Applying Evolutionary Theory to Understand Host-Microbiome Evolution:

New Tricks for Old Dogs

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ABSTRACT

All plants and animals are host to a community of microorganisms, their microbiomes, that have crucial influences on the life history and performance of their hosts. Despite the importance of such host-microbiome relationships, relatively little is known about the role microbiomes play in mediating evolution of the host as well as entire host-microbe assemblages. This knowledge gap is partly due to the lack of theoretical frameworks that generate testable predictions on the evolutionary dynamics of host-microbiome systems. In this Perspective, we argue that the foundation for such frameworks exists in evolutionary theory. We highlight four examples of theoretical models - niche construction, indirect genetic effects, maternal effects and multilevel selection – that capture important aspects of host-microbiome evolution. We outline how each of these frameworks can provide key insights into the evolution of host-microbiome systems while also suggesting expansions of current theory to incorporate processes unique to host-microbe assemblages, for instance focusing on nuances in microbiome transmission and ecological microbial community dynamics. Expanding evolutionary theory to accommodate host-microbiome systems is key for a more integrative understanding of evolution, which is undoubtedly impacted by the association with microorganisms, guiding future empirical research on the function and evolution of these omnipresent interactions.

Introduction

All multicellular organisms have long-standing, intimate relationships with microorganisms. These host-associated microbial communities (including bacteria, archaea, viruses, protists, and fungi, together termed the microbiome) are crucial for host performance, affecting host traits related to metabolism[1], pathogen resistance[2], immune development[3], disease[4]*, and behavior[5], among many others. Beyond its fundamental interest, the potential applications of the microbiome vary widely, ranging from human health[6], to sustainable agriculture[7], conservation biology[8], and adaptation to climate change[9].

There is no doubt that host-associated microbiomes can influence host performance. There is also increasing evidence that the association of host and microbiome can facilitate adaptation to new environmental conditions, either through changes in the microbiome, the host, or both[10–13]. There is likely variation among host systems in the exact processes mediating such host-microbiome evolution, including host-microbe coevolutionary interactions as

proposed by the hologenome theory of evolution[14], or less strict coevolutionary interactions [12,15–22]. Fidelity of microbiome transmission (Box 1), the multiple levels of selection and evolutionary interests possible in host-microbe associations[22] are all key determinants shaping the evolutionary dynamics of the association.

An in-depth appreciation of the exact characteristics of host-microbiome systems and their evolution greatly benefits from theoretical approaches[23]. While previous theoretical work has established novel frameworks to study host-microbe systems[24–27]*, the full breadth of well-established evolutionary theory has not been applied to understand the evolution of host-microbe associations.

We propose to to take advantage of existing theory in evolutionary biology to explore and dissect the evolution of host-microbiome systems. We highlight four existing frameworks that address key characteristics of host-microbiome evolutionary dynamics. We discuss how we may borrow useful elements from each of these frameworks, while also highlighting fundamental differences between host-microbe evolutionary dynamics and existing frameworks, pinpointing features of host-microbiome evolution that require the development of new theory. We point to important directions for future theoretical work, while emphasizing the importance of integrating theory and empirical work.

Box 1: Microbiome Inheritance

The fidelity of the microbiome across host generations is the most critical factor that determines whether microbes share the same evolutionary interests as their hosts, thus generally favoring beneficial interactions. Different mechanisms favor fidelity or infidelity in microbiome transmission across host generations (Figure B1).

One process that results in cross-generational host-microbiome fidelity is the vertical transmission of microbes from parents to offspring. Strict vertical transmission, akin to genetic inheritance, occurs through intracellular infection of germ cells, for example observed in aphid-*Buchnera*[28], or carpenter ant-*Blochmannia* interactions[29] as well as in more complex associations between sponges and part of their microbiome[30]*. However, even in the absence of such strict vertical transmission, 'intimate neighborhood transmission'[31] may result in the transmission from parents to offspring, for instance through the covering of eggs with microbes[32], through mode of delivery in humans[33], or through hosts shaping microbial community composition of their environment as a form of niche construction[34]*. Further, vertical microbiome transmission goes beyond direct transmission from parents to offspring: living in proximity (e.g., sharing the same household with relatives) can produce a so-called social microbiome that ultimately promotes microbiome fidelity[35]. Even in the absence of vertical transmission, host genotypes might directly influence the types of microbes that can be taken up and establish in a particular host, shaping microbiome composition and increasing cross-generational fidelity[36]. Environmental transmission can also result in host-microbe fidelity across generations, whenever hosts faithfully acquire the same microbes from the environment every generation, as found in the Bobtail squid–*Vibrio*[37] and stinkbug-*Burkholderia* associations[38]. Whenever the environmental microbial pool responds to selection on hosts, environmental acquisition alone can lead to cross-generational microbiome fidelity, through 'collective inheritance'[26]. A combination of these different processes can shape microbiome fidelity in single host systems, as shown in wood mice (vertical and social transmission)[39]* and sponges (direct and indirect vertical transmission)[30]*.

Despite all these different biological processes that may bolster microbiome fidelity, many host-associated microbes were proposed to lack cross-generational fidelity[22] and the exact degree of microbiome fidelity is often unknown for most host species. In other cases, certain interventions may compromise vertical microbe transmission. Examples include antibiotic therapy on newborns[40,41], or Caesarean section (i.e. delivery mode), which seems to have a transient effect on microbiome composition in newborns and is then further shaped by breast feeding (or its absence) and early life exposure to microbes[42–44].

To quantify the fidelity of microbiome transmission, one can estimate the heritability of microbiome composition, which is the proportion of microbiome variance (e.g., variance in relative abundance of a microbial taxon across hosts in a population) attributable to host genotypic variance. Microbiome heritability has been estimated for only a limited number of plant and animal host species[45], suggesting low microbiome heritabilities in general, although some were on par with heritabilities of important host traits.

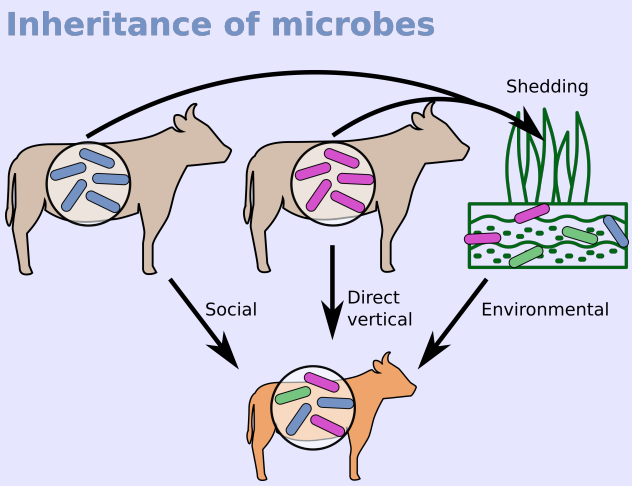

Figure B1: Three primary modes of microbial inheritance. The left mode indicates cross-generational transmission of microbes as a consequence of social acquisition of microbes. The middle mode indicates cross-generational transmission of microbes due to direct parent-offspring transmission. The right mode indicates microbial inheritance mediated by the environment where host offspring acquire environmental microbes that descend from microbes shed by hosts in a previous generation. Additional transmission routes via the environment can also ensure fidelity, as described above.

Adapting Evolutionary Frameworks for Host-Microbiome Systems

We define *host-microbiome evolution* as changes in allele frequencies in the host population and/or in associated microbial communities (through shifts in allele frequencies within microbial lineages or in the relative abundances of microbial taxa) occurring within or across host generations. When host trait variation is explained by genetic variation across either hosts or microbiomes, then host-microbiome evolution can result in changes of host traits over time. A few important processes contributing to host-microbiome evolution include microbial meta-community dynamics[46]*, microbe-microbe coevolution and host-microbe coevolution[47]*, and host dispersal. In general, we lack an understanding of the consequences of natural selection on host-microbiome systems and the inheritance of selected host-trait variation that is mediated by the inheritance of host-associated microbiomes. In the coming sections, we explore how four existing evolutionary frameworks may enhance our understanding of host-microbiome evolution over the timescale in which host microevolutionary dynamics occur (e.g., from a single to possibly thousands of host generations), each addressing different aspects of the evolution of host-microbiome systems, and where the choice of framework will depend on the study question. These frameworks are: (1) Niche construction, (2) Indirect genetic effects, (3) Maternal effects, and (4) Multilevel selection.

Each framework considers how microbiomes interact with the evolution of host traits, and treats host-associated microbiomes essentially as a form of non-genetic inheritance (NGI). NGI involves the transmission of other factors than the DNA (e.g., epigenetic patterns, cytoplasmic transmission, nutrient provisioning, and cultural inheritance), from parents (or other conspecifics) to offspring. Depending on how these non-genetic factors covary and interact

with genetic, environmental and/or stochastic factors, NGI can manifest itself as, for instance, maternal effects[48], ecological inheritance[49], indirect genetic effects[50], or possibly some combination of these effects. There exists a large body of literature on the implications of NGI for plant and animal evolution[51]. Framework 4, which describes how composites of individuals respond to selection that jointly acts on various scales of biological organization, also considers the evolution of microbial traits.

To illustrate concepts, we consider host traits that are jointly mediated by host genomes and host-associated microbiomes. More precisely, we build on previous quantitative genetic approaches by assuming host traits can be additively decomposed into genetic and microbial components[52,53]*. Here, microbiomes are summarized by their additive effect on a host trait, measured by a linear model that explains host trait variation in terms of microbiome composition[53–57]*. This is analogous to classical quantitative genetic methods for summarizing genomes by their additive genetic effects on traits[58]*.

Our focal frameworks are not mutually exclusive. Additionally, we introduce the concepts with mathematical terms to organize the main underlying principles, to enhance clarity of key relationships or dependencies within the frameworks, and to stimulate further theoretical work. Our symbolic representation can be and should be extended to account for additional factors contributing to host-microbiome evolution.

Niche Construction

The framework of niche construction is centered on the reciprocal dynamics of populations and their environment. Niche construction considers the indirect effects of organismal activity on their own or descendants' fitness through environmental modification, and it has been considered an evolutionary process in its own right[59]. There are two interpretations of niche construction, both applicable to host-microbiome systems (Figure 1): (A) Environmental modification by organismal activity (which may or may not have evolutionary consequences for either the host or associated microbes) and (B) an evolutionary process involving feedback between environmental change and organismal evolution.

Niche Construction as Environmental Modification

Niche construction as environmental modification by organismal activity applies to host-microbiome systems in at least two ways (Figure 1*). First, the microbiome of a host (such as microbiomes associated with the host's skin or gut) can be considered a part of the host's environment (Figure 1*, panel (1)). Host activity and host traits that result in microbe acquisition (e.g., through feeding, social behavior, or morphology), and host immune responses that result in selection of microbes, provide mechanisms of niche construction.

Second, the microbiome of a host's immediate surroundings (e.g., microbial communities associated with different food sources, or with surfaces the host comes into contact with) can be considered a part of the host's environment (Figure 1*, panel (2)). Niche construction then occurs when host activity alters the environmental microbiome composition, for instance by shedding microbes into the surroundings, by host-mediated structuring of the environment (e.g., nest building)[60], or the construction of other types of built environments[61]*, by nutrient provisioning (e.g., "priming" of soil microbes by plant roots)[62], and by "farming" activities (e.g., the cultivation of fungi by insects)[63]. Resulting altered microbiome composition or microbial activity may consequently affect host fitness, e.g., by increasing nutrient availability or suppressing pathogens, as has been observed in soil surrounding plant roots[64,65]. Furthermore, if host genotypes exhibit differential fitness in response to such environmental modifications, then niche construction can establish an evolutionary process, as discussed next.

Niche Construction as an Evolutionary Process

The interpretation of niche construction as an evolutionary process is more stringent, as it requires the maintenance of natural selection pressures across generations (Figure 1*, panels (3)-(4)). This process, called ecological inheri-

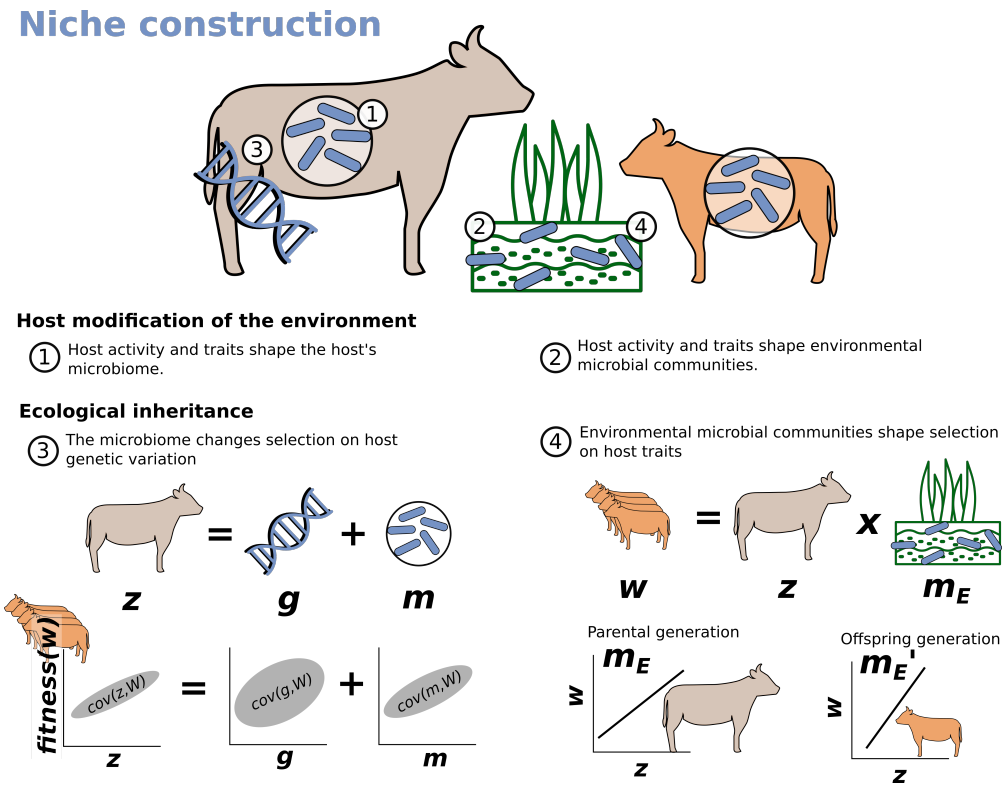

Figure 1. Conceptual overview for applying Niche construction (NC) to host-microbiome evolution. Points (1) and (2) focus on NC simply as environmental modification, and (3) and (4) consider NC as an evolutionary process with ecological inheritance. (1): Interpretation of a hosts microbiome as a component of the host environment modified by host activity (such as diet and immune response). (2): Interpretation of the microbiome in the hosts immediate surroundings as the host environment modified by activity such as shedding and priming. (3): Selection on microbiome-mediated host traits (quantified by the relationship between fitness W and phenotype z as illustrated at the bottom of panel (3)) decomposes into selection on genetic g and microbial m components. Even when selection consistently favors certain values of the host trait z, the resulting selection on host genetic variation depends critically on how microbiome variation is structured. (4): Microbiomes of the hosts immediate environment can shape selection on host traits (e.g., by competing with soil pathogens or altering nutrient availability). Here the slope of host fitness in relation to host trait is determined by the environmental microbiome m_E. In this example, host offspring encounter an environment with a steeper m'_E and consequently experience stronger directional selection.

tance, requires that the activity of a particular organism modifies the environment, and that these environmental modifications influence selection on this organism in subsequent generations[59,66]*. Host-associated microbiomes can establish modes of ecological inheritance in at least two ways.

First, by contributing to host trait variation, microbiomes can facilitate the inheritance of natural selection pressures on host genetic variation associated with that trait (Figure 1*, panel (3)). Consider a host trait z that is additively determined by a genetic component g and a microbiome component m such that $z = g + m$. This model of host trait architecture has previously been applied theoretically by[52], and expanded on by[53]*. Writing $W(z)$ for host fitness as a function of host trait, selection on the host trait is defined as the covariance of fitness and phenotype: $S_z = \mathrm{Cov}(W, z)$. This covariance can be partitioned into selection on host trait components g and m, given by $S_g = \mathrm{Cov}(W, g)$ and $S_m = \mathrm{Cov}(W, m)$, such that $S_z = S_g + S_m$ (panel (3) of Figure 1*). This illustrates that selection at the level of host trait results in indirect selection at the levels of host genotype and host microbiome. Rearranging these components shows that selection on host genotype is mediated by selection on host microbiome (i.e., $S_g = S_z - S_m$). Hence, in this case, the inheritance of microbiome variation across host generations establishes

a mode of ecological inheritance.

Second, microbiomes can modify selection pressures on host variation by altering the host environment, such as resource availability and habitat quality, (Figure 1*, panel (4)). These modified selection pressures can have long-term evolutionary consequences on host traits, such as immunological profiles, tissue structures, and physiological processes that influence specific microbial functions[18]. Summarizing the effect of the environmental microbiome on host fitness as m_E, we can include it as a parameter of the fitness function: $W(z|m_E)$. Then, the correlation of m_E between host generations maintained by host activity (such as shedding) results in the maintenance of selection pressures on host trait variation, and therefore establishes a second mode of ecological inheritance. We note that the focal host trait does not need to be mediated by the host microbiome. Empirically, m_E can be measured as the effect of the microbiome in the hosts immediate environment on host fitness obtained from a linear model that explains the variance of host fitness' both in terms of host traits and in terms of the environmental microbiome composition.

Indirect Genetic Effects

Indirect genetic effects (IGE) are the influence of an individual's genotype on the phenotype of another (typically conspecific) individual[50]. Because IGEs contribute to the expression and inheritance of phenotypic variation, they have important evolutionary consequences. A major application of IGE is to understand the evolutionary consequences of social interactions in social insects[67–70].

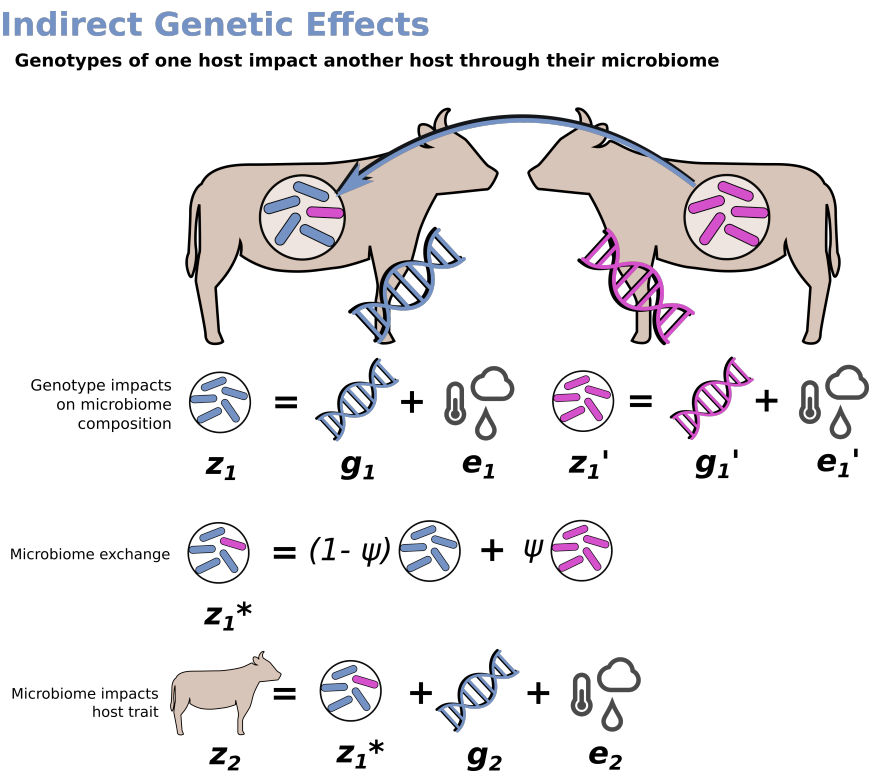

Figure 2. A model of a microbiome-mediated indirect genetic effect. Microbiome composition of a focal host and interacting partner (z_1, z_1' respectively) are mediated by host genetic effects (g_1, g_1') and environmental effects (e_1, e_1'). Updated compositions after microbes are exchanged via social contact, quantified by ψ, are denoted by z_1^* and z_2^*. Then a trait z_2 mediated by microbiome composition z_1^* (in addition to genetic effect g_2 and environmental effect e_2) is affected by the genetic factor g_1' in the interacting partner.

Host-associated microbiomes establish IGE between host individuals when three conditions are met: (1) host genes determine microbiome composition, (2) microbiome composition is transmissible between individuals, and

(3) microbiomes mediate a host trait. When these conditions are met, microbiome transmission (which may occur during social encounters[71]*) forms the mechanism for the genes of one host to influence the phenotype of another host (Figure 2*).

To illustrate this, consider microbiome composition as a host trait z_1 that is mediated by host genotype g_1 such that $z_1 = g_1 + e_1$, where e_1 is an environmental effect. Now consider another host trait that is mediated by host genes and microbiome composition; $z_2 = g_2 + e_2 + z_1$. In this scenario, z_2 is a quantitative host character that is partially mediated by its microbiome such that z_1 and g_2 are obtained as the additive microbiome and genetic effects given by a linear model, with e_2 taken as a standard error term[58]*. Furthermore, with measurements of z_1 across hosts in hand, g_1 and e_1 can then be obtained respectively as the host additive genetic effect and error term for a lineal model of z_1. This IGE model is similar to the starting point taken by[72] to derive their model for interactions with nonreciprocal effects.

Assuming that each individual engages in a single interaction with another randomly chosen individual, we denote by z_1', g_1', e_1' the microbiome trait and trait components for the non-focal interacting individual. Suppose that the social interaction results in an exchange of microbes, so that their microbiome composition traits become similar by $0 \leq \psi \leq 1$. Writing z_1^* as the microbiome composition of the focal individual after the interaction, we then have $z_1^* = (1-\psi)z_1 + \psi z_1'$. Assuming z_2 is expressed after microbe exchange, we can write $z_2 = g_2 + e_2 + (1-\psi)z_1 + \psi z_1'$. The coefficient ψ measures microbial transmission via social contact, quantifying an indirect genetic effect of the interacting partner's additive genetic value g_1' on the expression of the focal individual's trait value z_2.

The IGE framework has applications for understanding host-microbiome evolution, particularly for systems where social transmission of microbes (either through direct contact, or through spatial proximity) between unrelated individuals plays an important role. Because the IGE framework considers interactions between arbitrary individuals, and only incorporates non-random interactions mediated by trait covariances, and not necessarily relatedness[50], it requires additional assumptions to apply to systems with substantial parent-offspring microbe transmission. To model this complementary scenario more directly, the related framework of maternal effects has greater utility.

Maternal Effects

A maternal effect is the influence of a parental phenotype on an offspring phenotype, controlling for genetic variation, mediated by parent-offspring interactions such as maternal care[73]. Host-associated microbiomes establish maternal effects between host parents and host offspring when a host trait is mediated by its microbiome, and part of the host's microbiome is inherited from direct or indirect parent-offspring transmission (Box 1 and Figure 3*). For instance, the composition of mammalian milk is mediated by host microbiomes and is transmitted directly from parent to offspring[74]*.

Assume a host trait decomposes as $z = g + m$, where g is the additive host genetic effect, and m is the additive effect of host microbiome composition. Just as with the niche construction framework, m can be measured as the additive effect of microbiome composition on host trait obtained from a linear model[53,58]*. To account for microbiome transmission directly from parents to offspring, we model the offspring microbiome m' as depicted in Figure 3*, where ι is the proportion of the offspring microbiome inherited from its parent, and $(1-\iota)$ is the proportion acquired from the environment and unrelated hosts, together having the average composition ξ. δ is an ontogenetic differential of the microbiome that is independent of m.

With this, we can model microbiome-mediated maternal effects for a wide range of maternal care patterns (Box 1 for examples). The microbiome's contribution to a maternal effect is measured as the partial regression coefficient of offspring trait on maternal trait, holding genetic variation constant[48]. Then, writing the trait variance as $\mathrm{Var}(z)$, and $\mathrm{Var}(m)$ the component of $\mathrm{Var}(z)$ explained by microbiome variation, the maternal effect is then $\omega = \iota\,\mathrm{Var}(m)/\mathrm{Var}(z)$, which is the slope obtained from regressing the offspring trait z' on the parental trait z after controlling for genetic

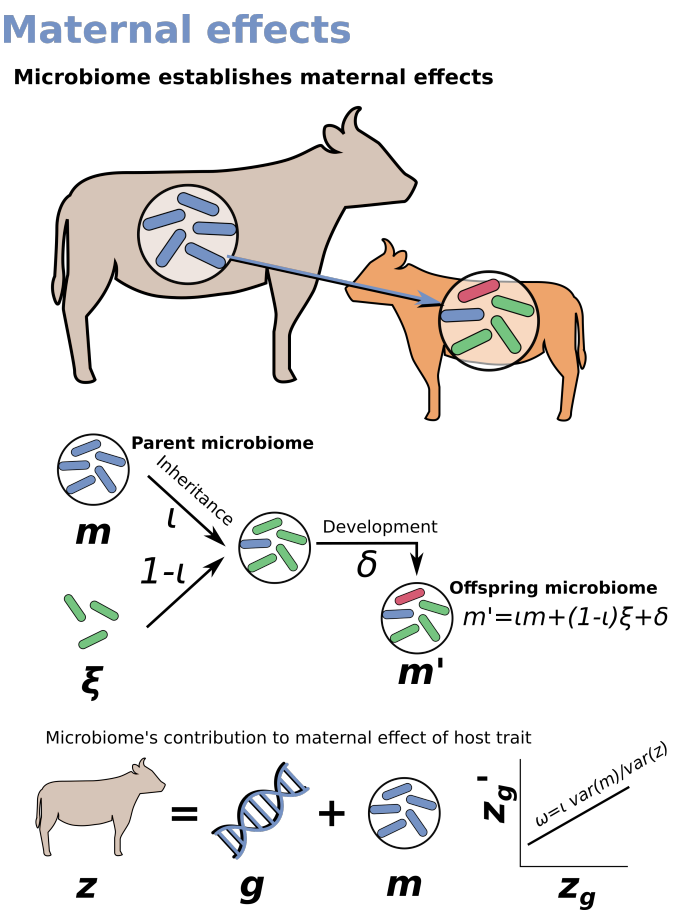

Figure 3. A model of parent-offspring microbiome transmission as a maternal effect. At birth the host offspring acquires a proportion ι of its microbiome from the parent, and the remaining proportion $1-\iota$ from the environment (with composition ξ). The offspring microbiome subsequently shifts by an amount δ throughout development. Quantification of the microbiome-mediated maternal effect is illustrated in the bottom section as the slope ω of the relationship between offspring trait value z'_g and parental trait value z_g, where the subscript $_g$ indicates host genetic variation is held constant.

variation (Figure 3*).

This demonstrates that host-associated microbiomes can be modeled as maternal characters. The application is limited to microbiome inheritance resulting from strict parent-offspring transmission. Furthermore, maternal effects on microbiome composition will likely depend on organism age and diminish as offspring acquire microbes from their environment and unrelated conspecifics[75]* and as within-host microbiome dynamics unfold[25]*. In contrast, estimates of maternal effects on some microbially mediated traits may actually become stronger with age as the early microbiome plays an important role in development[76]*.

Multilevel Selection

This last framework applies to host-microbiome systems in which either microbiome-mediated host traits or microbial traits are heritable and subject to natural selection. Heritable variation in the host, and microbiome fidelity across generations, are essential for the host-microbe system to collectively respond to natural selection (Box 1). In other words, Lewontin's conditions[77] must be met. Given these restrictive conditions, most host-microbiome systems are not considered evolutionary individuals.

Host-microbiome systems exhibit natural selection at the levels of individuals, kin, and as groups of unrelated organisms[78], all explicitly captured by multilevel selection (MLS) models. Because these models do not make a priori assumptions about inheritance, they are useful for understanding when a given inheritance mechanism establishes selection at higher levels of organization. Multilevel selection models have been categorized in two types. MLS1 explains differences among groups in their respective rates of production of contained individuals, while MLS2 explains differences among groups in their respective rates of production of distinct new groups[79,80]*.

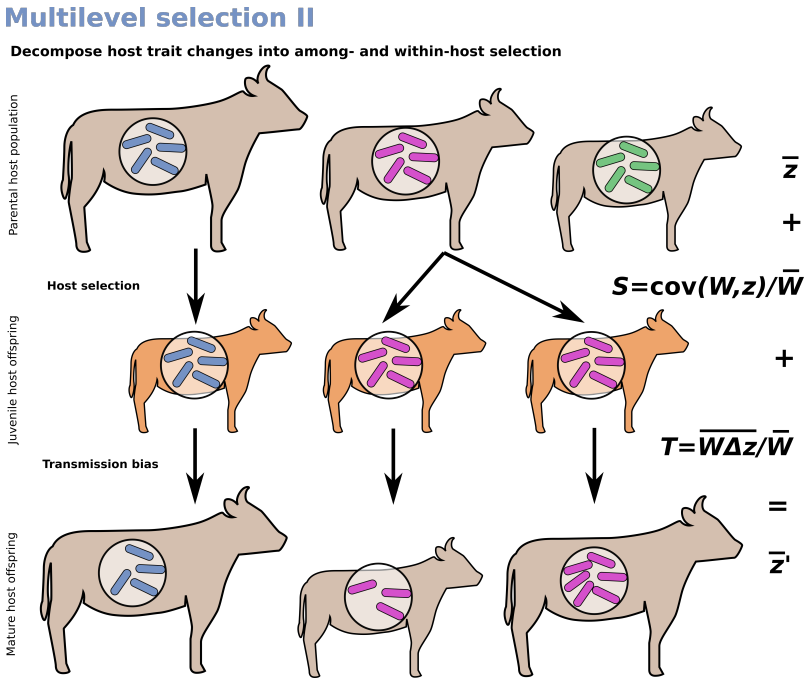

Figure 4. MLS2 in the context of host-microbiome systems. The top row represents the parent population with mean trait $\bar{z}$. Selection among hosts occurs via differential reproduction, forming host offspring in the middle row. Host-level selection is quantified by $S = \mathrm{Cov}(W,z)/\bar{W}$, where W is host fitness and $\bar{W}$ is mean fitness in the parental population. Subsequently, while offspring develop, transmission bias occurs due to within-host microbiome dynamics, and this leads to the mature offspring population (bottom row) with mean trait $\bar{z}'$. Transmission bias, capturing within-host selection, is quantified by $T = \overline{W\Delta z}/\bar{W}$, where Δz is the average ontogenetic differential (due to within-host microbiome dynamics) across offspring of a host parent and $\overline{W\Delta z}$ is the average of the product $W\Delta z$ across host parents.

By testing different MLS models, we can ascertain whether individual, kin, or group selection, or a combination, is the predominant force in the evolution of emergent host-microbe phenotypes. For instance, Hermsen[81]* recently introduced a framework for measuring MLS1 and MLS2 to analyze simulated data. Below, we describe how this general framework may be operationalized for the measurement of MLS1 and MLS2 in the context of host-microbe systems.

Multilevel Selection II

Due to its relative simplicity, we begin with MLS2 (Figure 4*), which focuses on the reproduction of groups. MLS2 occurs when host birth and death rates depend on microbiome composition. Focusing on a microbiome-mediated host trait z, the change in mean traits between parental and offspring generations can be expressed using the Price equation as

$$\Delta\bar{z} = S + T, \tag{1}$$

where $\Delta\bar{z} = \bar{z}' - \bar{z}$ is the difference between offspring mean trait $\bar{z}'$ and parental mean trait $\bar{z}$; host-level selection is captured by $S = \mathrm{Cov}(W, z)/\overline{W}$, with W ($\bar{W}$) host individual fitness (host mean fitness) and the covariance is taken across host parents. The term $T = \overline{W\Delta z}/\overline{W}$ is called *transmission bias*, and here accounts for trait differences resulting from within-host microbiome dynamics and differential colonization abilities of microbes. More precisely, Δz is the average ontogenetic differential across offspring of a given host parent so that $\overline{W\Delta z}$ is the average ontogenetic differential across all offspring[82]*. Following Hermsen's[81]* interpretation, in the context of host-microbiome systems T captures within-host selection on microbial variation. As one could measure microbiome-mediated host traits and host fitness, at least in principle, this approach is particularly amenable to empirical study. In particular, for cases where the mean trait differential $\Delta\bar{z}$ and host-level selection S are directly measurable, the transmission bias term can be inferred as $T = \Delta\bar{z} - S$. This provides an explicit method to partition host trait dynamics into components due to selection among hosts and selection within hosts.

Multilevel Selection I

MLS1, in this context, takes a microbe-centric approach and occurs when microbe strains or species exhibit differential fitness[81]*. It considers traits of different microbe taxa (e.g., strains or species), which we write as α_i for microbe taxon i. These traits may be any phenotype of the microbe, such as growth rates on different carbon sources or the additive effect on a host trait. Selection at different levels is then quantified using covariances that involve the microbial trait and a notion of microbe fitness (w_i for microbe i). We here focus on two measures of microbe fitness: host colonization rates and within-host proliferation rates.

First, we can quantify microbe colonization fitness for a given host by comparing the relative abundances of microbes in that host habitat (e.g., the substrate and other hosts) to the relative abundances of microbes inhabiting the host (top panel of Figure 5*). The relative abundance of microbe i in the host can be written as $\kappa_i' = w_i\kappa_i$, with κ_i being the relative abundance of microbe i within the host habitat. Because κ_i and κ_i' are, at least in principle, measurable, we can quantify microbe colonization fitness as $w_i^{(\kappa)} = \kappa_i/\kappa_i'$, where $^{(\kappa)}$ emphasizes that colonization is used as fitness measure.

Second, we can quantify microbe growth fitness within a given host by comparing the relative abundances of microbes at host adolescence to relative abundances at host maturity. We write γ_i for the relative abundance of microbe i in an adolescent host, and γ_i' for its relative abundance when that same host has matured (bottom panel of Figure 5*). Growth fitness of microbe i can be quantified as $w_i^{(\gamma)} = \gamma_i/\gamma_i'$.

Regardless of the exact fitness measure, MLS1 suggests that selection on microbe trait α (across the entire host-microbiome system) can be partitioned into two components: within and among host selection. Within-host selection (S_{within}), is found by first calculating the covariance of fitness and phenotypes across microbes within each host j separately ($\mathrm{Cov}_j(w, \alpha)$), and then this covariance is averaged across hosts so that

$$S_{\mathrm{within}} = \overline{\mathrm{Cov}(w, \alpha)}. \tag{2}$$

Among-host selection (S_{among}), is found by first averaging fitness and microbe traits across microbe taxa within each host separately (which we write respectively as $\bar{w}$ and $\bar{\alpha}$ for a given host) before computing their covariance across hosts such that

$$S_{\mathrm{among}} = \mathrm{Cov}(\bar{w}, \bar{\alpha}). \tag{3}$$

This approach allows us to connect levels of selection occurring simultaneously across distinct scales of biological organization. More than two levels of organization can be considered by generalizing Hermsen's[81]* framework, allowing for structured populations of host-microbe groups and within-host microbe groups, which is crucial for the

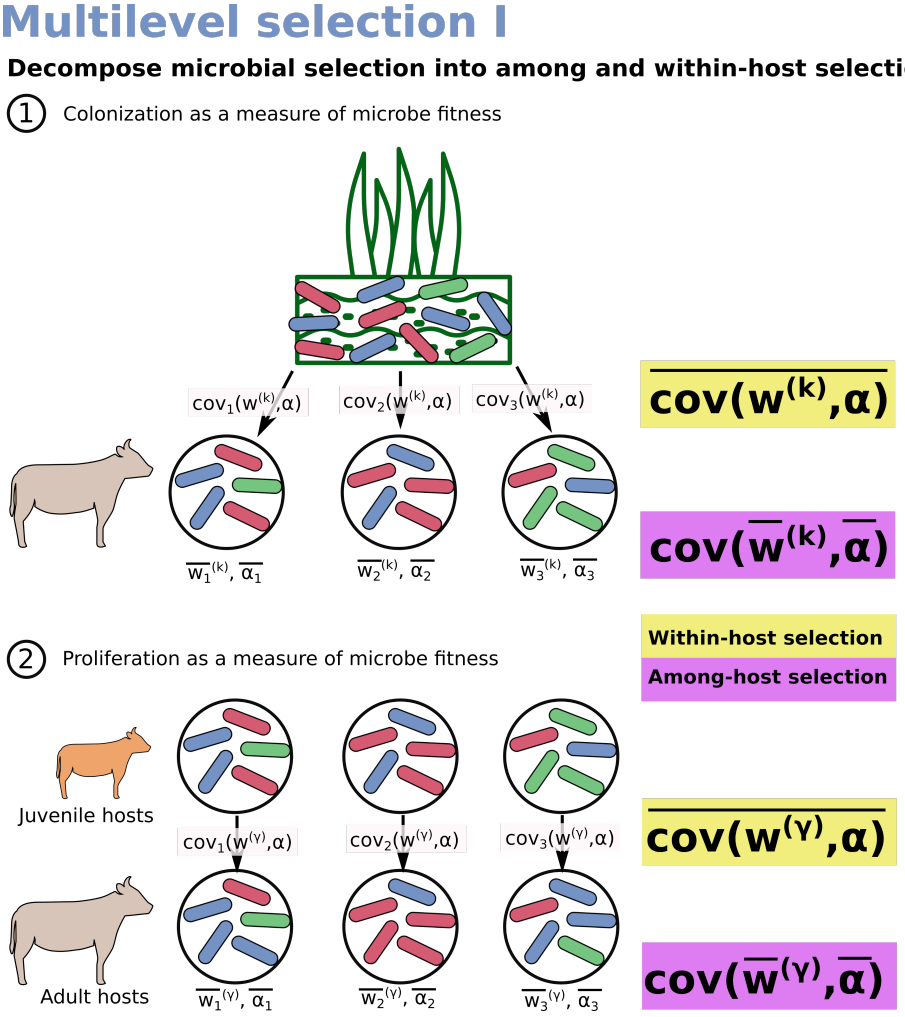

Figure 5. MLS1 in the context of host-microbiome systems. Circles represent host individuals. Microbe individuals are represented by capsule shapes, and their trait values α are indicated by color (red, blue, or green). The top panel (1) illustrates how selection at the levels of host and microbes can be measured when microbial fitness w is taken as the colonization fitness $w^{(\kappa)}$ (see main text). Here microbes in the immediate host environment (depicted by the green rectangle at the top of the figure) differentially colonize hosts (represented by arrows following microbes from the environment into host individuals). Microbe-level selection resulting from these differential colonization abilities for the j-th host is quantified by $\mathrm{Cov}_j(w^{(\kappa)},\alpha)$, and these selection indices are then averaged across hosts to obtain a global measure of microbe-level selection $\overline{\mathrm{Cov}(w^{(\kappa)},\alpha)}$ (highlighted in beige). Host-level selection is quantified by first averaging microbe fitness and trait values within each host (respectively, $\bar{w}_i$ and $\bar{\alpha}_i$ in host i) before taking their covariance across hosts $\mathrm{Cov}(\bar{w}^{(\kappa)},\bar{\alpha})$ (highlighted in magenta). The bottom panel (2) illustrates how these levels of selection may be quantified when using proliferation as measure of microbial fitness. Juvenile hosts are represented by the top row, and vertical arrows connect them to their matured selves. Changes in host microbiomes here are presumed to be a consequence of within host microbiome dynamics, quantified by the growth fitness $w^{(\gamma)}$ (see main text).

application of MLS to real-world systems.

Discussion

Limitations of Existing Frameworks

Each of the discussed pre-existing frameworks is useful for understanding specific cases of host-microbiome evolution, and one useful strategy is to select the framework that fits best to study question and model system used. At the same time, some fundamental properties of host-microbiome systems necessitate expanding these frameworks. Further, these frameworks are not mutually exclusive, and in real-world scenarios where multiple processes and mechanisms act simultaneously, different frameworks will likely interact. For example, niche construction has clear applications for understanding the relationship between host and environmental microbiomes, and consequential selection of host-microbiome systems. Modification of the social environment via microbiome transmission may be considered a form of niche construction, but this framework does not focus on microbiome transmission. The frameworks of indirect genetic effects (IGE) and maternal effects (ME) are particularly useful for understanding the evolutionary consequences of such social and parent-offspring transmission, respectively. In systems with mixed modes of microbiome transmission, both IGE and ME jointly impact the dynamics and could be combined to account for such mixed transmission. Still, IGE and ME treat the consequences of microbiome transmission as fixed effects, limiting their ability to incorporate microbiome community dynamics, host immune response, and variation of transmission other than what is explained by trait covariances.

Multilevel selection is useful as an overarching framework for understanding selection on complex host-microbiome systems. However, it is still under debate whether MLS1 and MLS2 are inequivalent, as the Price equation has supported their equivalency since the 1970s[80,83–85]*. Further, while some group-level phenotypes may have relevance at the individual level, not all will. For example, metabolic complementation between deep sea clams and their chemosynthetic gill endosymbionts for hemoglobin-transported sulfur-based carbon fixation is selected for in the host-microbe assemblage, but not in the individual organisms, because the individual bivalve and bacterial genomes lack genes to complete the pathway[86]*.

Opportunities for Developing Novel Frameworks

Host-microbiome systems provide a number of exciting opportunities for extending and developing theoretical approaches to describe features that are not sufficiently captured by our focal frameworks, as also apparent from the above described limitations of these frameworks. For example, microbiome composition varies over the course of a host's life[87,88]. Here, theory on ontogenetic changes in maternal and genetic contributions to host phenotypic variation[89] may provide useful insights to microbiome changes during host development and its implications for responses to selection. For instance, a maternal signal in microbiome composition that diminishes with host age[25,90]* could be captured by a negative relationship between host age and the contribution of maternal effects. Further, host microbiome composition is shaped by fluctuating microbial abundances resulting in within-host ecological interactions, but such interactions are ignored in the non-genetic inheritance and multilevel selection frameworks that we discussed. Further, microbiomes vary across host organs[90–92]*, but the frameworks we present do not account for this within-host spatial variation.

These frameworks can be extended to account for such ecological details by integrating models of microbial community dynamics into host trait architecture. These biotic interactions are even further complicated by the existence of multiple trophic levels within a microbiome community (e.g., interactions of bacteria with phages or predatory bacteria)[93–95]. Microbes can also show context-dependence in their contributions to host fitness, where they act as mutualists in one environment, while as pathogens in another[96–99]. Theory on fluctuating selection[100] could be used to assess host-microbiome evolution in such a case.

Additionally, there are many opportunities to expand theory of multilevel selection. For example, stochastic simulation of multilevel selection processes could enable the development of new MLS models and theories to

pinpoint the conditions required for cooperation among microbes and hosts to evolve[101]*. Spatial structure shapes the formation of groups that can respond to selection pressures[81,84,102,103], emphasizing the need to incorporate environmental parameters in MLS models. Genetic models for mapping trait selection onto the complex genetic basis for that trait[104] could be used to map group-level phenotypic selection onto individual genotypes. Incorporating genetic parameters into MLS models will enable the use of genome-wide datasets. Further, the impact of host versus symbiont population size and generation time on the rates of co-evolution should be considered[26,105].

Lastly, it may be useful to consider microbiome-mediated host traits as a form of phenotypic plasticity with host microbiome composition as the environmental factor. However, because microbiomes are transmissible between host individuals within a generation, and because theoretical approaches to study reaction-norm evolution have not focused on transmissible factors[106–110]*, there is a need to extend reaction norm theory to account for the complexity of host-microbiome systems. Microbiome transmission, either directly from parent to offspring as in the case of maternal characters or socially as in the case of IGE, can explain patterns of microbiome variation that then feedback on host-microbiome evolution[71]*. Moreover, microbiome-mediated plasticity can act at different levels and time scales[111]. For example, a new environmental challenge can be accommodated fastest by ecological changes in microbiome community composition, followed by evolutionary genetic changes in single microbial lineages. Such microbiome plasticity can further selectively favor hosts that either select the beneficial microbes from the environment or ensure their vertical transmission, as a kind of microbiome-mediated Baldwin effect[111]. Hence, application of the phenotypic plasticity framework to host-microbiome evolution would need to be extended to account for environmental factors that are transmissible between hosts within and across generations. Doing so will be useful for understanding microbiome-mediated adaptation to novel environments[11]* and microbiome-mediated local adaptation[10]*.

The Need for "Empirically Friendly" Theory

We feel that it is important to recognize that existing theoretical frameworks were often developed with biological systems in mind other than host-microbiome systems[112]*. Not only has this resulted in frameworks that lack key aspects of the biology of host-microbiome systems (as we discuss in the previous section), but it has also limited the application of these frameworks to host-microbiome systems in the laboratory and the field. To make these frameworks maximally useful, it is important that the validity of the underlying assumptions of these frameworks is determined empirically. It is also crucial that these frameworks are constructed in a way that makes their predictions empirically testable, given the technical limitations of empirical microbiome research. For example, for many host-microbiome systems, empiricists are limited to surveying relative abundance or presence/absence of microbial taxa, and for theory to be maximally useful it must generate predictions for these microbiome attributes. Tailoring theory in this way will likely require direct collaborations between theoretical and empirical microbiome scientists, to enable an iterative refinement of theory with information from actual host-microbiome systems. This requires host-microbiome systems that are sufficiently tractable to measure relevant quantities such as correlations among microbiome composition, host traits, and host fitness. Example systems include the water flea *Daphnia* (Box 2), the nematode *Caenorhabditis elegans*[13,113], the zebrafish *Danio rerio*[114], the bobtail squid *Euprymna scolopes*[115]*, or insect-*Wolbachia* associations[116].

Box 2: Testing Theory Using the Daphnia Host System

The water flea *Daphnia* (Figure B2), a freshwater crustacean, is a model organism in many biological fields, such as ecotoxicology[117], epidemiology[118], and eco-evolutionary dynamics[119]. Increasingly, it is also used to study host-microbiome evolution[120]. Several features make the *Daphnia*-microbiome system uniquely suited to test theoretical predictions:

- Its gut microbiome is relatively simple, has distinct core members, and is clearly separated from the surrounding aquatic microbiota[121].
- Microbiome composition is shaped by both environmental conditions (e.g., temperature[122]) and host genotype[123].
- The microbiome influences host fitness[124,125] and may mediate plastic responses to the environment[126].
- Many phenotypic traits—such as defense induction[127]*—are well-characterized and may be partly microbiome-mediated.
- Both horizontal and vertical microbiome transmission occur[128,129].
- Experimental manipulations (e.g., gnotobiotic rearing, microbiome transplants) are feasible, and host genotypes can be controlled via clonal lineages[125,130]*.

These advantages enable rigorous tests of theoretical frameworks. Below, we outline two illustrative experimental designs:

Testing Niche Construction Daphnia-microbiome associations can be evolved in mesocosms under three treatments (Figure B2 right panel):

1. **Environmental Microbiome Inheritance** Hosts and their microbial environment are maintained together, allowing microbial shaping of the environment and ecological inheritance via environmental modification.
2. **Vertical Microbiome Transmission Only** Hosts are transferred to fresh environments regularly, preserving vertical transmission but preventing environmental shaping.
3. **No Ecological Inheritance** Both host-associated and environmental microbes are excluded at each transfer; microbiomes are provided via inoculum from naïve Daphnia.

Each treatment is subjected to an environmental stressor (e.g., toxins, elevated temperature) to:

- Evaluate whether niche construction promotes adaptation (via host reproduction or population growth).
- Investigate ecological and molecular changes (e.g., microbiome dynamics, host/metatranscriptomic responses).

A follow-up transplant experiment can assess whether evolved hosts benefit specifically from shaped microbial environments.

Testing Multilevel Selection Microbiomes can be evolved under two selection regimes (Figure B2 left panel):

1. **Among-Host Selection** Microbes are transferred from the most reproductively successful hosts, favoring mutualistic strains that benefit both host and microbe.
2. **Within-Host Selection** Microbes are transferred from hosts with low reproductive output, favoring strains that enhance their own replication at a potential cost to the host.

After experimental evolution, microbial communities are introduced to a standardized host population. Differences in host fitness and microbial load can reveal the relative strength and outcome of different levels of selection.

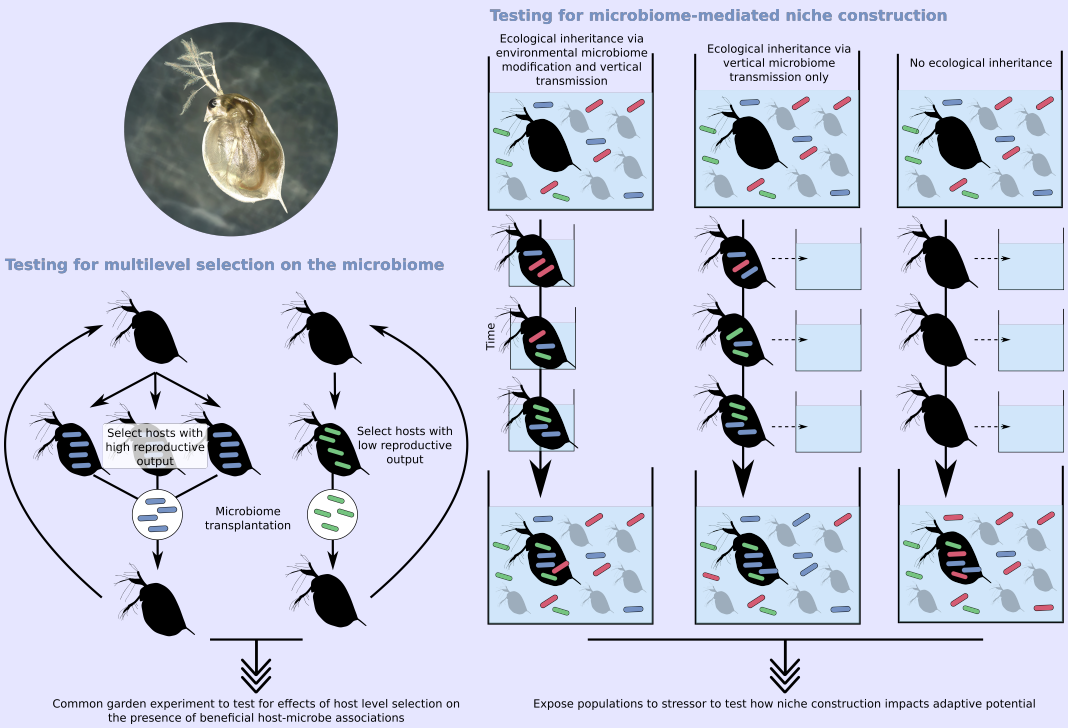

Figure B2: Experimental approaches for testing MLS1 (bottom left) and evolutionary niche construction (right) using the water flea *Daphnia* system. MLS1 can be tested by maintaining two treatments: (left) Microbes from the hosts with highest fitness are isolated and transplanted into subsequent host generation and (right) microbes from hosts with lowest fitness are isolated and transplanted into subsequent host generation. Evolutionary niche construction can be tested by maintaining three treatments: (left) Mesocosms are maintained including host-associated and environmental microbes, (middle) host-associated microbes are included but environmental microbes are excluded, and (right) both host-associated and environmental microbes are excluded. Subsequently, host populations are exposed to a stressor to test whether ecological inheritance via environmental modification (left) or vertical transmission (middle) promote host adaptation compared to the absence of ecological inheritance (right). Photo credit: Héléne Vanvelk and Maxime Fajgenblat (KU Leuven).

Conclusion

We presented four frameworks developed in the fields of evolutionary biology that help to generate new insights into host-microbiome evolution. In order to capture the biological diversity of such host-microbe systems and produce empirically testable predictions, these frameworks require thoughtful expansion and in some cases the development of novel theory, in close collaboration with empirical microbiome scientists. We envision that the initial result will be a mosaic of theoretical frameworks, each tuned to the set of processes considered and questions asked, with the initial goal of clarifying concepts. Such a mosaic could eventually lead to the identification of general principles underlying the interactions between microbes and their animal and plant hosts, greatly expanding our understanding of the evolutionary consequences of the host-microbe associations omnipresent across the tree of life.

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