Trends in Microbiology

CellPress

Review

Microbiome breeding: conceptual and practical issues

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Microbiome breeding is a new artificial selection technique that seeks to change the genetic composition of microbiomes in order to benefit plant or animal hosts. Recent experimental and theoretical analyses have shown that microbiome breeding is possible whenever microbiome-encoded genetic factors affect host traits (e.g., health) and microbiomes are transmissible between hosts with sufficient fidelity, such as during natural microbiome transmission between individuals of social animals, or during experimental microbiome transplanting between plants. To address misunderstandings that stymie microbiome-breeding programs, we (i) clarify and visualize the corresponding elements of microbiome selection and standard selection; (ii) elucidate the eco-evolutionary processes underlying microbiome selection within a quantitative genetic framework to summarize practical guidelines that optimize microbiome breeding; and (iii) characterize the kinds of host species most amenable to microbiome breeding.

Highlights

Microbiome breeding is a new technique to produce microbiomes that benefit hosts through artificial selection shaping the genetic composition of microbiomes, independently of any selection shaping host genomes.

Theory predicts that microbiome breeding is possible whenever microbiomes can be transmitted between hosts with sufficient fidelity; successful microbiome-breeding methods therefore optimize microbiome stability and transmissibility.

Microbiome-encoded genetic factors that influence host traits (e.g., health) are transmitted between hosts with different fidelities, ranging along a transmission–fidelity continuum; modeling a transmission continuum of microbiome-encoded genetic factors requires novel approaches.

Microbiome breeding improves host traits by leveraging transplantable, microbiome-encoded genetic effects, with applications in agriculture, medicine, and microbial engineering.

Microbiome transmission and microbiome breeding

To cure a patient from life-threatening infection by the gut pathogen *Clostridioides difficile*, one effective treatment is to purge the gut of all microbes with antibiotics, then reinoculate the gut with a microbiome transplanted from another individual [1]. What kind of donor individual should one choose as source of the transplanted microbiome? The microbiome donor should ideally be a healthy individual with a health-promoting gut microbiome, not an ailing individual who may carry an inferior microbiome. Likewise, when a honeybee worker is born, the newborn worker receives her initial gut microbiome as transplants from healthy nurse bees, not from ailing bees, such that the newborn worker's gut is seeded with a health-promoting microbiome [2,3]. These choices to preferentially transplant specific microbiomes with beneficial effects on host traits, while excluding from transplanting inferior microbiomes, have implications for the prevalence of health-promoting microbiome elements in host populations. We refer here to this differential microbiome propagation that depends on the microbiome's effects on host traits as **microbiome selection** (see Glossary). Such differential microbiome propagation can result in **microbiome evolution**, involving changes in frequencies of microbiome-encoded genetic factors that act to improve host traits (Figure 1).

One key practical issue determining the success of microbiome selection is the fidelity with which microbiome elements can be transplanted from one host to the next, specifically the transplant fidelity of microbiome-encoded genetic factors that affect host traits, including the so-called **microbiome function** that describes the cumulative effects of a microbiome on the host. Other fundamental issues are the extent to which variation in microbiome composition among hosts contributes to the overall phenotypic variation within a population of hosts (e.g., variation in host health or growth), and what fraction of this microbiome-associated variation for host traits is heritable and can respond to microbiome selection (Box 1). Our review aims to elucidate these key issues, **microbiome transmission** and

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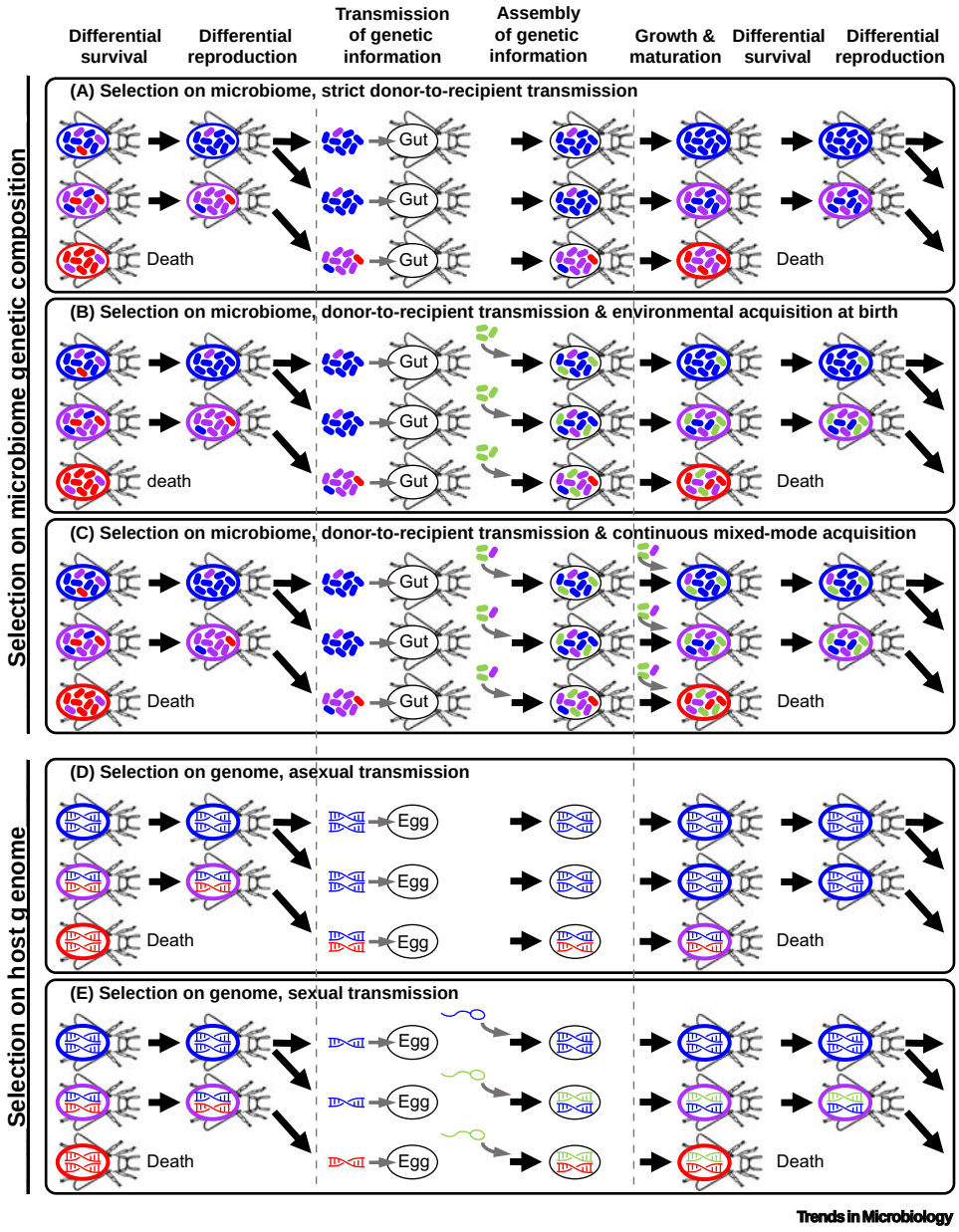

Figure 1. Comparison of microbiome selection that shapes genetic composition of microbiomes (A–C) versus natural selection that shapes host genomes (D,E). The timelines from left to right are linear representations of the kind of selection cycles shown in Box 3. Selection on the genetic composition of microbiomes (A–C). Blue microbes contribute beneficially to a host phenotype (blue bee body), red microbes contribute detrimentally to the host phenotype (red bee body), and purple microbes contribute intermediately. Changes in microbiome composition are illustrated for a microbiome that can be propagated with relatively high fidelity, such as the honeybee gut microbiome. Selection on microbiome-encoded genetic effects can occur under high-fidelity microbiome transmission from donor to recipient (A); under imperfect microbiome transmission because of some environmental acquisition of microbes (green microbes shown in B at the transmission/assembly stage); or under imperfect microbiome transmission and repeated acquisition from the environment or from other individuals throughout the recipient's life (C). Selection on host genomes (D,E). Differential survival and differential reproduction alter the frequencies of alleles between generations. Differential survival is illustrated as the selectively inferior red allele/chromosome that becomes extinct because it contributes detrimentally to the

(Figure legend continued at the bottom of the next page.)

Glossary

Co-propagation: linked replication of host and associated microbes between host generations (e.g., an endophytic fungus is inherited from the mother plant through a seed; a gut microbiome is inherited from parent to offspring).

Cycling: repeated transplanting of microbiomes between different generations of hosts or between different locations of the same host.

Ecological or environmental filtering: natural culling of microbe species that are unable to tolerate conditions in a particular environment.

Heritability versus heredity: heritability and heredity are very different concepts. Heredity refers to inheritance, the transfer of genetic information from parent to offspring. In contrast, heritability is a quantitative genetic parameter that refers to a specific population and specific trait. Heritability is the proportion of variation in a trait among individuals in a population that is due to transmissible genetic variation among individuals in that population.

Host control, partner choice, symbiont choice: capacity of a host to selectively recruit beneficial symbionts into symbiosis, selectively reward beneficial symbionts to amplify their beneficial effects, or selectively exclude or sanction ineffective symbionts to minimize negative effects; ability of a host to dictate ecological and evolutionary processes affecting a microbiome.

Microbiome assembly: process of establishing a microbiome, for example, when a newborn uninfected host is colonized by its first microbes. Assembly dynamics is predictable in some host–microbiome associations, with the same microbial taxa colonizing the uninfected host.

Microbiome engraftment: process of establishing a transplanted microbiome in a new host, for example, by grafting the transplanted microbiome into a resident microbiome.

Microbiome evolution: in the context of microbiome breeding, change in the genotype frequencies of the microbe populations that make up the microbiome, as a result of selection, horizontal gene transfer, immigration, mutation, or genetic drift.

Microbiome function: cumulative effects of a microbiome on the host, for example, metabolome properties of microbiomes that affect host phenotypes.

the **heritability** of microbiome effects on host traits (i.e., the proportion of total phenotypic variation in the host population that is due to variation in microbiome-encoded genetic factors that can be transmitted from one host to another; Box 2). We outline a quantitative genetic framework and summarize practical guidelines to optimize microbiome engineering through artificial selection on microbiomes.

Artificial selection on microbiomes

Microbiome selection is a new technique for generating microbiomes with beneficial effects on hosts [4–13]. Several methods of microbiome selection can be used (Box 3), each with potential advantages and disadvantages. These methods share that they (i) cycle microbiomes repeatedly through association with hosts (Box 3) and (ii) impose a selection step where desirable microbiomes are preferentially transplanted between hosts, while excluding from transplanting the undesirable microbiomes (Step 3 in the cycles shown in Box 3). This is the same kind of differential transplanting of microbiomes that humans perform when curing a patient from infection with *C. difficile*, except that the transplanting is done repeatedly in a controlled experiment involving large sample sizes of plant or animal hosts, as well as under rigorously standardized conditions, selection rules, and transplanting protocols. It is also possible to artificially select on microbiomes in the absence of a host (host-independent microbiome selection, e.g., microbiomes in flasks) [14–20], but we focus here on microbiome selection that improves host traits because of the strong potential for improving microbiomes of domesticated plants and animals.

When selecting microbiomes for transplanting between hosts, composition and functional properties of the microbiomes are typically not measured directly. Instead, microbiome functions are estimated indirectly by measuring some microbiome-influenced trait of the host (e.g., health, growth), which is easier to quantify and is also the focus of microbiome selection [4]. Like plant and animal breeding programs, microbiome selection is focused on achieving phenotypic outcomes in terms of the traits of plant and animal hosts, and is agnostic to the specific microbiome composition and combination of microbiome functional traits (e.g., microbiome metabolome) that contribute to the host traits of interest. Indeed, many microbiome compositions might be possible to generate similar microbiome functional traits and thus similar host-phenotypic outcomes, and several features of microbes (e.g., horizontal gene transfer between microbes [21]) may contribute to the change of functionally relevant genetic material of microbiome communities.

Most experiments using microbiome selection so far improved function of root-associated microbiomes affecting growth of plants [5–8,11,13,22]. It is also possible to select on microbiomes associated with animals [10,23], for example, to engineer gut microbiomes that protect bees against pesticide (Box 3). Microbiome selection can potentially also operate by **cycling** desirable microbiomes repeatedly through the same individual, or transplanting microbiomes between different parts of a modular organism (Box 3).

Microbiome breeding steers eco-evolutionary processes

Evolutionary and ecological processes under microbiome selection are highly intertwined such that it can become difficult to separate these processes (Figure 1). A critical feature of microbiome selection is that the entangled eco-evolutionary processes are partly under the influence of

Microbiome heritability: as an extension of the quantitative genetic concept of trait heritability, in the context of microbiome breeding, the heritability of microbiome effects on host traits describes the proportion of total phenotypic variation in a host population that is due to variation in microbiome-encoded genetic factors that are transmissible between hosts (see Box 2 for additional explanation).

Microbiome selection: differential retention and transplantation of microbiomes, depending on the effects that microbiomes have on host traits.

Microbiome stability and microbiome persistence: community-ecological processes maintaining constancy of microbiome function during association with a host, combining processes of microbiome resistance, resilience, robustness, and inertia (persistence) in the face of perturbation, as well as the ability of the host to steer these processes.

Microbiome transmission: perpetuation of a microbiome from donor host to recipient host, for example, from parent to offspring, between mates, or between siblings (e.g., between members of a social-insect colony).

Response to selection: the difference in average phenotype of a population before selection compared to the average phenotype of that same population after selection.

Strength of selection: the difference in average phenotype of a population before selection and the average phenotype of the subpopulation contributing offspring to the next generation, also called the selection differential.

Transfaunation: transfer of microbiomes from a healthy to a sick animal, to treat digestive disorders in ruminants (cattle, goat, sheep) or to increase the detox function of gut microbiomes to degrade plant toxins.

phenotype that is selectively inferior (red bee body). Differential reproduction is illustrated as the blue allele/chromosome that contributes beneficially to the selectively favored phenotype (blue bee body) and consequently out-reproduces other alleles/chromosomes. Selection on genomes can occur under asexual transmission of heritable information from parent to offspring (D), or under sexual transmission that mixes heritable information between parents (E).

Box 1. A quantitative genetic framework for microbiome selection

A-C: Brief tutorial of quantitative genetic partitioning of phenotypic variation and for visualizing heritability

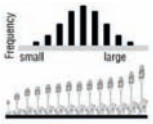

Figure I.A. Three sources contribute to total phenotypic variation in this plant population:
a. differences in genes carried by different plants;
b. differences in environments experienced by different plants;
c. different genotypes responding differently to environmental differences, so-called gene-by-environment interactions (e.g., a gene promotes growth in one environment, but not in a different environment).*

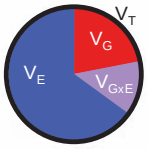

Figure I.B. Total phenotypic variation V_T of the population in Figure I.A can therefore be partitioned into:
a. V_G = variation due to genetic differences between plants;
b. V_E = variation due to environmental differences between plants;
c. V_{GxE} = variation due to differences in genotype-by-environment interaction.*
Different populations of the same species can differ in the contribution of genetic factors (V_G) to total phenotypic variation (V_T) (compare Figures I.B & I.C).

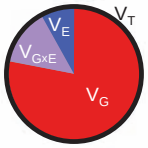

Figure I.C. Populations can therefore differ in heritability H^2, which is the proportion of V_T that can be attributed to differences in genes (V_G) carried by different individuals. That proportion H^2 is visualized here as the ratio of the red area relative to the area of the entire circle**, that is: heritability = $H^2 = V_G / V_T$. In the examples here comparing two different populations with high (Figure I.C) versus low heritability (Figure I.B), respectively, much versus little of the total phenotypic variation is due to genetic differences between individuals. In a selection experiment, the evolutionary response to selection is higher when heritability is high, because phenotypes of individuals are more reliable predictors of their genotypes if heritability is high.

D-G: Quantitative genetic variance-partitioning extended to microbiome selection

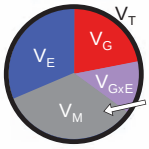

Figure I.D. If some of the phenotypic variation in the plant population in Figure I.A is due to differences in associated microbiomes, the phenotypic variation in host traits attributable to differences in microbiomes (V_M) can be partitioned out as one specific type of environmental factor.*

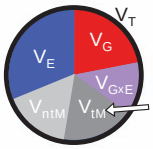

Figure I.E. Only some microbiome-encoded genetic factors can be transmitted with fidelity from one host generation to the next and can persist with fidelity throughout the lifetime of the host, so it is important to distinguish between phenotypic variation due to microbiome components that are transmissible (V_{tM}) and those that are non-transmissible (V_{ntM}). Only V_{tM}, the phenotypic variation due to transmissible microbiome-encoded genetic factors, can contribute to the evolutionary response to microbiome selection, and therefore V_{tM} / V_T would be the heritability relevant to microbiome selection.

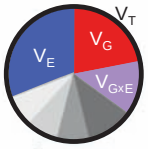

Figure I.F. More realistically, microbiome-encoded genetic factors differ in transmissibility along a continuum, ranging from high-fidelity transmission (dark grey) to low-fidelity transmission (light grey). Microbiome heritability is visualized here as the ratio of the grey areas to total phenotypic variation, weighted by relative microbe transmissibility (shadings of grey). Incorporating a transmissibility continuum in quantitative genetics requires special modeling approaches.

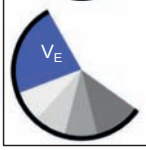

Figure I.G. To maximize response to selection in a microbiome-selection experiment, one strategy is to use a genetically invariable host population (e.g., inbred or clonal population), minimizing phenotypic variation due to variation in host genotype (i.e., V_G is very small or absent).*** Second, rigorously standardized conditions minimize the contribution of variation in environmental factors to total phenotypic variation (V_E is smaller). Both strategies maximize relative contribution of transmissible microbiome-encoded genetic factors (darker greys) to total phenotypic variation, which facilitates response to microbiome selection.****

Take-home messages: Microbiome selection is easiest if:
- there is no or little genetic variation of the host contributing to total phenotypic variation ($V_G \rightarrow$ zero)***
- microbiomes strongly affect phenotypic differences between hosts (V_M is large)
- microbiome-encoded genetic factors can be transmitted and perpetuated with high fidelity (V_{tM} is large)
- the host optimally affects microbiome assembly and stability through effective host control (V_{tM} is large).

* To simplify the pie charts, we do not show in any of the charts the contribution of measurement error to phenotypic variance; and in Figures I.D-G, we do not show the contributions of interactions between microbiome-encoded factors & host genes & environmental factors.

** To simplify, we visualize here broad-sense heritability H^2 that includes all kinds of genetic variation, although technically, in sexual populations, narrow-sense heritability h^2, which is restricted to additive genetic variation, is appropriate.

*** Under a standard view of selection, because V_G is experimentally reduced in Figure I.G to zero by using a genetically invariable host population, standard heritability is zero ($V_G / V_T = 0$), leading to the false conclusion that selection on phenotypic variation is not possible in this population. However, if microbiome-encoded genetic effects influence host traits, if these microbiome effects vary between individuals, and if these effects are transmissible between hosts, then heritability of microbiome effects on host traits is >0, and consequently microbiome selection can change average host phenotype between host generations.

**** Minimizing genetic variation of the host ($V_G \rightarrow$ zero) obviously limits the chance for G_{host} x $G_{microbiome}$ interactions (i.e., different host genotypes respond differently to different microbe genotypes); therefore, including variable host genotypes in a selection experiment could potentially increase response to selection beyond what would occur with invariable host genotypes.

the host (**host control**), and the challenges of host-mediated microbiome breeding are therefore fundamentally different from microbiome breeding in the absence of a host [14–20,24]. Figure 1 illustrates how microbiome selection differs from standard natural selection and entangles evolutionary and ecological processes:

(i) Ecological processes that change microbiome composition. Microbiome communities undergo ecological changes during a cycle of microbiome selection (Figure 1 and Box 3), for example, when different microbial species compete with each other within hosts; when population size, density, or relative abundance of specific microbes changes; or when microbial communities assemble in an uninfected new host under **ecological filtering** [25–28]. Microbial species may also enter a microbiome from external environmental sources, or may become extinct in a microbiome, and microbiomes may therefore undergo some turnover that may, or may not, alter microbiome function and phenotypic effects on the host. Moreover, the functional traits expressed by microbes making up the microbiome (e.g., microbial metabolome) and their effects on host traits may depend on the relative and absolute abundances of other microbes, their network-interaction structure, and the specific environments they experience. These ecological factors and processes can potentially operate at any step in the cycles of microbiome selection shown in Box 3, but do not preclude microbiome breeding if critical microbiome components and functions are preserved over time with adequate fidelity.

(ii) Evolutionary processes that change the genetic makeup of microbe populations of individual species comprising the microbiome community. Populations of the same microbial species evolve within microbiomes whenever the frequencies of genetic variants in such a population change over time [29,30], due to natural selection, genetic drift, mutation, immigration, or horizontal gene transfer. For example, natural selection may favor microbial genotypes that are better adapted to the host, to the rhizosphere–soil environment in the case of plants, or to the microbiome transplanting method. This is evolution changing allele frequencies within populations of the same microbial species, operating potentially at any step in the cycle of microbiome selection.

(iii) Evolutionary processes that operate at the microbiome level and that simultaneously change the genetic makeup of many components comprising a microbiome. A second kind of evolutionary change acts on entire microbiomes when microbiomes are chosen, or are eliminated, before transplanting microbiomes between hosts. Unlike the processes summarized under (i) and (ii) above that potentially operate at any step of the cycle of microbiome selection, process (iii) is stage-specific and operates only at Step 3 in the cycles shown in Box 3. This is the key selection step of microbiome breeding, the selective perpetuation or termination of microbiomes, depending on each microbiome's collective properties and its effects on host traits. This differential transmission of microbiomes to new hosts is also the step in the multilevel selection process when a microbiome breeder can minimize any detrimental variants that may spread in microbiomes of some hosts (e.g., by choosing microbiomes for transmission from only those hosts that exhibit the very best trait values). Just as the evolution of individual microbe species involves change in allele and genotype frequencies, evolution at the level of microbiomes is ultimately manifested by changes in the relative frequencies of microbial genotypes and functional–genetic units that together make up the microbiome.

An important distinction between standard selection that causes changes in host genomes (Figure 1D,E) versus selection that causes changes in the genetic composition of host-associated microbiomes (Figure 1A–C) is that host genomes are comparatively stable. Except for mutation, recombination, and segregation, host genomes typically do not change dynamically during a selection cycle, and host genomes can therefore be categorized as having high within-generation stability (i.e., high persistence fidelity) and high between-generation transmission fidelity. In contrast, the

Box 2. What is heritability relevant to microbiome breeding?

The heritability of a trait quantifies the degree to which trait variation in a population is due to variation in transmissible genetic factors. The estimated heritability of a trait can be used to predict short-term evolutionary responses to natural or artificial selection [38].

Importantly, heritability is always defined with respect to a specific trait (e.g., size, health), but the commonly used phrases 'heritability of the microbiome' or 'microbiome heritability' are often used imprecisely because traits are inadequately specified, or heritability estimates are used to infer incorrectly an inheritance by microbiome transmission between hosts. Figures IA–F clarify these issues.

In the simplest quantitative genetic model, an individual organism's phenotype depends only on its own genotype and the environment it experiences (Figure IA). At the population level, the heritability of one of the organism's traits is then defined as the proportion of trait variation among individuals in the population that is caused by transmissible genetic variation among individuals.

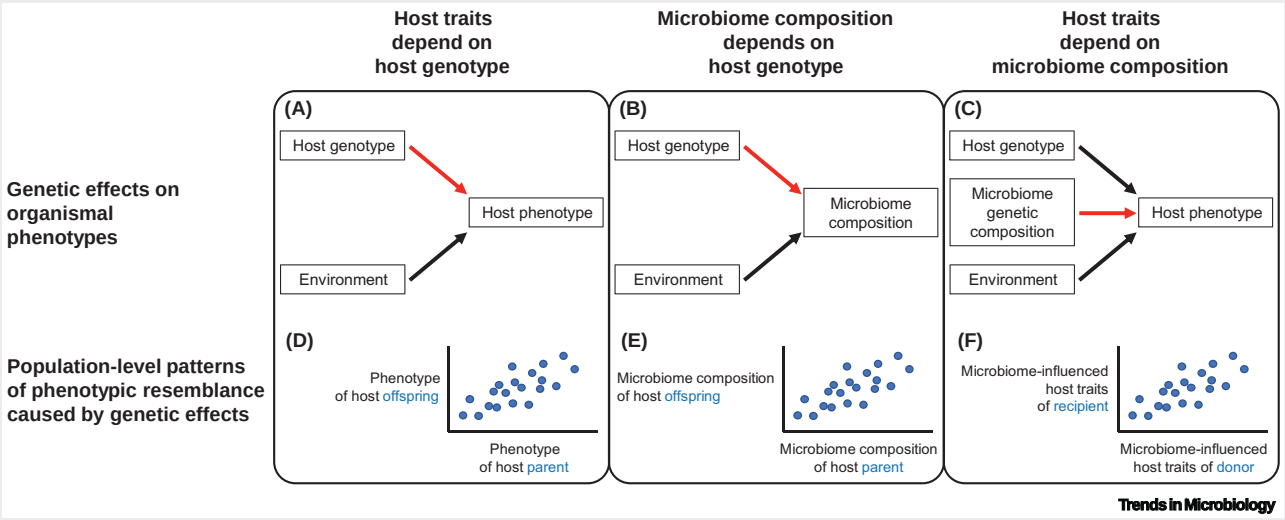

Figure I. Genetic effects on organismal phenotypes (A–C), and corresponding population-level patterns of phenotypic resemblance caused by these genetic effects (D–F).

The composition of a host's microbiome can be treated as a property or trait of the host (Figure IB), affected by host genotype and environment, just like any other host trait (Figure IA). Many publications define 'microbiome heritability' in this way (e.g., [28,39,41,76–78]), effectively asking whether host genotype predicts microbiome composition. This definition of 'microbiome heritability' can be more clearly labeled as 'heritability of host effects on microbiome composition', or in short, 'heritability of microbiome composition'. This definition does not imply that any component of the microbiome is transmitted vertically from parent to offspring host, but simply that there is an association between host genotype and microbiome composition [33,41,60]. Such an association could mechanistically arise each generation from scratch, in complete absence of microbe transmission between hosts, if certain host genotypes favored colonization by certain acquired microbes. Also, heritability of microbiome composition does not necessarily imply any functional impact of the microbiome on host phenotype.

An individual host's phenotype may also depend to some degree on the genetic composition of its microbiome (Figure IC). If so, microbiome-encoded genetic effects on host traits can contribute to phenotypic variation among hosts. However, for these microbiome-based genetic effects on host traits to respond to selection on host traits – and potentially contribute to microbiome breeding to improve host traits – they must be transmissible from donor to recipient host. Genetic components of the microbiome that cannot be faithfully transmitted between hosts may still influence host traits but cannot be shaped by microbiome breeding for host traits. We can define 'heritability of microbiome effects on host traits' as the proportion of total host phenotypic variance that is caused by transmissible microbiome-based genetic effects on host traits.

We can estimate this heritability of microbiome effects on host traits by quantifying the phenotypic resemblance between donor and recipient hosts (Figure IF), while experimentally or statistically controlling effects of host genotype and environment. Thus, the heritability of microbiome effects on host traits requires (i) mechanistic links between microbiome genetic composition and host phenotype, such that variation in microbiome genetic composition contributes to phenotypic variation among hosts; and (ii) sufficiently faithful transmission of these microbiome-based genetic effects on host traits from donor to recipient host.

Take-home messages:. 'Heritability of microbiome effects on host traits' (Figure IC,F) is different from the 'heritability of host effects on microbiome composition' (Figure IB,E) that has been most frequently discussed and estimated in the literature. The latter definition treats microbiome composition as a property of the host and is useful to evaluate the degree to which microbiome composition depends on host genotype. Only the former definition is useful for microbiome breeding seeking to improve microbiome-influenced host traits, where the host phenotype is treated as a property of the microbiome that can be shaped by selection acting on the genetic composition of the microbiome.

Box 3. Methods of microbiome transplanting for microbiome selection

Selection on host-associated microbiomes is possible using several microbiome transplanting schemes:

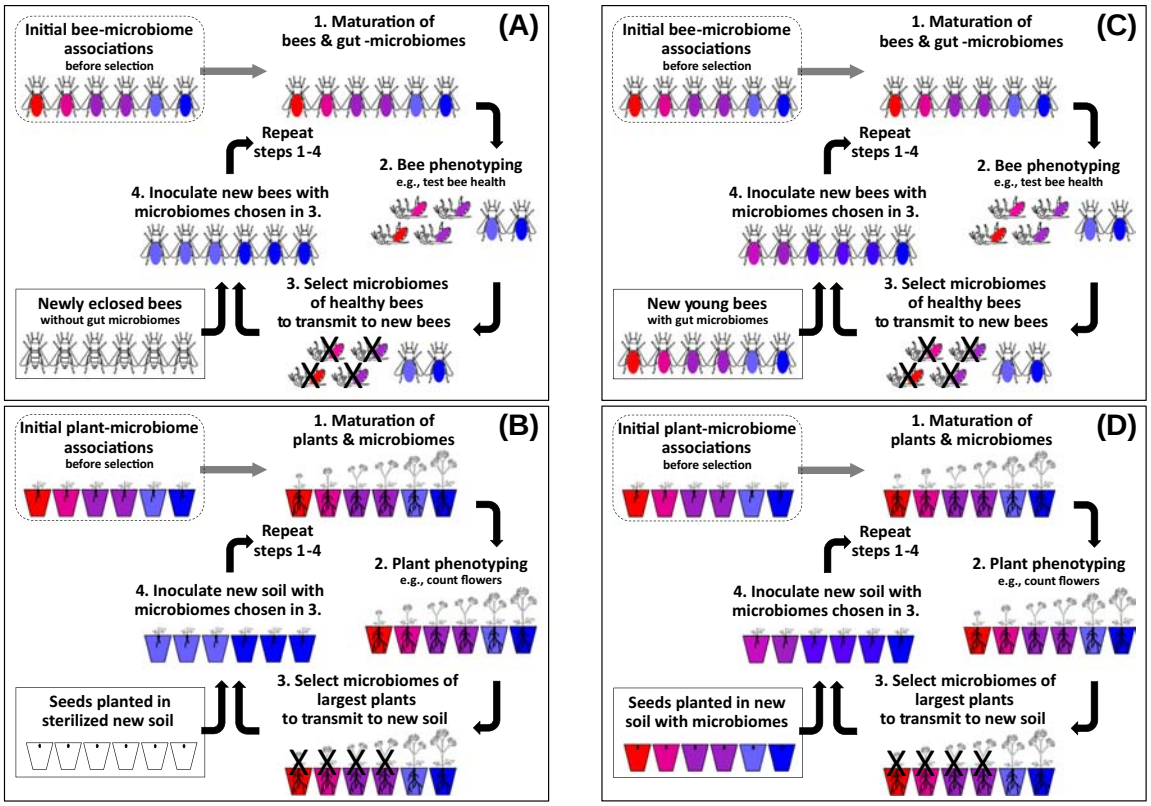

Figure I.A&B. Microbiome transplant from infected host to uninfected host. Microbiomes can be transplanted from infected to uninfected hosts, e.g., to uninfected hosts such as newborn bees **(A)** or to amicrobial hosts such as surface-sterilized seeds in sterile soil **(B)**. An advantage of this method is that microbiomes are more likely transplanted with sufficient fidelity because of transfer to microbe-free hosts; a disadvantage is that a source of uninfected hosts is necessary for a microbiome-selection experiment. Natural microbiome transmission in social insects is most similar to this transplanting scheme, for example when newborn bees or newly-molted termites acquire microbiomes from nestmates. All experiments published so far on host-mediated microbiome selection used this microbiome-transplanting scheme.

Figure I.C&D. Microbiome transplant from infected host to infected host (microbiome superposition, engraftment). When microbiomes are transplanted from infected to infected hosts, microbiomes are superimposed on, or **engrafted** into, resident microbiomes, leading to microbiome mixing and potential microbiome coalescence. A disadvantage of this method is that microbiomes are transplanted with less fidelity, because a transplanted microbiome has to coalesce with a microbiome that is already established, and successful transfer may require several inoculations [68]. An advantage is that no source of uninfected hosts is necessary for such a microbiome-selection experiment. No experiments have been published so far that used microbiome superposition or engraftment for microbiome selection.

Within-individual microbiome transplanting, cycling microbiomes through the same host, or between different modules of a modular host: In theory, it may be possible to repeatedly cycle gut microbiomes differentially through the same host, or transplant microbiomes differentially between different modules of the same host (e.g., between different leaves of the same plant). This is a so-far untested but intriguing possibility, and we include this method here for completeness.

genetic composition of microbiomes can potentially change dynamically at microbiome transmission and **microbiome assembly**, as well as while associated with a host between successive microbiome transmissions. Transmission and persistence fidelities are consequently lower for the genetic composition of the microbiomes than for host genomes (Figure 1), but fidelities can also be moderate to high for some specialized microbiomes of social animals (see below). A key question for microbiome breeding is, therefore, what minimum levels of microbiome transmission and persistence fidelities (**microbiome stability**) are sufficient to enable an evolutionary **response to selection** (here, response to microbiome selection)?

Microbe transmission fidelity, microbiome heritability, and response to microbiome selection

Not all microbiome components are relevant to host-mediated microbiome selection, but only those microbiome-encoded genetic factors that affect host traits, that contribute to trait variation among hosts, and that are transmissible between hosts (Boxes 1 and 2). For most microbiomes, there likely exists a gradation of transmission fidelity for different microbiome components (see Figure IF in Box 1). Only those microbiome-encoded genetic factors on the fidelity gradients that transmit and persist with sufficient fidelity can reliably contribute to a response to artificial selection on the effect of the microbiome on host phenotype [31,32].

Mitochondria, chloroplasts, plasmids, transovarially propagated bacteria of insects (e.g., *Wolbachia*, *Blochmannia*, *Sodalis*), and trans-seed-propagated endophytic fungi of grasses represent microbial symbionts that are reliably transplanted with high fidelity between hosts. In these cases, the genomes of these microbial symbionts are transplanted by **co-propagation** with the host nuclear genome, and therefore such microbial symbionts add extranuclear genetic material to the nuclear genome of the host [33,34]. Whenever genes encoded by these transmissible microbial symbionts have significant effects on the host phenotype, and whenever the genetic composition of these symbionts vary among hosts, these symbiont-encoded genetic effects on host traits are heritable (Box 2) and can therefore be shaped by host-mediated selection [33–35]. Other microbial genomes are co-propagated with the host genome with somewhat lesser fidelities, such as the gut microbiomes of bees and termites; these can still be shaped by host-mediated microbiome selection, although the same **strength of selection** is expected to engender a reduced **response to selection** (i.e., there will be a lesser change in microbiome genetic composition, microbiome functional effects, and host phenotype between rounds of selection). More generally, the various host-associated microbes that make up the microbiome show a range of cotransmission with the host genome, going from high cotransmission in some endosymbiotic bacteria (e.g., *Wolbachia*), to incomplete but appreciable cotransmission in some microbiomes (e.g., gut microbiomes of honeybees), to very weak cotransmission in most environmentally acquired symbionts with poor between-host transmission. A simple rule-of-thumb is that microbe components that are more reliably transplanted between hosts, and that persist with greater fidelity while associated with a host, should more readily contribute to heritability for microbiome effects on host traits and more readily respond to microbiome selection.

A typical microbiome is therefore not an evolutionarily cohesive unit in the sense that an organism is [36]. Whereas an organism's genome is reliably transmitted across generations, similar reliable transmission of microbiomes between hosts is true only for some genetic elements of the total genetic content of the microbiome, and different microbiome-encoded genetic elements will respond differently to host-mediated microbiome selection, depending on their relative transmissibility between hosts and their persistence while associated with a host. It is therefore inappropriate to view an entire host-associated microbiome as a cohesive and so-far overlooked

Box 4. Conditions for host-mediated microbiome selection

The conditions for evolution by natural selection are often abbreviated as 'heritable variation in fitness' [33]. Here, we formulate the conditions in greater detail to delineate similarities and differences between evolution of host traits by standard natural selection, caused by genetic change in host genomes (Table I, left), compared to the evolution of host traits by microbiome breeding, caused by genetic change in host-associated microbiomes (Table I, right).

Table I. Comparison of conditions for natural selection and for microbiome selection

Conditions for evolution by natural selection (conditions for evolution of host-genetic effects on host traits by natural selection)	Conditions for evolution by microbiome selection (conditions for evolution of microbiome-genetic effects on host traits by microbiome selection)
Genetically encoded trait variation	
Mechanistic link between individual host's genotype and individual host's phenotype such that variation in genotype among individuals causes trait variation among individuals	Mechanistic link between an individual host's microbiome-genetic composition and the individual host's phenotype such that variation in microbiome-genetic composition among individuals causes phenotypic trait variation among individuals
Fitness consequences of trait variation	
Mechanistic link between individual host's phenotype and individual host's fitness, such that variation in phenotype among individual hosts causes variation in the survival and/or reproductive success among individual hosts (Figure 1D,E)	Mechanistic link between individual host's microbiome-mediated phenotype and individual host's fitness, such that variation in microbiome-mediated phenotype among individual hosts causes variation in survival and/or reproductive success among individual hosts (Figure 1A–C)
Transmission of genetic information	
Sufficiently faithful transmission from parent to offspring of the genetic makeup of individual hosts	Sufficiently faithful transmission of the genetic makeup of microbiomes from donor host to recipient host (i.e., components of the microbiome genetic makeup that affect host traits persists sufficiently across time within the host and during transmission)

The trait-variation condition (1) and the fitness-variation condition (2) in Table I are true for many host-microbiome associations [32,33,41], but the degree to which the transmission condition (3) is satisfied in general is less clear. Because of differences in transmission and persistence fidelities between standard versus microbiome selection, the mechanics of microbiome selection can differ substantially from standard natural selection. Under standard selection (i) genomic makeup is propagated largely intact between generations to preserve genome–phenotype correlations despite possible recombination (e.g., in genomes that replicate sexually); (ii) the mechanics of segregation and recombination follow simple rules (e.g., Mendelian rules); (iii) genomes change by simple mutational rules, but genomes typically do not change dynamically during ontogeny of an organism. Under microbiome selection, genomic makeup of microbial strains within a microbiome can change by horizontal gene transfer, recombination, and mutation, just as under standard natural selection, but microbiome genetic makeup can undergo additional dynamic changes between and within generations. Despite these dynamic changes, however, some microbiome components can have sufficient transmission and persistence fidelities over time to preserve some microbiome-encoded genetic effects on host fitness and thus preserve microbiome–host–phenotype correlations with sufficiently fidelity. Standard natural selection and microbiome selection therefore depend on different fidelities of transmission and preservation of correlations between inherited genetic information and host phenotype.

second genome of a host that can be shaped as a whole by selection. Such a view greatly oversimplifies the complexities of the dynamics of an unsequestered and unbounded microbiome community [19,37]. Instead, it seems more appropriate to acknowledge that only a portion of the genetic makeup of a microbiome can be shaped by microbiome selection, and only under very specific conditions (Box 4); and moreover, that this shaping by microbiome selection will exhibit a more complex response to selection when compared to standard selection.

Under standard natural selection, the magnitude and direction of the short-term evolutionary response to natural selection depend only on trait heritability and the strength and direction of selection, as described by Breeder's Equation [38]. In theory, the evolutionary response to microbiome selection should also depend only on the strength of selection and a properly defined (Box 2) heritability of microbiome effects on host traits. That said, because microbiome stability within hosts, transmission between hosts, and the heritability of microbiome effects on host

traits are complex [31–33,39,40] (Box 4), response to selection is more difficult to predict [41]. Instead of trying to directly estimate the heritability of microbiome effects on host traits, in practice, it may often be easier and of more interest for microbiome breeders to estimate the so-called realized heritability, estimated from the observed response to selection and the imposed strength of selection. Furthermore, modeling using diverse approaches [12,32,41–43] that incorporate various components of the complex ecological and evolutionary processes affecting microbiomes will be useful to elucidate how exactly these processes can contribute to microbiome breeding.

Maximizing response to microbiome selection

Despite the complex ecoevolutionary processes that govern microbiome dynamics within hosts and the transmission of the microbiome between hosts, the pragmatic quantitative genetic perspective that has proven hugely successful for plant and animal breeders is also promising for microbiome breeding. Boxes 1 and 2 emphasize how variation in causal genetic factors, whether encoded by the host or by the microbes that make up the microbiome, can contribute to heritable variation for host phenotypes and for the composition of the microbiome. Depending on the precise rules of microbiome transmission, the heritable variation in microbiome-encoded factors can contribute to evolutionary responses to selection on host traits, and it is therefore possible to derive guidelines for effective microbiome breeding.

Maximizing the relative contribution of microbiome-encoded genetic effects on host traits

A first experimental strategy is to minimize or eliminate the genetic contribution of the host to total phenotypic variation of the host trait under selection (see Figure IG in Box 1), for example, by using a highly inbred, near-clonal host population, as was done in all host-mediated microbiome-selection experiments conducted so far with plants [5,6,8,11,13,22]. In the absence of variation in host-encoded genetic effects to the total phenotypic variation of the host, contributions of microbiome-encoded genetic effects between hosts to total phenotypic variation will be more prominent relative to other factors contributing to this variation (see Figure IG in Box 1). Consequently, hosts with the most beneficial microbiomes can be identified more efficiently for microbiome harvesting and transplanting, facilitating response to selection. However, microbiome breeding experiments that include genetically variable hosts plus variation in the genetic composition of microbiomes among hosts may capture all possible contributions of microbiomes to phenotypic variation of the host, whenever the effects of microbiome genetic composition depend on host genotype.

Microbiome transmission

Experimental strategies seeking to increase microbiome transmission of microbiome-encoded genetic effects on a host should focus on those microbes that contribute significantly to host phenotype. These strategies include harvesting microbiome components that are mechanistically most closely linked to the host phenotype to be improved, techniques to prevent loss of such microbes during transfer between hosts, and strategies to faithfully reassemble a beneficial microbiome community after transplanting. Optimizing priority effects after transplanting or manipulating resource abundances [44–47] are effective mechanisms to steer microbiome reassembly and thus increase transmissibility between hosts.

Microbiome stability

A third strategy is to experimentally facilitate microbiome stability, thereby increasing **microbiome persistence** and constraining microbiome dynamics while a microbiome is associated with a host. For host-independent microbiome selection, both modeling and experimentation have shown that uncontrolled dynamic changes reduce fidelity of microbiome function and are therefore a major

obstacle to obtain a significant response to microbiome selection [19,48]. Adding experimental protocol steps to stabilize the microbiome community has therefore been one strategy to obtain a response to host-independent microbiome selection [19,48], for example, by first allowing microbiomes to adapt to experimental conditions, then starting differential microbiome propagation after this stabilization phase. Host-mediated microbiome selection, in contrast, is thought to be less subject to uncontrolled dynamic changes, because the host actively stabilizes its microbiomes by attracting, recruiting, regulating, retaining, or sanctioning specific microbial components and thus steering microbiome assembly and microbiome resilience (e.g., by controlling essential or private resources [49]). These combined processes have been variously called partner choice, symbiont choice, host control, or ecological filtering controlled by the host [46,50–52]. Strategies to optimize host-dependent microbiome selection should therefore optimize effective host control of microbiome composition and microbiome dynamics.

Because of the important role of host control for host-mediated microbiome selection, microbiome selection should be easier with host species that exert effective host control. Hosts that evolved to effectively control and shape their microbiomes are predicted to enable a rapid and strong response to microbiome selection than hosts with poor control over their microbiomes; and any within-host-population variation in host control should affect the response to host-mediated microbiome selection (e.g., hosts with more resources or energy stores may be better able to control microbiome assembly and stability).

The nature of microbiome selection

Levels of organization, meta-organisms, and coevolution

An ongoing debate revolves around the prerequisites of microbiome selection (Box 4), for example, whether microbiome selection is possible only if a microbiome forms a unit of selection [19,53,54]; only if microbiome plus host transition first to a higher level of organization and form an evolutionary unit (meta-organism, super-organism, or holobiont built by a hologenome) [31,53,55]; or only if there is sufficient vertical transmission between co-propagating microbe genomes that coevolve with the host genome as a conjoined extended genome [12,32,33,37] such that conflict between genomes is reduced or becomes self-limiting [36]. These are clearly interesting evolutionary questions, and only a few of these have simple answers. For example, coevolution between host and microbiome components is definitely not a requirement for host-mediated microbiome selection because microbiome selection in the absence of host–microbe coevolution is documented by the successful microbiome-selection experiments that used genetically invariable, non-evolving host populations [5,8,11,13]. Because host control facilitates microbiome selection, however, co-evolution between host and some microbiome components likely emerges whenever microbiome selection operates in a host population that is genetically variable for host control.

From a practical perspective, addressing questions of synergisms, coevolution, extended genome, or level of organization – all interesting research dimensions – is initially unlikely to be helpful for a microbiome breeder when designing and optimizing microbiome selection. To optimize selection on host-associated microbiomes, a pragmatic breeder's mindset is most useful, focusing on (i) understanding all the sources of variation that contribute to overall variation of phenotypes in a host population, and (ii) designing strategies to maximize that transmissible portion of variance in host traits that is due to microbiome-encoded genetic effects, as discussed in Boxes 1 and 2.

Emerging frontier: modeling and theory of microbiome selection

Recent modeling analyses that explored the eco-evolutionary dynamics operating under artificial selection on microbiomes confirmed that microbiome selection can be effective to breed beneficial microbiomes [12,32,41–43,56,57], but that there are also constraints (e.g., [9,42,57]). Additional

models capture specific aspects of the eco-evolutionary dynamics of microbiome selection, for example, to identify network modules within a microbiome that can potentially be developed into synthetic microbiome modules useful for agriculture or biotechnology [56]. Other models address the relative importance of direct parent–offspring transmission of microbes versus acquisition of microbes from a shared microbe pool to which many parents contribute (collective microbiome inheritance) [31,32]; or elucidate the consequences of mixing parental with environmental microbiome contributions (mixed-mode transmission) to explore potential advantages of such mixing that increases variation in host–genotype–microbiome associations among offspring [12]. Clearly, to optimize microbiome breeding, additional theoretical work remains to be done [17–19,23,33,57,58], specifically analyses that elucidate microbiome selection under evolving microbiome transmission and persistence fidelities.

Emerging frontier: microbiome selection in animal hosts

To further elucidate general principles of microbiome breeding, it may be possible to learn from organisms that evolved to transmit complex microbiomes under conditions that are conducive to microbiome selection. Of special interest are organisms that (i) exert effective host control over composition and stability of their microbiomes, (ii) have phenotypes that critically depend on microbiome function, (iii) live in microbially stable environments (e.g., nests), and (iv) evolved mechanisms to reliably transmit microbiomes. Humans do not meet these criteria well because transgenerational microbiome transmission is relatively unfaithful in humans, with the possible exception of a few bacterial genera [59]; transmission occurs in variable environments; and microbiomes change dynamically during human ontogeny [26,60–62]. Microbiome selection in humans therefore may be possible for microbiomes beneficial under specific stresses, for specific athletic performance [63], or at specific ages [64], but it will likely be challenging.

Some mammals transmit microbiomes transgenerationally more faithfully than humans and they do so in microbially stable environments (e.g., sequestered nests of rodents) [65]. For mice, therefore, it has been possible to optimize gut microbiomes for specific biochemical functions while perpetuating microbiomes between mouse generations under stable laboratory conditions [66]. Transplants of gut microbiomes have been used for centuries to try to cure ruminal microbiome dysbiosis in cattle, sheep, and goats (ruminant **transfaunation**) [67,68], and recently also to evaluate transfaunation as a way to enhance milk production in cows [69], but a microbiome breeding experiment aiming to improve gut-microbiome communities of domesticated animals has so far not been attempted, presumably because of logistic challenges. Fish may represent the most tractable vertebrates for microbiome breeding because fish have short generation times and because it was possible to evolve a single bacterial strain by transplanting such a single-strain microbiome repeatedly from infected to uninfected guts of zebrafish [70].

The animals that seem most likely to have evolved adaptations facilitating microbiome selection are social organisms caring for brood in stable nest environments, such as eusocial insects. Honeybees have a simple gut microbiome (six to ten bacterial species-complexes account for >95% of the bacterial diversity in the bee gut), and newborn honeybees inherit a specialized portion of their gut microbiomes from nurse bees, and another portion from a stable nest environment [2,3]. Young termites inherit from nestmates far more complex microbiomes (specialized communities of bacteria, Archaea, ciliates, flagellates, etc.), but microbiomes of the termite hindgut are voided with each molt during development, and termites therefore need to receive new microbiome transplants from nestmates after each molt [71]. In each colony of honeybees and termites, therefore, hundreds to millions of microbiomes are reliably transplanted each day between siblings, generating great potential for microbiome selection driven by worker behavior. Cumulative differential microbiome propagation should therefore generate a response to

selection on microbiome components that affect host phenotypes, in particular because transmission occurs from a population of microbiome donors that are genetically relatively homogeneous (because donors are part of the same family, V_G is small or negligible; see Figure IG in Box 1) and the donors live in a stable nest environment (V_E is small; see Figure IG in Box 1). These are exactly the conditions that facilitate microbiome selection. Selection on gut microbiomes of social insects should therefore be experimentally easier than selection on the root-associated microbiomes of plants that have been the focus of microbiome-selection experiments so far.

Concluding remarks and future perspectives

If humans were like honeybees or termites, transplanting microbiomes would be a normal feature of human development, and medical research would have begun centuries ago to understand methods of differential microbiome transplanting to optimize microbiome breeding. Humans are not like social insects, however, and we are only now beginning to understand the basic principles of microbiome transmission and host-mediated microbiome selection. For successful microbiome breeding, practical issues matter, for example, the choice of a suitable experimental system involving ideally a host capable of effective host control over microbiome assembly and stability; whether variation in composition of host-associated microbiomes drives variation in host phenotype (Boxes 1 and 2); and whether microbiome transmission and persistence fidelities are adequate in a microbiome-breeding experiment to yield an acceptable response to selection. To fully develop the potential of microbiome breeding (see Outstanding questions), future research could take advantage of established techniques of directed evolution [9,19,72,73], and pair microbiome-breeding experiments with analyses of the molecular basis of microbiome effects on host traits, metabolomics, and microbiome-plus-host genome-wide association studies (mGWAS [74,75]).

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Declaration of interests

There are no interests to declare.

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Outstanding questions

Can domesticated plants and animals be bred to facilitate microbiome breeding? What breeding designs best enable integrative breeding of hosts together with transmissible and selectable microbiomes?

How exactly does microbiome transmissibility evolve during microbiome selection? Does microbiome selection improve transmissibility for most microbes in a microbiome, or does transmissibility become bimodal within microbiomes because some cotransmitted microbes are captured effectively in the selection process and others are excluded?

Do environmentally acquired microbiome components facilitate microbiome selection, because they increase microbiome variation among host offspring and allow new microbes to be captured in the process of microbiome selection? If so, what is the optimal mix between transmitted versus environmentally acquired microbe contributions to offspring?

What is the optimal host control to facilitate microbiome breeding? Could extreme host control over microbiome assembly reduce variation in microbiomes between hosts because all hosts assemble the same microbiome, and would therefore a less-than-extreme host control be best for microbiome assembly, but extreme host control be best for microbiome stability after assembly?

How does microbiome selection shape specific traits of specific microbes and simultaneously also emergent functions of the microbial community?

What are the precise molecular mechanisms by which microbe-encoded genetic factors under microbiome selection interact with complex biotic and abiotic factors to influence host traits?

Does horizontal gene transfer mediated by viruses within microbiome communities facilitate microbiome selection, potentially adding to microbiome selection a viral dimension that can be shaped by concurrent artificial selection on viromes?

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