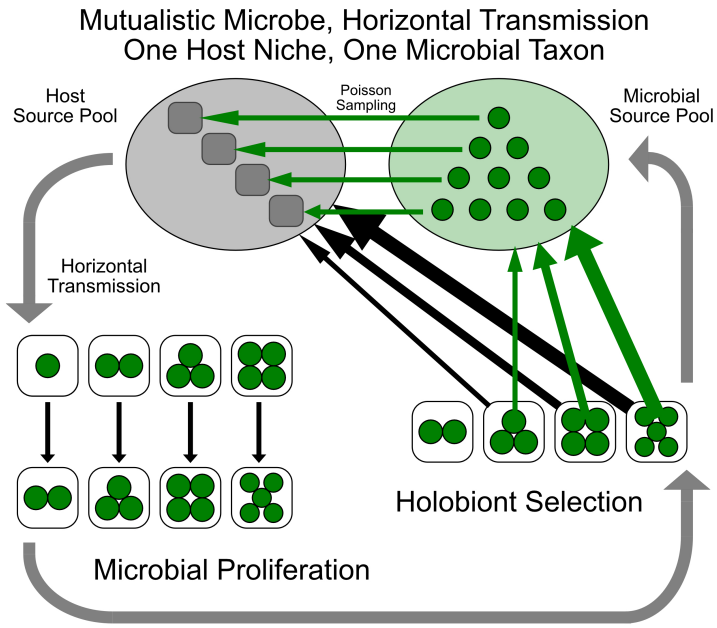


Figure 7: Sequence of processes with horizontal transmission of mutualistic microbiome.

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. 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.

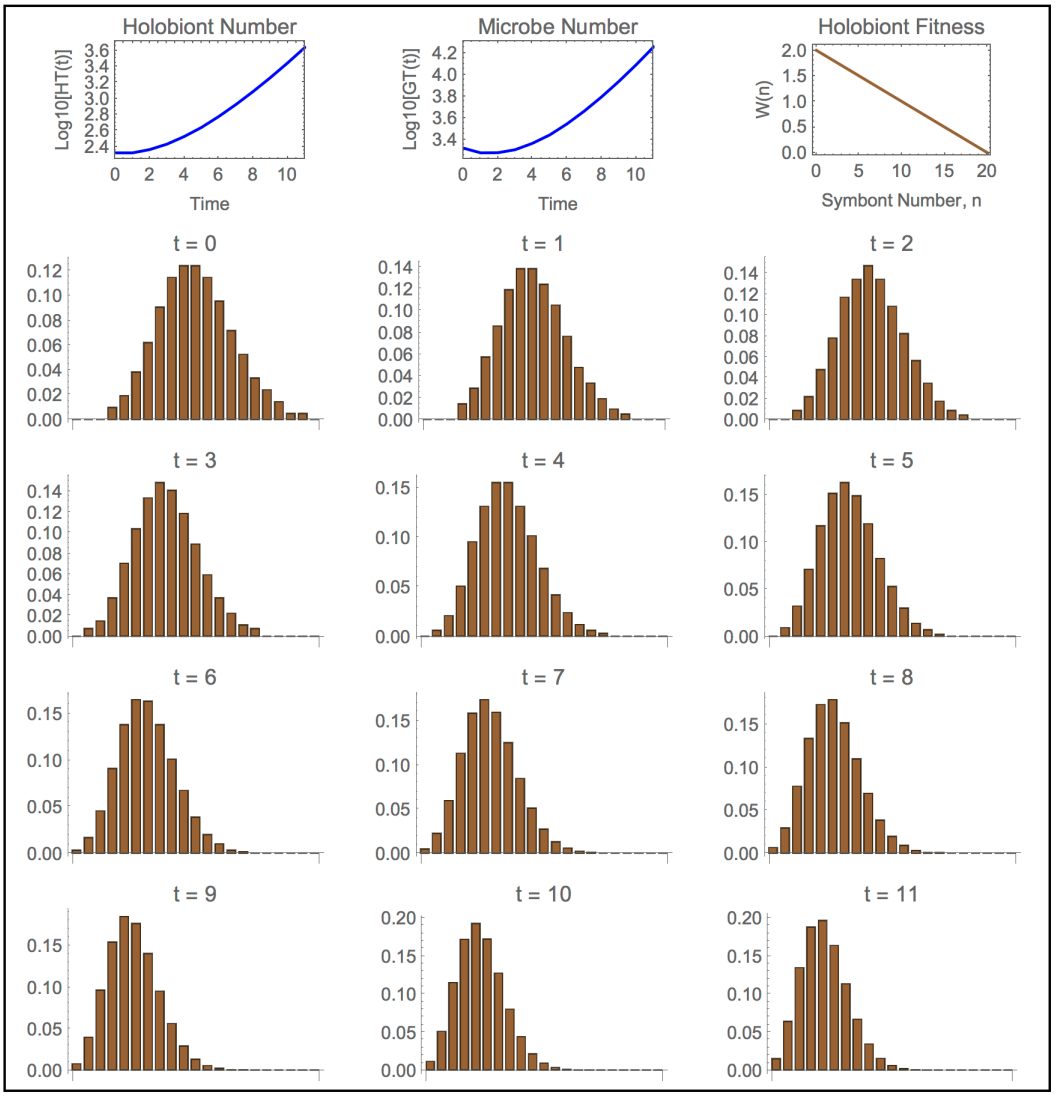


Figure 8: Results with horizontal transmission of parasitic microbiome.

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

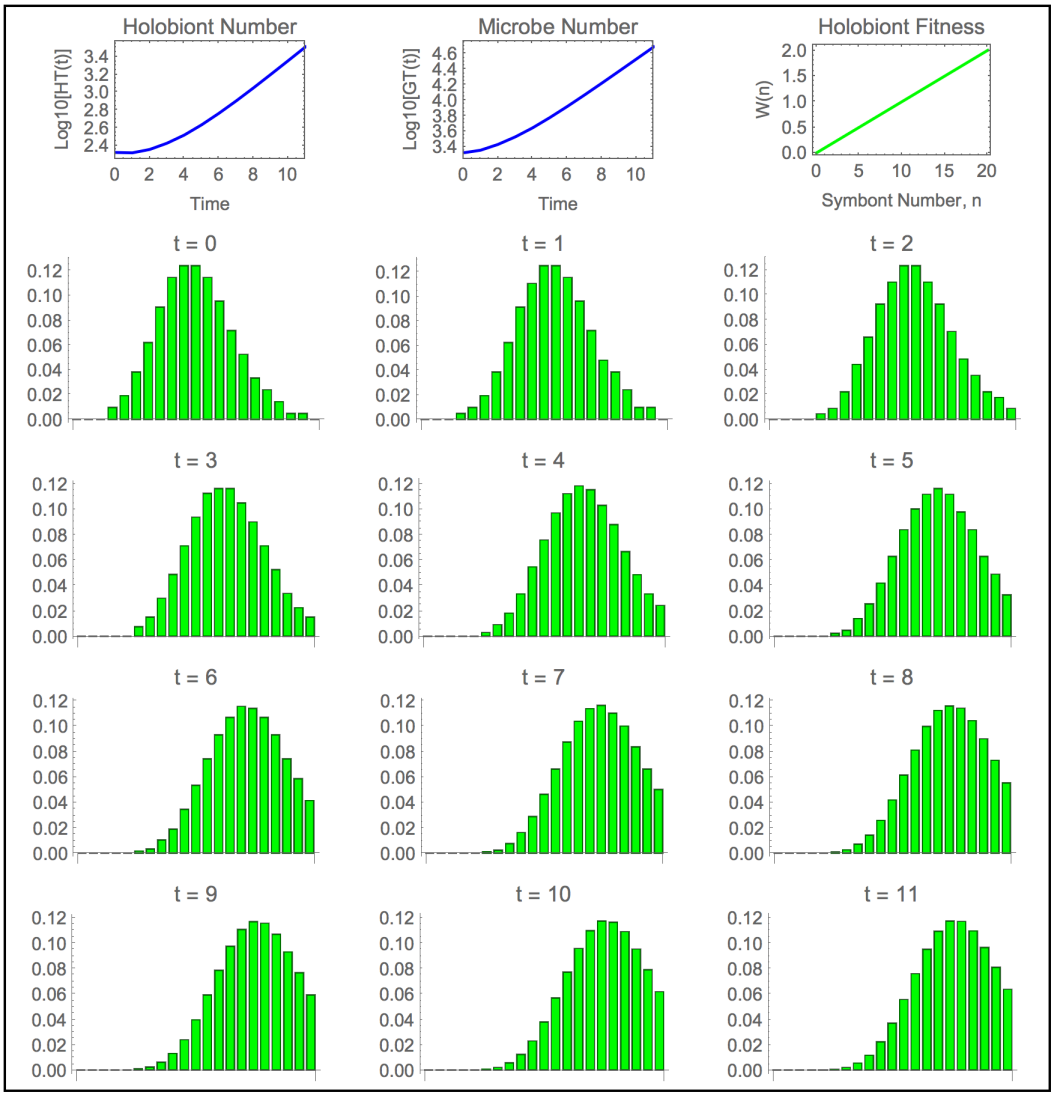


Figure 9: Results with horizontal transmission of mutualistic microbiome.

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-

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.

Discussion

Although the qualitative outcome of holobiont selection is similar with both horizontal and vertical transmission, the underlying evolutionary processes are different.

In vertical transmission, microbes incorporate into a host lineage as an extension of the host's genome. Microbial mixing loosens the microbe/host association, reducing the impact of the holobiont selection.

In horizontal transmission, beneficial microbes flood the microbial source pool leading to assembly of the next generation's holobionts with an increase in microbe number because the ratio of microbes to hosts has increased. Conversely, the limited production of parasitic microbes dilutes the microbial source pool leading to holobiont assembly 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 microbial abundance is inherited by lineal descent, albeit not as a Mendelian trait. In contrast, the process of hologenome assembly from the combined parental microbe production might be dubbed "collective inheritance".

Evolution by two modes of holobiont selection is reminiscent of evolution by two modes of group selection, one based on limited migration among demes in a metapopulation (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 because holobiont selection is a selection process acting on individuals. However, evolution *via* holobiont selection is not neoDarwinian because neoDarwinism is founded on Mendelian inheritance (Wright 1931).

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).

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.

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

In 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.

Conclusion

Holobiont selection is plausibly an important evolutionary force with both vertical and horizontal microbiome transmission.

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,

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

where $P_{\mu(t)}(n) = \frac{e^{-\mu(t)}\,\mu(t)^n}{n!}$ for $n \geq 0$.

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.

Literature Cited

Babcock RC, Bull GD, Harrison PL et al (1986) Synchronous spawnings of 105 scleractinian coral species on the Great Barrier Reef. Marine Biol 90:379–394

Booth A 2014 Symbiosis, selection, and individuality. Biol Philos 29:657–673

Bordenstein SR, Theis KR. 2015 Host biology in light of the microbiome: ten principles of holobionts and hologenomes. PLoS Biol 13(8):e1002226

Burke C, Steinberg P, Rusch D, Kjellberg S, Thomas T (2011) Bacterial community assembly based on functional genes rather than species. Proc Natl Acad Sci USA 108(34):14288–14293

Chiu L & G Eberl 2016 Microorganisms as scaffolds of biological individuality: An eco-immunity account of the holobiont. *Biol Philos* 31: 819-837.

Doolittle WF, Booth A 2017 It's the song, not the singer: an exploration of holobiosis and evolutionary theory. Biol Philos 32(1):5–24

Douglas AE, Werren JH 2016 Holes in the hologenome: why host-microbe symbioses are not holobionts. MBio 7(2):e02099-15. doi:10.1128/mBio.02099-15

Dubilier N, McFall-Ngai M, Zhao L, 2015. Microbiology: Create a global microbiome effort. *Nature*. 526(7575):631–4.

Eshel I. 1972. On the neighborhood effect and the evolution of altruistic traits, Theor Popul Biol 3:258–277

Fraune S, Bosch TCG. 2010 Why bacteria matter in animal development and evolution. Bioessays 32:571–580

Gilbert SF, Sapp J, Tauber AI. 2012 A symbiotic view of life: we have never been individuals. Q Rev Biol 87:325–341

Gilbert SF, Rosenberg E, Zilber-Rosenberg I. 2017. The holobiont with its hologenome is a level of selection in evolution. In: Gissis SB, Lamm E, Shavit A (eds) Landscapes of collectivity in the life sciences. The MIT Press, London, pp 305–324.

Guerrero, Ricardo, Lynn Margulis, Mercedes Berlanga 2013, Symbiogenesis: the holobiont as a unit of evolution. *International Microbiology* (2013) 16:133–143

Hester ER, Barott KL, Nulton J, Vermeij M, Rohwer F. 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.

Hurst GDD 2017 Extended genomes: symbiosis and evolution. *Interface Focus* 7: 20170001. http://dx.doi.org/10.1098/rsfs.2017.0001.

Jefferson R. 1994. The hologenome. Agriculture, Environment and the Developing World: A Future of PCR. Cold Spring Harbor, New York

Lederberg J, McCray AT. 2001. "Ome Sweet Omics"—a genealogical treasury of words. Scientist. 15:8.

Lloyd E 2017 Holobionts as units of selection: Holobionts as interactors, reproducers, and manifestors of adaptation. In: Gissis SB, Lamm E, Shavit A (eds) Landscapes of collectivity in the life sciences. The MIT Press, London, pp 351–367

Mao, J., & Lu, T. (2016). Population-Dynamic modeling of bacterial horizontal gene transfer by natural transformation. Biophysical journal, 110(1), 258-68.

Margulis, L. 1991. Symbiosis as a source of evolutionary innovation: speciation and morphogenesis. In: Margulis L, Fester R, editors. Symbiogenesis and Symbionticism. Cambridge: MIT Press. p. 1–14.

McFall-Ngai, M., M. G. Hadfield, T. C. Bosch, H. V. Carey, T. Domazet-Lošo, 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 NA, Sloan DB 2015 The hologenome concept: helpful or hollow? PLoS Biol 13(12):e1002311

Osmanovic D, Kessler DA, Rabin Y, Soen Y. 2018. Darwinian selection of host and bacteria supports emergence of Lamarckian-like adaptation of the system as a whole. Biology Direct. in press. https://doi.org/10.1186/s13062-018-0224-7

OSTP (The White House Office of Science and Technology Policy) 2016. Fact Sheet: Announcing the National Microbiome Initiative. United States Government. https://obamawhitehouse.archives.gov/the-press-office/2016/05/12/fact-sheet-announcing-national-microbiome-initiative. (Accessed December 10, 2018)

Queller DC & JE Strassmann 2016 Problems of multispecies organisms: endosymbionts to holobionts. *Biol Philos* 31: 855-873.

Rosenberg E, Koren O, Reshef L, Efrony R, Zilber-Rosenberg I. 2007. The role of microorganisms in coral health, disease and evolution. Nature Reviews Microbiology. 5(5):355–62

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

Rosenberg and 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. , Sharon, G. and Zilber-Rosenberg, I. (2009), The hologenome theory of evolution contains Lamarckian aspects within a Darwinian framework. Environmental Microbiology, 11: 2959-2962. doi:10.1111/j.1462-2920.2009.01995.x

Roughgarden J 2017. Model of holobiont population dynamics and evolution: a preliminary sketch. Pp 325—350 in: Gissis SB, Lamm E, Shavit A (eds) Landscapes of collectivity in the

life sciences. Vienna series in theoretical biology. MIT Press, Cambridge

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

Schluter J, Foster KR. 2012. The evolution of mutualism in gut microbiota via host epithelial selection. *PLoS Biol* 10(11): e1001424. doi:10.1371/ journal.pbio.1001424

Skillings D 2016 Holobionts and the ecology of organisms: Multi-species communities or integrated individuals? *Bio Philos* 31: 875-892.

Trench RK (1993) Microalgal-invertebrate symbioses: a review. Endocytobiosis Cell Res 9:135–175

Turnbaugh, P. J., R. E. Ley, M. Hamady, C. M. Fraser-Liggett, R. Knight and J. I. Gordon. 2007. The human microbiome project. *Nature* **449**:804-810.

Wilson DS. 1975. A theory of group selection. Proc. Nat. Acad. Sci (USA). 72:143—146.

Woese CR. 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

Zeng, Q., Wu, S., Sukumaran, J., & Rodrigo, A. (2017). Models of microbiome evolution incorporating host and microbial selection. Microbiome, 5(1), 127. doi:10.1186/s40168-017-0343-x

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