Opinion piece

Cite this article: Kolodny O, Schulenburg H. 2020 Microbiome-mediated plasticity directs host evolution along several distinct time scales. *Phil. Trans. R. Soc. B* **375**: 20190589.
<http://dx.doi.org/10.1098/rstb.2019.0589>

Accepted: 25 May 2020

One contribution of 16 to a theme issue 'The role of the microbiome in host evolution'.

Subject Areas:

evolution, behaviour, ecology, genetics, microbiology

Keywords:

microbiome-mediated evolution, Baldwin effect, genetic accommodation, genetic assimilation, phenotypic plasticity

Authors for correspondence:

Oren Kolodny
e-mail: oren.kolodny@mail.huji.ac.il
Hinrich Schulenburg
e-mail: hschulenburg@zoologie.uni-kiel.de

Microbiome-mediated plasticity directs host evolution along several distinct time scales

Oren Kolodny¹ and Hinrich Schulenburg²

¹Ecology, Evolution, and Behavior, The Hebrew University of Jerusalem, Giv'at Ram, Jerusalem, Israel

²Evolutionary Ecology and Genetics, Kiel University, Am Botanischen Garten 9, 24098 Kiel, Germany

OK, 0000-0002-0095-693X; HS, 0000-0002-1413-913X

Host-associated microbiomes influence their host's fitness in myriad ways and can be viewed as a source of phenotypic plasticity. This plasticity may allow the host to accommodate novel environmental challenges and thus influence the host's evolutionary adaptation. As with other modalities of phenotypic plasticity in phenomena such as the Baldwin effect and genetic assimilation, the microbiome-mediated plasticity may influence host genetic adaptation by facilitating and accelerating it, by slowing it down, or even by preventing it. The dynamics involved are likely more complex than those of previously studied phenomena related to phenotypic plasticity, and involve different processes on each time scale, such as acquired recognition of newly associated microbes by the host's immune system on single- and multiple-generation time scales, or selection on transmission dynamics of microbes between hosts, acting on longer time scales. To date, it is unclear if and how any of these processes shape host evolution. This opinion piece article provides a conceptual framework for considering the processes by which microbiome-mediated plasticity directs host evolution and concludes with suggestions for key experimental tests of the presented ideas.

This article is part of the theme issue 'The role of the microbiome in host evolution'.

1. Introduction

Phenotypic plasticity has been suggested to direct, to precede and to mediate genomic adaptation via mechanisms such as the Baldwin effect and genetic assimilation [1–6]. On the other hand, plasticity has also been proposed to slow down genomic adaptation [4,7]. Both of these general scenarios have been supported by extensive modelling and demonstrated in natural and experimental settings that span a broad range of categories of plasticity, from behavioural to physiological and from environmentally induced to stochastically occurring variation [5,8,9].

The standard sequence of events through which plasticity influences evolution may be simplistically sketched thus: a species is faced by a novel environmental challenge; phenotypic plasticity allows some individuals to accommodate the challenge better than others (henceforth, *phenotypic adaptation*), thus placing them closer to the new adaptive peak in the fitness landscape. Other individuals do not survive, or leave few progeny, as a direct result of their failure to accommodate the novel challenge (hard selection) or indirectly, following intra-species competition (soft selection). At this point, one scenario is that the phenotypically adapted individuals are found at an optimum or very near to it, thus preventing selection from further acting on genomic variation, or slowing down such selection in comparison with its rate had there not been plasticity that provided the phenotypic adaptation (evolutionary slowdown; [6]). The alternative scenario is that the individuals that are near the adaptive peak are exposed to directional selection that is strong enough to select for genomic adaptation, either acting directly on the genomic underpinnings of the plasticity and

Box 1. Terminology

We here define several terms that are widely used in the context of microbiome-mediated responses and/or the evolutionary consequences of plasticity. In those cases, where distinct definitions are used for a particular term in the literature, we focus on one of the most common concepts.

Evolution—change in inheritable information across time within the population of organisms, including either genetic changes, epigenetic changes, or changes in other transmissible information such as microbes.

Evolutionary adaptation—as above, yet the change additionally provides an increased evolutionary fitness (i.e. higher relative reproductive rate) within the population of organisms in the context of the current environmental conditions.

Microbiome—the total of all microbes and their genomes, which is associated with a particular host species.

Phenotypic plasticity—environmentally induced phenotypic change, leading to phenotypic variation of single genotypes as a consequence of distinct reaction norms towards different environmental conditions, whereby the expressed reaction norms are assumed to be genetically determined (i.e. manifested in the genome of the considered organism).

Plastic response—the same as above, yet the expressed, environmentally induced response need not be genetically determined but can also be mediated by the microbiome or epigenetic mechanisms.

Genetic accommodation—the general effect of a plastic trait on evolutionary adaptation of an organism, including the five phenomena below [3,10,13,14].

Baldwin effect—evolution of an optimized plastic response from the originally expressed plastic response, thereby increasing evolutionary fitness in the context of the current environmental conditions; we here use a broad definition of the Baldwin effect, not only restricted to behavioural responses but including any phenotypically plastic trait [3,15].

Genetic assimilation—evolution of a constitutive/fixed expression of the originally plastic trait, thus ensuring high fitness in the given environment.

Microbiome-mediated Baldwin effect—evolution via heritable changes in the host that ensure an enriched presence of beneficial microbes, for example through vertical transmission and/or preferential uptake of the microbes from the environment, leading to enhanced fitness in the current environment.

Microbiome-initiated genetic assimilation—evolution via heritable changes in the host that cause constitutive expression of a trait, which covers a function originally provided by the microbiome, thereby ensuring high host fitness independent of the microbiome (and possible changes within the community). This may include cases in which the host achieves this function via a different molecular mechanism from that provided by the microbiome (e.g. the microbiome may have originally provided protection against a pathogen through colonization resistance, while the host counters this pathogen via the evolution of an improved immune response).

Microbiome-driven reverse-Baldwin effect—a fully adapted host loses functionality of the genes encoding a particular advantageous trait if this trait is provided by the microbiome; this effect does not depend on a change of environment, and may occur even under constant conditions.

reducing the variation in the plastic trait in a way that is optimized to the new fitness landscape, or acting on other traits that replace the need for the original plasticity (e.g. by dealing with the challenge in an altogether different way from the way made possible by the original plasticity) [6,10].

An important case of the latter scenario is one in which the environmental challenge is in the form of an ecological niche that may or may not be interacted with by individuals, such as a case in which a novel food source is to be found in the environment. Initial plasticity may allow some individuals to make use of this food source, causing them to interact with it and paving the way for genomic adaptation that would improve the utilization of the resource. This selection would not have had the opportunity to act in the absence of the initial plasticity, because individuals would not have repeatedly interacted with the novel food source if they could not derive some nutritional value from it. This is sometimes referred to as ‘the needle in the haystack’ scenario, because of the shape of the fitness landscape in the absence of plasticity [11,12].

The host-associated microbiome (henceforth ‘microbiome’, box 1) can generate and contribute to plastic responses. Microbiome composition and abundance are characterized by high variation in time and among individuals; these dynamics are driven by stochastic processes and/or variation in microbial

fitness, yet they can potentially influence many dimensions of the host’s phenotype. These microbiome-mediated changes can be induced by the environment and thus produce effects equivalent to what is traditionally considered phenotypic plasticity. In turn, the microbiome-mediated responses should similarly influence host evolution. To date, however, the microbiome’s impact on the host’s evolution is largely unexplored. We propose that, like other forms of plasticity, plasticity conferred by the microbiome may give rise both to acceleration and to deceleration of genomic adaptation, significantly influencing hosts’ evolution. Importantly, the dynamics of this scenario may be more interesting and complex than scenarios studied so far, potentially spanning a range of time scales, including feedback loops that do not exist in other scenarios and involving different extents of non-genomic heritability across generations. In particular, environmental challenges that are accommodated by microbiome-provided plasticity may produce selective pressures on host–microbe interaction that would not have occurred otherwise. Moreover, we expect that the microbiome-mediated effects are of general importance for the evolution of organisms, because all more complex organisms originated in a ‘sea’ of microbes, they all adapted to the omnipresence of the microbial cohabitants, and most likely all multicellular organisms are also host to an associated microbial community [16].

The aim of this article is to develop a conceptual framework for the influence of the microbiome on host adaptation, with a special emphasis on the involved evolutionary and ecological processes. For clarity and simplicity, we focus throughout this article primarily on the scenario described above, in which a single environmental change occurs and subsequently persists, for example, the appearance of a new pathogen, a new dietary resource, or a new habitat niche. We discuss the ecoevolutionary processes that may be triggered by this change and may allow the host to accommodate it via microbiome-mediated plasticity. These processes range across time scales, from within-generational effects, possibly playing out within hours in some cases, to very long processes, spanning hundreds or thousands of host generations. For simplicity, we assume that the host species was initially at a fitness optimum with respect to the environment prior to the environmental change. Our views are related to inspiring previous opinion and perspectives articles [17–19]. In our article, we specifically consider currently neglected processes, especially different eco-evolutionary dynamics and feedbacks in host-associated microbial communities and how they affect distinct evolutionary responses in the host over a range of time scales, including both adaptive and maladaptive changes. It is highly likely that the microbiome influences host adaptation across different organizational levels and time scales. We currently lack the data to assess which of these time scales and/or levels may be more influential. In fact, their importance may vary among host–microbiome systems and environmental context. We anticipate that the developed framework will help future research to more precisely query the role of different time scales and organizational levels in microbiome-mediated host adaptation. Below, we begin by describing different types of changes that can occur within the microbiome across different time periods and which are the basis of any subsequent effect on host adaptation (§2). Subsequently, we explore in the core section of our article (§3) in what way the microbiome may influence host adaptive responses across distinct time scales. We emphasize that the microbiome may also have detrimental effects on host adaptation (§4). Several important ramifications arise from our framework (§5), which clearly deserve future attention, including a re-adjustment of current models of fitness landscapes or the consideration of less simplistic environmental scenarios, such as repeated changes of environmental conditions that yield a highly dynamic context for the microbiome-mediated responses. We conclude by outlining key study approaches for future research, in order to dissect the microbiome's influence on host adaptation (§6). We additionally use boxes 2 and 3 to point to two specific aspects of the involved dynamics, which we find particularly exciting in our understanding of the evolution of host–microbiome interactions.

2. Microbiome changes as a basis for subsequent effects on host adaptation

The microbiome can mediate plastic responses of the host across different organizational levels and time scales. On extremely short time scales—of the order of minutes or hours—microbes may respond to new environmental challenges through changes in physiology and thereby influence the host phenotype. For example, members of the gut microbiome can rapidly change their individual metabolism and thereby switch between the exploited energy sources on the

advent of new nutrients, as reported for *Bacteroides* bacteria from human or mouse gut microbiomes [23–25]. These physiological or metabolic responses are most likely to have evolved because they enhance microbial fitness. Yet, they may also benefit the host if the microbial responses help to make nutritional resources more accessible for the host (i.e. by-product mutualism; [26]). Moreover, the physiological microbial changes may trigger a response from the host, for example, hosts may alter their behaviour, immune system activity or metabolism in response to microbes' metabolic output or spatial configuration [27] in ways that increase host fitness (e.g. reviewed in [28,29]).

Individual microbiome members may also evolve and thus change genetically in response to a new environmental challenge. Such changes were documented for single microbial strains with the help of controlled evolution experiments and occurred within a few weeks, possibly faster. For example, a selected member of the zebrafish microbiome, a strain of the Gram-negative bacterium *Aeromonas veronii*, was able to adapt in several steps to a specific host line under the experimental conditions, including initially improved colonization of the fish from the environment, and enhanced host-to-host transfer, followed later by intra-host adaptation and increased competitive ability [30]. Similarly, an experimental symbiont of the nematode *Caenorhabditis elegans*, a strain of the Gram-positive bacterium *Enterococcus faecalis*, rapidly adapted to provide increased levels of host protection from a pathogenic bacterium [20,21,31] (see box 2). In addition, microbes were also shown to adapt genetically within a community [32]. For example, using experimental evolution with gnotobiotic mouse hosts, a gut-colonizing *Escherichia coli* was demonstrated to adapt through changes in anaerobic respiration to the presence of a single additional microbiome member, while increased consumption of specific amino acids was selected when *E. coli* was alone [33]. In a related experiment with distinct *E. coli* strains, adaptation of the focal *E. coli* strain was enhanced by horizontal gene transfer [34], highlighting the importance of a process that is particular to communities and that can speed up adaptation through uptake of favourable genes or gene variants. The latter results are consistent with recent longitudinal metagenomic studies of the human gut microbiome. In detail, the employment of high-throughput chromosome conformation capture (Hi-C) to reconstruct bacterial genomes from two host individuals and two time points revealed adaptive evolution in essential genes combined with extensive horizontal gene transfer in the persistent bacterial lineages [35]. Similarly, a metagenomic and culture-based population genomic analysis of *Bacteroides fragilis* identified signatures of intra-host adaptive evolution in cell-envelope biosynthesis and polysaccharide utilization pathways [36]. As above, these genetic changes occur because they maximize fitness of the microbial lineages. Yet, they can simultaneously benefit the host if the bacterial changes provide an advantage to the host, as most convincingly demonstrated for the evolved immune-protective *E. faecalis* in the *C. elegans* host (box 2).

The microbiome may also respond to new environments through changes in its composition. These compositional changes constitute a different organizational layer compared with the physiological or genetic response of individual microbes and they may themselves be determined by distinct processes across different levels. In particular, the compositional changes may occur for different genotypes within a species or across the species (e.g. [35,36]); they may affect

Box 2. Experimental evolution of the microbiome-mediated Baldwin effect

Evolution experiments are an informative tool to assess if and how the microbiome influences host adaptation. While such controlled experiments have not yet been performed with complex microbiome communities, first insights were obtained with single microbiome members. One example includes the nematode *Caenorhabditis elegans* as a model host and a strain of the Gram-positive symbiont *Enterococcus faecalis* as a model microbiome member that benefits the host by providing protection against a pathogen. During the evolution experiment, both host and symbiont were co-passaged over 14 host generations, allowing them to co-adapt to one another, either in the presence or in the absence of a non-evolving pathogen [20]. The symbiont adapted by increasing its protective effect against the pathogen (figure 2, left panel), possibly through elevated production of antimicrobial superoxide [21,22]. By contrast, the host did not adapt by producing a direct response to the pathogen: under standardized conditions without the microbiome, hosts from the pathogen- and the control treatments showed similar levels of pathogen susceptibility (figure 2, left panel, square symbols). Most importantly, the hosts from the pathogen treatment did adapt by accommodating a significantly increased number of the protective symbiont, especially if co-adapted (figure 2, right panel), thereby achieving higher survival rates in the presence of the pathogen (figure 2, left panel; [20]). Even though the exact genetic changes underlying this phenotype are currently unknown, the effect is genetically determined [20] and thus represents an example of a Baldwin effect initiated by a microbe-mediated response. A particular challenge for the future is to dissect the molecular mechanisms responsible for the increased accommodation of the symbiont and to explore to what extent similar effects are produced when a complex microbiome accounts for the beneficial effect.

Figure 2. Example of a microbiome-mediated Baldwin effect. Figure adapted from [20].

relative or absolute abundances of particular species/genotypes (e.g. [37]; and they may concern only the currently resident microbes or additionally be influenced by uptake of microbes from the environment (e.g. [38,39]). The above two layers (physiological and genetic changes in individual microbes) may have a direct impact on compositional changes, since physiologically or genetically adapted microbial lines are likely to increase in abundance. The time scale on which any of the above species' abundances change may be very short, within hours or days (e.g. compositional changes within the gut microbiome in response to a certain meal), or on a scale of weeks or months (e.g. in response to changes in the diet; reviewed in [28]). They are again driven by microbial fitness variation alongside stochastic processes.

The changes in community composition are likely to represent the most influential plastic response, because they can come with large potential benefits for the host. On the one hand, they can occur across a variety of different time scales, including the very short time spans that are likely key for a beneficial effect for the host. On the other hand, and likely more importantly, they can account for fundamental changes in function, especially if they are based on uptake of microbes from the environment, including taxa or at least gene/gene repertoires not present in the resident microbiome (e.g. [38,39]). Microbiome-mediated functional changes have

been reported for a variety of host taxa and environmental contexts. For example, dietary fluctuations in humans are met by corresponding changes in microbiome composition with important shifts in the availability of carbohydrate-active enzymes [28,40]. Similarly, cooked versus raw plant food leads to rapid compositional microbiome community changes in mice, which were demonstrated to improve starch digestion and degradation of harmful compounds, thereby modulating host energy status [41]. A variety of insects counter the use of pesticides through compositional changes in their microbiomes, which become enriched in bacteria able to degrade the toxic substances [39,42]. This latter effect can be enhanced through the selective uptake of toxin-degrading bacteria from the environment, as demonstrated for stinkbugs [39]. Furthermore, corals are apparently able to survive increases in temperature and CO₂ levels through functionally relevant alterations in their microbiomes [19,43,44].

These compositional changes may not always be easy to reconstruct or predict, primarily because they can involve numerous microbial strains, all with many possible interactions among each other, between each of them and the host, and also between the host-associated and environmental microbes. In the end, the compositional changes are driven by dynamics and processes well known from community ecology [45–47]. They may thus be influenced by purely neutral dynamics,

characterized by stochastic community changes and non-selective migration of the microbes [45,48]. They may be affected by different direct interactions among the microbes, ranging from cooperation (e.g. cross-feeding of essential nutrients), to competition (e.g. reliance on the same nutrients), or exploitation [47]. They may be shaped by microbes with different general strategies, for example, those defined by the universal adaptive strategy theory, which includes ruderals, competitors and stress-tolerators as key life-history strategies [49]. Importantly, these ecological community dynamics are influenced by an additional hierarchical level, here imposed by the host or the population of host organisms. To account for these ecological relationships across organizational levels, microbiome metacommunity models have recently been proposed [50,51]. These models are a first step towards development of a more general metacommunity framework that captures and more precisely predicts the dynamics in microbiome community composition involving complex interactions and the diverse plastic responses towards novel environmental challenges.

3. The impact of microbiome-provided plasticity on host adaptation: multiple processes across different time scales

The microbiome may provide its host with adaptive plasticity much in the same way that adaptive plastic responses to environmental challenges may be in the form of host learning, gene expression, or behavioural changes. However, as opposed to these processes, microbiome-provided plasticity is expected to involve and to trigger a plethora of more complex (and more interesting) eco-evolutionary dynamics. In this section, we outline such dynamics, presented in the chronological order in which they come into play following the emergence of an environmental challenge and according to the time scale on which they play out. Examples of such challenges are (1) loss of access to a necessary metabolite (e.g. following movement to a new environment), (2) appearance of a new pathogen, or (3) the presence of a new food source that requires metabolic processing for utilization.

(a) Short-term dynamics: the within-host-generation time scale

Each of the various modes in which short-term microbiome plasticity may come about—from changes in relative microbial species' abundance to genetic adaptation on their part—can influence a host's ability to cope with environmental change, mediating the host's accommodation of the new challenge, as outlined in §2 and illustrated in figure 1*a*. Moreover, initial variation among host individuals in the composition of their microbiomes may in itself allow certain individuals to cope with the evolutionary challenge better than others. Whether such inter-individual differences subsist or increase, or whether the microbiome-mediated accommodation of the challenge occurs across the hosts' population in a coordinated manner, depends on many factors. Most interestingly, if horizontal transmission of microbes between individuals is prevalent, then the hosts' fates may be closely coupled.

The short generation time of microbes compared with that of most hosts may create interesting feedback loops that do not have a direct parallel in previously studied dynamics of

Figure 1. Schematic illustration of the main processes by which the microbiome directs host evolution across different time scales. (a) Upon a new environmental challenge (light red background colour), microbiome-mediated plasticity ensures host survival and thus persistence under the new conditions. Subsequently, the host may adapt to specifically harbour the beneficial microbes (i.e. microbiome-mediated Baldwin effect), either through their selective uptake from the environment (left side of top middle individual) or their efficient vertical transmission (red arrow on right side of top middle individual). In the long run, the host may evolve to constitutively express the microbiome-mediated trait (i.e. microbiome-initiated genetic assimilation). As an alternative, the microbiome-mediated plasticity prevents (or minimizes) host adaptation. (b) A different scenario is the microbiome-driven reverse-Baldwin effect: If the microbiome can provide the selectively favoured trait, then a fully adapted host may lose the corresponding gene. This effect does not depend on a new environmental condition, but can be expressed under constant conditions. For further details see text.

environmental challenges being accommodated via phenotypic plasticity. Thus, for example, microbes that break down complex carbohydrates found in a novel food resource might facilitate increased consumption of this resource by the host, leading in turn to an increase in abundance of these microbes in the host's gut, creating positive feedback and increasing this resource's consumption. This might also lead eventually to microbial genetic adaptation and further specialization in this role as metabolizers of the particular novel resource. Thus, the microbial community responds to the environmental change, thereby influencing the host and the microbially provided plastic response. This type of feedback loop, playing out over multiple generations of microbes but within a single generation of the host, is absent from most conventional accounts of phenotypic plasticity. However, interestingly it may find a parallel in some classic scenarios of the Baldwin effect that involve learned behaviour. It may be that within an individual's lifetime, learned behaviour may allow accommodation of an environmental challenge, and that this accommodation can improve over time via secondary learning

phases, made possible through the continued interaction with the novel challenge or ecological niche.

An additional aspect of the host–microbiome interaction creates qualitatively different dynamics from those seen in other cases of phenotypic adaptation: the host may actively influence which microbes compose its microbiome in different ways, many of which involve the immune system. The adaptive immune system is a phenotypically responsive system itself, which may potentially ‘learn’ to recognize and support the species that contribute to host adaptation, magnifying over time the extent of the microbially provided phenotypic adaptation.

(b) From mother to son: early between-generation dynamics

In the host generations following the appearance of the environmental challenge, a few factors may come into play that are not necessarily found in other plasticity-providing mechanisms. The individuals whose microbiome provided adaptive accommodation of the environmental change enjoy increased fitness and more offspring; it may even be that the only individuals to survive are those that happened to have an adaptive microbiome composition, e.g. in the case that the microbiome provides defence from a novel pathogen. Thus, second-generation hosts, and following generations, may benefit from an early exposure to an adaptive microbiome composition. This may have two implications: first, beneficial microbial species may capitalize on a priority effect [52], colonizing the host early in infancy, establishing, and preventing establishment of other microbial species that occupy a similar ecological niche. Second, early establishment may facilitate recognition and positive interactions between the phenotypic adaptation-providing microbe and the host’s immune system, especially in higher vertebrate animals, in which the adaptive immune system matures during development, most likely instructed by the microbes present at the time [29,52,53]. These dynamics may serve to amplify the microbially provided contribution to the host fitness compared with this contribution in the preceding generation. Of course, they rely on the details of microbiome inheritance, such as the extent to which the microbiome is vertically acquired and the extent of horizontal microbial transfer among host lineages [54,55], which may lead to host phenotypic adaptation at a group level. Importantly, both such vertical and horizontal transmission may occur indirectly, i.e. via the environment: host individuals typically shed bacteria to their direct environment in large quantities, making them available for uptake by young hosts.

Some conventional scenarios of phenotypic plasticity that are not microbially provided may also be optimized across generations in a similar manner. One example is in cases where young individuals can learn advantageous behavioural responses from their parents or other adults, and potentially even improve over their parents’ performance thanks to early adoption of the behaviour, increased interaction with the niche in which it is useful, or non-random choice of an adult individual to learn from. This requires that the ability itself to transmit the plastic trait be already present in the population (e.g. a tendency of young to learn from adults) or arise and spread in it. On the other hand, this would typically not be the case with phenotypic plasticity in physiological traits, for example. Perhaps most interestingly, and relatively under-

appreciated in discussion of Baldwin effects, plasticity via learning and plasticity via microbiome changes share the possibility of non-vertical transmission of the trait, namely a behaviour can be learned from non-parental individuals, just as microbes may be horizontally acquired.

(c) Intermediate time scale: multiple generations following the rise to the challenge

On a time scale of tens to hundreds of host generations, processes that are somewhat analogous to genetic accommodation may take place, acting to cement the relation between the host and the phenotypic adaptation-providing microbe: to increase its robustness and fitness gain, from the host’s perspective. This is expected to occur via selection on the host’s genetic underpinnings of the host–microbiome interactions, selecting for genetic variants that would support successful colonization, establishment and maintenance of the adaptation-providing microbial species or strain. These variants might be in genes related, for example, to the immune system, to setting the skin/gut pH, or to provisioning by the host of certain nutrients in the gut mucous layer, through the diet, or in breast milk. Similarly, selection may favour on this time scale behaviours that increase faithful vertical transmission from parent to offspring or that facilitate successful uptake of the microbial partner from the environment. We refer to this scenario as the microbiome-mediated Baldwin effect (see glossary in box 1, an example in box, and also figure 1).

Notably, these dynamics may lead to the replacement of the microbial species/strains that provided the initial adaptive plasticity with strains that bestow a greater fitness benefit to their host. This would require what may be viewed as second-order selection, i.e. the strains that provide higher fitness to the host do not directly experience positive selection thanks to this service, but benefit from their increased contribution to the host via an increased likelihood that their host will survive for longer or reproduce more successfully, thus providing them with more opportunities to survive and spread [55,56]. Such replacement of microbial strains in favour of those that most benefit the host is likely to occur only if different microbial partners lead to large differences in fitness among individual hosts.

(d) Long-term evolution

(i) Scenario 1: genetic adaptation of the host to the environmental challenge

On long time scales, the microbiome-mediated plasticity may eventually be replaced by genetic adaptation of the host to the environmental conditions. As opposed to the traditional concept of genetic assimilation, where the initial plasticity is replaced by a less plastic or a fixed phenotype that best accommodates the environmental challenge, the genetic adaptation of the host in our case would necessarily be via a different mechanism or trait. Thus, for example, it may be that the original accommodation of a pathogen was via uptake of a microbial species that outcompetes the pathogen in its physical niche (i.e. colonization resistance; [57]), while the genetic accommodation of the challenge would be via changes in the immune system that would protect from the pathogen directly. We refer to this scenario as microbiome-mediated genetic assimilation (box 1 and figure 1).

Box 3. Why have a complex microbiome? The merits of outsourcing

The complexity of some host-associated microbiomes is perplexing, and is surprising when compared across body sites and across taxa. There are plenty of examples in which organisms seem to successfully limit their microbiome to be composed of a select number of species. The gut microbiomes of many vertebrates, for example, thus present a puzzle. Why permit such hard-to-control mayhem, which sometimes comes at a high cost, such as the establishment of pathogens or the risk that a regular resident becomes virulent?

We suggest that the complexity of many animal gut microbiome compositions may be the result of massive outsourcing of high-cost–low-gain ‘services’, which—just as in industries in which outsourcing is common—it is not worthwhile for the host to regularly maintain. Thus, for example, a major service provided by a large number of gut bacteria seems to be the metabolizing of complex carbohydrates, a task that generally yields low rewards and requires maintenance of many metabolic and regulatory pathways.

In particular, outsourcing of specialized functions is beneficial if those functions are very diverse and needed only sporadically: if, for example, an organism primarily feeds on a particular resource, then it is perhaps worthwhile to specialize in its breakdown. If, however, the organism’s diet varies constantly and/or is highly diverse, such specialization might not be worthwhile or may be unfeasible: it requires maintenance of complex genetic and metabolic networks and their regulation. Instead, recruitment of bacteria for a task can be done flexibly, on a short time scale: even a microbe that is initially found in the gut in extremely low abundance may increase in population size by many orders of magnitude within a few days or even less time, if the substrate that it uses becomes abundant. The microbiome thus constitutes a highly modular, self-regulating mechanism, whose recruitment on short time scales comes at a cheap price. Moreover, the necessary microbes need not always be searched for and collected from across diverse habitats; the host might sometimes be able to take advantage of the bacteria that inhabit the food source and are adapted to break it down. Thus, in the case of sporadic food sources, the host is likely to have a selective advantage if it can exploit the locally present and adapted microbes.

Furthermore, once a species’ evolutionary trajectory had been set and the species ‘committed’ (from an evolutionary perspective) to maintaining a complex microbiome, accumulation of further species or services that it provides might be ‘cheap’. For example, for a species without an associated microbiome, accepting a commensal microbe that provides an added yield of, say, 1% to the species’ caloric intake from its diet might have low value, if at all, considering the costs and risks associated with carrying a microbial organism. However, if the species already carries a microbiome with dozens or hundreds of species in it, adding yet another might increase risk or costs only negligibly compared with a 1% added caloric value from the diet. A useful metaphor for this process is in the management of a restaurant: the major expenses are on infrastructure and operation; once the business is running, the added cost of accommodating an additional customer or of adding a dish to the menu are almost negligible.

(ii) Scenario 2: slowed-down genetic adaptation to the challenge, or no genetic adaptation at all

The microbiome-mediated plasticity may also have the opposite effect: it may be that the fitness benefit provided via the microbiome brings individuals to a fitness peak such that a genetic adaptation would not provide a further fitness increase. A similar outcome would result when the microbially provided adaptation brings individuals close to the fitness peak in the adaptive landscape, such that the differential of selection is too weak to generate effective selection in favour of genetic accommodation of the challenge by the host. In this scenario, genetic adaptation might never occur (figure 1), or might occur much slower than it might have occurred in the absence of the initial adaptive plasticity provided by the microbiome. Notably, this rate-comparison might often be a somewhat meaningless thought experiment: initial plasticity that allows accommodation of the challenge is sometimes imperative for the species’ survival (i.e. without the microbially provided plasticity the host population would have gone extinct), or for recurring interaction with the novel niche, a requirement for selection to act on adaptation to that niche (e.g. attempts to use a novel food source).

Whether microbiome-mediated adaptation would eventually be replaced by genetic adaptation depends not only on the eventual distance from the fitness peak that a host reaches thanks to its microbiome, but also on the process that leads each individual to that point. If the process, within an

individual’s developmental trajectory, is long or is prone to errors, e.g. some individuals pick-up suboptimal strains and fail to arrive at (or near enough to) the fitness peak, then the differential of selection between genetically adapted lineages and lineages that rely on microbiome-mediated adaptations may be large enough to replace the latter with the former.

4. The microbiome-driven reverse-Baldwin effect

In the Baldwin effect, plasticity allows accommodation of a novel challenge and an eventual genetic adaptation to it. We propose that selection, coupled with activity of the host-associated microbiome, can drive an opposite trend, the microbiome-driven reverse-Baldwin effect (figure 1).

As opposed to the scenario discussed in the rest of this article, consider a population of hosts that is well adapted to its environment. The microbiome may influence the host phenotype in many ways, including protection from pathogen establishment or in break-down of complex carbohydrates in the gut. What would happen if these were functions that were also already carried out by the host itself?

There is a possibility that the redundancy between the microbiome’s actions and those of the host would lead to reduced selective pressure on these host functions, making them vulnerable to mutation-driven deterioration that is not purged by purifying selection, until eventual loss of function. The final result is the opposite from that of the Baldwin

effect: here, the host loses a genetic adaptation that had previously existed.

Loss of a host pathway in this way makes the host permanently dependent on its microbiome. It would drive, in turn, selective pressures that would increase the likelihood of reaching an appropriate microbiome composition that would provide the required functions, making the host–microbe relationship closer to an obligatory one, at least from the host’s perspective. These would potentially increase selection in favour of behaviours that ensure vertical transmission such as coprophagy or parental smearing of the eggs with their own microbiome.

Notably, the loss of the host function as a result of reduced purifying selection may turn out in the long run to be an adaptation or a maladaptation, depending, as is often the case, on the details: avoiding the need to control a genetic pathway may be beneficial, as it saves time, energy and resources, but the dependency it creates on the associated microbiome may be disadvantageous in multiple respects: the risk of reaching a suboptimal microbiome composition with respect to crucial functions that it must provide, or the cost of microbiome maintenance, the cost of policing to prevent establishment of pathogens within the resident microbiome and the risk that residents would develop pathogenicity.

Both dynamics of microbiome-mediated accommodation of an environmental challenge and those of reverse-Baldwin effects may lead to massive ‘outsourcing’ of necessary functions to the microbiome (box 3).

5. The consequences of microbiome-provided phenotypic adaptation for the host’s evolutionary trajectory

The comparison between microbially provided plasticity and conventional modes of phenotypic plasticity, which we have pointed out intermittently, yields the observation that learning shares similarities with microbially provided plasticity quite often, while other forms of phenotypic plasticity do less so. This is particularly true with respect to dynamics on intermediate time scales, i.e. within-generation of the host, and across a number of generations: as learned behaviour can improve in its effectiveness in accommodation of an environmental challenge with time and experience, so can the host–microbiome dynamics allow for secondary selection of microbial partners after initial accommodation of the challenge. As later generations may benefit from learning the adaptive behaviour from their seniors, so can adaptation-providing microbes be picked up early in a population that benefits from a certain microbe that provides adaptive plasticity, allowing individuals’ immune systems to recognize and accept it early and allowing that species to benefit from early colonization and associated priority effects. Part of this similarity is related to the role of transmission in both of these modalities—learning and microbiome, which does not exist in a similar form in the case of genetically determined or environmentally induced phenotypic plasticity. The joint consideration of learning and microbiome as modes of adaptive plasticity may thus be fruitful. Moreover, it may highlight intermediate time scale dynamics that emerge in learning-mediated plasticity that have been under-considered in the discussion of the Baldwin effect, while being treated in other contexts, such as in accounts

of cultural evolution [58]. Of course, as highlighted above, the microbially provided plasticity also triggers dynamics that do not find an analogue in learning nor in other conventional forms of phenotypic plasticity.

One consideration that we have not discussed at depth is the robustness and consistency of microbially provided plasticity that allows accommodation of a challenge, compared with the robustness and consistency of conventional forms of phenotypic plasticity. The multiplicity of ‘players’ in microbially provided plasticity may create a large difference in this respect: the broad range of possible microbiome compositions allows a respectively broad breadth of plastic responses to challenges; however it also means that a stable state is rarely achieved. Microbiome compositions change constantly, driven by a combination of many forces, from stochastic effects to selective dynamics, from competition between microbial species to coevolution of cross-feeding relations between consortia of species. These changes imply, from the host’s perspective, that accommodation of an environmental challenge that is mediated by the microbiome might not be easily reproducible, may deteriorate over time, and might even be lost, within the lifetime of an individual and even more so across generations. In other words, microbially provided plasticity may be very flexible compared with conventional forms of phenotypic plasticity thanks to the many species involved, but this may come at the expense of robustness and consistency, as each of these species is a separate target of selection dynamics. This is in stark difference from genetically provided plasticity, where selection may act directly to favour hosts with an adaptive plastic response and maintain it once achieved. We noted earlier that whether a microbially provided phenotypic adaptation will subsist or be replaced by a genetic adaptation of the host depends on the location in the adaptive landscape to which the microbial adaptation leads. Of course, other factors that are regularly considered in such questions also come into play: the ruggedness of the landscape, the availability of genetic variation, and factors related to population dynamics, such as population size and population structure. However, in the case of microbially provided plasticity, a number of non-traditional factors are involved as well. It may be important to consider a more explicit definition of the adaptive landscape; traditional definitions associate a fitness with each genotype or phenotype. Our earlier use of fitness landscape invokes the latter. However, how to best define a landscape that relates a microbiome-provided adaptation to fitness is not obvious; should it consider the fitness of an individual that reached a particular microbiome composition, or the mean fitness reached by hosts in that individual’s lineage, considering those individuals that reached somewhat different microbiome compositions? Should it consider the fitness over a host’s lifetime, including partially associated costs, such as the probability that one of the microbes becomes virulent? How should it take into account variation in the microbiome compositions over different phases of the microbial succession process, and variation in these phases’ lengths? Answers depend on the particular question being asked, and we leave elaboration of this topic to future research.

The question of when a microbially provided adaptation subsists and is not replaced by genetic assimilation is partially analogous to the question of when a plastic response, such as a learning ability, is selectively favoured over an innate function [59–61]. The answer often depends on the variability of the environment: a highly variable environment

(i.e. with recurrent changes in key parameters) calls for an ability to respond flexibly, even at the costs of occasional failure (such as unsuccessful learning) or a costly process such as a long or risky period of trial and error. The same is true for microbially provided adaptations: the considerations mentioned with respect to constructing the fitness landscape should be supplemented by consideration of the extent of environmental variation in the rate in which the adaptive function is required. This variation might rely not only on variation of the external environment, but also on host behaviour, such as the diversity of the host diet, a factor that is partially dictated by host ecology and partially determined by the host individual's choices. Together, assessing these empirically may prove challenging. Not only may the nature of the environmental variation determine whether microbially provided adaptation is maintained or replaced by genetic assimilation but it is also expected to influence many other dynamics outlined in this article and drive additional processes. Thus, for example, seasonal variation in diet may dictate a constant need for metabolic plasticity that may be accommodated via changes in microbiome composition [62], but at the same time these microbiome changes might disrupt other adaptive aspects of microbially provided plasticity, such as protection from certain pathogens. Consideration of such more complex scenarios of environmental change are beyond the scope of the current article.

A related perspective to consider focuses on the ability of selective dynamics to maintain a genetic adaptation: even if some of the microbially provided functions could instead be carried out by genetically encoded mechanisms of the host itself, purifying selection may fail to operate effectively in purging arising deleterious mutations if these functions are required only rarely, such as the breakdown of a rare complex carbohydrate or the detoxification of an uncommon toxin.

Finally, an open question is whether reliance on the microbiome for provisioning of adaptive functions increases or decreases a species' long-term extinction probability. One might imagine that developing reliance on other organisms that may be promiscuous or might not be recruited successfully is a risky strategy, prone to error, that might lead sooner or later to disaster. Alternatively, one might envision this as a risk-averse strategy, that spreads risks in an underappreciated way: a complex microbiome can be viewed as a highly modular system with much redundancy in many of the functions that it can provide and with huge potential for rapid accommodation of new challenges, even from existing variation among the microbiome's species, strains and functions. These may be crucial in the face of environmental change.

6. The need for experimental tests of microbiome-mediated host evolution

A particular challenge for the future will be to dissect precisely to what extent and in what form microbiome-mediated plastic responses really influence evolution of the host. The inference of cause–effect relationships is key and thus a rigorous experimental approach is needed. Two types of experiments are likely most instructive: evolution experiments and transplantation experiments. Controlled evolution experiments under laboratory conditions allow us to directly compare the influence of alternative conditions on the evolutionary outcome. Host adaptation to a new environmental context can thus be

studied either in the presence or in the absence of a microbiome-mediated plastic response, as recently exemplified for a model association between the nematode *C. elegans* and symbiotic *E. faecalis* (box 2; [20]). Experimental and genetic analysis of the evolved material will then permit us to analyse the emergence of host adaptive responses related to genetic assimilation, the Baldwin effect, or genomic deterioration. Time-shift experiments and/or controlled competition experiments with the evolved organisms may further allow us to obtain information on selection differentials and at least some part of the fitness landscape, which may then help us to identify specific trait combinations and/or specific host–microbe associations that are favoured by selection. This experimental evolution approach requires availability of a suitable host system with (i) a short generation time, (ii) accessibility to controlled experimentation in the laboratory, (iii) a microbiome that can be manipulated effectively, including generation of sterile hosts and subsequent controlled recolonization, and (iv) available databases and tools for informative genomic analysis and functional genetic validation of host changes. Possible hosts that fulfil these criteria may include the traditional genetic model systems, such as the mouse, the fruit fly *Drosophila melanogaster*, the nematode *C. elegans* or the thale cress *Arabidopsis thaliana*. Thanks to advances in CRISPR/Cas technology for genetic analysis and gnotobiotic methods, a variety of other host taxa, such as turquoise killifish [63], zebrafish [64], *Hydra* [65], *Nasonia* [66] and honeybees [67], will soon become suitable for such an approach. The dissection of the underlying genetic changes will benefit from application (and further development) of quantitative genetic models, which explicitly consider the contribution of the microbiome (or individual microbes) to the overall phenotypic variance, as recently proposed (e.g. [18]).

As an alternative, microbiome transplantation experiments can help under certain conditions to uncover the involved evolutionary processes, especially for host taxa that are not amenable to the performance of evolution experiments (e.g. hosts with long generation times). Two main conditions have to be fulfilled for the approach to be informative: (i) the host–microbiome association needs to have adapted to at least two distinct conditions (e.g. high versus low temperature), yielding different adaptive outcomes, which can then be contrasted in the transplantation experiment, and (ii) the microbiome of this host taxon can be manipulated, ideally including—as above—the generation of gnotobiotic hosts and subsequent controlled recolonization (i.e. as a prerequisite to being able to perform a transplantation experiment). These conditions may be given for certain host systems with historical records, such as domesticated plants or animals, or material from previous evolution experiments, for which the microbiome could have influenced the adaptive outcome even if it had not been the focus of the experiment. A full factorial design, similar to a common garden set-up and based on reciprocal microbiome transplantation and exposure to the relevant alternative environmental conditions, should then permit us to infer whether adaptation is due to the microbiome alone, the host alone, or specific host–microbiome combinations. Functional genetic and genomic analysis can subsequently be performed, in order to assess whether the host evolved to express a new beneficial trait that may have originally been produced by the microbiome, thus indicating what we term microbiome-initiated genetic assimilation; or whether the host evolved to harbour specific microbes that express the

favourable trait, through either vertical transmission or specific environmental uptake of the microbes, thus indicating a microbiome-dependent Baldwin effect; and/or whether it shows signatures of genetic degeneration of genes relevant for the interaction with the tested environmental condition.

These experimental approaches are essential to test whether the microbiome is indeed the cause of changes in host evolution. In addition, a more precise description of the involved processes may already be an advance, including for example: characterization of the expression of different types of beneficial traits by single microbes or the community of microbes (Which microbes or interacting microbes can provide benefits? What type of benefits under which conditions are possible?); the metacommunity dynamics underlying microbiome-mediated plastic responses (Where do the beneficial microbes come from? With which do they interact within the community and how? How do they spread within single hosts and within the meta-community?); or the transmission dynamics of different types

of microbes with either beneficial or non-beneficial effects (Are beneficial microbes preferentially transmitted vertically or can they be specifically taken up from the environment?). Such descriptions will help to identify the likely most influential processes determining microbiome-mediated host evolution.

Data accessibility. This article has no additional data.

Authors' contributions. Both authors developed the concepts and jointly wrote the article.

Competing interests. We declare we have no competing interests.

Acknowledgements. We thank the Kolodny and Schulenburg groups for helpful discussions and advice. We also thank Sharon Greenblum, Marcus Feldman and David Relman's laboratory members for insightful comments on early versions of the outlined ideas. We are grateful for funding from the Gordon and Betty Moore Foundation (GBMF9341, <https://doi.org/10.37807/GBMF9341>; O.K.), the German Science Foundation within the CRC 1182 (projects A1.1 and A4.3, H.S.) and also under Germany's Excellence Strategy—EXC 22167–39088401 (Excellence Cluster Precision Medicine in Chronic Inflammation; H.S.).

References

1. Simpson GG. 1953 The Baldwin effect. *Evolution* **7**, 110–117. (doi:10.1111/j.1558-5646.1953.tb00069.x)
2. Waddington CH. 1953 Genetic assimilation of an acquired character. *Evolution* **7**, 118–126. (doi:10.1111/j.1558-5646.1953.tb00070.x)
3. Crispo E. 2007 The Baldwin effect and genetic assimilation: revisiting two mechanisms of evolutionary change mediated by phenotypic plasticity. *Evolution* **61**, 2469–2479. (doi:10.1111/j.1558-5646.2007.00203.x)
4. Price TD, Qvarnström A, Irwin DE. 2003 The role of phenotypic plasticity in driving genetic evolution. *Proc. R. Soc. Lond. B* **270**, 1433–1440. (doi:10.1098/rspb.2003.2372)
5. Ghalambor CK, Hoke KL, Ruell EW, Fischer EK, Reznick DN, Hughes KA. 2015 Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature. *Nature* **525**, 372–375. (doi:10.1038/nature15256)
6. Robinson BW, Dukas R. 1999 The influence of phenotypic modifications on evolution: the Baldwin effect and modern perspectives. *Oikos* **85**, 582–589. (doi:10.2307/3546709)
7. Sznajder B, Sabelis MW, Egas M. 2012 How adaptive learning affects evolution: reviewing theory on the Baldwin effect. *Evol. Biol.* **39**, 301–310. (doi:10.1007/s11692-011-9155-2)
8. Ancel LW. 2000 Undermining the Baldwin expediting effect: does phenotypic plasticity accelerate evolution? *Theor. Popul. Biol.* **58**, 307–319. (doi:10.1006/tpbi.2000.1484)
9. Grether GF. 2005 Environmental change, phenotypic plasticity, and genetic compensation. *Am. Nat.* **166**, E115–E123. (doi:10.1086/432023)
10. West-Eberhard MJ. 2003 *Developmental plasticity and evolution*. Oxford, UK: Oxford University Press.
11. Hinton GE, Nowlan SJ. 1987 How learning can guide evolution. *Complex Syst.* **1**, 495–502.
12. Smith JM. 1987 When learning guides evolution. *Nature* **329**, 761–762. (doi:10.1038/329761a0)
13. Ehrenreich IM, Pfennig DW. 2016 Genetic assimilation: a review of its potential proximate causes and evolutionary consequences. *Ann. Bot.* **117**, 769–779. (doi:10.1093/aob/mcv130)
14. Levis NA, Pfennig DW. 2019 Phenotypic plasticity, canalization, and the origins of novelty: evidence and mechanisms from amphibians. In *Sem. Cell Dev. Biol.* **88**, 80–90.
15. Scheiner SM, Holt RD. 2012 The genetics of phenotypic plasticity. X. Variation versus uncertainty. *Ecol. Evol.* **2**, 751–767. (doi:10.1002/ecs3.217)
16. McFall-Ngai M *et al.* 2013 Animals in a bacterial world, a new imperative for the life sciences. *Proc. Natl Acad. Sci. USA* **110**, 3229–3236. (doi:10.1073/pnas.1218525110)
17. Alberdi A, Aizpurua O, Bohmann K, Zepeda-Mendoza ML, Gilbert MTP. 2016 Do vertebrate gut metagenomes confer rapid ecological adaptation? *Trends Ecol. Evol.* **31**, 689–699. (doi:10.1016/j.tree.2016.06.008)
18. Henry LP, Bruijning M, Forsberg SKG, Ayroles JF. 2019 Can the microbiome influence host evolutionary trajectories? *bioRxiv*, 700237. (doi:10.1101/700237)
19. Webster N, Reusch T. 2017 Microbial contributions to the persistence of coral reefs. *ISME J.* **11**, 2167–2174. (doi:10.1038/ismej.2017.66)
20. Rafaluk-Mohr C, Ashby B, Dahan DA, King KC. 2018 Mutual fitness benefits arise during coevolution in a nematode-defensive microbe model. *Evol. Lett.* **2**, 246–256. (doi:10.1002/evl3.58)
21. King KC *et al.* 2016 Rapid evolution of microbe-mediated protection against pathogens in a worm host. *ISME J.* **10**, 1915–1924. (doi:10.1038/ismej.2015.259)
22. Ford SA, Kao D, Williams D, King KC. 2016 Microbe-mediated host defence drives the evolution of reduced pathogen virulence. *Nat. Commun.* **7**, 13430. (doi:10.1038/ncomms13430)
23. Sonnenburg JL, Xu J, Leip DD, Chen C-H, Westover BP, Weatherford J, Buhler JD, Gordon JL. 2005 Glycan foraging in vivo by an intestine-adapted bacterial symbiont. *Science* **307**, 1955–1959. (doi:10.1126/science.1109051)
24. Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon JL. 2009 The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. *Sci. Transl. Med.* **1**, 6ra14. (doi:10.1126/scitranslmed.3000322)
25. Townsend GE, Han W, Schwalm ND, Raghavan V, Barry NA, Goodman AL, Groisman EA. 2019 Dietary sugar silences a colonization factor in a mammalian gut symbiont. *Proc. Natl Acad. Sci. USA* **116**, 233–238. (doi:10.1073/pnas.1813780115)
26. Martino ME, Joncour P, Leenay R, Gervais H, Shah M, Hughes S, Gillet B, Beisel C, Leulier F. 2018 Bacterial adaptation to the host's diet is a key evolutionary force shaping *Drosophila-Lactobacillus* symbiosis. *Cell Host Microbe* **24**, 109–119. (doi:10.1016/j.chom.2018.06.001)
27. Natan E, Fitak RR, Werber Y, Vortman Y. 2020 Symbiotic magnetic sensing: raising evidence and beyond. *Phil. Trans. R. Soc. B* **375**, 20190595. (doi:10.1098/rstb.2019.0595)
28. Zmora N, Suez J, Elinav E. 2019 You are what you eat: diet, health and the gut microbiota. *Nat. Rev. Gastroenterol. Hepatol.* **16**, 35–56. (doi:10.1038/s41575-018-0061-2)
29. Al Nabhani Z, Eberl G. 2020 Imprinting of the immune system by the microbiota early in life. *Mucosal Immunol.* **13**, 183–189. (doi:10.1038/s41385-020-0257-y)
30. Robinson CD, Klein HS, Murphy KD, Parthasarathy R, Guillemin K, Bohannan BJM. 2018 Experimental bacterial adaptation to the zebrafish gut reveals a primary role for immigration. *PLoS Biol.* **16**, e2006893. (doi:10.1371/journal.pbio.2006893)
31. Ford SA, Williams D, Paterson S, King KC. 2017 Co-evolutionary dynamics between a defensive

- microbe and a pathogen driven by fluctuating selection. *Mol. Ecol.* **26**, 1778–1789. (doi:10.1111/mec.13906)
32. Sousa A, Frazão N, Ramiro RS, Gordo I. 2017 Evolution of commensal bacteria in the intestinal tract of mice. *Curr. Opin. Microbiol.* **38**, 114–121. (doi:10.1016/j.mib.2017.05.007)
 33. Barroso-Batista J, Pedro MF, Sales-Dias J, Pinto CJG, Pereira H, Demengeot J, Gordo I, Xavier K. 2019 Specific eco-evolutionary contexts in the mouse gut reveal *Escherichia coli* metabolic versatility. *Curr. Biol.* **30**, 1049–1062. (doi:10.1016/j.cub.2020.01.050)
 34. Frazão N, Sousa A, Lässig M, Gordo I. 2019 Horizontal gene transfer overrides mutation in *Escherichia coli* colonizing the mammalian gut. *Proc. Natl Acad. Sci. USA* **116**, 17 906–17 915. (doi:10.1073/pnas.1906958116)
 35. Yaffe E, Relman DA. 2020 Tracking microbial evolution in the human gut using Hi-C reveals extensive horizontal gene transfer, persistence and adaptation. *Nat. Microbiol.* **5**, 343–353. (doi:10.1038/s41564-019-0625-0)
 36. Zhao S, Lieberman TD, Poyet M, Kauffman KM, Gibbons SM, Groussin M, Xavier RJ, Alm EJ. 2019 Adaptive evolution within gut microbiomes of healthy people. *Cell Host Microbe* **25**, 656–667. (doi:10.1016/j.chom.2019.03.007)
 37. Vandeputte D *et al.* 2017 Quantitative microbiome profiling links gut community variation to microbial load. *Nature* **551**, 507–511. (doi:10.1038/nature24460)
 38. Carrier TJ, Reitzel AM. 2017 The hologenome across environments and the implications of a host-associated microbial repertoire. *Front. Microbiol.* **8**, 802. (doi:10.3389/fmicb.2017.00802)
 39. Itoh H, Hori T, Sato Y, Nagayama A, Tago K, Hayatsu M, Kikuchi Y. 2018 Infection dynamics of insecticide-degrading symbionts from soil to insects in response to insecticide spraying. *ISME J.* **12**, 909–920. (doi:10.1038/s41396-017-0021-9)
 40. Di Rienzi SC, Britton RA. 2019 Adaptation of the gut microbiota to modern dietary sugars and sweeteners. *Adv. Nutr.* **11**, 616–629. (doi:10.1093/advances/nmz118)
 41. Carmody RN *et al.* 2019 Cooking shapes the structure and function of the gut microbiome. *Nat. Microbiol.* **4**, 2052–2063. (doi:10.1038/s41564-019-0569-4)
 42. van den Bosch TJM, Welte CU. 2017 Detoxifying symbionts in agriculturally important pest insects. *Microb. Biotechnol.* **10**, 531–540. (doi:10.1111/1751-7915.12483)
 43. Ziegler M *et al.* 2019 Coral bacterial community structure responds to environmental change in a host-specific manner. *Nat. Commun.* **10**, 1. (doi:10.1038/s41467-018-07882-8)
 44. Ziegler M, Seneca FO, Yum LK, Palumbi SR, Voolstra CR. 2017 Bacterial community dynamics are linked to patterns of coral heat tolerance. *Nat. Commun.* **8**, 14213. (doi:10.1038/ncomms14213)
 45. Costello EK, Stagaman K, Dethlefsen L, Bohannan BJM, Relman DA. 2012 The application of ecological theory toward an understanding of the human microbiome. *Science* **336**, 1255–1262. (doi:10.1126/science.1224203)
 46. Koskella B, Hall LJ, Metcalf CJE. 2017 The microbiome beyond the horizon of ecological and evolutionary theory. *Nat. Ecol. Evol.* **1**, 1606–1615. (doi:10.1038/s41559-017-0340-2)
 47. Coyte KZ, Schluter J, Foster KR. 2015 The ecology of the microbiome: networks, competition, and stability. *Science* **350**, 663–666. (doi:10.1126/science.aad2602)
 48. Sieber M *et al.* 2019 Neutrality in the metaorganism. *PLoS Biol.* **17**, e3000298. (doi:10.1371/journal.pbio.3000298)
 49. Fierer N. 2017 Embracing the unknown: disentangling the complexities of the soil microbiome. *Nat. Rev. Microbiol.* **15**, 579. (doi:10.1038/nrmicro.2017.87)
 50. Miller ET, Svanbäck R, Bohannan BJM. 2018 Microbiomes as metacommunities: understanding host-associated microbes through metacommunity ecology. *Trends Ecol. Evol.* **33**, 926–935. (doi:10.1016/j.tree.2018.09.002)
 51. Miller ET, Bohannan BJM. 2019 Life between patches: incorporating microbiome biology alters the predictions of metacommunity models. *Front. Ecol. Evol.* **7**, 276. (doi:10.3389/fevo.2019.00276)
 52. Sprockett D, Fukami T, Relman DA. 2018 Role of priority effects in the early-life assembly of the gut microbiota. *Nat. Rev. Gastroenterol. Hepatol.* **15**, 197–205. (doi:10.1038/nrgastro.2017.173)
 53. Laforest-Lapointe I, Arrieta M-C. 2017 Patterns of early-life gut microbial colonization during human immune development: an ecological perspective. *Front. Immunol.* **8**, 788. (doi:10.3389/fimmu.2017.00788)
 54. Roughgarden J, Gilbert SF, Rosenberg E, Zilber-Rosenberg I, Lloyd EA. 2018 Holobionts as units of selection and a model of their population dynamics and evolution. *Biol. Theory* **13**, 44–65. (doi:10.1007/s13752-017-0287-1)
 55. Van Vliet S, Doebeli M. 2019 The role of multilevel selection in host microbiome evolution. *Proc. Natl Acad. Sci. USA* **116**, 20 591–20 597. (doi:10.1073/pnas.1909790116)
 56. Daybog I, Kolodny O. 2020 Simplified model assumptions artificially constrain the parameter range in which selection at the holobiont level can occur. *Proc. Natl Acad. Sci. USA* **117**, 11 862–11 863. (doi:10.1073/pnas.2004737117)
 57. Buffie CG, Pamer EG. 2013 Microbiota-mediated colonization resistance against intestinal pathogens. *Nat. Rev. Immunol.* **13**, 790–801. (doi:10.1038/nri3535)
 58. Creanza N, Kolodny O, Feldman MW. 2017 Cultural evolutionary theory: how culture evolves and why it matters. *Proc. Natl Acad. Sci. USA* **114**, 7782–7789. (doi:10.1073/pnas.1620732114)
 59. Johnston TD. 1982 Selective costs and benefits in the evolution of learning. In *Adv. Stud. Behav.* **12**, 65–106.
 60. Stephens DW. 1991 Change, regularity, and value in the evolution of animal learning. *Behav. Ecol.* **2**, 77–89. (doi:10.1093/beheco/2.1.77)
 61. Bergman A, Feldman MW. 1995 On the evolution of learning: representation of a stochastic environment. *Theor. Popul. Biol.* **48**, 251–276. (doi:10.1006/tpbi.1995.1029)
 62. Smits SA *et al.* 2017 Seasonal cycling in the gut microbiome of the Hadza hunter-gatherers of Tanzania. *Science* **357**, 802–806. (doi:10.1126/science.aan4834)
 63. Smith P, Willemsen D, Popkes M, Metge F, Gandiwa E, Reichard M, Valenzano DR. 2017 Regulation of life span by the gut microbiota in the short-lived African turquoise killifish. *eLife* **6**, e27014. (doi:10.7554/eLife.27014)
 64. Burns AR, Guillemin K. 2017 The scales of the zebrafish: host–microbiota interactions from proteins to populations. *Curr. Opin. Microbiol.* **38**, 137–141. (doi:10.1016/j.mib.2017.05.011)
 65. Klimovich AV, Bosch TCG. 2018 Rethinking the role of the nervous system: lessons from the *Hydra* holobiont. *Bioessays* **40**, 1800060. (doi:10.1002/bies.201800060)
 66. Dittmer J, van Opstal EJ, Shropshire JD, Bordenstein SR, Hurst GDD, Brucker RM. 2016 Disentangling a holobiont – recent advances and perspectives in *Nasonia* wasps. *Front. Microbiol.* **7**, 1478. (doi:10.3389/fmicb.2016.01478)
 67. Zheng H, Steele MI, Leonard SP, Motta EVS, Moran NA. 2018 Honey bees as models for gut microbiota research. *Lab. Anim.* **47**, 317–325. (doi:10.1038/s41684-018-0173-x)