Holobiont Evolution: Model with Lineal *vs.* Collective Hologenome Inheritance

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The intimate integration of microbiomes with their hosts might imply that the holobiont is a unit of evolution and that selection on holobionts is an important evolutionary force. Skeptics argue that vertical transmission of microbes is too uncommon for holobiont selection to be evolutionarily important. What has been missing is a simple mathematical model to assess how holobiont selection may operate. Here I offer two variants of such a model. In one variant, juvenile hosts obtain microbiomes from their parents (lineal inheritance). In the other variant, microbiomes of juvenile hosts are assembled from source pools representing the combined microbiomes of all parents (collective inheritance). According to both variants, holobiont selection causes evolutionary change in holobiont traits and thus is plausibly an important evolutionary force.

Holobiont selection as an important evolutionary force is supported by mathematical model.

Introduction

A *holobiont* is a composite organism consisting of a host together with its microbes (*1*). A *microbiome* is the "ecological community of commensal, symbiotic, and pathogenic microorganisms" that shares the "body space" of a host (*2*). A holobiont is a host plus its microbiome. A *hologenome* is the union of all host genes with all genes from its microbes (*3-4*). *Holobiont selection* is the differential reproduction and survival of hosts, including their microbiomes, depending on their hologenomes.

The intimate anatomical, physiological, developmental and immunological integration of many microbiomes with their hosts (*5*) implies to some that a holobiont is a unit of evolution and that holobiont selection is a major evolutionary force (*6-15*). Others are skeptical, arguing that vertical transmission of microbes across host generations is too uncommon for holobiont selection to be evolutionarily important (*16-24*).

What has been missing is a simple mathematical model to assess the plausibility of holobiont selection as a force in evolution. Here I offer two variants of such a model. In one variant (vertical colonization), the microbes from parental hosts directly colonize offspring, or are transmitted through host gametes, leading to a lineal inheritance of the hologenome. In the other variant (horizontal colonization), the microbiomes of juvenile hosts are assembled from source pools of microbes in the environment that come from the combined microbiomes of all the parents, leading to a collective inheritance of the hologenome. According to both variants, holobiont selection causes the evolution of holobiont traits.

This study responds to the demand from medical and conservation constituencies for understanding the microbiome/host relationship (*25-27*).

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 functional roles (*28*) or physical locations. 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 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 frequencies for a holobiont population.

To compare the evolutionary outcomes of vertical and horizontal colonization, 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 Colonization

Model

Three processes occur as sequential stages within each host generation (*29-30*), as shown in the top panels of Figure 2. 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:

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 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 parent. In the case of microbial parasites on the left, the holobionts with the lowest number of microbes leave the largest number of progeny, and conversely for microbial mutualists on the right. 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, screen shots, and instructions for how to download the model from the web appear in the supplementary material.

Results

The bottom panels of Figure 2 show screen shots of the histogram through time of the hologenotype frequencies for parasitic and mutualistic microbes. In both panels 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.

On the left, holobiont selection leads to a preponderance of holobionts with fewer parasites than initially. On the right holobiont selection leads to a preponderance of holobionts with more mutualists than initially. In the model's picture display (*cf.* supplementary material) the pictures on the left change through time from 50% brown to almost no color and on the right 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.

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 (*31*).

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.

In reality, much of the microbiome does not colonize vertically. For example, in corals a few species transmit zooxanthellae within their eggs (*32*) but the vast majority do not (*33-34*). Evidently substantial mutualism evolves even in the absence of vertical microbe transmission. 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 colonization can be introduced to the model. This sampling produces hologenotypic variation across holobionts and leads to the following formulation:

Horizontal Colonization

Model

Three processes occur as sequential stages within each host generation, as shown in the top panels of Figure 3. In detail:

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

Results

The bottom panels of Figure 3 show screen shots of the histogram through time of the hologenotype frequencies where the microbes are parasitic (left) or mutualistic (right). Overall, the results are similar in this model variant to those in the vertical colonization 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 colonization, a stationary distribution is attained when the holobiont selection balances the variability caused by the Poisson sampling of the source pools during colonization. (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 outcome of holobiont selection with horizontal microbe colonization is similar to that with vertical colonization, the underlying evolutionary processes are entirely different.

In vertical colonization, microbes incorporate into a host lineage as an extension of the host's genome. Successful host lineages have few deleterious and many beneficial microbes similar to having few deleterious and many beneficial mutations in the host genome. Microbial mixing loosens the microbe/host association, decoupling the fate of the microbes from the fate of the hosts they reside in, thereby dissolving the impact of the holobiont selection.

In horizontal colonization, beneficial microbes from successful holobionts flood the microbial source pool leading to reassembly of the next generation's holobionts with an average increase in microbe number because the ratio of microbes to hosts has increased. Conversely, the limited production of parasitic microbes from successful holobionts dilutes the microbial source pool leading to holobiont reassembly with an average 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 colonization destroys hologenotypic variation whereas the sampling process in horizontal colonization produces hologenotypic variation.

With vertical colonization, a holobiont's microbial abundance is inherited by lineal descent, albeit not as a Mendelian trait. Lineal inheritance is the inheritance mechanism that, together with holobiont selection, accounts for holobiont evolution in cases where the transmission of microbes is primarily vertical.

The process of hologenome assembly from the combined parental microbe production might

be dubbed “collective inheritance”. Collective inheritance provides the inheritance mechanism which, together with holobiont selection, accounts for holobiont evolution in cases where the transmission of microbes is primarily horizontal.

Thus, holobiont traits respond to holobiont selection with both lineal and 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 (*35*) and the other based on trait groups in a population with a dispersal phase (*36*).

Previous investigators have noted that holobiont evolution is, in a sense, Lamarckian because holobiont selection affects acquired rather than inherited traits (*37-38*). With horizontal colonization 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 (*39*).

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 (*40*). Also, collective inheritance might prove valuable to understanding the evolution of bacteria and other microbes that feature large amounts of horizontal gene transfer (*41*).

The model here and its framework are extensible and scalable. A possible roadmap for future theoretical research is to investigate how host-microbe coordination evolves, 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 colonization.

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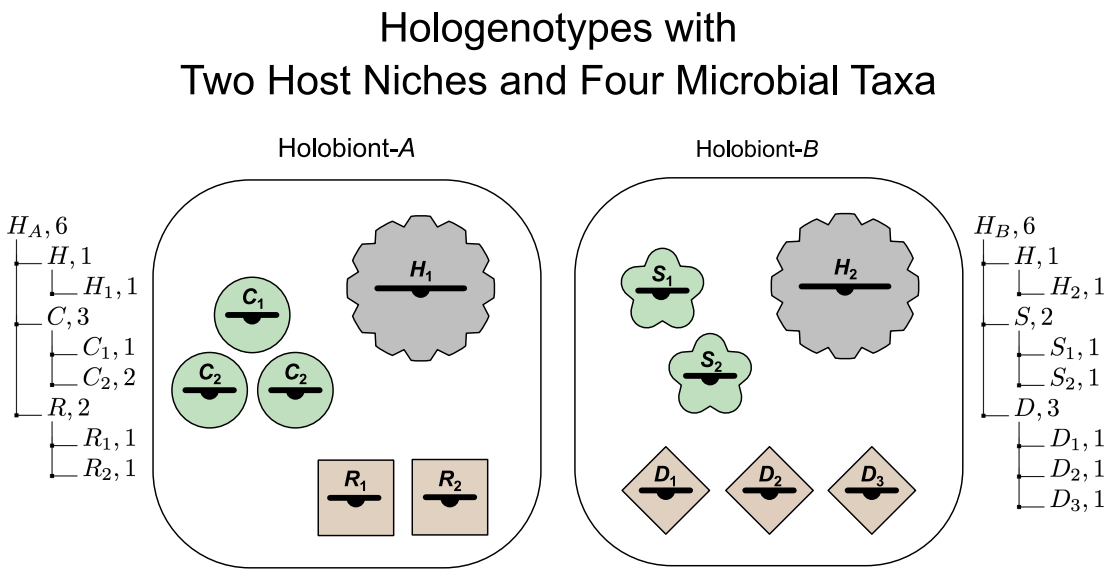
Figures



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

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

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.

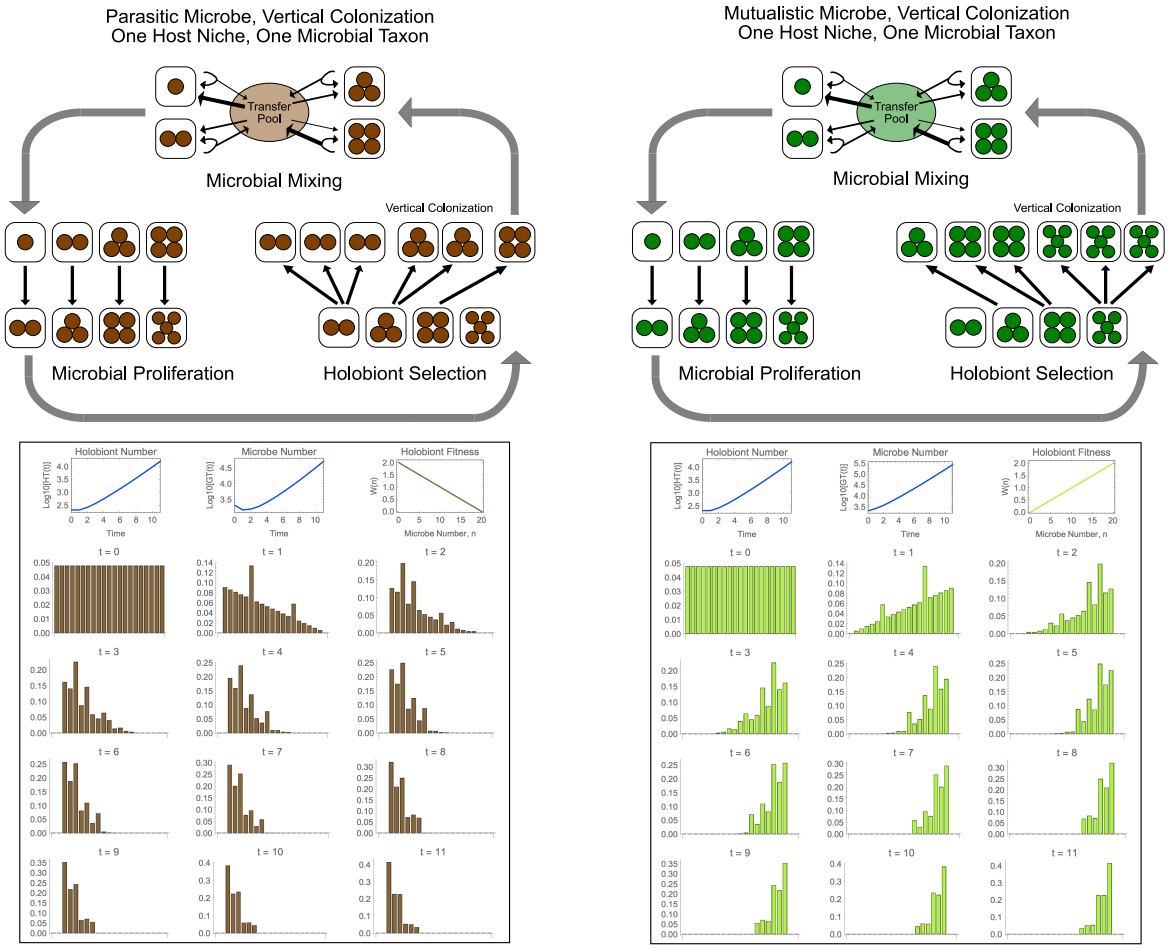


Figure 2. Outcome of holobiont selection with vertical transmission of microbes from parental to juvenile hosts. Left panel is for parasitic microbes, right for mutualistic microbes. Within each panel, top left and middle graphs show log of total holobiont and microbe numbers through time and top right graph shows holobiont fitness as function of number of microbes in host. Histograms show distribution of hologenotype frequencies through time. The horizontal axis is number of microbes per host and vertical axis is fraction of holobiont population.

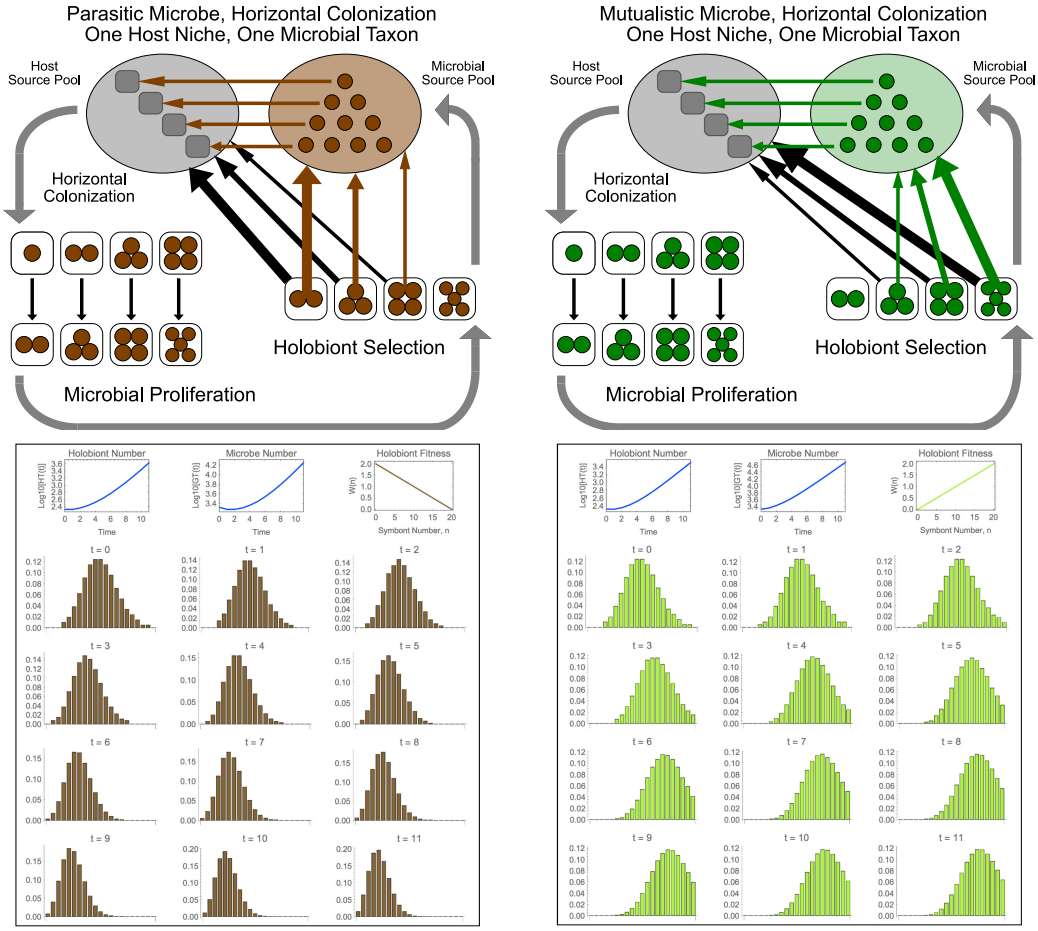


Figure 3. Outcome of holobiont selection with colonization of juvenile hosts from source pool of microbes in environment. Layout of graphs same as Figure 2.

Supplementary Material
Holobiont Evolution: Model with Lineal *vs.* Collective Hologenome Inheritance

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Introduction

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,

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

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

$$G_T(t) = \sum_{n=0}^{K} n\, H(t,n) \tag{2}$$

Model Equations—Vertical Colonization

Here are the three stages within a macro time step for the vertical-colonization 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.

$$n'(t,n) = (1-m)\, n + m\, G_T(t)/H_T(t) \tag{3}$$

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,

$$n'(t,n) = \lfloor (1-m)\, n + m\, G_T(t)/H_T(t) \rceil \tag{4}$$

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'.

$$H'(t,n) = \sum_{i=1\,|\,n=n'(t,i)}^{K} H(t,i) \tag{5}$$

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

$$n''(n) = F^{t_m}(n) \tag{6}$$

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

$$n''(n) = \lfloor F^{t_m}(n) \rceil \tag{7}$$

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''.

$$H''(t,n) = \sum_{i=1\,|\,n=n''(i)}^{K} H'(t,i) \tag{8}$$

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

$$H(t+1,n) = W(n)\, H''(t,n) \tag{9}$$

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

$$H(t+1,n) = \lfloor W(n)\, H''(t,n) \rceil \tag{10}$$

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

Model Equations—Horizontal Colonization

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

Horizontal Colonization 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

$$\mu(t) = \frac{G_T(t)}{H_T(t)} \tag{11}$$

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,

$$H'(t,n) = \lfloor \frac{P_{\mu(t)}(n)}{\sum_{n=0}^{K} P_{\mu(t)}(n)} H(t,n) \rceil \tag{12}$$

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

$$n''(n) = \lfloor F^{t_m}(n) \rceil \tag{13}$$

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''.

$$H''(t,n) = \sum_{i=1\,|\,n=n''(i)}^{K} H'(t,i) \tag{14}$$

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

$$H(t+1,n) = \lfloor W(n)\, H''(t,n) \rceil \tag{15}$$

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 recolonization 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,

$$H_T(t+1) = \sum_{n=0}^{K} H(t+1,n) \tag{16}$$

and

$$G_T(t+1) = \sum_{n=0}^{K} n\, H(t+1,n) \tag{17}$$

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 colonization 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.

Here are some representative screen shots from the programs' output.

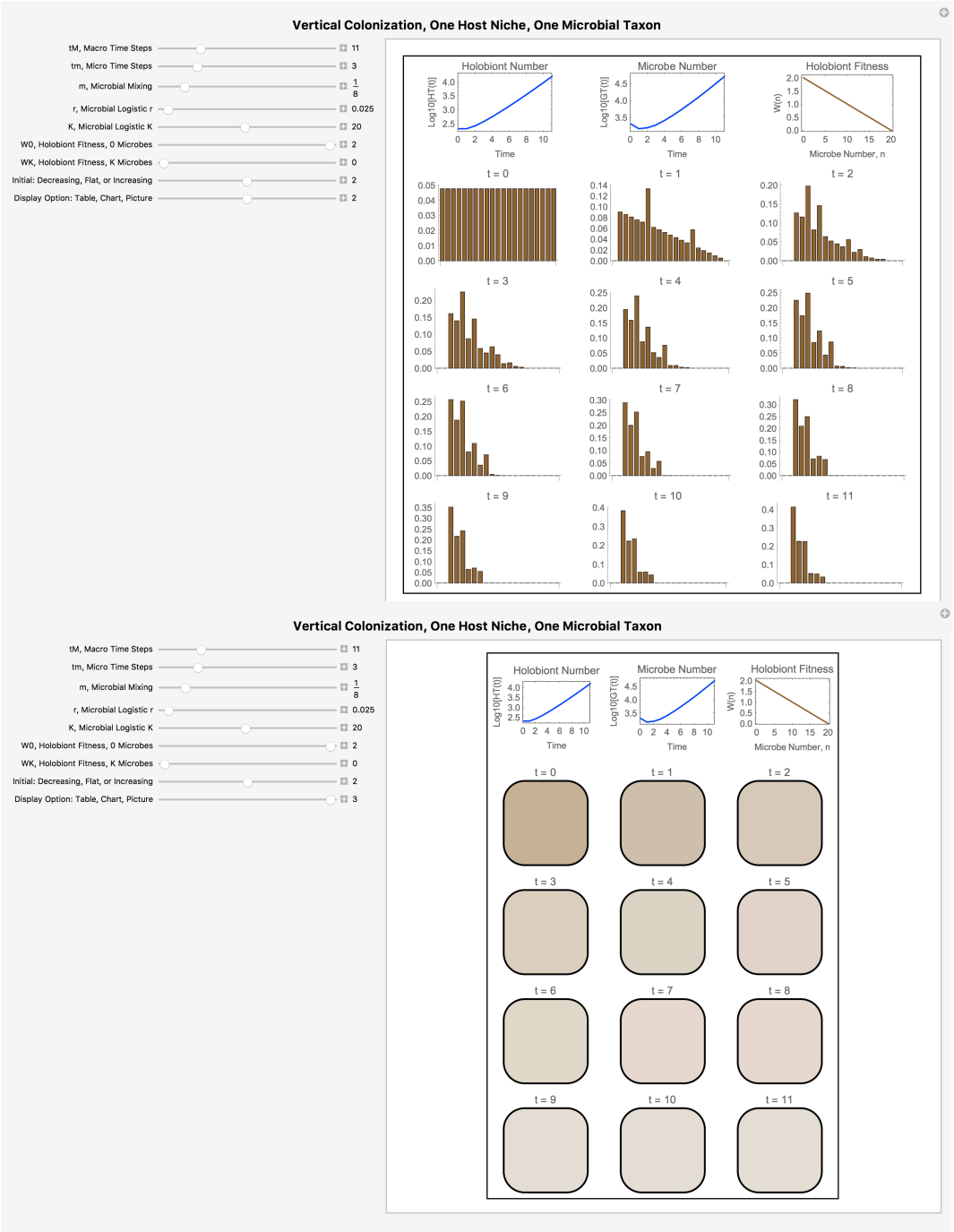

Figure S1. Examples of chart and picture styles of output. Notice holobionts become lighter in color as holobiont selection reduces the average parasite density.

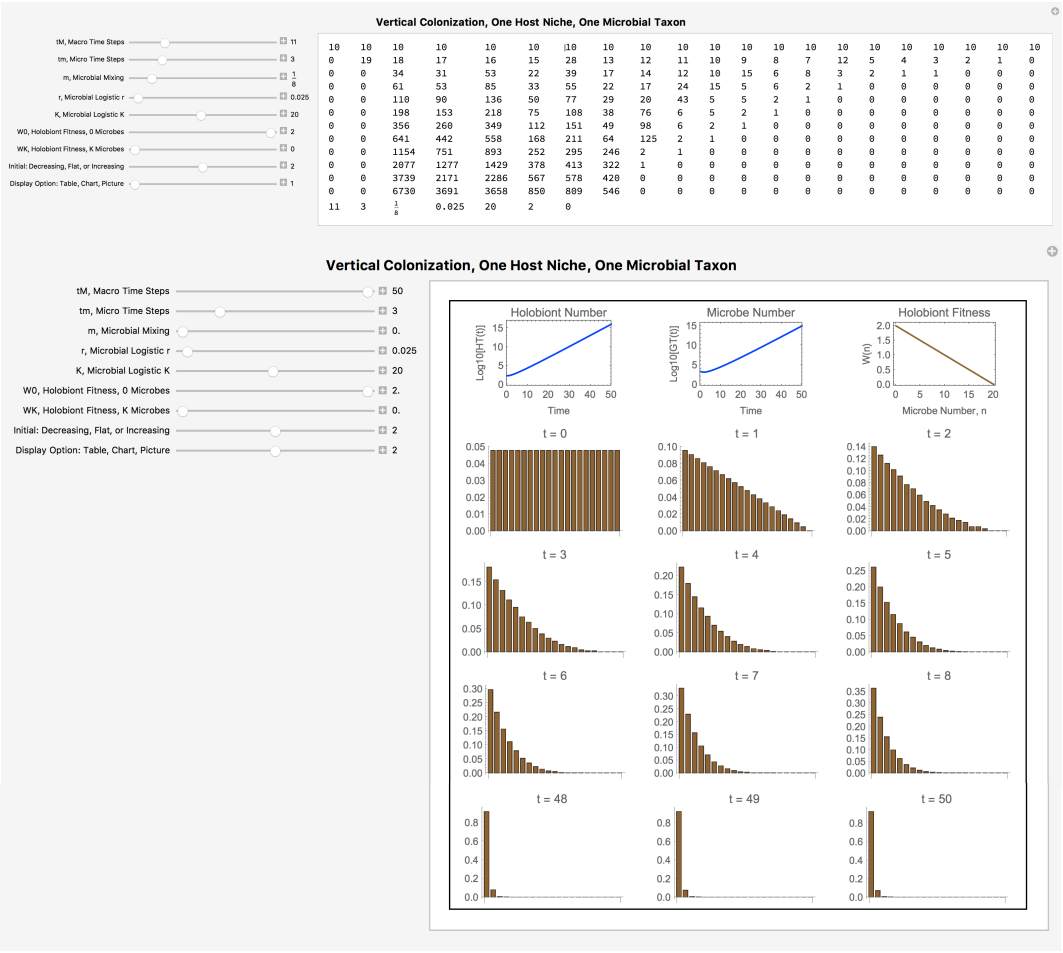

Figure S2. Top. Example of table style of output. Bottom. With zero mixing, $m = 0$, holobiont selection eventually eliminates the parasites. Here, number of macro time steps at end of iteration, t_M, is 50. A still longer iteration shows the total elimination of the microbes. Thus, the presence of mixing ($m > 0$) prevents the total loss of the parasites leading to a stationary distribution where the force of holobiont selection balances the force of mixing. Exactly where this balance occurs depends on the initial condition, as the next figure shows.

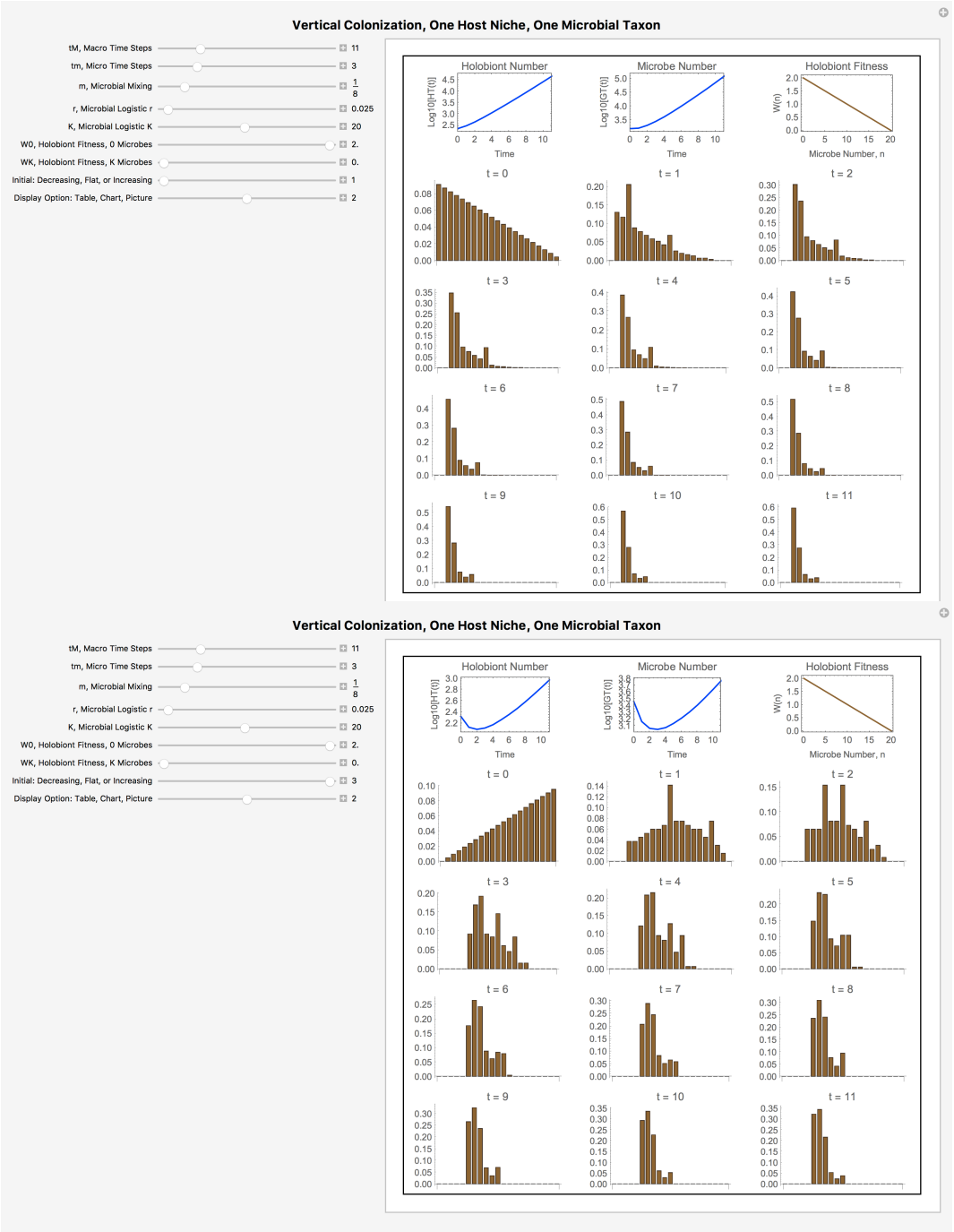

Figure S3. Different initial conditions lead to different stationary distributions. In every case, holobiont selection reduces average parasite density relative to initial condition.

Figure S4. Increasing number of micro time steps, t_m, per macro step from 3 to 13 causes the parasite virulence to overpower holobiont selection leading to holobiont extinction.

Figure S5. Microbe is a mutualist. Values of W_0 and W_K are transposed relative previous figures. Here, holobiont fitness increases with number of microbes.

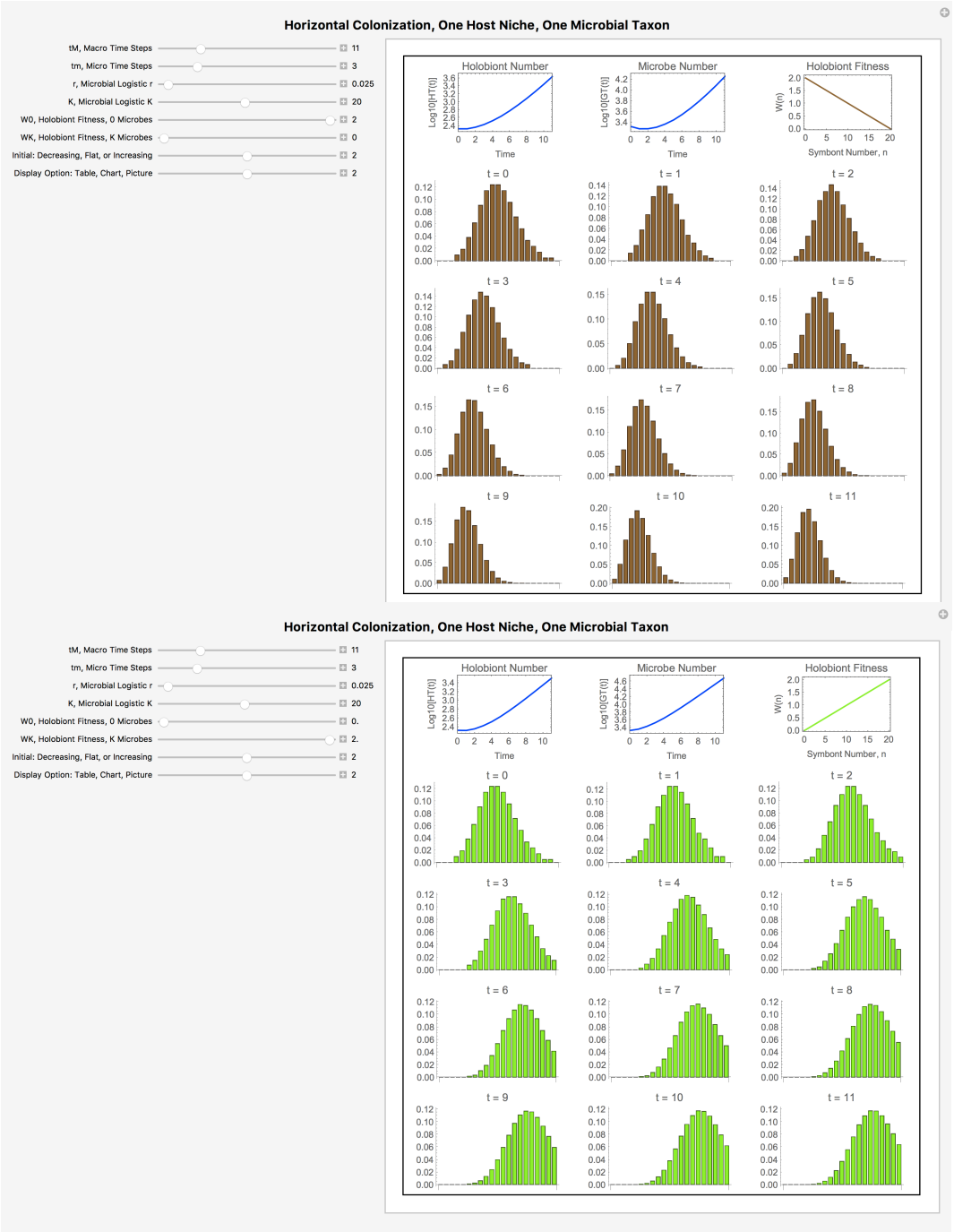

Figure S6. Horizontal colonization with parasitic (top) and mutualistic (bottom) microbes.