The Evolution of the Host Microbiome as an Ecosystem on a Leash

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The human body carries vast communities of microbes that provide many benefits. Our microbiome is complex and challenging to understand, but evolutionary theory provides a universal framework to analyse the microbiome and its health impacts. Here we argue that understanding evolutionary function – why a particular microbiome feature has evolved – is fundamental to progress. Symbiont features commonly evolve for competition within the host ecosystem, while many host features evolve to keep the ecosystem on a leash. We suggest that the health benefits of the microbiome should be understood, and studied, as a by-product of microbial competition and host control.

Introduction

Humans carry massive and diverse communities of symbiotic microbes[1,2]. These cells colonise almost every surface of the body: the skin, teeth, airways and, particularly, the epithelial surfaces of the gastrointestinal tract. They impact nutrition[4], tissue and immune development[5], pathogen resistance[6,7] and even our behaviour[8]. The importance of the human microbiome – defined here as the microbes (microbiota) plus the host environment[9] – is matched by its complexity. At each body site, many different species and strains occur, each with the potential to interact with the host via metabolism and the immune system that is itself a vastly complex biological system[5]. Moreover, each species has the potential to exert diverse effects on neighbouring microbes[10]. Some species kill others with dedicated toxins[11-13], but others invest in enzymes that feed other species for mutual benefit[14].

The microbiome is thus a complex and dynamic ecosystem, in which species are in continual flux. It is now clear that tools from theoretical ecology will play a central role in understanding and predicting the dynamics of the microbiota[1,15-18]. What has been less clear is the value in applying evolutionary thinking to understand the form and function of the whole microbiome (Box 1). The relationship between mammalian hosts and microbes is just one of a myriad of evolved symbioses that date back to the dawn of multicellular life[18]. Many of these have striking similarities to our own microbiome, indicative of general evolutionary principles to be discovered and applied (Figure 1).

Here we use the predictions of evolutionary theory to interpret microbiome data, with a particular focus on the intensely studied mammalian microbiome and the benefits it provides to the host. Our goal throughout is to understand evolutionary function: why did a particular feature of interest evolve (Box 1)? To apply this reasoning, we reduce microbiome complexity to three classes of effect, each of which has its own evolutionary characteristics: *microbe-to-host*, *host-to-microbe* and finally, *microbe-to-microbe*. Many studies have focused on how the microbiota affects human health (microbe-to-host). However, here we argue that it is the other effects (host-to-microbe and microbe-to-microbe) that explain why particular functions evolve within the microbiome. Specifically, natural selection on the microbiota alone is not expected to make them beneficial to the host; rather,

microbiota evolution is dominated by the need for each species to compete and persist within the host[10]. However, at the same time there is potential for strong natural selection on hosts to force their microbiota to be beneficial. Thus, rather than being intrinsically helpful, the microbiome is a dynamic microbial ecosystem held on an ever-evolving leash by the host (Figure 2). We discuss the implications of our perspective for microbiome health and disease, and lay out key next steps for microbiome research.

Microbe to Host: the problem of a diverse microbiome

We first consider the evolutionary origins of the best-studied aspect of the microbiome, the beneficial effects of the microbes on the host. Interactions between hosts and their microbiota appear to include many examples of biological mutualism: interactions which provide fitness benefits to all the species involved[19]. However, a degree of caution is required; while benefits to the host from their microbiota have been well documented, it can be extremely difficult to demonstrate the reverse - that microbes benefit from host association, as we typically do not know if better alternatives exist for microbes outside of the host[20]. If microbes do not benefit, the prediction is that natural selection will favour strategies to escape from the microbiome, or adaptions that increase within-host fitness, even if this harms the host. Nevertheless, for many species in environments like the human gut, the combination of relatively stable conditions, nutrients and warmth is likely to improve microbial fitness relative to host-free environments.

For microbes that benefit from living inside a host, it might seem inevitable that natural selection would favour the evolution of microbe-to-host benefits. Evolutionary theory, however, shows otherwise. Consider a bacterial strain that generates a nutrient for the host, but must divide more slowly to do so. If this is the only strain within the host, then it can persist and continue to help its host, as this slow-growing strain will have no microbial competitors. However, in a diverse microbiota, the slow growing strain runs the risk of being outcompeted by other faster growing genotypes that do not make the nutrient (Box 2). As a result, natural selection will tend to favour microbes that invest in their own reproduction, rather than help the host[3,21,22].

Crucially then, we cannot assume that the host and microbiota are a single evolutionary unit acting with a common interest, as is sometimes done in applications of the "holobiont" or "superorganism" metaphors[23] (although see[24]). Rather, the host and each individual microbial strain are distinct entities with potentially divergent selective pressures. The potential for divergent interests is made abundantly clear by the existence of pathogens within the microbiome, such as *Clostridium difficile*[7] and more transient species like *Salmonella enterica*[25]. However, these pathogens appear to be exceptions, with the microbiota dominated by helpful, or at least neutral, microbial species. We are left again with the fundamental question: why do so many members of the microbiota benefit the host?

Host to microbe: the importance of host control

An answer to why microbes help their hosts is again offered by general evolutionary theory. Here, the question of what drives between-species cooperation – a trait that evolves because of a beneficial effect on another species – has received a lot of attention. A recurring theme throughout evolutionary literature is the importance of control of one species by the other[21]. Applying this finding, we predict that hosts are under strong natural selection to control their microbiota[3], an idea also raised by some

applications of the holobiont metaphor[24]. Host control over the microbes (as opposed to microbial control of the host) is predicted because there is only one host in the interaction, in contrast to the myriad microbes. Thus, unlike individual microbes, a host can easily influence the entire microbiome, and benefit from doing so.

Evolutionary theory then predicts that host-to-microbe effects – rather than the much studied impacts of microbe on host – are critical for microbiome form and function. Here we discuss evidence for four key aspects of host control: immigration, compartmentalisation, monitoring and targeting.

Immigration and Compartmentalisation

Ecosystems are often shaped by which species happen to arrive from a global pool of species. The order of species arrivals is particularly important when there are priority effects, such that early arriving species resist invasion by later ones[26]. A host can thus exert control by influencing which microbial strains and species it encounters, and which make it to each epithelial surface. This can occur at the cognitive and behavioural level, such as by conditioned avoidance of rancid foods containing harmful microbes[27]. Once eaten, stomach acid can further reduce microbial diversity and abundance, and protect against pathogens[28], although the primary evolutionary function of acidity is typically assumed to be digestion.

Vertical transmission from parents to offspring will promote immigration of particular species, and active transmission has evolved in species including leafcutter ants[29] and the beewolf[30]. The importance of parent-offspring transmission remains unclear for the mammalian microbiome[31]. However, the infant microbiome is strikingly similar to that of the mother's vagina with natural birth, but not with caesarean, suggesting a role for parent-offspring transmission in early colonisers of infants. Similarly, breast milk contains large amounts of oligosaccharides that the baby cannot metabolise but the microbiota can, which might cement transmission of specific symbionts[32]. Picking up a beneficial microbe from a parent, however, does not guarantee it will remain beneficial throughout a host's life as the microbiota can rapidly evolve (Box 2). A host needs additional mechanisms of control.

Hosts also exert control over their microbiota by compartmentalisation, which keeps some regions largely microbe free[4,5]. In many species, the epithelial barrier greatly limits tissue invasion by microbes (although less so in plants than animals[4]). In animals, mucus also plays a key role at this barrier by permitting access to host tissue for many diffusive molecules, while limiting both the access and colonisation of microbes[33]. The secretion of mucilage in plants may play a similar role[34,35]. Finally, antimicrobial factors, including proteins such as RegIIIγ[36] and Lypd8[37], also appear to be central in maintaining the epithelial barrier by discouraging microbial growth too close to the epithelial surface (Figure 1).

Monitoring and Targeting

A host can benefit from monitoring the species in its microbiota, and targeting species to either promote or hinder their proliferation. In the mammalian intestine, microbe-specific features, for example lipopolysaccharide or flagellin, are detected by pattern recognition receptors such as toll-like receptors. The host response to these structural features – often known as Pathogen Associated Molecular Patterns (PAMPs) – is strongest where detection indicates intestinal breach, and can include both tissue repair and anti-microbial factor secretion[36,38,39]. Hosts can detect when microbes enter a cell using pattern recognition receptors inside endosomes and other locations[40]. Hosts also monitor

cell damage, such that the effects of bacterial toxins activate the inflammasome[41], and hosts detect common effects of pathogens on their cells, including actin polymerization[42]. Such surveillance is not restricted to mammals. Toll-like receptors are important for microbiome composition in more basal animals (Cnidarians)[39], and innate immunity and pattern recognition has convergently evolved in plants[43] (Figure 1).

Another host strategy is to monitor incoming benefits. Legumes house and monitor rhizobial bacteria in root nodules. If the bacteria do not fix nitrogen, the plant cuts off nutrients to the nodule[22,44]. In the vertebrate gut, short chain fatty acids such as butyrate and acetate provide energy for colonic epithelial cells[45]. Strikingly, butyrate also has potent anti-inflammatory properties in regulation of inflammatory gene expression and induction of regulatory T cells[46]. We speculate that butyrate monitoring, and specifically recognizing its absence, may drive host responses to restructure the microbiota in order to restore butyrate availability (Box 1). More generally, we predict that monitoring will occur for other nutrients, both within mammalian systems and further afield.

Detecting harmful and beneficial traits (trait-based discrimination) is a robust way for a host to monitor a microbiota that may rapidly evolve. Should a beneficial microbe evolve to be harmful, a host can detect this and respond. In this way, a host can link a microbe's fitness to the benefits it provides, and thereby generate natural selection for desirable phenotypes[47]. The evolution of such pleiotropic links is a general way to promote cooperation that may feature commonly in host-microbiome evolution[48]. The immune system can do this via localised responses – like the inflammatory response - that target harmful microbes at the position where harm is detected or inferred[41]. However, this is fallible, as shown by the ability of *Salmonella* to prosper in the face of the inflammatory response in the gut[25]. An alternative to trait-based discrimination is to monitor or target microbial genotypes via unique chemical moieties (genotype-based discrimination). There is significant variability in antimicrobial peptides both within and between host species, suggesting that the secretion of these peptides from the host epithelium helps determine which microbial genotypes prosper[36,39]. Genotype discrimination is limited by an inability to detect a beneficial strain that evolves a pathogenic phenotype, or vice versa. The adaptive immune system can solve this problem by learning new associations during infection – combining trait and genotypic information – and adaptive immunity can impact the microbiota[47,49]. However, it is not yet clear whether this results in effective control of the microbiota at epithelial surfaces.

The study of immune responses naturally leads to a focus on negative selection, but there is also the intriguing possibility that hosts target *beneficial* traits or genotypes. Recent theory predicts that feeding of the microbiota from the host epithelium is a powerful mechanism of positive selection[3]. Fed strains will bloom and push other strains and species away from the surface. How though can a host target specific strains? One trait-based mechanism is to provide substrates that are most easily used by microbes with desirable metabolic capabilities. Vertebrate hosts feed their microbiota with diverse glycans that must be removed from mucin (Figure 1), which is expected to favour bacteria with enzymes to digest complex carbohydrates, such as the Bacteroidaceae[3,50].

Mucus also has the potential to select particular symbionts in corals, where an amazing 20-45% of photosynthate is released as mucus[51] that houses a diverse microbiota[52]. Plants appear to have convergently evolved a comparable system to the animals (Figure 1). They release 25% of photosynthate into the soil, much of it as root mucilage that houses and feeds rhizosphere microbes[34,35]. Interestingly, plant mucilage contains large amounts of "arabinogalactan" proteins[53], which show striking structural and functional similarities to mucins (also glycoproteins). Symbiotic microbes not only feed on arabinogalactan proteins and mucins, they also attach to them[53-55]. Theory suggests that a host can make use of this attachment by secreting specific glycoproteins, and

molecules like IgA, that help beneficial strains to colonise[54]. This raises the possibility that adaptive immunity – via IgA – is used to hold onto beneficial symbionts (and not just inhibit harmful ones).

Microbe to microbe: surviving the microbiome jungle

While hosts may have evolved multiple means to regulate their microbiota, control of all strains is challenging and - in the face of vast microbial diversity - likely impossible. Thus, which of the microbial species persist will not only depend on host control, but also on their ability to compete in the microbiome jungle[56]. In order to understand the function of microbial traits, therefore, we must understand what it takes for a strain to succeed in the microbiome.

Evolution within the microbiota

A key determinant of evolutionary function is resource competition. Host diet has a major impact on the available resources within animals and, accordingly, which microbial species and types of metabolism can dominate[57]. Resources also come from a host's attempts to exert control, such as via mucin secretion[3,50]. Competition over resources in an evolving microbial population can drive rapid evolutionary radiations, and character displacement[58,59]. This suggests that we each carry strains that are tuned to our specific set of niches, which may in turn promote colonisation resistance by ensuring that invading strains are less evolutionarily adapted. Availability of a resource to exploit, however, is not sufficient for persistence. Microbial cells influence each other in many ways[10] and these interactions can determine whether a given strain can persist[7] and, more generally, the traits needed to compete in a given community[60].

Microbes use diverse ways to compete with other members of the microbiota[7,60]. This includes resource acquisition as just discussed but also through physical properties such as adhesiveness[67], production of antibiotics and bacteriocins, or the poisoned molecular spears of the type VI secretion system[11,13]. These weapons function to eliminate competitors and can be central to explaining which strains persist in the gut[11,12]. More subtle competition occurs by monitoring and manipulating signalling molecules of competitors, as appears to occur for the quorum sensing molecule autoinducer 2 (AI-2)[68]. There are also many phage[69], which can promote microbial diversity by limiting common strains[15] and driving horizontal genetic transfer (HGT). The evolutionary impacts of HGT, by phage and other means, is an important area for microbiome research. Even when rare, HGT can have major effects[70,71] and move a single function, like antibiotic resistance, horizontally through competing strains and species[70].

Many microbes also employ cooperative traits to remain competitive within communities. Cells secrete enzymes that break down complex molecules, siderophores that scavenge iron, and quorum sensing molecules to communicate information to others such as cell density, diffusion conditions and genetic mixing[10]. In vitro and genomic studies indicate that host-associated microbes carry many such phenotypes[61,62], but inferring the function of extracellular enzymes can be challenging. *Bacteriodes thetaiotaomicron*, an abundant species in the human gut microbiome, carries extracellular enzymes that break down complex carbohydrates. However, experimental work found little evidence that these enzymes function to help other cells (cooperation)[14]. The import of breakdown products is so effective that little is actually shared with others, which renders breakdown a largely private function.

Indeed, the evolution of microbial cooperation is only expected under certain conditions. Genotypes that benefit from cooperative traits but do not provide them (sometimes called "cheaters") can invade cooperator populations and replace them, rendering cooperation fragile. This invasion can be prevented if cells grow in single-genotype patches where one strain cannot use the resources of another. As a result, we expect that spatiogenetic structure is fundamental to how microbes evolve within microbiome communities[10,63,64]. We still understand little of spatiogenetic structure in microbiomes, but recent work revealed striking structure in dental communities[65] and (more modest) structuring in gut communities[66]. While cell-cell benefits often evolve to help clonemates[10], cooperation can also evolve *between* species. *B. ovatus* breaks down the carbohydrate inulin, at a cost, to feed other species that provide benefits in return[14]. More simply, one species can benefit from the waste products of a second species. However, waste production is not formally a cooperative function; waste is a metabolic byproduct and did not evolve to benefit other cells (Box 1). This distinction is important for understanding the robustness of communities because cooperative functions can be fragile and waste production not[10,63,64].

The microbiome is an evolving system. Competition between microbes drives both resource specialisation and the evolution of extreme weaponry. Microbes also possess many traits that function to help other cells, particularly clonemates. But how do these diverse microbial functions combine at the level of the whole microbiome, and what is the effect on the host? Here, we must turn to ecology.

Ecology of the microbiome: consequences of microbe-microbe interactions

Ecological theory provides a map between the properties of individual species and the properties of the whole community[1,15]. The mammalian microbiota often responds robustly to perturbations allowing a host to keep key species for long periods[72,73]. Theory suggests that the key to this stability lies in how species interact with one another. Weak and competitive interactions are stabilising[15] - they limit positive feedbacks and the possibility that, if one species goes down, it will take others with it. Another key property is productivity - the efficiency of converting resources into energy. Here, it is cooperative interactions that can improve a community by preventing wasteful functions like antibiotic competition[10]. A host then may face a tension between communities that are highly productive and those that are stable[15].

Another community-level property is redundancy, which can protect against the extinction of a beneficial symbiont by ensuring that another is there to take its place. There is evidence for considerable functional redundancy in microbiome systems, including the bovine rumen[74] and the polysaccharide utilizers of the human intestine[62]. However, redundancy may not need host intervention. Theory predicts that redundancy will naturally evolve via a combination of competition and stochastic processes that allows highly-similar species to persist together for long periods[75]. In conclusion, a greater focus on microbe-to-microbe interactions will be central to understanding both the functions and systems-level properties of the microbiota[15].

An ecosystem on a leash

The mammalian microbiome comprises an ecosystem within which microbes must compete to survive and persist. What makes it so fascinating is that all of this complexity occurs inside a living host, that is itself evolved. Unlike a rainforest or river ecosystem, therefore, the microbiome is not only driven from the bottom up by species interactions. Rather, the host is under strong natural selection to shape

the microbiota from the top down and keep them as beneficial as possible. We arrived at this characterisation of the microbiome - an ecosystem held on leash by the host – by applying evolutionary and ecological theory to microbiome data (above, Boxes). But what are the plausible alternatives to our perspective and, looking forward, what predictions distinguish our model from these alternatives? We discuss this next and, in the final section, we consider the implications of the leash model for microbiome health and disease.

Alternative models of host-microbiota systems

We contrast the leash model to three alternatives; a *host-control* model, a *symbiont control* model, and an *open ecosysten* model (Figure 2). While many permutations of the models are possible, these alternatives satisfy two key criteria. First, they are consistent with evolutionary theory – their evolution is predicted given certain conditions – and, secondly, each model appears supported by real-world examples.

Host control that tightly regulates microbial phenotypes is most feasible when a host individually monitors one or a few strains[21,22]. Though all hosts will also interact with microbes they cannot control, such fine-scale host control has evolved. One example is legumes, which hold nitrogen-fixing bacteria in root nodules (above)[44]. Host control also dominates the beautiful mutualism between the bobtail squid and its luminescent bacteria, wherein the host uses multiple "winnowing" mechanisms to ensure that only one strain of light-producing *Vibrio fischeri* grows in each crypt of its light organ[76,77] (Figure 2).

Contrasting host control is the possibility of *symbiont control,* where a microbe alters global host phenotypes - such as reproduction, survival, or behaviour - in order to increase its own fitness. Like host control, symbiont control is predicted when one or a few strains interact with the host[78]. One reason for this prediction is that a single strain has to be abundant enough to influence host phenotypes. The key reason though is that a strain must invest resources into host manipulation without being outcompeted by other strains not investing the resources[78] (Box 2). A master manipulator of insect hosts is the endosymbiotic bacteria *Wolbachia*, which by living inside cells avoids competition and ensures it can affect all regions of the insect. *Wolbachia* can benefit the host, for example augmenting the host's metabolism[79] but it also performs dramatic reproductive manipulations, such as killing males, that benefit the endosymbiont but not the host[80]. More dramatic still is the fungal parasite *Ophiocordyceps unilateralis*, which influences ant behaviour for its own grim ends[81] (Figure 2C). There is no clear evidence yet for such manipulation by mammalian symbionts[8], nor indeed is it typically predicted given the high diversity[78].

Finally, we consider an *open ecosystem* model where the host exerts little control over the microbiome. *Ad absurdum*, this can be envisaged by a dead or dying host that is simply a resource for the microbiota. In healthy systems, limited host control is most likely when the microbiota has weak effects on host fitness. This appears consistent with bromeliads[82], and perhaps some pitcher plants, whose leaves create a rainwater pool containing microbes[83]. In pitchers, the host may affect the microbiota via its digestive enzymes and hydrogen ions that digest prey. However, beyond compartmentalising the microbes in the pool, there is not yet evidence of evolved mechanisms of control[83].

Predictions of the leash model

How can the leash model be distinguished from these alternatives? A key approach is to compete a symbiont with a host-benefitial trait, e.g. vitamin production, with an isogenenic mutant lacking the trait. There are many potential complexities but, in general, the leash model predicts that such a trait

help the symbiont to persist in the microbiome and therefore that the mutant will be outcompeted. The reason that the mutant loses might be either due to the leash (host control) or the ecosystem (the inability to compete within the microbiota), or both at the same time. By contrast, the expectation of symbiont control is that the mutant will win, so long as it can save the resources used to manipulate the host and reinvest them into growth[78]. The host control and open ecosystem models, like the leash model, predict mutant loss but they are more restrictive on why. Host control predicts it is due to host manipulation, while the open ecosystem predicts a general inability to compete in the microbiome ecosystem that is independent of host control.

We can apply these predictions to data on *B. ovatus* that breaks down complex carbohydrates in the mammalian intestine[14], which is thought to benefit the host nutritionally (Box 2). Here, symbiont control is unlikely because a competitive benefit to carbohydrate-utilisation was found (which occurs via cross feeding another species)[14]. Moreover, this benefit was seen *ex vivo* and in a mouse model suggesting that host control is not central to the outcome. While one cannot reject the open ecosystem model, therefore, the data are consistent with the leash model. In another example, light-producing *V. fischeri* outcompete dark mutants in the squid light organ, again against symbiont control. Moreover, this is thought to occur due to host-produced enzymes, against the open ecosystem model (Figure 2B). However, no other bacterial species are found in the light organ, which rejects the leash model, where the ecosystem and species interactions are important. Host control, therefore, appears to be the best model for the squid symbiosis.

In contrast to the squid system, species interactions are known to affect the mammalian microbiome[11,14]. How is it possible to hold such a diverse dynamic community on a leash? Unlike the host-control model, the leash model does not predict near-faultless control. Rather, hosts might focus on certain hub species[84] that are important for community functioning. More generally, hosts will benefit from influencing the global properties of the microbiota. We should expect hosts to act as ecosystem engineers that influence not only individual species but community-level properties – like stability and productivity – in their pursuit of evolutionary benefits[15]. Possible mechanisms of this control include the immune system and epithelial mucus secretion, which can weaken ecological interactions by regulating species density and increasing spatial structure[15]. Interrupting the immune system or mucus secretion, therefore, may lead to a less stable, and therefore less diverse, microbiome. Any mechanism of control also has to be insured against easy escape. This is why we predict that microbes who mutate to pull against the leash will typically sustain a fitness cost within the dynamic ecosystem (see Targeting discussion above).

The importance of host control does not imply that community composition will remain static. Omnivorous hosts, in particular, may benefit from a flexible microbiota that can respond to changing metabolic demands. The fact that microbiome communities can shift strongly with host diet[57], therefore, is not in itself evidence that a host is powerless to influence communities. Indeed, we display a remarkable ability to keep major microbial lineages within our microbiota[72,73] to the extent that several bacterial lineages appear to have cospeciated with us[85]. Like many other hosts then, there is growing evidence that humans have evolved to create a microbiome environment that selects for specific bacterial lineages. Severe perturbations, however, will force a host to deal with extinctions followed by stochastic recolonisation as new species arrive at random. This potential for recolonisation is expected to promote trait-based discrimination in a host, which applies general selection for microbes based on their benefits rather than targeting specific genotypes (Host control section).

Coevolution

Our conceptual model includes the possibility of *coevolution*, which is distinct from the potentially related process of cospeciation[85]. Coevolution describes reciprocal evolutionary adaptations in different species in response to one another[86]. A classic example is the Bullhorn Acacia that gets its name from the horns it evolved to house ants. The ants fiercely defend the plant, both from insects and other plants that contact the acacia[87]. This defence is so effective that the plant appears, in turn, to have lost the normal defences against herbivory: without ants, the plant suffers severe defoliation and death. To our knowledge, there is not yet comparable evidence of such reciprocal adaptation between mammalian hosts and their beneficial microbiota. The hypothesis that there has been some degree of coevolution is a reasonable one. However, it remains possible that hosts control their microbiota and that the microbiota has evolved in response, but that the evolution in diverse microbial species is too weak for a coevolutionary feedback (which would drive further adaptation in the host). Distinguishing this null model from more elaborate, and true, coevolutionary dynamics is an important empirical goal for the future. The evolution, and coevolution, of host-microbe interactions is also a rich area for new theoretical work, especially given the complexity and rapid evolution of the microbiota.

Treating and engineering the human microbiome

There is an ongoing effort to identify individual species within the human microbiome that have particular health benefits. While this pursuit has great value, our evolutionary approach suggests that too much focus on microbe-to-host effects can be misleading. If we are correct, the benefits that microbes provide are typically by-products of them striving to persist in the microbiome; the benefits are not evolutionary functions (Boxes 1 and 2). To better understand the form and function of the microbiome, therefore, we should focus more on the evolutionary and ecological challenges that face the microbiota. These challenges come both from competition with other microbes and from the selective influences of the host, including the effects of innate and inducible immune systems. We close then by considering how the study of these effects – microbe to microbe and then host to microbe – can help with treating and engineering the human microbiome.

The list of benefits provided by the microbiota is ever increasing and includes improved nutrition[4], colonisation resistance[6,7], immune system function[5] and even mental health[8]. However, it is an open empirical question how many species actually provide benefits. Moreover, to improve health outcomes, we need to understand if species provide unique benefits and, linked to this, the functional basis of the benefit provided. For example, certain microbial strains appear to have unique effects on immune system development, which might be interpreted as individual strains evolving specific benefits for a host[88] (Box 1). However, this specificity might be better explained by host evolution and the general, but complex, actions of the immune system. Consistent with such general effects, a virus, acting through innate cytokine mediators such as type I interferons drives immune maturation of the mouse, similar to the bacterial microbiota[89].

Certainly, evolutionary theory does not predict that each symbiont strain will provide a benefit, unique or otherwise, but it does predict that all will be good competitors (Boxes 1 and 2). For decades there have been attempts to introduce particularly cellulolytic bacteria into the bovine rumen in order to improve energy yield, but despite massive doses they are often outcompeted and lost[74]. In order to design probiotics, therefore, we need to better understand how bacteria compete, be it via metabolism[60], adhesion[54,67] or compounds that inhibit other strains in its niche[7,11,12]. Understanding

symbiont competitiveness, however, faces further challenges. The rapid diversification of microbes is expected to personalise some symbionts to each person's biology and microbiota. This threatens any one-size-fits-all probiotic, because if a strain is not competitive in all ecosystems, it will only sporadically perform its desired function. One potential solution comes from exploiting the same evolutionary processes that are the barriers to colonisation. Repeated large introductions of a strain will increase the chances that the probiotic will adapt to the host in a way that allows it to persist. However, even when a strain is well-adapted to a niche there is no guarantee it will invade if there is a competing strain in place to exclude it.

Another key question, therefore, is when and how do similar species avoid competitive exclusion? This question is much discussed in theoretical ecology[75], and may help to explain much of the functional redundancy in the microbiome. Ecological theory can also be used to identify species important for microbiome function[84] or stability. Theory shows that, even if a species provides no direct benefit to the host, it may matter for overall community stability but we need more data on species interactions to develop and apply the theory[15]. Indeed, while ecological stability is typically seen as a desirable trait, it can be detrimental when attempting to introduce a probiotic, or when trying to reconstitute a healthy microbiome during domination of a dysbiotic yet stable community. In addition to understanding what makes a strain competitive, therefore, we need to understand how to make a community temporarily susceptible to invasion. Faecal microbiota transplantation, which pits large sets of strains against each other, represents a major ecological manipulation that may provide valuable insights into these questions.

In seeking to control the microbiome, we are faced with its daunting complexity. However, there is solace in the fact that diverse host species have faced, and largely overcome, the same challenge over evolutionary time. We might, therefore, spend a little less time fixated on how our symbionts affects us, and more on how our biology affects them. What is the role for adaptive immunity? Does a host typically employ positive or negative selection? What types of traits and species are targeted? By understanding the way that a host exerts control over its microbiome, we might engineer communities with traits, species or communities that are most easily controlled by a host. The long evolutionary history of host control also raises the possibility of harnessing this control as part of treatment. If a host already has powerful ways to regulate the microbiota, augmenting these mechanisms may sometimes work better than targeting the microbes themselves.

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Box 1. Identifying evolutionary function in the microbiome

"The question inevitably arises as to how such an abundance of misinterpretation has arisen. I believe that the major factor is that biologists have no logically sound and generally accepted set of principles and procedures for answering the question: "What is its function?" In practice this question is answered on the basis of a variety of criteria, some of which are of value, but their use is largely dictated by taste and intuition and their value obscured by terminological inconsistencies." GC Williams, 1966[90]

Darwin provided a unified framework to understand biological functions based upon the question: why did a trait evolve? The peacock's tail attracts mates, honeybees collect nectar to feed their colony, male polar bears fight over females. However, evolutionary theory is often neglected when assigning function[90]. Assigning function can also be challenging, particularly when traits are influenced by multiple individuals or species[48], as occurs in both the vertebrate immune system[91] and the mammalian microbiome. Nevertheless, evolutionary function is fundamental to understanding the organisation of biological systems[48], including which party ultimately controls a trait, and how best to manipulate a system[92]. Here we discuss the evolutionary function of several oft-discussed benefits of our microbiota:

Colonisation resistance. Microbial symbionts often protect against pathogens[4,6,7,52]. Is host protection an evolved function of symbionts? Under some conditions it may be[93]. However, in diverse communities, symbionts are often strongly affected by competition from other microbes (Box 2). Host protection may, therefore, arise as a fortunate by-product of natural selection on microbes to avoid being replaced by other species[26]. Once in place, host mechanisms may evolve to promote the most protective microbes, which would make colonisation resistance a composite of two adaptations, basic microbial competition and host control of the microbiome.

Polysaccharide digestion and butyrate production. Digestion of carbohydrates by bacteria like the Bacteroidales is thought to provide us with nutritional benefits. The digestion products feed other bacteria which, in turn, make butyrate and other products that we can use[45]. Why evolve to break down polysaccharides? Again, competition within the microbiota appears central; cells are feeding themselves, their clonemates, or less commonly another species that provides help in return[14]. Why make butyrate? Butyrate is a metabolic waste product and so may have arisen due to colonization by an environmental butyrate producer[94]. Combined with the weak evolutionary incentive for microbiota species to help the host (Box 2), this suggests that butyrate production in the gut arose as a by-product of microbial metabolism, which was then reinforced by host evolution. Consistent with host adaptation, gut epithelial cells directly take up butyrate[45] and a lack of butyrate is associated with immune dysregulation in the intestine[95] (main text).

Development of the immune system and tolerance. The microbiota facilitates normative immune system development[5,96]. Have then symbionts evolved to influence immune processes and limit negative impacts on the host (increasing host tolerance) *because* this improves host fitness[88]? Evolutionary theory suggests not (Box 2). Natural selection may favour symbionts that influence host immunity but, again, a likely function is to improve competitiveness and niche occupation within the microbiome. From the host's perspective, a long association with symbionts can drive the evolution of tolerance to symbionts but also "evolved dependence"[19], whereby immune system development evolves to rely on certain microbial phenotypes (but without the bacteria needing to evolve dedicated functions for the host).

Box 2 The problem of evolved cooperation from microbiota to host

"*If it could be proved that any part of the structure of any one species had been formed for the exclusive good of another species, it would annihilate my theory, for such could not have been produced through natural selection*" Charles Darwin[97].

We now understand many examples of evolved cooperation between species[21], including between members of the mammalian gut microbiota[14]. However, it is less clear why our symbionts help us. The problem is simple, the mammalian microbiota comprises many strains and species subject to continual turnover[3,54]. Consider a new mutation in a focal microbe that benefits the host but comes at a cost to the microbe. The mutant strain will divide slightly less rapidly than the parent and, even for small costs, the strain can be rapidly lost (Figure B1). This effect is expected to greatly constrain the potential for a symbiotic microbe to evolve a cooperative trait that helps the host.

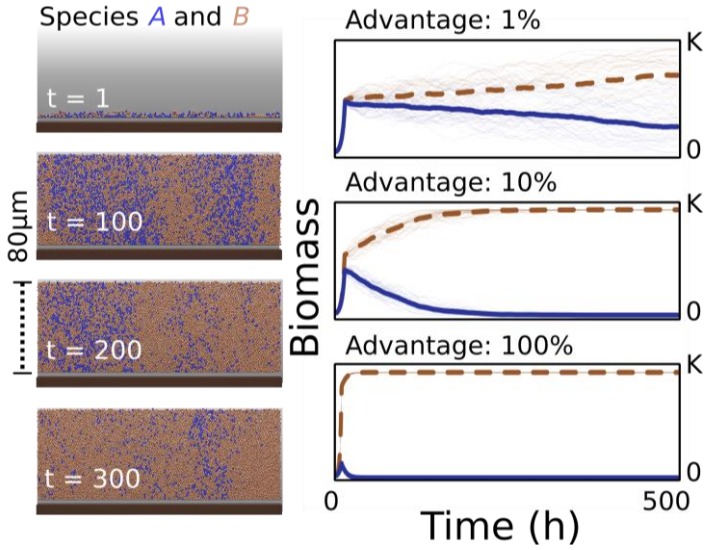

Figure B1. Simulation of bacterial growth on host epithelium. In the left hand images, brown bacterial cells (strain *B*) have a 1% growth rate advantage over blue bacterial cells (strain *A*). Even with a modest growth rate advantage, strain *B* succeeds and strain *A* is washed out in a few days. The right hand side shows plots of thirty independent simulations of bacterial competition. Development of biomass of strain *B* (brown dashed) and *A* (blue) with growth rate advantages for strain *B* of 1%, 10%, and 100% and environmental capacity K. The thick lines are mean values. From[3].

There are three potential exceptions to this conclusion. First is if the short-term natural selection to be competitive within the microbiota (within-group selection) can be outweighed by the negative effects on the host (between-group selection)[98]. *Ad absurdum*, if the lack of a cooperative trait in one strain always led to immediate host death, this trait may be selected within the microbiota. However, the focal strain must have a very strong impact on host fitness in amongst the hundreds to thousands of other microbial genotypes[26]. Transmission from parents to offspring might improve the prospects of the symbiont, as it can now also benefit by helping the host to have offspring[30]. However, it is difficult to see how these effects can protect against loss of a less competitive microbe[23].

Second, if strains differ in their benefits to the host, but not in competitive ability, host-level selection has more potential to favour beneficial strains. Consider a hypothetical case where only some hosts carry a strain that synthesises a vitamin in order to grow and compete within the microbiota; the vitamin-producing strain might increase in frequency during a famine that kills vitamin-deficient hosts. This would mean that the evolutionary function of vitamin production is both for microbial competition *and* host benefit. Such selection might even occur at the community scale if traits of multiple species interact to create a host benefit[99,100]. However, the requirement for strains to differ strongly in host benefits but not in competitive ability appears stringent and may rarely be satisfied.

Finally, as discussed in the main text, the host appears to have evolved mechanisms that encourage beneficial traits in the microbiota, such as immune suppression of harmful microbes and preferential feeding of beneficial strains. When effective, the microbiota may thereby evolve increased investment into benefitting the host. The result can be that the two sides of the interaction - host and microbiota - both benefit, and thus conform to the definition of an evolved mutualism[19]. We believe this to be, by far, the most important of the three exceptions for species-rich microbiota.

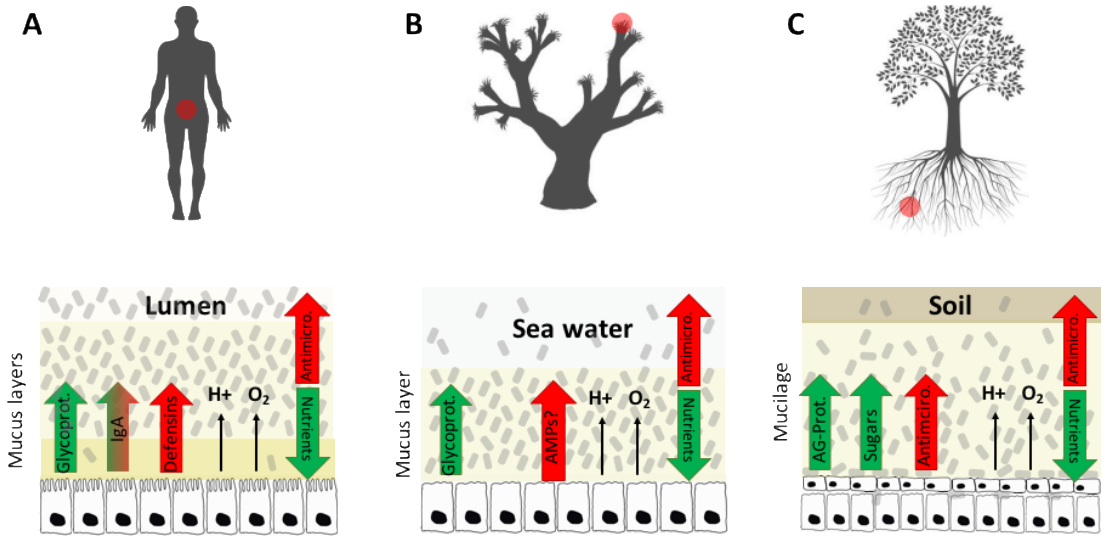

Figure 1. Convergent evolution of the host epithelial interface with the microbiota. While the study of the mammalian microbiome is most developed, diverse animals (A, B) and plants (C) possess epithelial surfaces where a diverse microbiota grows. In these systems, the host releases nutrients, antimicrobials, and a slimy matrix of mucus or mucilage, which are all thought to help control the microbiota (see Host Control section). In return, the symbionts may provide nutrients and protection from pathogens via antimicrobial release and other mechanisms[4,7,34,45,101]. Strikingly, the common ancestor of plants and animals is a single-celled organism[102], which means that these adaptations have evolved convergently after multicellularity evolved in the two lineages. Convergence is an indicator of common evolutionary principles across diverse systems. A) Human large intestine. The host secretes glycoproteins, such as mucins, which certain microbes attach to and feed on. Large amounts of immunoglobulin A are released[5], which may both help and harm symbionts by affecting adhesion[54]. Defensins (antimicrobial peptides), acids and oxygen release also shape the symbiotic community. B) Coral epidermis. Corals have many of the same features as the mammalian intestine, including mucins containing microbes[52], acids, and oxygen[51]. Whether antimicrobial peptides are released from the epidermis is not yet clear but the more general role of the innate immune system in shaping the epithelial microbiota has been established in the coral relative *Hydra*[39]. C) Plant root epidermis. Plants release mucilage containing arabinogalactan-conjugated proteins[53] (which appear functionally similar to mucins) but also sugars and other carbon sources in large quantities, which all provide nutrients for the root microbiota [34,35] The release of oxygen, antimicrobials, and particularly organic acids, also shapes the microbiota of the rhizosphere[4,34].

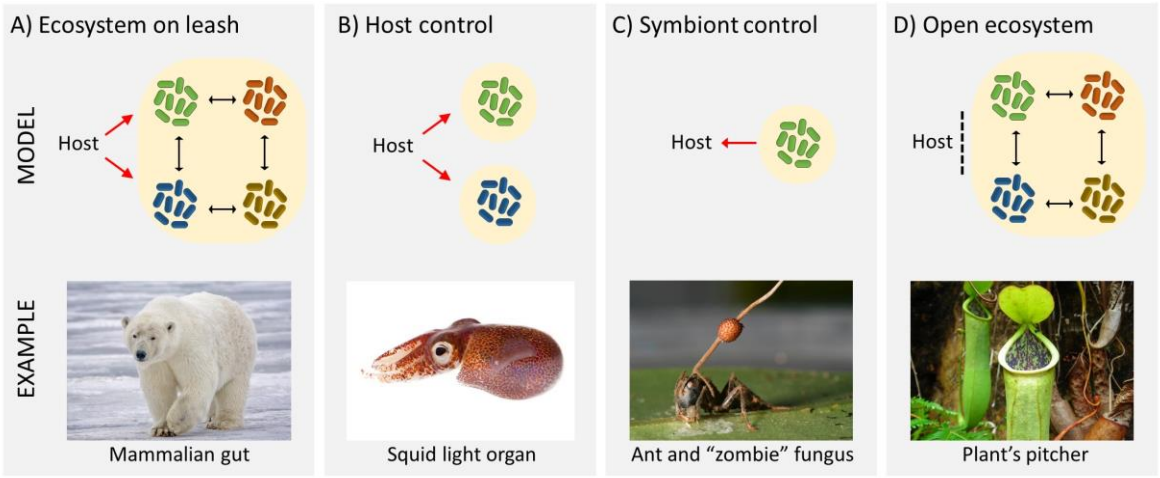

Figure 2. Models of host-microbiome interaction. Black arrows represent ecological interactions within the microbiota, red arrows indicate mechanisms of control. A) Ecosystem on a leash. When host species interact with a diverse but beneficial microbiota, as occurs in mammals, evolutionary theory predicts that the microbial functions will centre on persistence in the microbiome ecosystem, while the host will attempt to control the microbiota, hence the "leash" (Boxes 1 and 2). B) Host control. In interactions involving few microbial strains, ecological complexity is reduced and microbes are primarily shaped by the host environment. Natural selection on the host, therefore, can result in the phenotypes of beneficial microbes being strongly shaped and controlled. The bobtail squid has specialist light organs which control both the access and light production of the symbiotic bacteria that grow inside[76,77]. For example, one hypothesis is that host enzymes generate bacteriocidal compounds from substrates that become available if the bacteria do not perform the light-producing reaction[77]. C) Symbiont-control. Low microbial diversity also increases the potential for microbes to affect global host traits – including survival, reproduction and behaviour - and receive a fitness benefit from doing so (Box 2). This may select for adaptations that function to increase host fitness, such as enzymes that feed the host but slow microbial growth. However, this can also enable symbiont manipulation of the host, such as fungi whose infection causes ants to move to a position ideal for fungal development[81]. D) Open ecosystem. A host carries a complex ecosystem without evolved control mechanisms beyond compartmentalization. This is most likely if the microbiota is rarely either a threat or a benefit. Pitcher plants use pools of water to kill and digest prey. While plants do regulate the pool by releasing enzymes and acids to promote digestion, there is currently little evidence that the plants have dedicated mechanisms to regulate the pool microbiota[83]. Polar bear by Alan D. Wilson and pitcher plant by P. J. Ding used under Creative Commons Licence. Photo of *Ophiocordyceps unilateralis* and ant by David Hughes, Penn. State, photo of *Euprymna scolopes*, the Hawaiian bobtailed squid by Margaret McFall-Ngai, PBRC, University of Hawaii-Manoa, both used with permission

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