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Why do hosts malfunction without microbes? Missing benefits versus evolutionary addiction

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Abstract

Microbes are widely recognized to be vital to host health. This new consensus rests, in part, on experiments showing how hosts malfunction when microbes are removed. More and more microbial dependencies are being discovered, even in fundamental processes such as development, immunity, physiology, and behavior. But why do they exist? The default explanation is that microbes are beneficial; when hosts lose microbes, they also lose benefits. Here I call attention to evolutionary addiction, whereby a host trait evolves a need for microbes without having been improved by them. Evolutionary addiction should be considered when interpreting microbe-removal experiments, as it is a distinct and potentially common process. Further, it may have unique implications for the evolution and stability of host-microbe interactions.

What's wrong with the beneficial microbiome narrative?

Host organisms often exhibit defects when their microbiome is disrupted or removed entirely. This phenomenon has been known for decades [1–3] and supports the now-generally accepted notion that microbes are fundamentally important to host biology (e.g., [4–6]). The malfunctioning of microbe-free hosts—those deprived of specific symbionts or the entire microbiome—encompasses a wide range of host diversity and of host traits, including key processes such as development, metabolism, nutrition, physiology, immunity, and behavior [7–10]. Detailed molecular mechanisms have emerged for many microbial effects in model hosts, and major efforts are underway to leverage this knowledge for microbiome engineering.

The malfunctioning of microbe-free hosts is of enormous fundamental and applied interest, but why does it occur in the first place? This question is rarely examined through an evolutionary lens. The most common interpretation is what I call "missing benefits": performance of trait X in a microbe-free host is compromised because microbial services that benefit X are missing. The microbiome is often considered to be beneficial to hosts. For example, the literature is full of statements such as "...beneficial interactions between the host and its associated microbiota are key requirements for host health" [8].

The concept of benefits is frequently used to convey something more than just higher host fitness or trait performance with versus without microbes. It also implies novelty, utility, or improvement: microbes have enabled hosts to perform X, or to perform it more effectively. The microbiome literature bears this out. Hosts "receive a multitude of benefits from their microbial communities, such as enhanced nutrition and protection from enemies" [11] and gain "new physiological abilities as a result of contributions from their microbial partners" [5]. Microbes "extend the host genome" [12] and

“perform metabolic processes that the animal or plant cannot carry out without the microbiota” [6]. Microbe-free host malfunctioning may also be taken to indicate that the microbes in question were adaptive, increasing host fitness because of improvements to X. Indeed, microbiomes are widely recognized as key contributors to host adaptation (e.g., [6,13–15]).

Often, missing benefits (hereafter MB) easily explains the data. In these cases, microbes provide unambiguously beneficial services that expand or improve host functionality. Rhizobia fix nitrogen, benefitting the nutrition of leguminous plants. Gut bacteria in herbivorous animals break down plant polysaccharides with unique digestive enzymes, releasing useful byproducts such as short-chain fatty acids. Photosynthetic or chemosynthetic symbionts provide organic carbon to marine invertebrates. In these examples microbes provide a useful service that their host cannot perform on its own. MB would be a justified interpretation of experiments showing, for example, reduced growth in nitrogen-poor soil of a microbe-free legume. Further, it is reasonable to infer that these symbioses were adaptive for hosts.

The difficulty comes when MB is used to explain microbial dependencies of endogenous functions—those that a host used to, or should, be able to perform on its own (Box 1). This form of dependence is common. For example, microbe-free hosts often show behavioral defects, such as a lack of motivation to feed or move; physiological defects, such as abnormal digestive processes and barrier maintenance; an inability to activate signaling pathways that control key steps in development; and reduced expression of genes involved in immunity and other processes [1,16–19]. (Side effects of antibiotics or other methods for eliminating microbes may sometimes explain these phenomena but will not be discussed further).

MB transforms defects of microbe-free hosts into positive microbial functions, using terms that convey active, beneficial, and potentially adaptive roles in host biology: microbes regulate, induce, activate, stimulate, mediate, modulate, promote, teach, and train. But in these examples, what novel or useful services are microbes offering? Were these microbes adaptive in providing hosts with the necessary motivation to feed? Or in helping hosts figure out how to regulate their genes? In contrast to cases like rhizobia or photosymbionts, here there is no obvious currency being provided to hosts. Microbially mediated regulation of endogenous processes does have the potential to be beneficial (Box 2). But benefits are not the only, or even most likely, explanation for these kinds of defects.

Evolutionary addiction can explain host-microbe dependencies

A different perspective is that hosts which evolved in the presence of certain microbes will malfunction when those microbes are suddenly removed. Malfunctioning occurs because, over evolutionary time, the microbes have

become integrated into key aspects of host biology. This dependence can be thought of as an addiction [20]: something becomes needed, but has not expanded or improved host capabilities. For example, I need coffee to perform basic functions, but I don't perform them any better now than before the addiction began. I need coffee just to get back to normal. The same process can occur with host-microbe symbioses: a dependence evolves without an improvement in functionality. This general state has been termed evolved dependence and discussed with regard to mutualists [21], parasites [22], and selfish genetic elements [23].

Herbivory provides an example of how dependence can arise without novelty, utility, or improvement. Some plants produce lower biomass and fewer flowers, fruits, and seeds when deprived of herbivores (e.g., [24]). So are herbivores actually plant mutualists rather than parasites? Do herbivores benefit plants by teaching them to grow and reproduce? The paradox can be resolved by rethinking how we define benefits and mutualism [21]. Organisms adapt to features of the environment—including other organisms—that they frequently encounter. The absence of a habitual partner can lower an organism's fitness simply because it is an abnormal state, one lacking cues and inputs that the organism has incorporated into its biology.

In the realm of host-microbe symbiosis, evolved dependence has been labeled evolutionary addiction (EA) [20,25,26]. EA occurs when microbes become integrated into, and necessary for, a host trait (X) without benefitting it—i.e., without adaptively improving or expanding host functionality relative to an earlier state (Fig. 1). A necessary precondition for EA is that the microbes are persistently associated with the host. This may occur if the microbes benefit other host traits besides X, are themselves under selection to spread among hosts (e.g., parasites), or are simply ubiquitous in the host's local environment**.**

Though EA has empirical support and is occasionally discussed in the literature [20,25–27], it is not widely considered in the microbiome field. Researchers may be unaware of it, find mutualistic and adaptive narratives more intuitive, or just consider it a fringe phenomenon. Rather than an oddity, EA should be expected to generate trait-microbe dependencies whenever hosts evolve in the continual presence of microbes. One would expect an organism to perform poorly with climatic conditions, light/dark cycles or food that it never experiences in nature, and the same expectation should extend to microbes. These are recurring features of the environment in which hosts evolve. When they are suddenly and drastically changed, a lot can go wrong.

A relatively well-studied example of EA concerns the wasp *Asobara tabida*, which is chronically infected with the bacterial endosymbiont *Wolbachia*. *A. tabida* requires *Wolbachia* to carry out oogenesis [28]. When experimentally

deprived of *Wolbachia*, the wasps exhibit extensive apoptosis of nurse cells, which are needed for oocyte maturation [29]. Other *Asobara* species that are not chronically infected with *Wolbachia* (and some that are) do not need them for oogenesis [28,30]. Relative to the other wasp species, the ability of *A. tabida* to make eggs has not been improved by *Wolbachia*.

How did microbial dependence evolve in this case? One potential pathway is adaptive. *Wolbachia* are common reproductive manipulators [31], and in ancestral *A. tabida* wasps they may have interfered with apoptosis in host ovaries (where *Wolbachia* are most abundant) [29]. The resulting harm to host reproduction could then have selected for wasps that integrated *Wolbachia*'s manipulative effects into how it regulates ovarian function. In the process, the wasp's reproductive system became reliant on it. Another potential pathway is neutral. If *Wolbachia* produce a redundant oogenesis-mediating factor, relaxed selection would allow loss-of-function mutations to accumulate in the host version [29]. These two mechanisms of EA are discussed at a more general level below.

Adaptive accommodation

An evolutionary addiction can arise when a host trait adapts to accommodate microbes, in the process becoming dependent on them to function (Fig. 2). Adaptive accommodation occurs because, especially in the early stages of a host-microbe symbiosis, there is a lot of room for improvement. This is clear in the case of a purely parasitic interaction. Alternatively, symbionts may provide a benefit but also come with harmful side effects. For example, they may grow excessively or in the wrong place, induce inflammation, manipulate the host to further their transmission, or antagonize other beneficial microbes. More generally, the symbiosis is in a suboptimal state. For example, the microbe's microhabitat within the host may not be well-adjusted for its growth, or existing transmission mechanisms do not transmit the microbe with high fidelity.

Selective pressures such as these favor adaptive host responses, which may be induced or otherwise dependent on the microbes themselves. For example, some mammalian gut bacteria degrade the mucus layer lining the colon [32]. A sensible host response is to create a mucus production system that is activated when potentially mucus-degrading bacteria are detected. This process can explain why germ-free rodents produce a thinner mucus layer than conventional rodents, and why normal mucus can be restored by adding peptidoglycan or lipopolysaccharide [33]. Those molecules are structural components of bacterial cells and do not directly contribute to building the mucus layer. Microbes are often said to maintain gut homeostasis [8] or promote gut barrier function [34]. Such phrasing is not inaccurate, but may convey a microbially mediated improvement that has not actually occurred.

Adaptive accommodation may contribute to the widespread dependence of endogenous immune function on microbial symbionts [16,35–37]. Imagine a new symbiosis between a host and a non-pathogenic microbe. Hosts experience some inflammation as a side effect of new microbial cells consistently colonizing their bodies. The elevated baseline of inflammation-inducing microbes selects for a less-sensitive immune response, thereby increasing infection tolerance. (A similar mechanism may partly explain why reservoir hosts of zoonotic pathogens are often highly tolerant of them, with little sign of morbidity when infected [38]). The host now possesses a regulatory system that requires the microbes for normal immune function. It is commonly said that microbes activate, induce, or stimulate the host immune system, but in this scenario, they have not benefitted it. Note that there are other adaptive pathways to immune dependence on symbionts. For example, hosts may experience inflammation if deprived of coevolved symbionts that produce immunomodulatory molecules [39].

Developmental functions may become dependent on microbes via adaptive accommodation. For example, mosquito larvae depend on hypoxia generated by the growth of aerobic gut microbes to induce molting [17,40,41]. In theory, this could be because insect molting serves as a useful way to clear excess microbes from the gut, as well as a critical developmental process. By triggering molting when excess numbers of microbes have accumulated—sensed by hypoxia—a normal developmental pathway has become microbe-dependent. The observation that germ-free mice have slower gastrointestinal transit [42] could have a similar explanation: they have adapted to regulate bacterial overgrowth through microbially induced transit. In pigs, gut transit is stimulated by parasitic worms [43], a presumably adaptive response to clear infections.

Compensated trait loss

Another way EA can arise is compensated trait loss (CTL; [44]), where: 1) microbes perform a similar function as the host, 2) selection on host function is relaxed because of microbial compensation, 3) host function is lost neutrally (Fig. 2). The microbes have not improved the trait in taking it over. In fact, the end result could be worse for hosts; for example, there are long-term costs to relying on microbial partners for essential functions [45,46]. Note that as a mechanism of evolutionary addiction, CTL is distinct from outsourcing [47] or Black Queen dynamics [48], where relinquishing a task to microbes is adaptive because it reduces costs or provides other advantages.

The concept of constructive neutral evolution, formulated to explain intermolecular dependencies [49,50], illustrates how CTL could operate. Take the process of subfunctionalization. After gene duplication, both copies compensate for each other, allowing the accumulation of degenerative

mutations that create interdependence without any improvement to the ancestral function. The same compensation can occur between organisms. For example, *Wolbachia* can restore fertility to otherwise-sterile *Drosophila* mutants [51], showing how deleterious host mutations could persist in the presence of compensatory symbionts.

Jeon and colleagues' experimental evolution of a protist-bacteria symbiosis illustrates how a metabolic dependence could be generated by CTL. At first, the protist grows fine on its own. After co-culture with pathogenic bacteria, it evolves to tolerate and then depend on them [52]. The dependence is linked to the enzyme S-adenosylmethionine (SAM) synthetase, a highly conserved enzyme encoded by both host and symbiont [53]. SAM is integral to many metabolic processes. The symbiont-evolved protists no longer express the enzyme; when deprived of the symbionts, they die [54]. The symbiont's version of S-adenosylmethionine synthetase appears to have compensated for the host's version, allowing the latter to incur regulatory defects. However, the possibility of other underlying mechanisms cannot yet be ruled out.

CTL has been suggested to explain why maturation of the host immune system requires microbial cues [55]. Microbial cues are often needed to "prime" immunity, but they are not necessarily better than endogenous ones. If microbial cues are consistent and compensatory, they can relax selection on endogenous cues, allowing the latter to be lost (or never gained) [55]. This process could also operate on other endogenous pathways regulated by redundant inputs, including those mediating growth and reproduction. For example, CTL has been proposed to underlie the obligate dependence of fungal sporulation (in *Rhizopus microsporus*) on bacterial symbiont-produced factors [56].

CTL can operate on host defense, as shown experimentally in *Drosophila melanogaster* [57]. Virus resistance in *D. melanogaster* is controlled to a large degree by the gene *pastrel*. *Wolbachia* contribute to (and thus compensate for) virus resistance. When flies are experimentally evolved with just the virus, a virus-resistant *pastrel* allele rapidly reaches fixation. When evolved alongside *Wolbachia* as well, selection on *pastrel* is relaxed, slowing spread of the resistant allele and resulting in a more virus-susceptible fly population [57]. A similar "divestment" of endogenous defense has been shown to occur when *C. elegans* nematodes are experimentally evolved with a defensive bacterial symbiont [58].

Other mechanisms and features of evolutionary addiction

Adaptive accommodation and compensated trait loss are not the only ways by which host-microbe dependencies can arise. Microbes might actively impose addictions to promote their persistence. Proof of concept comes from

plasmids and other mobile genetic elements (MGEs) that encode coupled toxin-antitoxin systems wherein the toxin persists longer than the antitoxin [59]. The host cell is protected from poisoning only so long as the MGE is present and expressing the antidote, effectively forcing it to maintain the MGE. In theory, the same dynamic could occur in host-microbe symbioses. One potential case is the protist *Bodo saltans*, which rapidly dies when deprived of its bacterial endosymbiont. The endosymbiont genome encodes multiple toxin-antitoxin systems but no metabolic functions with discernible value for the host [60].

Divisions between EA and MB, and between the various mechanisms of EA, may not be clear-cut. Multiple processes may operate on different traits in the same host. For example, the fungus *Rhizopus microsporus* requires its symbiont for the endogenous process of sporulation, but also benefits from a uniquely symbiont-synthesized toxin [56]. Multiple process may also operate within the same trait in the same host, depending on how broadly the trait is defined. For example, both EA and MB can explain the susceptibility of *Wolbachia*-free *Drosophila* to viral infection [57].

Different mechanisms of dependence may interact. For example, EA may counter-balance MB. Returning to the analogy of a coffee addiction, one could argue that there is a moderate gain in alertness—a benefit—with initial consumption. But once the addiction sets in, continued consumption is needed to avoid a much larger loss of alertness. And one process may engender the other. A microbe providing an adaptive function can be expected to spread among hosts, facilitating the subsequent evolution of dependence. Conversely, parasitic or commensal microbes to which hosts are addicted might be co-opted for novel, beneficial functions.

Evolved dependencies can be narrow or broad. Some examples involve specific, coevolving symbionts, such as *Wolbachia*. Others appear to be diffuse, where a variety of taxa (or broadly conserved microbial molecules) rescue defective phenotypes of microbe-free hosts. For example, colonic mucus production in germ-free rodents can be restored by feeding lipopolysaccharide or peptidoglycan, as discussed above [33]. Development of axenic mosquito larvae can be restored by a broad range of bacteria or eukaryotes, including taxa not normally present in the microbiome [40,61]. But in most cases, it is not known exactly which microbes are required for a given host trait to function. Colonizing microbe-free hosts with isolates or defined communities is restricted to hosts with culturable symbionts. Dependencies may also involve interactions among microbes (e.g., [62]), which will be challenging to tease apart in hosts with complex microbiomes.

Why evolutionary addiction matters, and how to study it

By largely ignoring EA, the microbiome field has missed a plausible and likely common evolutionary explanation for microbially dependent host traits. Its probable commonality is due to the fact that any host organism is a complex, internally interconnected system; the absence of a microbe that has been integrated into it, like a cog in a machine, will cause components to malfunction. EA and MB are fundamentally different processes, though they can lead to the same experimental result (Fig. 1).

Are there real-world implications of whether a host trait depends on microbes due to MB versus EA? One may be the evolutionary potential of host traits following microbiome disturbance. Consider the aforementioned wasp *Asobara tabida*. If *Wolbachia* disappeared, *A. tabida* could likely adapt from standing genetic variation [30] or new mutations and gain the ability to make eggs on its own. The dependence is relatively recent, and derived within *Asobara*; closely related wasps can make eggs without *Wolbachia* [28,30]. Now consider a legume that depends on rhizobia to fix nitrogen. If rhizobia disappeared, it is extremely unlikely that the host could gain the ability to fix nitrogen on its own (considering that no plant has ever been able to do this)**.** EA-generated dependencies could thus be more reversible, though obviously more work is needed to formalize and test this hypothesis. Reversibility matters when we consider disruptions of long-associated microbial symbionts. Which traits, in which hosts, will evolution be able to rescue in the microbes' absence?

EA could be considered as an eventual outcome when we create new and persistent host-microbe associations. This may occur unintentionally, such as when wild hosts are brought into captivity and exposed to novel microbes, or with the intentional introduction of probiotics. In the latter case, unexpected dependencies could evolve in traits beyond those which the probiotic was originally intended to benefit. We may even consider EA in probiotic engineering if we want to better lock in the new symbiosis. For example, harmful side effects or compensatory features are not normally desirable properties of a probiotic, but they might have stabilizing effects in the long term.

The standard experimental design does not distinguish MB from EA (Fig. 1) and doing so is not trivial. In theory, one way to do so is to compare trait function of hosts evolved with the microbes, in the presence of those microbes, to hosts evolved without the microbes, in the absence of those microbes [21]. This approach—though not broadly feasible—would reveal whether a trait has been expanded or improved by microbes. Conceivably, one could follow host evolutionary responses to natural introductions of microbes over time—especially for hosts that can be collected over time, stored, and revived for experiments. Or, one could analyze host-microbe interactions over space, using a mosaic of host populations with heterogenous microbial histories [63]. For hosts with short generation times,

new symbioses can be experimentally evolved. This approach would allow a host trait to be directly compared between pre-microbe ancestors and post-microbe descendants.

Mapping microbial associations and dependencies onto the host phylogeny can also guide inference [22]. If one host is naturally infected with a microbe and requires it to perform endogenous function X, while a second, closely related host naturally lacks the microbe and can perform X without it, EA is likely to have operated on X in the first host. The derived dependence of *Asobara tabida* oogenesis is a good example. A dependence has been gained, but the function has stayed the same.

Even with just the standard experimental design and the data we already have, we can acknowledge EA as a viable interpretation of microbe-free host malfunctioning. EA is less familiar and less intuitive than MB but is arguably a less onerous explanation in some cases. This is particularly true for endogenous processes (Box 1), like regulation of host gene expression (Box 2), for which there is no evidence of a hypothetical missing benefit. EA should also be considered likely when the defective function can be rescued with microbial structural components that, in and of themselves, probably do not do anything useful for that function.

Concluding Remarks

It will be difficult to empirically distinguish EA from MB for particular microbe-dependent traits in particular hosts. An alternative approach is to develop theory determining the conditions under which EA is most likely to arise. A recent theoretical model shows that life cycle overlap—i.e., shared habitat preferences and dispersal routes—can cause a microbe to become enriched in the host's local environment without it necessarily having provided any benefit to the host [64]. In turn, enrichment sets the stage for EA. Attention should also be paid to the types of traits that are most likely to evolve dependence on microbes. Microbe-free hosts are not completely defective in all respects. On the contrary, some traits are unaffected or improved in the microbe-free condition (e.g., [1,2]). It should also be noted that some hosts appear not to need microbial symbionts at all [45].

More generally, we need a better understanding of the EA process itself. Among other unknowns (see Outstanding Questions), what is the role of coevolution in EA? I have taken the host's perspective, considering microbes as just another feature of the environment. But microbes also evolve and may coevolve with hosts. Coevolution is not required for EA to arise, only persistence of the host-microbe association, which may be highly asymmetrical or generalized. But when it occurs, coevolution could change the dynamics of EA, particularly when mutual dependencies evolve in both partners.

Evolutionary addiction complicates the common narrative that microbes are "important" to animals, plants, and other hosts. They are certainly important, but the reasons why matter. Are hosts and microbes "friends" (e.g., [65])? That view accords well with MB, but not EA. Similarly, the standard classification of host-associated microbes as mutualists, commensals, or parasites based on their contemporary fitness effects does not easily accommodate the nuances of EA. The microbial histories of individual traits in individual hosts are varied and will be difficult to fit neatly into boxes. If we are to achieve a broadly representative conceptual model of host-microbe interactions, we should not only measure microbial effects on hosts and study their mechanisms, but also further explore why they exist at all.

Endogenous functions in a microbial world (Box 1)

Evolutionary addiction involves endogenous functions: those that hosts should be able to perform independently. Consider a hypothetical host function whose performance depends on certain microbes. The dependence exists in a contemporary host population that has evolved with the microbes in question. The function can be considered endogenous if it was fully executable before the host acquired those microbes. Or, if there was no pre-microbe state—e.g., as with gut bacteria generally—we can think of endogenousness in terms of functionality: are the microbes needed for their unique contribution to the function, or only because their presence has been integrated into the regulatory systems that control it?

Does it even make sense to talk about a currently microbe-dependent yet endogenous function, given that hosts evolved in a microbial world [4]? If they have always interacted with microbes, can any organism do anything on its own? I argue that it does if we keep in mind that microbial associations and dependencies are highly heterogeneous across hosts. Even just focusing on animals, many taxa either do not need microbial symbionts at all [45], or only need them for a specific function, like bioluminescence or biosynthesis of certain nutrients. Their existence implies that fundamental host processes —such as behavior, development, physiology, and defense—are, in a very general sense, viable without microbial symbionts.

Why be regulated by microbes? (Box 2)

Defects of microbe-free hosts are often regulatory in nature. For example, expression of metabolic or immune genes, developmental pathways, or physiological processes may be under-activated. These cases are likely to have arisen through EA because the function being regulated does not fundamentally require unique microbial services. This is especially apparent when structural components of microbial cells restore the function in

microbe-free hosts (e.g., [33,66]). Through the EA lens, microbe-associated stimuli or cues become woven into the host's regulatory fabric; removing them rips holes in the fabric. There is no expanded functionality of the trait that becomes microbially regulated. Take epigenetic programming, bodily contractions, or circadian rhythms, which are disrupted in some microbe-free animals [67–69]. Though microbially dependent now, it is not clear how those functions have been improved by symbiosis. This is distinct from whether they have adapted to better manage the symbiosis (see "adaptive accommodation" in the main text).

The MB lens frames regulatory defects as evidence that microbes provide benefits such as "teaching", "training", or "promoting homeostasis" (e.g., [8,70,71]). There are certainly scenarios where microbial regulation could be adaptive for hosts. Microbes might serve as biosensors, providing unique and useful information about the host's internal or external environment. Hosts could leverage that information to, for example, decide where and when to best complete development and reproduce [25,72,73]. But overall, EA-generated regulatory dependencies might be as, if not more, common.

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References

1. Wostmann, B.S. (1981) The Germfree Animal in Nutritional Studies. *Annual Review of Nutrition* 1, 257–279
2. Hale, M.G. *et al.* (1973) Gnotobiotic culture of plants and related research. *Bot. Rev* 39, 261–273
3. Buchner, P. (1965) *Endosymbiosis of animals with plant microorganisms*, John Wiley & Sons
4. McFall-Ngai, M. *et al.* (2013) Animals in a bacterial world, a new imperative for the life sciences. *PNAS* 110, 3229–3236
5. Lynch, J.B. and Hsiao, E.Y. (2019) Microbiomes as sources of emergent host phenotypes. *Science* 365, 1405–1409
6. Rosenberg, E. and Zilber-Rosenberg, I. (2016) Microbes Drive Evolution of Animals and Plants: the Hologenome Concept. *mBio* 7, e01395-15
7. Smith, K. *et al.* (2007) Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. *Seminars in Immunology* 19, 59–69
8. Sommer, F. and Bäckhed, F. (2013) The gut microbiota — masters of host development and physiology. *Nat Rev Microbiol* 11, 227–38
9. Morais, L.H. *et al.* (2021) The gut microbiota–brain axis in behaviour and brain disorders. *Nat Rev Microbiol* 19, 241–255
10. Hooper, L.V. *et al.* (2012) Interactions between the microbiota and the immune system. *Science* 336, 1268–73

11. Scheuring, I. and Yu, D.W. (2012) How to assemble a beneficial microbiome in three easy steps. *Ecol Lett* 15, 1300–1307
12. Müller, D.B. *et al.* (2016) The Plant Microbiota: Systems-Level Insights and Perspectives. *Annu. Rev. Genet.* 50, 211–234
13. Shapira, M. (2016) Gut Microbiotas and Host Evolution: Scaling Up Symbiosis. *Trends in Ecology & Evolution* 31, 539–549
14. Alberdi, A. *et al.* (2016) Do Vertebrate Gut Metagenomes Confer Rapid Ecological Adaptation? *Trends in Ecology & Evolution* 31, 689–699
15. Kolodny, O. *et al.* (2020) The role of the microbiome in host evolution. *Phil. Trans. R. Soc. B* 375, 20190588
16. Round, J.L. and Mazmanian, S.K. (2009) The gut microbiota shapes intestinal immune responses during health and disease. *Nat Rev Immunol* 9, 313–323
17. Coon, K.L. *et al.* (2017) Bacteria-mediated hypoxia functions as a signal for mosquito development. *PNAS* 114, E5362–E5369
18. Zheng, H. *et al.* (2017) Honeybee gut microbiota promotes host weight gain via bacterial metabolism and hormonal signaling. *PNAS* 114, 4775–4780
19. Dohnalová, L. *et al.* (2022) A microbiome-dependent gut–brain pathway regulates motivation for exercise. *Nature* 612, 739–747
20. Moran, N.A. (2002) The Ubiquitous and Varied Role of Infection in the Lives of Animals and Plants. *The American Naturalist* 160, S1–S8
21. de Mazancourt, C. *et al.* (2005) Understanding mutualism when there is adaptation to the partner. *J Ecology* 93, 305–314
22. Fellous, S. and Salvaudon, L. (2009) How can your parasites become your allies? *Trends in Parasitology* 25, 62–66
23. Werren, J.H. (2011) Selfish genetic elements, genetic conflict, and evolutionary innovation. *PNAS* 108, 10863
24. Paige, K.N. and Whitham, T.G. (1987) Overcompensation in Response to Mammalian Herbivory: The Advantage of Being Eaten. *The American Naturalist* 129, 407–416
25. Moran, N.A. *et al.* (2019) Evolutionary and ecological consequences of gut microbial communities. *Annu. Rev. Ecol. Evol. Syst.* 50, 451–475
26. Douglas, A.E. (2010) *The Symbiotic Habit*, Princeton University Press
27. Sullivan, W. (2017) *Wolbachia*, bottled water, and the dark side of symbiosis. *Molecular Biology of the Cell* 28, 2343–2346
28. Dedeine, F. *et al.* (2001) Removing symbiotic *Wolbachia* bacteria specifically inhibits oogenesis in a parasitic wasp. *PNAS* 98, 6247–6252
29. Pannebakker, B.A. *et al.* (2007) Parasitic inhibition of cell death facilitates symbiosis. *PNAS* 104, 213–215
30. Dedeine, F. *et al.* (2005) *Wolbachia* requirement for oogenesis: occurrence within the genus *Asobara* (Hymenoptera, Braconidae) and evidence for intraspecific variation in *A. tabida*. *Heredity* 95, 394–400
31. Werren, J.H. *et al.* (2008) *Wolbachia*: master manipulators of invertebrate biology. *Nature Reviews Microbiology* 6, 741–51
32. Cani, P.D. *et al.* (2022) *Akkermansia muciniphila*: paradigm for next-generation beneficial microorganisms. *Nat Rev Gastroenterol Hepatol* 19, 625–637
33. Petersson, J. *et al.* (2011) Importance and regulation of the colonic mucus barrier in a mouse model of colitis. *American Journal of Physiology-Gastrointestinal and Liver Physiology* 300, G327–G333
34. Leonardi, I. *et al.* (2022) Mucosal fungi promote gut barrier function and social behavior via Type 17 immunity. *Cell* 185, 831-846.e14

35. Kwong, W.K. *et al.* (2017) Immune system stimulation by the native gut microbiota of honey bees. *R. Soc. Open Sci.* 4, 170003
36. Shalev, O. *et al.* (2022) Commensal *Pseudomonas* strains facilitate protective response against pathogens in the host plant. *Nat Ecol Evol* 6, 383–396
37. Weiss, B.L. *et al.* (2011) Tsetse Immune System Maturation Requires the Presence of Obligate Symbionts in Larvae. *PLoS Biol* 9, e1000619
38. Mandl, J.N. *et al.* (2015) Reservoir Host Immune Responses to Emerging Zoonotic Viruses. *Cell* 160, 20–35
39. Graham, A.L. *et al.* (2022) The evolution of powerful yet perilous immune systems. *Trends in Immunology* 43, 117–131
40. Valzania, L. *et al.* (2018) Both living bacteria and eukaryotes in the mosquito gut promote growth of larvae. *PLOS Neglected Tropical Diseases* 12, e0006638
41. Valzania, L. *et al.* (2018) Hypoxia-induced transcription factor signaling is essential for larval growth of the mosquito *Aedes aegypti*. *Proc. Natl. Acad. Sci. U.S.A.* 115, 457–465
42. Kashyap, P.C. *et al.* (2013) Complex Interactions Among Diet, Gastrointestinal Transit, and Gut Microbiota in Humanized Mice. *Gastroenterology* 144, 967–977
43. Masure, D. *et al.* (2013) The Intestinal Expulsion of the Roundworm *Ascaris suum* Is Associated with Eosinophils, Intra-Epithelial T Cells and Decreased Intestinal Transit Time. *PLoS Negl Trop Dis* 7, e2588
44. Ellers, J. *et al.* (2012) Ecological interactions drive evolutionary loss of traits. *Ecology Letters* 15, 1071–1082
45. Hammer, T.J. *et al.* (2019) Not all animals need a microbiome. *FEMS Microbiol. Lett.* 366, fnz117
46. Bennett, G.M. and Moran, N.A. (2015) Heritable symbiosis: The advantages and perils of an evolutionary rabbit hole. *PNAS* 112, 10169–10176
47. Kolodny, O. and Schulenburg, H. (2020) Microbiome-mediated plasticity directs host evolution along several distinct time scales. *Phil. Trans. R. Soc. B* 375, 20190589
48. Morris, J. *et al.* (2012) The Black Queen Hypothesis: Evolution of Dependencies through Adaptive Gene Loss. *mBio* 3, e00036-12
49. Stoltzfus, A. (2012) Constructive neutral evolution: exploring evolutionary theory's curious disconnect. *Biol Direct* 7, 35
50. Muñoz-Gómez, S.A. *et al.* (2021) Constructive Neutral Evolution 20 Years Later. *J Mol Evol* 89, 172–182
51. Starr, D.J. and Cline, T.W. (2002) A host–parasite interaction rescues *Drosophila* oogenesis defects. *Nature* 418, 76–79
52. Jeon, K.W. (1972) Development of Cellular Dependence on Infective Organisms: Micrurgical Studies in Amoebas. *Science* 176, 1122–1123
53. Jeon, T.J. and Jeon, K.W. (2003) Characterization of sams Genes of *Amoeba proteus* and the Endosymbiotic X-Bacteria. *J Eukaryotic Microbiology* 50, 61–69
54. Choi, J.Y. *et al.* (1997) Evidence for Symbiont-induced Alteration of a Host's Gene Expression: Irreversible Loss of SAM Synthetase from *Amoeba proteus*. *J Eukaryotic Microbiology* 44, 412–419
55. Selosse, M.-A. *et al.* (2014) Microbial priming of plant and animal immunity: symbionts as developmental signals. *Trends in Microbiology* 22, P607-613
56. Partida-Martinez, L.P. *et al.* (2007) Endosymbiont-Dependent Host Reproduction Maintains Bacterial-Fungal Mutualism. *Current Biology* 17, 773–777
57. Martinez, J. *et al.* (2016) Addicted? Reduced host resistance in populations with defensive symbionts. *Proc. R. Soc. B.* 283, 20160778

58. Bates, K.A. *et al.* (2021) A globally ubiquitous symbiont can drive experimental host evolution. *Molecular Ecology* 30, 3882–3892
59. Jurėnas, D. *et al.* (2022) Biology and evolution of bacterial toxin–antitoxin systems. *Nat Rev Microbiol* 20, 335–350
60. Midha, S. *et al.* (2021) *Bodo saltans* (Kinetoplastida) is dependent on a novel *Paracaedibacter*-like endosymbiont that possesses multiple putative toxin-antitoxin systems. *ISME J* 15, 1680–1694
61. Coon, K.L. *et al.* (2014) Mosquitoes rely on their gut microbiota for development. *Molecular Ecology* 23, 2727–2739
62. Gould, A.L. *et al.* (2018) Microbiome interactions shape host fitness. *PNAS* 115, E11951–E11960
63. Thompson, J.N. (2005) *The geographic mosaic of coevolution*, University of Chicago Press
64. Sieber, M. *et al.* (2021) On the evolutionary origins of host–microbe associations. *PNAS* 118, e2016487118
65. Robinson, J. (2023) *Invisible Friends: How Microbes Shape Our Lives and the World Around Us*, Pelagic Publishing
66. Schwarzer, M. *et al.* (2023) Microbe-mediated intestinal NOD2 stimulation improves linear growth of undernourished infant mice. *Science* 379, 826–833
67. Krautkramer, K.A. *et al.* (2016) Diet-Microbiota Interactions Mediate Global Epigenetic Programming in Multiple Host Tissues. *Molecular Cell* 64, 982–992
68. Brooks, J.F. *et al.* (2021) The microbiota coordinates diurnal rhythms in innate immunity with the circadian clock. *Cell* 184, 4154-4167.e12
69. Murillo-Rincon, A.P. *et al.* (2017) Spontaneous body contractions are modulated by the microbiome of *Hydra*. *Sci Rep* 7, 15937
70. Honda, K. and Littman, D.R. (2016) The microbiota in adaptive immune homeostasis and disease. *Nature* 535, 75–84
71. Cani, P.D. *et al.* (2019) Microbial regulation of organismal energy homeostasis. *Nat Metab* 1, 34–46
72. Hadfield, M.G. (2011) Biofilms and Marine Invertebrate Larvae: What Bacteria Produce That Larvae Use to Choose Settlement Sites. *Annual Review of Marine Science* 3, 453–470
73. Metcalf, C.J.E. *et al.* (2019) Why Evolve Reliance on the Microbiome for Timing of Ontogeny? *mBio* 10, e01496-19

Figures

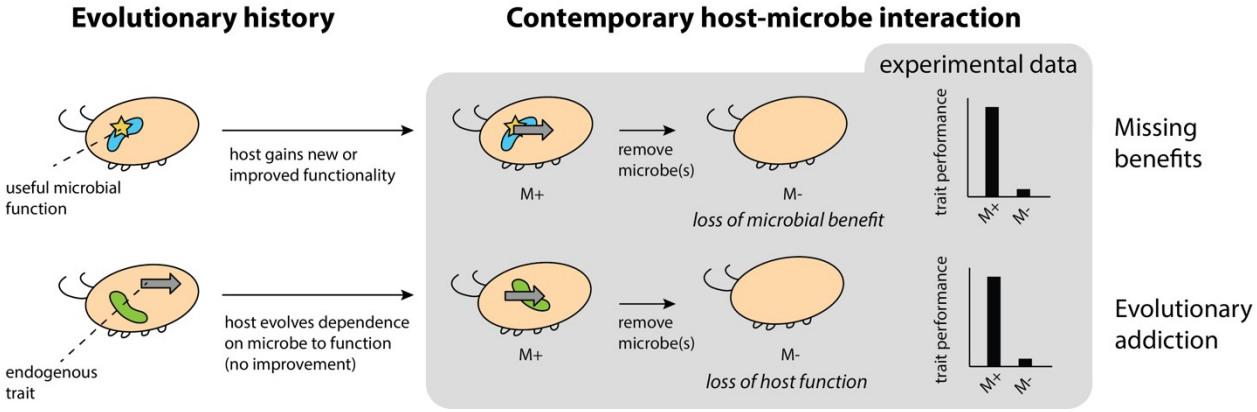

Figure 1. The same experimental result can be explained by missing benefits (MB; top pathway) or evolutionary addiction (EA; bottom pathway). A model host with only one symbiont (blue or green cell) is shown, but other microbes are expected to be present. The gray box shows the organisms we have available for experiments today. Note that the green symbiont (bottom) may or may not have functions useful to traits besides the focal one (grey arrow). Also note that EA is not mutually exclusive with MB, but they are depicted separately for clarity.

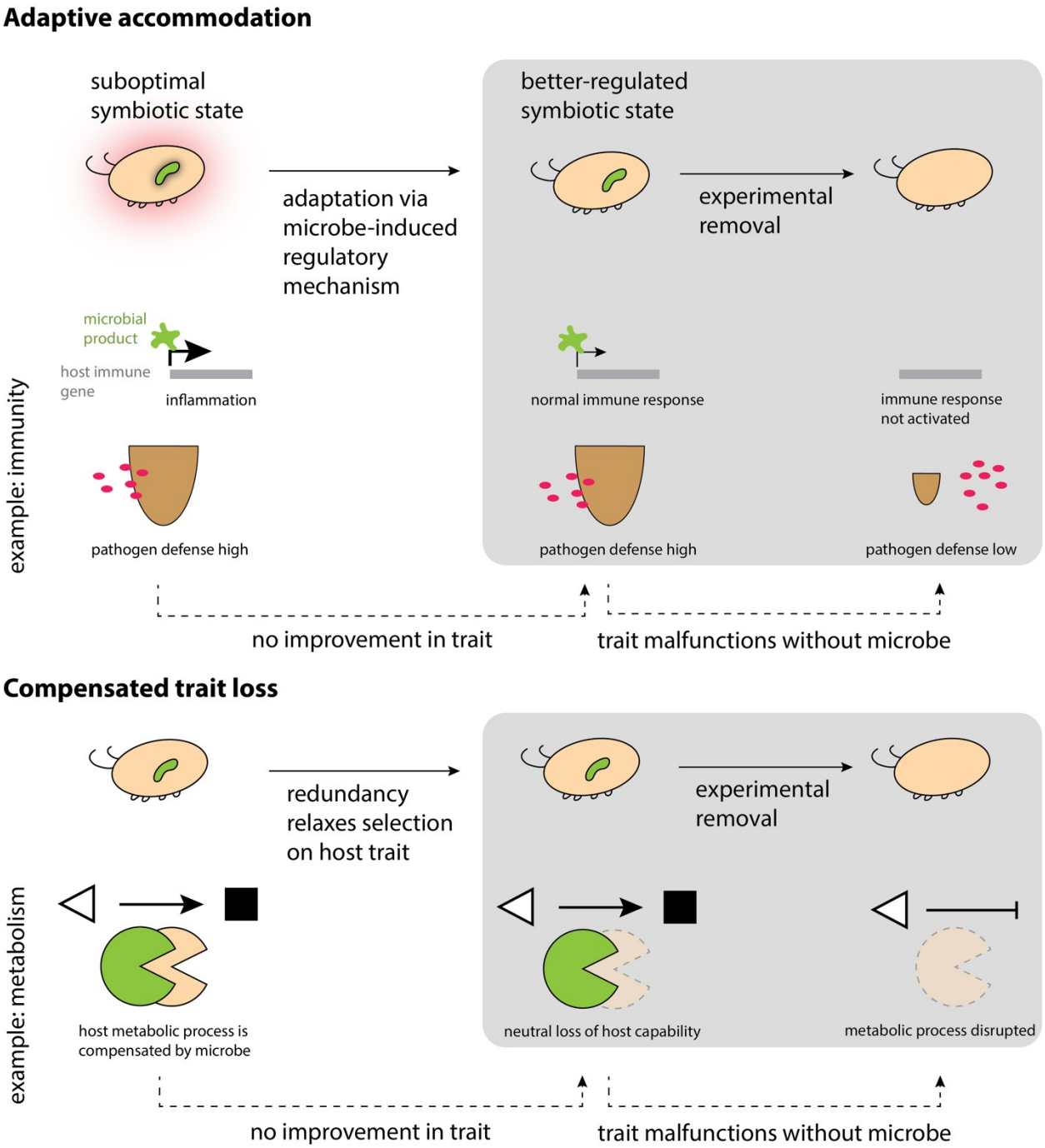

Figure 2. Two paths by which an endogenous trait can become addicted to a focal microbe (shown as green cells). The gray boxes show the organisms we have available for experiments today. For adaptive accommodation, endogenous immune function is used as an example. For compensated trait loss, metabolism is used as an example. The focal microbe may or may not have beneficial effects on other traits besides the focal one, and other microbes are expected to be present (not shown).