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Evolutionary and ecological consequences of gut microbial communities

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Abstract

Animals are distinguished by having guts: organs that must extract nutrients from food while barring invasion by pathogens. Most guts are colonized by non-pathogenic microorganisms, but the functions of these microbes, or even the reasons why they occur in the gut, vary widely among animals. Sometimes these microorganisms have co-diversified with hosts; sometimes they live mostly elsewhere in the environment. Either way, gut microorganisms often benefit hosts. Benefits may reflect evolutionary "addiction" whereby hosts incorporate gut microorganisms into normal developmental processes. But benefits often include novel ecological capabilities; for example, many metazoan clades exist by virtue of gut communities enabling new dietary niches. Animals vary immensely in their dependence on gut microorganisms, from lacking them entirely, to using them as food, to obligate dependence for development, nutrition, or protection. Many consequences of gut microorganisms for hosts can be ascribed to microbial community processes and the host's ability to shape these processes.

Keywords

codiversification; coevolution; host filtering; microbiome; symbiosis

INTRODUCTION

A distinguishing feature of animals is a digestive cavity, or gut: a tube or pocket where food is digested and absorbed. These structures can be considered as microbial incubation or culture chambers, colonized by ingested microorganisms that are sustained with food and host-produced molecules. Most animals harbor microbial communities in their guts, and these communities usually make up the vast majority of microorganisms associated with a host. For example, an estimated 99% of microorganisms associated with a human individual are in the gut, with the total number of bacterial cells approaching that of the somatic cells in the human host (Sender et al. 2016). Animal guts must overcome the dual challenges of

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extracting nutrients from environmental materials, with possible assistance from microorganisms, while barring microorganisms in the gut lumen from invading host cells or tissues. As habitats for microorganisms, guts pose distinctive challenges, including a lack of nutrients (which are efficiently absorbed by host cells), host immune factors (such as antimicrobial peptides, reactive oxygen species and extreme pH), and antagonistic interactions with other community members.

Gut microbial communities were virtually ignored by biologists until about 20 years ago, but this has changed dramatically. Thousands of papers on gut communities have appeared in the past decade. This shift is due primarily to high-throughput sequencing technologies that allow even complex communities to be surveyed deeply and inexpensively. These technological developments, accompanied by computational approaches for handling the resulting large datasets, enable us—at last—to sample, categorize, and often completely census the microbial life in diverse natural habitats, including deep ocean hydrothermal vents, soil, the open ocean—and animal intestines (Knight et al. 2018; Thompson et al. 2017,). The resulting exploratory surveys, based mostly on deep sequencing of diagnostic regions of ribosomal RNA genes, have yielded an enormous increase in knowledge of the composition of microbial gut communities. Experimental work and functional genomics studies have complemented these surveys and have revealed a myriad of effects of these communities on hosts (e.g., Gilbert et al. 2018; Leitäo-Gonçalves et al. 2017).

A strong motivator for expanded microbiota research (and a primary driver for funding) is relevance to medicine. Composition of the human gut microbiota correlates with a variety of lifestyle and health conditions (Gilbert et al. 2016; Schmidt et al. 2018), and at least some of these patterns likely result from causal effects of gut microbiota on hosts. For example, experimental work in mouse models has demonstrated substantial effects of the gut microbiota on development, immune function, nutrition and susceptibility to disease (*e.g.*, Kim et al. 2017; Ng et al. 2013; Stappenbeck et al. 2002; Ubeda et al. 2017). The success of gut microbiota transplantation as a treatment for digestive tract pathogenic infection (van Nood et al. 2013) has proven that therapies based on microbiota manipulation can indeed improve health. In some cases, success or failure of drug-based therapies appears to depend on modulation of gut communities (*e.g.*, Forslund et al. 2015; Maier & Typas 2017). It has been proposed that many modern ailments, such as late-onset diabetes and autoimmune disorders, stem from disruption of our own microbiota through antibiotic use and unfavorable diets (Blaser 2018; Cho & Blaser 2012; Sonnenburg & Sonnenburg 2014; Sonnenburg & Bäckhed 2016). The notion that maintaining health depends on the care of our gut microbiota is now widespread and is driving explosive growth of the probiotics industry, already worth billions of US dollars annually.

In this review, we address the roles of gut microbiota in animal ecology and evolution. Animals vary immensely in diet, gut structure, and immune systems, and, as is now clear, this variation extends to their gut communities. We first summarize the basic approaches to characterizing these communities and what has been learned regarding gut community features, including diversity, size, consistency among hosts, extent of host-restriction, transmission routes, and evolutionary histories with host lineages. We then turn to our major focus, which is whether and how gut communities are relevant for host ecology and

evolution. Here, a central distinction is that gut microorganisms may matter only because hosts have evolved dependence on their presence (evolutionary "addiction" (Douglas 2010; Moran 2002); these would still be categorized as mutualists since they confer fitness benefits though they don't extend the original ecological range of the host. Alternatively, gut microorganisms may confer new capabilities entirely lacking in the ancestral host, and thus expand their host's ecological range and evolutionary success. We give examples of the two main categories of new capabilities conferred by gut microbiota: (*i*) improved utilization of sub-optimal foods, and (*ii*) resistance to pathogens and parasites. Finally, we address how selection acts on host and microbial lineages, and how this depends on transmission mode. Our emphasis is on implications of the gut microbiome for host ecology and evolution and, therefore, give little attention to the community ecology of the microbes themselves, which has been comprehensively reviewed elsewhere (Nemergut et al. 2013; Zhou & Ning 2017). Also, gut communities can contain eukaryotic, archaeal, or bacterial cells, but are typically dominated by bacteria, so these are our main focus in this review. We hope that this summary will be helpful in clarifying major conceptual issues and advances, for readers who might be new to the emerging research on the gut microbiome.

CHARACTERIZING GUT COMMUNITIES

Determining Taxonomic Composition

Among the first challenges in understanding evolutionary and ecological aspects of gut microbiomes has been to simply identify which and how many microbes are present in a given animal, and how these communities vary across host species, individuals, and time. Using a comparative, phylogenetic framework, such surveys of gut community composition can reveal whether host guts simply collect microbes from environmental sources, whether particular hosts have characteristic microbial communities, and whether particular lineages of gut microbes share an evolutionary history with their hosts.

Beginning nearly 15 years ago (Sogin et al. 2006), high throughput sequencing and complementary data analysis pipelines have enabled thousands of surveys of gut microbiota composition, for animals from diverse phyla and lifestyles. These are part of an even broader wave of discovery-based research using molecular markers to profile microbial communities in diverse habitats. Before molecular methods were available, microbiologists relied on laboratory culture and microscopy, methods that can give valuable information on microbial localization, abundance and physiology, but that yield a highly skewed and generally depauperate picture of community diversity and composition. In the 1980's, biologists combined PCR, cloning and Sanger sequencing to achieve a first look at the uncultured microbial world. They settled on the small subunit (16S) ribosomal RNA (rRNA) gene for distinguishing taxa and reconstructing their relationships, but these methods were too expensive for characterizing complex communities (Hugenholtz 2002).

Rapid and inexpensive community profiling using high-throughput sequencing of rRNA genes has become the standard approach for characterizing microbial communities in the environment, including guts. For studies of gut communities, nucleic acids (usually DNA) are sampled and extracted from whole animals, whole guts, particular gut compartments, or feces. Next, the 16S rRNA gene is amplified using "universal" PCR primers and the

resulting amplicons are deeply sequenced using high-throughput methods. Most 16S rRNA-based surveys focus on bacteria, but for communities with archaeal or eukaryotic components, alternative primers can target those organisms. The resulting sequence reads are clustered into groups (Operational Taxonomic Units, or OTUs) of near-identical sequences, corresponding to taxa, which are assigned to known species or higher taxonomic units using curated databases (*e.g.*, McDonald et al. 2012). The numbers of reads per taxon or OTU are used to generate a profile of relative abundances within the community. These steps are readily carried out using ever-improving computational tools (Callahan et al. 2016; Knight et al. 2018), which also can be used to quantify community diversity within samples (alpha diversity) and divergence of communities among host species or individuals (beta diversity). Multivariate statistical analyses can enable comparisons of communities and tests of specific hypotheses relating composition to a known variable, such as geographic location, host diet, or host species.

Surveys applying these methods have allowed astonishing advances in our understanding of gut communities, but they do have methodological limitations. As described further below, this approach neglects absolute abundance, or biomass, of individual gut microbes or the whole community. Contamination with DNA from reagents, human skin or other sources has also been a problem in some studies, especially for low biomass samples (Eisenhofer et al. 2019; Salter et al. 2014; Weiss et al. 2014). Another issue is the erroneous assignment of reads from samples in the same sequencing run and other problems affecting replicability (Nelson et al. 2014). For non-invasive studies of humans and other vertebrates, feces are usually sampled to represent gut communities, possibly skewing results. Finally, sample storage, DNA extraction, and PCR primers can all introduce biases. Though technical issues are not a focus of our review, awareness of potential artifacts is important to the interpretation of results.

Inferring Metabolic Capabilities

The importance of gut communities lies in large part in their metabolic capabilities, which potentially can be exploited by hosts to expand ecological range. Examples of such capabilities are digestion or detoxification of food components, use of novel energy sources, and production of toxins that affect the host or pathogenic organisms. Some information on metabolic capabilities can be inferred from sequencing data, to frame hypotheses about the roles of particular bacteria in hosts.

A major approach, which imparts both taxonomic assignments as well as information on functional capabilities, is direct sequencing of whole community DNA (or RNA)—termed “metagenomics” (or “metatranscriptomics”)—and is used to ascertain what genes are present or what genes are being expressed. Because metagenomic studies assay sequences directly from the sample and lack an intermediate amplification step, they give a relatively unbiased representation of genomes present and divulge the complement of functional genes that may play a part in host biology, such as genes encoding enzymes that interact with food or toxins that may target the host or pathogens in the gut. Sequencing of community DNA samples is straightforward, and homology of sequenced genes to enzymes of known

function is readily ascertained using bioinformatic pipelines and databases that are constantly being improved (Franzosa et al. 2018).

While extremely useful for generating a broad picture of community function, sequencing approaches alone cannot directly demonstrate function, which depends on experimental results, usually with laboratory models. Functions are generally inferred by homology to genes that have been evaluated experimentally in a model system, but confidence in inferring function by homology varies across gene families and across taxa, and functional roles are entirely unknown for a large proportion of encoded proteins. Other challenges for metagenomic studies include the strain variation within communities, which hampers sequence assembly, particularly when using short-read technologies. Nonetheless, metagenomic methods offer a relatively easy and robust way to identify likely community functions. Complementary experiments, such as those using proteomic and metabolomic data, are increasingly used to complement metagenomics to strengthen evidence for particular metabolic activities within a community.

Compared to rRNA barcoding, metagenomics requires massively more sequencing (and expense) per sample, and is thus not currently feasible for studies incorporating large numbers of gut microbiome samples. As a shortcut, projections regarding metabolic function can be generated from 16S rRNA community profiling. Metabolic capabilities for particular bacterial taxa, identified by rRNA sequences, are inferred from experimental studies or from genome sequencing and homology-based analyses and are used to project the community metabolic potential (Langille et al. 2013). This approach has uncertainties; for example, bacterial genomes undergo frequent horizontal gene transfer, sometimes resulting in different functional abilities for close relatives. Using taxonomic assignments to infer community metabolism works best when community members have well-characterized relatives.

Direct knowledge of metabolic activity of genes comes from biochemical experiments on cultured microbial strains and through “functional metagenomics”, in which genomic fragments from uncultured organisms are cloned and expressed in laboratory model organisms to ascertain gene functions (Schloss & Handelsman 2003). Both culturing and heterologous gene expression can be challenging for non-model organisms, including most gut bacteria, but new approaches, such as those that exploit high-throughput sequencing in the context of lab experiments, show promise (Dantas et al. 2013).

Microbial Community Size and Numbers

Though rarely measured, total abundance is a key aspect of a gut community and can vary by orders of magnitude among individuals within a host species, as documented for humans (Vandeputte et al. 2017), and between host species, as documented, for example, for different species of ants (Sanders et al. 2017) and bees (Kwong et al. 2017) (Table 1). This belies a shortcoming of PCR-amplicon-based community profiling. Although the most commonly applied method to study the microbiome, it reveals only relative abundances of taxa within a host or sample. Thus, an acute limitation of many gut microbiome studies is the lack of quantification of absolute numbers (or absolute densities) of organisms present in a host or sample.

Even shifts in relative abundances cannot be reliably interpreted without estimates of absolute numbers. An increase in the proportional representation of a taxon can reflect either an increase in abundance of that taxon or decreases in abundance of other community members (*e.g.*, Vandeputte et al. 2017). Further, community size is important in itself, as most effects of gut communities on hosts, such as digestion of food components, production of nutrients, and protection against pathogenic invaders, are heavily dependent on cell numbers. Estimates of absolute numbers are also required for studying establishment of gut communities during development. Almost all animal embryos start with a microbe-free gut, and are colonized after birth or hatching through ingestion of environmental materials followed by growth and establishment of the gut community. As discussed below, variation in community size among host groups is linked to very different roles of microbes and microbiomes. Thus, assessing absolute numbers, using methods such as quantitative PCR, microscopy, or live colony counts (for cultivable organisms), is crucial for making strong inferences about the roles of gut microbiota.

Microbial Gut Communities Vary Enormously

The sequence-based methods described above have produced a flood of new data on microbial gut communities. One of the most evident broad conclusions is that animal species have extremely different kinds of gut communities. These differences include community size (or density of microbial biomass within the host), composition, constancy of composition within individuals and between individuals, functional roles of microbes in host biology, degree of microbial restriction to the host gut environment, and evidence for shared evolutionary history of gut microbial lineages with host lineages. Here we provide examples illustrating such differences for a selection of well-studied animal species (Table 1).

Phylosymbiosis: Pattern Versus Underlying Process

In many animal groups, including nematodes, numerous insect clades, fish, mammals generally, and hominids specifically, phylogenetic relatedness has been shown to correlate with gut community similarity, as inferred from similarity clustering of community profiles from rRNA amplicon analysis (Amato et al. 2018; Anderson et al. 2012; Brooks et al. 2016; Brune & Dietrich 2015; Kwong et al. 2017; Moeller et al. 2012; Nishida & Ochman 2018; Ochman et al. 2010; Tai et al. 2015). However, this pattern, sometimes called “phylosymbiosis”, of more similar community composition in more closely related hosts can result from completely different underlying processes (Figure 1). One possibility, referred to as “host filtering”, is that guts of related hosts are more likely to permit colonization by similar sets of bacteria present in food or other environmental sources. Most microorganisms present in the environment cannot live in the host gut, and gut community composition thus reflects a strong filtering process for those that can persist and replicate there. Filters are imposed by host behaviors such as choice of diet, physicochemical properties of the gut environment and host immune systems, and these tend to be more similar in more closely related host species (Moran & Sloan 2015). Thus, host filtering could be a primary driver of the general pattern of phylosymbiosis, which does not imply that microbes coevolve with hosts (Mazel et al. 2018).

By itself, a pattern of phylosymbiosis does not imply that the host gut is a significant habitat for the constituent microorganisms. For example, the nematode *Caenorhabditis elegans* feeds on bacteria and is colonized by a subset of the bacterial taxa present in its food (Samuel et al. 2016). Populations at different geographic locations and on different substrates select (through behavior and/or gut physiology) similar sets of bacterial taxa, and thus possess similar sets of bacteria in their guts (Zhang et al. 2017). These correspond to fast-growing bacterial taxa with opportunistic lifestyles, that are able to live in many environments other than nematode guts (Berg et al. 2016). Likewise, in caterpillars, dietary preferences can yield an apparent pattern of phylosymbiosis in the absence of a functional host-gut microbe association (Hammer et al. 2017).

In contrast, some animals harbor gut microorganisms that have evolved with host lineages over evolutionary time (Figure 1A). In these cases, microbial adaptations for living in the host are likely to occur. How can a shared evolutionary history of host and microbial associates be tested? The most definitive test is co-diversification of microbial and host lineages, that is, matching of phylogenies for the partner organisms. This pattern has been observed for many cases of intracellular symbionts that have co-diversified with insect hosts (Moran et al. 2008), and also for some highly specialized gut bacteria that are maternally transferred, as in some stinkbugs (Hosokawa et al. 2006). Unfortunately, the most commonly available data for gut bacteria, short-read rRNA sequences, lack sufficient phylogenetic signal for testing whether particular bacterial groups show matching phylogenies with animal hosts (Sanders et al. 2014). The 16S rRNA gene evolves at a rate of ~1–2% per 100 million years (Kuo & Ochman 2009), so short regions will differ at only a few sites even for bacterial lineages that have co-diversified with very old metazoan clades (such as mammals or orders of insects, which date to about 200 Mya). As described above, 16S rRNA amplicons are useful for assignment to taxa and comparing community profiles, and they often show that host phylogeny is a predictor of gut community composition (Amato et al. 2018). But to determine whether bacteria have evolved with related hosts since the time of their shared ancestor, finer scale markers are needed (Sanders et al. 2014), from longer or more variable sequences, with sufficient information for reconstruction of lineage phylogenies.

The appropriate data and analyses are available for a few cases: In great apes, including humans, chimpanzees, gorillas and orangutans, markers from protein-coding genes (sensitive enough to discriminate strains diverging over a few million years) provide evidence for co-diversification of hosts and some lineages of gut bacteria, implying long-term vertical association (Moeller et al. 2016a). This shared evolutionary history likely reflects longstanding transmission among conspecifics, including maternal transmission. However, other gut bacterial taxa that were assayed did not show a pattern of parallel phylogeny with great ape hosts. Similarly, in social corbiculate bees (honey bees, bumble bees, and stingless bees), five core lineages of the gut microbiota show phylogenies mostly matching those of hosts, supporting co-diversification over about 80 million years (Kwong et al. 2017). However, some of these bee host lineages have lost or gained gut bacteria over this period, and a few members of the bee gut microbiota appear to be opportunistic environmental bacteria or pathogens. To date, evidence for co-diversification is limited to a few cases, but this is potentially due to a lack of searching using suitable sequence markers.

A pattern of co-diversification supports a shared evolutionary history of lineages; it is consistent with, but does not imply, both coevolution (reciprocal evolutionary changes of partners due to their selective effects on one another) and vertical inheritance (transmission of symbionts from parent to progeny).

Environmental Acquisition versus Inter-host Transmission of Gut Microbiota

A central distinction among gut communities is whether constituent organisms are found in non-gut environments—in other words, whether they are host-restricted (Figure 2). Animals such as termites, social bees and mammals have characteristic gut communities, dominated by bacterial lineages that are never or rarely detected outside their hosts. Host-restricted microbes such as these are more likely to adapt to the particular guts they inhabit and to share an evolutionary history with their host lineages. But in some animals, the gut community is derived from the bacterial community ingested with food. For example, in laboratory-raised *Drosophila melanogaster*, gut communities are acquired from food sources, with lower titers in the host gut than in the food itself (Blum et al. 2013). Likewise, in wild *D. melanogaster* populations, communities are highly variable among individual flies and are dominated by bacterial species common in non-gut environments (Wong et al. 2013). Even individuals of the same strain of *D. melanogaster*, reared on the same food, can show divergent gut communities between laboratories (Chandler et al. 2014). Similarly, in wild *Caenorhabditis elegans*, gut communities are a subset of the environmental bacteria that serve as food (Zhang et al. 2017), and mosquito gut communities are subsets of the bacteria present in the water (Coon et al. 2016). In contrast, mammalian gut communities are often dominated by host-restricted bacteria within the phyla Firmicutes, Bacteroidetes, Actinobacteria, and Verrucomicrobia (Nishida & Ochman 2018). Communities in non-mammalian vertebrates (fish, reptiles, amphibians and birds) have been studied less, but often contain a high incidence of genera in the Proteobacteria, some of which are widespread in the environment (Colston & Jackson 2016; Kohl et al. 2017; Roeselers et al. 2011; Sullam et al. 2015). Interestingly, high proportions of Proteobacteria have also been reported from giant pandas and bats (Xue et al. 2015; Phillips et al. 2012).

Gut microbes are generally acquired after birth or hatching, from conspecific hosts and/or from other environmental sources, depending on the animal species. Gregarious or social animals have more opportunities for direct acquisition from conspecifics than do solitary species. In turn, frequent direct transmission provides greater opportunity for gut bacteria to specialize on the gut niche and to lose the ability to replicate outside of the gut environment.

Correspondingly, social or other group-living species are expected to have a higher proportion of host-restricted gut microorganisms. This effect of host sociality is evident among bee species, almost all of which have similar diets (nectar and pollen). Guts of highly social bees are dominated by specialized gut bacterial species that live nowhere else, while guts of non-social bees are dominated by bacterial species present in nectar and other environments (Kwong et al. 2017; Martinson et al. 2011; McFrederick et al. 2012). Experimental trials in honey bees show that direct social contact of colony members is required for establishment of a normal microbiota (Powell et al. 2014). Gut-restricted microorganisms are also prevalent in other social or gregarious animals, including termites

(Bourguignon et al. 2018) and some ants (Hu et al. 2018; Łukasik et al. 2017), but are not found other ant groups (Sanders et al. 2017) even though all ants are social. Sociality, including extended maternal care, may also explain the prevalence of host-restricted gut bacterial in many mammals (Groussin et al. 2017; Ley et al. 2008; Muegge et al. 2011; Nishida & Ochman 2018).

Studies in baboons and in chimpanzees indicate that rates of transfer are higher for host individuals engaging in more social interactions, indicating direct contact as a route of transmission (Moeller et al. 2016b; Tung et al. 2015). The bacterial taxa dependent on direct contact were largely non-spore-forming anaerobes, and thus unable to persist or grow in external environments. Additionally, mammalian mothers are an important initial source of gut bacteria for offspring, and the strains acquired from mothers can persist, as recently documented for humans using molecular markers sensitive to strain-level differences (Ferretti et al. 2018; Yassour et al. 2018). Experiments on inbred lines of mice housed together or separately showed that transmission of some gut bacteria is predominantly vertical at least for short time periods: individual lines tended to retain their ancestral bacterial strains even when sharing cages with other lines (Moeller et al. 2018). This offers contrast to zebrafish, in which co-housing resulted in horizontal transmission rates that essentially erased ancestral microbiota signatures (Burns et al. 2017).

An underappreciated aspect of transmission patterns is the extent to which they are shaped by selection on the microbial partners themselves. Lacking opportunities for direct transmission between hosts, microbial lineages are likely to retain the ability to live and replicate outside the host gut. However, once direct transmission is easily achieved, lineages that specialize on the gut environment can be favored and may dominate. An interesting study on a host-adapted strain of *Acetobacter* living in wild *Drosophila melanogaster* illustrates this possibility (Pais et al. 2018).

EFFECTS OF GUT COMMUNITIES ON ANIMAL ECOLOGY AND EVOLUTION

The potential for impact on host biology is the primary incentive for studying gut communities, both for researchers from biomedical fields and for those studying ecology and evolution. The rapid expansion of research on gut microbiomes has been driven by findings that gut bacteria often do matter for hosts. Specifically, many studies have documented fitness deficits in animals experimentally deprived of a normal gut microbiota, either with antibiotics or by rearing subjects in sterile environments to prevent colonization. The most common experiments compare hosts lacking a gut microbiota to hosts colonized by a normal or otherwise defined microbiota, then determine consequences for host phenotypes such as aspects of development, physiology, or behavior. Experiments are often complemented by correlative studies that establish whether microbial gut communities show expected correlations with fitness measures within populations.

How have gut communities shaped large scale patterns in animal ecology and evolution? Effects on hosts can be divided into two broad categories. First, hosts may evolve dependence on presence of gut bacteria as a persistent part of their environment over evolutionary time, such that removal of the normal gut microbiota represents an abnormal

environment that leads to abnormal development or behavior, and usually to lowered fitness. Second, gut microbes may confer new capabilities, resulting in ecological expansion and potentially evolutionary diversification. These alternatives, and examples of each, are considered next.

Evolutionary Addiction to Gut Microbiota

Host developmental pathways may evolve dependence on the presence of colonizing gut communities, simply because these have been ubiquitous elements in the environment. Since their origin more than half a billion years ago, metazoans have dwelt in environments in which microorganisms have been ubiquitous and diverse (McFall-Ngai et al. 2013). Thus, experiments that eliminate or perturb microbial gut communities impose abnormal environments. In animals normally harboring a specific, specialized set of gut-dwelling taxa, exclusion of those particular taxa is abnormal. Hosts are expected to evolve dependence on the presence of an evolutionarily persistent association, even if the associated microorganism was neutral or even deleterious when the association originated. This dependence can be described as evolutionary “addiction” (Douglas 2010; Moran 2002), a dependence that can evolve in the absence of any fitness benefit of initial acquisition of symbiotic partners. For example, mammalian guts are dependent on mucin-consuming bacteria such as *Akkermansia muciniphila* (Verrucomicrobia) for regulation of the mucus layer that is a critical barrier against microbial invasion of intestinal epithelial cells (Everard et al. 2013), and perturbation of these populations, through antibiotics, other drugs, or fiber-poor diets, can lead to disease states in hosts (Belzer et al. 2017; Wu et al. 2017). Yet, the original association was likely a parasitic one, in which these lineages evolved ability to exploit mucin as an energy source.

In mammals (Stappenbeck et al. 2002), *D. melanogaster* (Shin et al. 2011), and honey bees (Zheng et al. 2017), normal gut development and metabolic homeostasis are impaired in microbiota-deprived hosts. Honey bee workers deprived of their microbiota fail to gain weight at the normal rate, and at least part of this effect likely reflects depressed insulin signaling in microbiota-free individuals, resulting in lowered appetite (Zheng et al. 2017). Many of these effects likely reflect evolved dependence on microbial colonization, which itself represents a normal step in development. For example, in zebrafish, a minor member of the microbiome secretes a protein that sequesters intestinal inflammation, thereby suppressing the host immune response to bacterial infection and colonization as well as helping bacteria to survive (Rolig et al. 2018). Larval mosquitoes cannot complete development unless their gut lumen is colonized by living aerobic microorganisms that deplete gut oxygen (Coon et al. 2014; Valzania et al. 2018). Potentially, bacterial activity is simply part of the normal environment and has been incorporated into the developmental cycle, as a form of evolutionary addiction. Alternatively, growing populations of aerobic organisms may serve as indicators of a nutrient-rich environment conducive to further development and adult eclosion. Microbial populations are often fast-growing and quick to detect and respond to environmental shifts; thus, they provide useful sources of information for animals, with their longer life cycles and limited sensory capabilities.

Ecological or Evolutionary Expansion Enabled by Gut Communities

The alternative to addiction is that recruitment of particular gut microbial strains or communities expands the ecological capabilities of hosts or otherwise provides a direct fitness advantage to individual hosts. For example, gut bacteria may enable use of new foods or protect against negative impacts of abiotic or biotic forces (Figure 2). In these scenarios, microbial partners bring new services and potentially increase the evolutionary success of individuals, populations or whole lineages. Animals comprise a relatively recently derived clade and descend from an ancestral lineage that underwent extensive loss of genes underlying metabolic capabilities, including pathways required for biosynthesis of molecules essential to life. This limited metabolic toolkit is relatively constant across animal species, reflecting loss in shared ancestors followed by low rates of acquisition of novel genes through horizontal transfer.

In contrast, species of bacteria and other microorganisms collectively possess a vast array of pathways for consuming and creating organic molecules, along with sophisticated molecular machines for delivering their gene products so as to influence nearby organisms. Furthermore, individual microbial lineages are constantly shuffling metabolic capabilities amongst themselves, through horizontal gene transfer, which is rampant in bacteria and in most other microbial lineages. In bacteria, gene acquisition is one of the principal routes towards metabolic innovation, and thus, microbial partners offer animal hosts the potential for ecological expansion (see section below). One means by which gut microbiota can expand host ecological range and improve fitness is through the improvement of a sub-optimal diet. Animals are unusual in depending on external sources for many essential molecules, including amino acids and vitamins and in being highly mobile, thus having intensive energy needs; a major role of gut microorganisms in many species is to improve nutrition of their hosts.

Digesting plant polysaccharides.—Earth’s most abundant carbon substrates are complex polysaccharides present in terrestrial plants and in marine algae. These substrates, such as hemicellulose, cellulose, and pectin, contain a huge array of chemical bonds requiring specific enzymatic machinery for their breakdown and fermentation. Gut bacteria are key for making these abundant energy sources available to animals, and many animal groups have evolved specialized guts to exploit these microbial capabilities. Indeed, the greatest impact of gut communities in shaping the ecological roles and evolutionary success of animals may lie in their roles as fermenters of plant carbohydrates. Animals that rely on microbial breakdown of dietary polysaccharides often have the largest and most diverse (Nishida & Ochman 2018) gut communities (Table 1). In some cases, such as termites and ruminant mammals, these digestive feats are accomplished through massive modification of the gut to accommodate host-restricted communities containing diverse bacterial species and strains encoding enzymatic machinery for carbohydrate utilization (Brune & Dietrich 2015; Flint et al. 2008). In humans, most of the gut microbiota is located in the large intestine where recalcitrant carbohydrates are digested by secreted enzymes from dominant members of the human gut microbiota, such as *Bacteroides* and *Prevotella* species. A contrasting example of a gut modification promoting microbial breakdown of use of plant polysaccharides is the specialized system in the leaf-eating tortoise beetle *Cassida*

rubiginosa, which have foregut crypts that house a host-specific bacterial symbiont that secretes pectinase into the lumen (Salem et al. 2017). Digestion of pectin and other polysaccharides can also result in the release of indigestible or toxic sugars; some gut bacteria are able to neutralize and utilize these byproducts, as exemplified in honey bee gut bacteria in the genus *Gilliamella* (Zheng et al. 2016b).

Biosynthesis of limiting nutrients.—Gut microbiota also possess biosynthetic pathways and thus can provide limiting nutrients to hosts, including essential amino acids and vitamins that animals cannot produce themselves. Obligate intracellular symbionts of insects often provision such nutrients (Shigenobu & Wilson 2011), and gut bacteria also can provide B vitamins, as in the case of pyrrhocorid bugs (Salem et al. 2014), or amino acids, as in the case of termites (Brune & Dietrich 2015). Indeed, gut bacteria can bolster the overall nitrogen supply by recycling nitrogenous waste products (such as uric acid or ammonia) to increase the supply of protein amino acids, or by fixing atmospheric nitrogen. A dependence on gut bacteria to upgrade a nutrient-poor diet could favor establishing efficient transmission routes and a more consistent gut community. For example, while many ants are omnivorous and have erratic gut communities (Sanders et al. 2017), herbivorous turtle ants (genus *Cephalotes*) depend on nitrogen-poor foods such as nectar, and they consistently harbor conserved bacterial gut symbionts that have the capacity to upgrade waste products to enhance the nitrogen supply (Hu et al. 2018). Wood is a particularly low-nitrogen food, and gut bacteria capable of nitrogen fixation are found in wood-feeding termites (Zheng et al. 2016a) and in at least one species of wood-feeding catfish (McDonald et al. 2015).

Neutralization of dietary toxins.—One barrier to using many potential food sources is toxicity to animals, and bacteria in the gut sometimes neutralize dietary toxins (Freeland & Janzen 1974). For example, oxalate is present in many plants and is a potent mammalian toxin; it can be degraded by bacterial gut isolates, potentially enabling the use of otherwise toxic plants (Kohl et al. 2014). A remarkable example of gut symbiont-mediated detoxification protects the bean bug (*Riptortus pedestris*) from the insecticide fenitrothion: the insects protect themselves by adopting environmental bacteria able to degrade the toxin (Kikuchi et al. 2012). A conserved gut microbiota of carrion beetles (Silphidae) helps to detoxify and preserve the carrion food resource for developing progeny (Shukla et al. 2018). Detoxifying gut bacteria have been proposed to allow some insects to feed on chemically defended plants, although examples are currently few (Engel & Moran 2013; Hammer & Bowers 2015; Itoh et al. 2018). To benefit hosts, detoxification must occur before toxins act, and this may limit the potential for bacteria in the distal gut (colon or hindgut) to provide this service.

The gut as a battlefield—Colonization resistance.—One of the most widely documented benefits of gut communities to hosts is enhanced resistance to pathogens and parasites, a phenomenon that has been experimentally verified in vertebrate and invertebrate hosts. Many of these experiments are based on parasite challenge of hosts lacking microbiota versus hosts possessing a conventional or defined microbiota. For example, adult bumble bees (*Bombus terrestris*) challenged with the trypanosomatid parasite *Crithidia bombi* receive a high degree of protection from their gut microbiota (Koch & Schmid-

Hempel 2011). Sometimes specific microbiota types or specific bacterial strains are shown to underlie protection: in a later study, the microbiota from different *B. terrestris* colonies were found to confer specific levels of protection against particular strains of *C. bombi* (Koch et al. 2012), and similar specific effects were documented in another *Bombus* species (Mockler et al. 2018).

While pathogen protection is often found to be a benefit of an intact microbiota, the underlying mechanisms for this protection are often not identified. Most of the more definitive studies on mechanisms concern human pathogens studied in mammalian models, in which a wide range of processes underlie protection against various pathogens (Ubeda et al. 2017). These processes can be roughly sorted into two categories: First, gut microbiota species may be required to stimulate the host's own immune system, thereby activating immune defenses against invading pathogens; these may involve innate immune pathways or elements of the vertebrate adaptive immune system. For many such cases, the gut microbiota can be viewed as contributing to the normal development of the immune system: depriving hosts of a normal microbiota essentially imposes an abnormal environment in which this development is thwarted, as described for evolutionary addiction above. These influences on the host's own immune defenses are diverse, ranging from the modeling of the mucus layer lining the mammalian intestine (Johansson et al. 2015) to stimulating production of anti-microbial peptides in insect systems.

A second broad category of symbiont-conferred defense includes cases in which gut-dwelling microorganisms bring their own weaponry to kill or inhibit invading pathogenic species. "Colonization resistance", the ability to exclude invading microbial strains, may be the primary basis for stability and resilience of a gut community, attributes that may in turn improve host health (Sommer et al. 2017). Colonization resistance is documented for many cases, including both mammalian and invertebrate examples (Foster et al. 2017; Dillon et al. 2005; Koch & Schmid-Hempel 2011). This phenomenon is thought to arise from intense competition between resident and invader for limiting nutrients or colonization sites, or from antagonistic interactions. Warfare among strains or species is well documented in guts of humans (Wexler et al. 2016) and honey bees (Steele et al. 2017) and is often mediated by bacteriolytic toxins delivered at close range (Antunes et al. 2014; Russell et al. 2014; Verster et al. 2017). In other cases, colonization resistance is imposed by diffusible metabolic products, such as acetate, which is generated by many gut bacteria and which inhibits growth of some pathogenic species (Fukuda et al. 2011).

Gut microbiota as a portal for genetic novelty through horizontal gene transfer.—By excluding the entry of novel microorganisms, colonization resistance could limit the ecological and evolutionary novelty provided by gut communities. On the other hand, the resident gut community may act as a portal for the acquisition of novel genes, as successful horizontal gene transfer is far more frequent in bacterial genomes than in animal genomes (Shterzer & Mizrahi 2015). For example, lineages established as gut bacteria have acquired antibiotic resistance loci in the context of selection by antibiotic exposure, and these loci are subsequently exchanged within gut communities (Guo et al. 2017; Ludvigsen et al. 2017; Stecher et al. 2013; Tian & Moran 2016). Upon introduction of novel dietary substrates or dietary toxins, gut bacterial lineages potentially can acquire genes encoding

enzymes for using or degrading these molecules; these genes are often present in ingested food, in the genomes of bacteria adapted to use those same dietary components. The result can be enzymatic activity in the gut that is useful to hosts for digestion. For example, *Bacteroides* strains in the gut microbiota of Japanese individuals have acquired enzymes from marine bacteria able to digest porphyrin and other polysaccharides. These compounds are abundant in marine red algae, which are frequently consumed in Japan (Hehemann et al. 2010). The selective force driving maintenance of such horizontally acquired genes could be the availability of a dietary substrate that other bacteria cannot use, thus providing a distinct niche for those that can. Experiments with mice verified that dietary porphyrin can enable establishment of *Bacteroides* encoding the corresponding enzyme (Larsbrink et al. 2014). In turn, the expanded digestive abilities potentially benefit hosts.

HOW DOES SELECTION ACT TO SHAPE EFFECTS OF GUT COMMUNITIES ON HOSTS?

As our brief summaries show, gut communities can benefit hosts, raising the question of whether this benefit arises from selection for mutualistic effects themselves, or from selection on the individual microbial strains and species, with host benefits as an added consequence, or from selection on hosts to control their microbial associates. The answer clearly varies across animal species.

One possibility is that selection acts on the host and its symbionts as a unit favoring mutually beneficial features (Rosenberg & Zilber-Rosenberg 2018). However, even low incidence of inter-host horizontal transfer will dissociate evolutionary fates of host alleles and symbiont associations. Thus, selection for increasing host fitness, i.e., for mutualism, is expected to be the exception for gut bacteria (Douglas & Werren 2016; Moran & Sloan 2015). Such exceptions exist: some highly specialized gut bacteria behave essentially as heritable elements, passed from mother to progeny with high fidelity (Hosokawa et al. 2006; Salem et al. 2017). In such cases, natural selection on the bacteria will act strongly to favor host-beneficial features. At the other extreme, different gut bacteria are harvested from the environment every generation or are simply food, as in the cases of mosquitoes and *C. elegans*. Such organisms share no evolutionary history, and, usually, no fitness interests, with host lineages. Somewhere in between are host-restricted gut microbes that are passed among individuals of the same host species; these include many social animals, such as mammals, bees, and termites. In such cases, highly pathogenic effects will often be negatively selected, as killing the host destroys the current habitat, but some deleterious effects may arise from within-host competition among members of the microbiome.

A second possibility is that selection and community processes acting directly within gut communities can benefit hosts. Colonization resistance, one of the most common benefits of gut communities, reflects inter-strain competitive interactions (Foster et al. 2017). Likewise, a microbe's ability to utilize a specific dietary substrate may provide access to an ecological niche that allows a strain to invade a community, thereby benefitting the host by enabling it to use a newly available food (*e.g.*, Larsbrink et al. 2014). But community processes can also be detrimental to hosts. If hosts depend on colonization from the environment, they will be

vulnerable to consequences of community shifts due to random priority effects based on the timing of colonization, as well as to intermittent disturbances, such as those imposed by antibiotics or dietary shifts. For example, antibiotic exposure or elimination of particular substrates from the diet can cause extinctions that later restrict the host's ability to use particular foods or to defend against pathogenic invaders (Sonnenburg et al. 2016).

An interesting issue is why selection acts on some bacterial lineages to shift into the gut habitat. Potentially, guts simply provide stable resources, but another possibility is that hosts provide transport among resource patches. As mentioned, bacterial titers are lower within guts than within food for lab-grown *D. melanogaster*; bacteria usually do not adhere to the gut wall and are sustained by constant replenishment but nonetheless benefit from presence of flies (Blum et al. 2013; Storelli et al. 2018). A recent study on bacteria in guts of wild *D. melanogaster* populations revealed an exception—an *Acetobacter* strain able to adhere to the gut wall and to form stable gut populations; this feature benefits the bacteria by facilitating transport to new resources and benefits flies by reliably speeding fruit decay (Pais et al. 2018). Thus, selection acting separately on both could result in mutually beneficial adaptations that stabilize the association, with no role of selection on the host and gut bacteria as a unit. Similar processes may be acting on other gut-restricted bacteria that are common in wild *Drosophila* species living in mixed species groups on localized substrates such as rotting fungi or plants (e.g, species of Orbaceae (Martinson et al. 2017a,b).

A third route through which selection shapes gut communities is selection on hosts themselves. A suite of host adaptations influences gut community composition, size, and spatial distribution. These include behaviors such as diet choice and coprophagy or its avoidance, digestive physiology such as pH, oxygen level, rate of flow, and structures such as paunches or crypts, and immune responses including a wide range of effectors. Indeed, the expanded appreciation of gut microbiota has led to a new view of the immune system, as having evolved not only to thwart certain microorganisms, but also to encourage and foster others (McFall-Ngai et al. 2013).

Thus, consequences for hosts reflect ecological processes within gut communities, as they assemble themselves within hosts and undergo subsequent perturbations. Understanding these processes and the means by which hosts have evolved to control them is a key challenge for studies of gut communities (Coyte et al. 2015; Foster et al. 2017).

Animal Lacking Functionally Significant Gut Communities

Clearly, animal species vary massively in how selection has shaped their associations with gut microbiota (*e.g.*, Table 1). Some animals, such as humans and termites, host huge numbers of specific, host-restricted gut microbes that play critical roles in their host's growth and survival. At the other extreme are animals with few or no resident gut microbes and which may not rely on gut microbes for any aspect of their biology. For example, the guts of caterpillars, many (but not all) ants, and stick insects have extremely low abundances of microbes; those that occur are likely ingested with food and only transiently present (Hammer et al. 2017; Sanders et al. 2017; Shelomi et al. 2013). It may simply be more profitable for these insects to obtain nutrition, ward off disease and complete development with endogenous mechanisms, and avoid paying the costs of hosting symbionts. Mammals

belonging to the Carnivora, including the herbivorous red panda, also have low-abundance and Proteobacteria-rich gut microbiota relative to other mammals (Contijoch et al. 2018). Possibly, these species and other vertebrates with similar gut microbiota represent a middle ground in between the two extremes. Greater adoption of quantitative and experimental approaches will help differentiate resident microbes important to host fitness from environmental transients and laboratory contaminants, and thus likely identify additional animal lineages with weak or no reliance on gut microbiota.

EVOLUTIONARY DIVERSIFICATION AND GUT MICROBIOTA

Gut communities can confer specific abilities to exploit niches that would otherwise not be available. For example, ruminants and most termites are wholly dependent on their gut communities for extracting nutrients from plant cell wall components. These groups would simply not exist without their microbial associations. Thus, some clades of animals are dependent on gut communities for ecological and evolutionary success.

An additional route through which gut communities might spur diversification is by directly limiting gene exchange between diverging populations during early stages of speciation. Potentially, if hosts rapidly coevolve with specific, host-restricted gut communities, it is possible that hybridization between recently diverged host populations would result in genetic incompatibilities among host genes and/or among members of the communities (Brucker & Bordenstein 2012). This process would be unlikely in hosts that frequently exchange bacteria among individuals or populations, as it depends on fidelity of particular associations between bacteria and hosts.

Another possible route through which gut microbes potentially could speed reproductive isolation and rates of speciation is through direct effects on mate choice. Specifically, microbial associates might cause hosts to favor mates that harbor the same microbial types; the resulting assortative mating might lead to genetic divergence of host sub-populations. This possibility was investigated for lab strains of *D. melanogaster*; however, an effect of the gut microbiota on mating could not be replicated (Leftwich et al. 2017, 2018). In fact, *D. melanogaster*'s gut microbiota is highly variable among individuals (Wong et al. 2013) and derived from food each generation (Blum et al. 2013), so reproductive isolation through associations with specific gut communities seems especially unlikely. However, in other hosts with specific microbiota that are largely vertically inherited, such as appears to be the case in house mice (Moeller et al. 2018), this process is conceivable and potentially enhances rates of reproductive isolation and speciation.

CONCLUSIONS AND FUTURE DIRECTIONS

For ecologists and evolutionary biologists, the newfound ability to study the gut microbiota has opened a vast and exciting research frontier. Some authors have even argued that nothing can be understood about animal biology without studying associated microbiota, and that this development demands a fundamental revision of our understanding of how evolution works. For example, Bang et al. state "Symbiotic relationships are the signature of life on earth, and evolutionary biology has to include the species community within the

metaorganism as a central unit of selection" (Bang et al. 2018). The above discussion and examples indeed show that gut microorganisms are central to the lives of many animals, and that they have had far-reaching consequences for the ecological range and evolutionary success of many species and clades. However, the importance of gut microbiota varies among animal taxa, and their specific ecological roles vary even more. The two central functions of guts, to extract nutrition from the environment and to prevent invasion by pathogenic organisms, are also the two arenas in which gut microbiota contribute new capabilities: for digesting, detoxifying, and biosynthesizing biomolecules involved in nutrition, and for excluding invasive microorganisms that may otherwise colonize hosts to their detriment.

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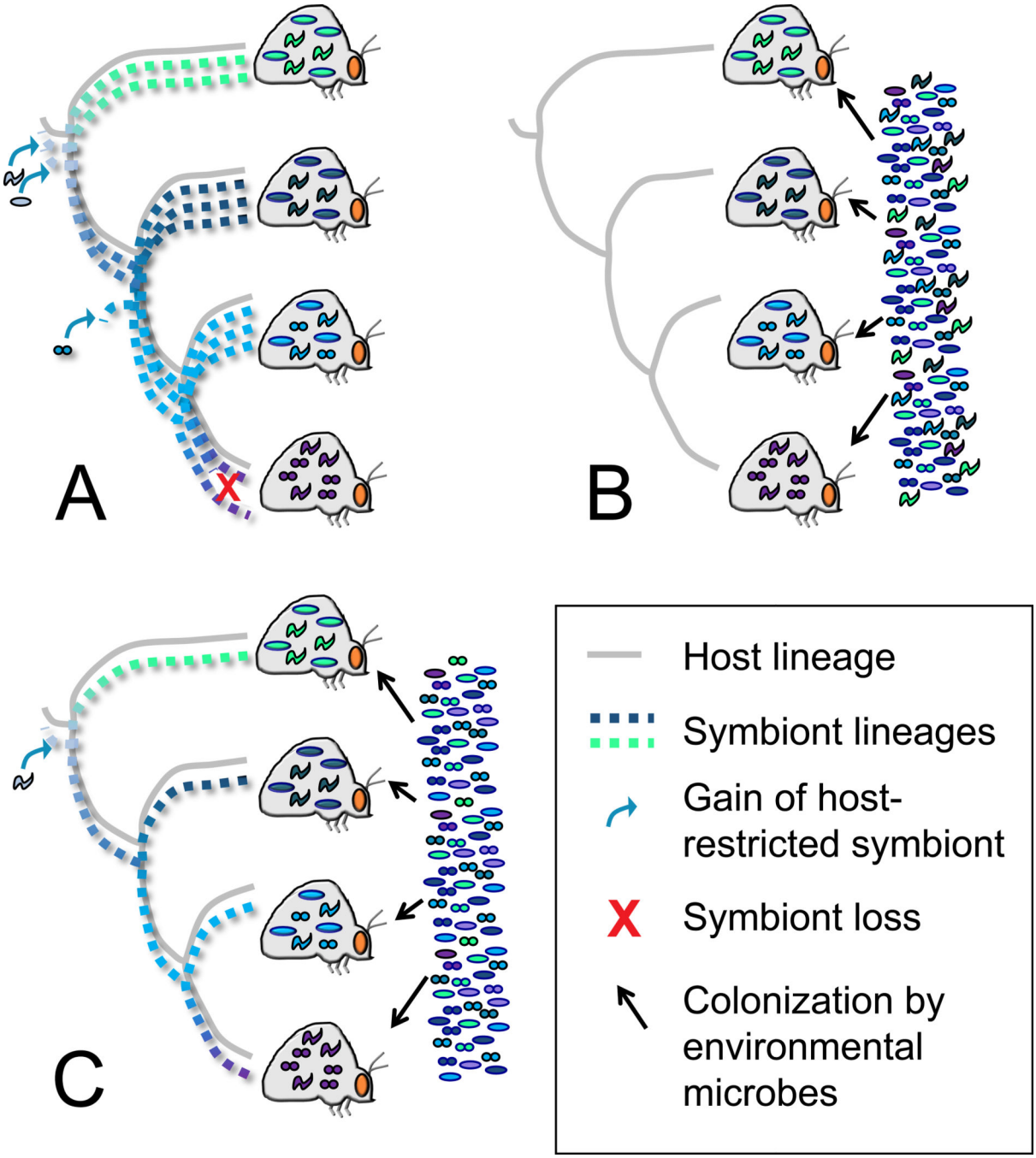

Figure 1.
Different processes giving rise to similar gut communities in related hosts. A. Shared evolutionary history of hosts with gut bacteria, evidence by parallel phylogenies. B. Host filtering, whereby related hosts are colonized by similar microbial types. C. A mix of shared evolutionary history and host filtering.

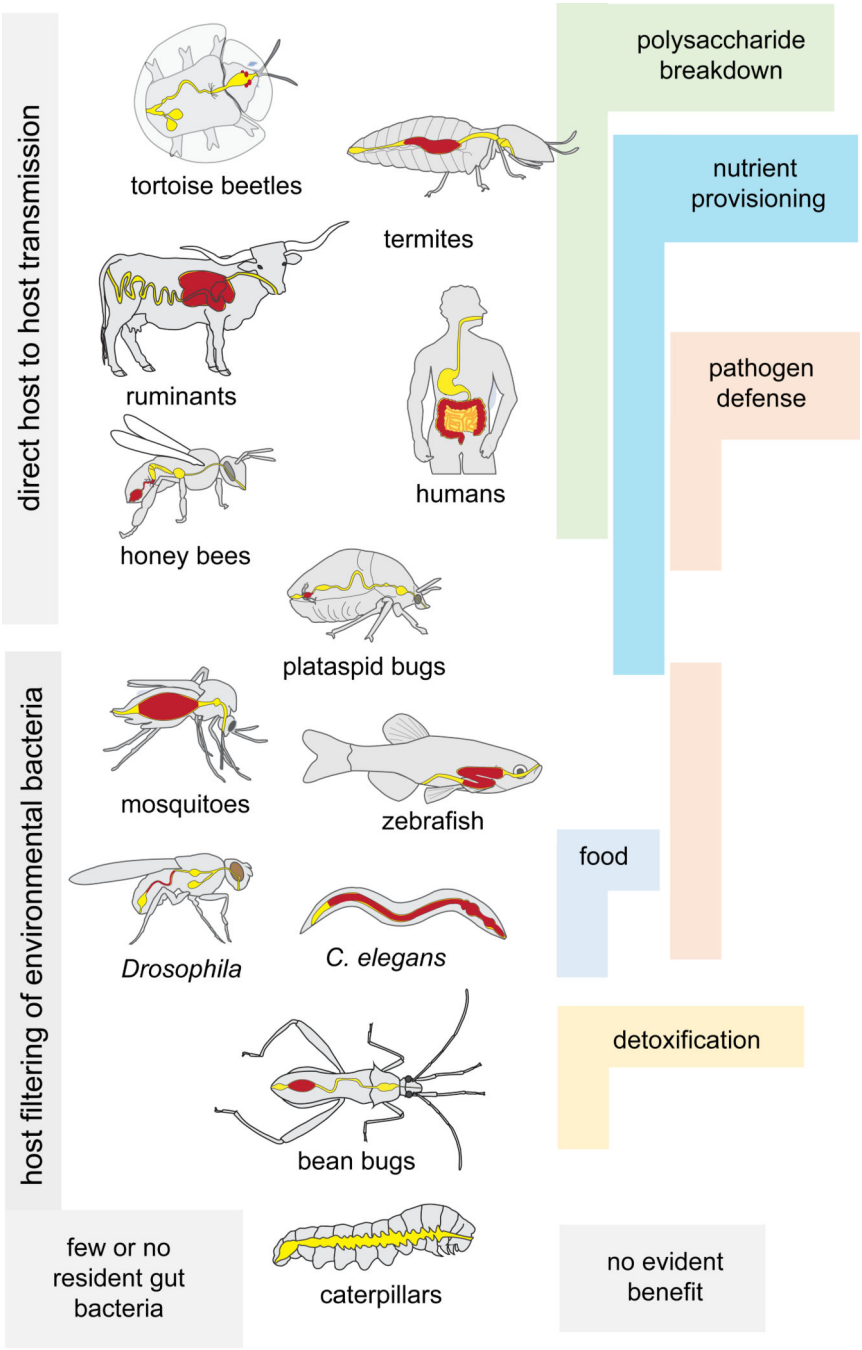

Figure 2.
Animal gut microbiomes are acquired from different primary sources and provide different primary benefits to hosts.

Table 1.

Variation in gut microbial associations across diverse animal hosts.

Animal	Community size (microbes per g)[a]	Alpha diversity[b]	Dominant microbe(s)	Diet	Main site of colonization	Transmission mode	Microbe(s) host-restricted?	Main documented benefit(s) to host	References
Humans	10^{11}	>1000	Bacteroidetes, Firmicutes, some Proteobacteria, some methanogenic archaea	Omnivorous	Hindgut (large intestine)	Social interactions	Yes	Digestion, nutrient synthesis, toxin metabolism, pathogen protection	*1–5*
Termites	10^{7} – 10^{11}	100s to >1000	Spirochetes, Bacteroidetes, Proteobacteria, methanogenic archaea, flagellates (lower termites only)	Wood, grass, dung, or soil	Hindgut (expanded paunch)	Social interactions	Yes	Digestion, nutrient synthesis	*6–7*
Ruminants	10^{11}	>1000	Bacteroidetes, Firmicutes, Fibrobacter, some Proteobacteria, some Spirochetes, ciliates, methanogenic archaea, fungi	Herbivorous (foliage)	Foregut (rumen)	Social interactions	Yes	Digestion, nutrient synthesis, toxin metabolism	*8–11*
Honey bees	10^{9} – 10^{10}	5–10	*Lactobacillus* (Firmicutes), *Bifidobacterium* (Bacteroidetes), *Snodgrassella* (Betaproteobacteria), *Gilliamella* (Gammaproteobacteria)	Pollen and nectar	Hindgut (surface and lumen)	Social interactions	Yes	Digestion, toxin metabolism, pathogen protection	*12–16*
Camponotus ants	10^{9} per ant[c]	1	*Blochmannia* (Gammaproteobacteria)	Omnivorous	Midgut (within specialized bacteriocytes)	Transovarial (via egg)	Yes	Nutrient synthesis	*17–19*
Riptortus bugs	10^{7} per bug[c]	1	*Burkholderia* (Betaproteobacteria)	Herbivorous (seeds)	Midgut (lumen of specialized crypts)	Acquired from environment	No	Metabolism of toxins, putative role in nutrient synthesis	*20–21*
Drosophila melanogaster	10^{7} – 10^{8}	1–30	Variable; often Acetobacteraceae, *Lactobacillus*, yeasts	Saprophytic (microbes in fermenting fruit)	Foregut (in adults)	Among individuals via shared feeding and breeding sites	Variable	Food source, nutrient synthesis, pathogen protection	*22–26*
Pandas	ND[d]	Tens	Gammaproteobacteria, *Streptococcus* (Firmicutes), some Clostridia	Herbivorous (bamboo)	Not known	Not known	Not known	Not known	*27–29*
Ectatomma, Azteca, Crematogaster ants	10^{5} – 10^{6}	Tens	Highly variable; mainly Proteobacteria	Omnivorous or herbivorous	No significant, consistent colonization	Likely transiently sourced from diet	No	Not known	*30–31*
Caterpillars	10^{4} – 10^{5}	Tens	Highly variable; mainly diet-associated Proteobacteria, Firmicutes	Herbivorous (foliage), some carnivorous, other substrates	No significant, consistent colonization	Transiently sourced from diet	No	Not known	*32–33*

[a] Density of microbial cells or genomes per gram host feces or gut tissue, rounded to nearest order of magnitude. Densities measured per ml gut were converted to a per gram using 1 g/ml (humans, termites, ruminants).

[b] Phylotype richness typically reported by studies with comparable methodology

[c] Weight of gut unavailable

[d] Comparable value unavailable, but red pandas were found to have the lowest gut microbial densities among all mammals surveyed in ref. (Contijoch et al. 2018)

1: Sender et al. 2016

2: Flint et al. 2008

3: Moeller et al. 2016a

4: Walter & Ley 2011

5: Vandeputte et al. 2017

6: Bourguignon et al. 2018

7: Brune & Dietrich 2015

8: Groussin et al. 2017

9: Flint et al. 2008

10: Jami & Mizrahi 2012

11: Mackie et al. 2000

12: Kwong & Moran 2016

13: Zheng et al. 2016

14: Raymann et al. 2017

15: Zheng et al. 2017

16: Kwong et al. 2017

17: Wolschin et al. 2004

18: Degnan et al. 2004

19: Feldhaar et al. 2007

20: Kikuchi et al. 2012

21: Takeshita & Kikuchi 2017

22: Blum et al. 2013

23: Wong et al. 2013

24: Broderick & Lemaitre 2012

25: Obadia et al. 2017

26: Storelli et al. 2018

27: Xue et al. 2015

28: Guo et al. 2018

29: Contijoch et al. 2018

30: Sanders et al. 2014

31: Sanders et al. 2017

32: Hammer et al. 2017

33: Whitaker et al. 2017