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COMMENTARY

A place for host–microbe symbiosis in the comparative physiologist's toolbox

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ABSTRACT

Although scientists have long appreciated that metazoans evolved in a microbial world, we are just beginning to appreciate the profound impact that host-associated microbes have on diverse aspects of animal biology. The enormous growth in our understanding of host–microbe symbioses is rapidly expanding the study of animal physiology, both technically and conceptually. Microbes associate functionally with various body surfaces of their hosts, although most reside in the gastrointestinal tract. Gut microbes convert dietary and host-derived substrates to metabolites such as short-chain fatty acids, thereby providing energy and nutrients to the host. Bacterial metabolites incorporated into the host metabolome can activate receptors on a variety of cell types and, in doing so, alter host physiology (including metabolism, organ function, biological rhythms, neural activity and behavior). Given that host–microbe interactions affect diverse aspects of host physiology, it is likely that they influence animal ecology and, if they confer fitness benefits, the evolutionary trajectory of a species. Multiple variables – including sampling regime, environmental parameters, host metadata and analytical methods – can influence experimental outcomes in host–microbiome studies, making careful experimental design and execution crucial to ensure reproducible and informative studies in the laboratory and field. Integration of microbiomes into comparative physiology and ecophysiological investigations can reveal the potential impacts of the microbiota on physiological responses to changing environments, and is likely to bring valuable insights to the study of host–microbiome interactions among a broad range of metazoans, including humans.

KEY WORDS: Microbiome, Microbiota, Gut microbes, Ecophysiology

Introduction

Research over the past ∼15 years has led to an explosion of awareness and new knowledge of the role of microbial symbionts (see Glossary) in nearly all aspects of animal biology, and we are just beginning to appreciate the breadth and depth of these relationships. A fundamental premise underlying this development is the recognition that because animals evolved in a microbial world, they have been influenced by microbial activity since the earliest stages of their evolution (McFall-Ngai et al., 2013). Given this, and the fact that all metazoans share their bodies with hundreds to trillions of indigenous microbes (see Glossary), it stands to reason that multiple aspects of animal biology are functionally tied to the activity of their microbial symbionts (McFall-Ngai, 2015). The microbiota (see Glossary) associated with animals' bodies include Bacteria, Archaea, Eukarya and viruses. Various body surfaces (e.g. skin, anal glands, lung, vagina) are colonized by microorganisms, with most microbes residing in the gastrointestinal tract. The idea that resident microbes perform processes that benefit host animals has long been appreciated for ruminants. In these obligate nutritional symbioses, rumen microbes degrade plant structural carbohydrates and produce metabolites that the host can use for energy and nutrition. We now know that non-ruminant animals also benefit nutritionally from the ability of their gut microbes to degrade otherwise indigestible carbon sources, and there is increasing evidence that gut symbionts can influence diverse aspects of animal structure and function – from development to cellular and organ system physiology, and even behavior. Indeed, many physiological processes and adaptations that have long been regarded as originating in an animal's genome may actually involve components that are directly or indirectly influenced by genes within the animal's microbiome (see Glossary). This implies that the integration of host–microbe symbioses into animal physiology extends to the ecological and evolutionary implications of these ancient partnerships.

In this Commentary, we provide an overview of the ways in which animal–microbe symbioses can influence host physiology. While a substantial body of work has been conducted in invertebrate hosts (Graf, 2016; McFall-Ngai, 2014) we frame our discussion primarily around the intestinal microbiota of terrestrial vertebrates. We first discuss factors that shape the structure and function of the microbiome and then provide examples of ways in which gut microbes may influence the physiology and ecology of their hosts. We end by making the case that host–microbe symbioses should have a place in the physiologist's toolbox, and offer suggestions on how physiologists might incorporate symbiosis studies into their research.

Determinants of microbiome structure and function

The structure and function of gut microbiomes are closely linked to the biology of the host. There is some evidence that development of the microbiota in vertebrates may begin *in utero* or *in ovo* (Funkhouser and Bordenstein, 2013), although whether this mode of transmission is significant is still unclear (Lauder et al., 2016). Transfer of vaginal microbes from mother to neonate during parturition leads to rapid development of the offspring's microbiota, with additional input from the local environment (Merrifield et al., 2016). Host genetics, maternal nutrition and the function of the maternal immune system during gestation sculpt the microbiome's development during early life (Gomez de Aguero et al., 2016; van Best et al., 2015). As the host matures, physical properties of the intestinal environment further influence microbial colonization and persistence; these include the availability of oxygen, organic carbon and other nutrients, and pH. For most vertebrates, the dietary transition that occurs during postnatal maturation through

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Glossary

Commensals
Microorganisms that benefit from their hosts but have no known effects on them.

Community structure
The composition of a microbial community and the relative abundance of its members.

Germ-free animals
Animals born under and raised in sterile isolators that exclude microorganisms.

Holobiont
A eukaryotic host and its resident microbes.

Metabolome
The set of small molecules produced by the microbiota and host.

Microbiome
The assemblage of microorganisms and their collective genes.

Microbiota
The collection of microorganisms in an environment (e.g. gut); can include bacteria, archaea, eukarya and viruses.

Mutualists
Members of a symbiotic association in which the microorganisms and the host both benefit.

Operational taxonomic units (OTUs)
Clusters of microbial sequences with similar 16S rRNA sequence identity (or other genetic markers). Varying sequence identity thresholds (99%, 97%, etc.) can be used to differentiate sequences into clusters.

Resident (or indigenous) microbes
Organisms native to a particular habitat.

Symbionts
Microorganisms that live in association with a host; encompasses mutualists, commensals and parasites.

metamorphosis or weaning plays a major role in shaping the adult microbiota (Kohl et al., 2013; Pantoja-Feliciano et al., 2013).

One of the largest influences on gut microbial community structure (see Glossary) is diet. Among mammals, herbivores, omnivores and carnivores harbor microbial communities with distinct taxonomic representation and metabolic functions (Ley et al., 2008; Muegge et al., 2011). Similarly, diet-related differences in microbial communities have been observed in fish (Miyake et al., 2015) and ants (Russell et al., 2009). The amount of food consumed, its macromolecular composition and the frequency of feeding all influence the structure of microbial communities and, consequently, the microbiome's influence on host physiology (Zarrinpar et al., 2014). In fact, in mice, diet can have a larger influence on microbial diversity than host genotype (Carmody et al., 2015). The dominant effect of diet on the microbiota is an important consideration for physiologists who introduce dietary changes into their experiments, and for ecologists and conservation biologists studying the effects of habitat loss or fragmentation on food resources (Amato et al., 2013).

Theoretical and experimental evidence suggests that host animals can suppress colonization of certain microbial taxa and, conversely, select taxa that are beneficial to them (Donaldson et al., 2016). Hosts suppress colonization of potentially harmful species through the secretion of mucus, immunoglobulin A and antimicrobial peptides, along with maintenance of the intestinal epithelial barrier (McLoughlin et al., 2016; Schluter and Foster, 2012). An elegant study conducted in *Hydra* demonstrated that antimicrobial peptides were responsible for host species-specific structuring of microbial communities (Franzenburg et al., 2013). A host's defensive repertoire requires a sophisticated system of direct sensing of the microbiota, mediated through microbial metabolites generated in the lumen or close to the mucosal epithelium (Rooks and Garrett, 2016). Mechanisms of host selection of bacterial taxa include the secretion of nutrients, such as mucin glycans from epithelial cells, that are used as primary or alternative energy sources by certain bacteria (Derrien et al., 2010). Some of these 'dedicated' mucin-degraders have intimate relationships with host cells and promote processes associated with host health, such as the maintenance of gut barrier function (Reunanen et al., 2015). Retention of beneficial microbes that can degrade host-derived nutrients also promotes ecological stability within the gut community during nutritional deprivation, such as acute or predictable fasts, when metabolic activity of some bacteria is compromised because of an inadequate supply of diet-derived nutrients (Costello et al., 2010; Dill-McFarland et al., 2014; Kohl et al., 2015). Species that can degrade host-derived nutrients release intermediate metabolic substrates that other members of the community can use as energy, nitrogen and carbon source – so-called 'cross-feeding' (Seth and Taga, 2014).

Other aspects of a host's internal and external environments can affect gut microbiomes. Rearing tadpoles at two environmental temperatures (28°C vs 18°C) alters gut microbiota structure, with higher inter-individual heterogeneity at warmer tank temperatures (Kohl and Yahn, 2016). Exposure of laboratory mice to low ambient temperatures affects their gut microbial communities and thereby alters biological functions – including gene expression and other biochemical processes – in host tissues that are responsive to microbial activity (Chevalier et al., 2015; Ziętak et al., 2016). Microbiome structure and function can also be influenced by host sex (Bolnick et al., 2014; Liang et al., 2015), circadian rhythms (Liang et al., 2015; Thaiss et al., 2014), exercise (Allen et al., 2015; Mika et al., 2015), psychological stress (Galley et al., 2014) and social interactions (Moeller et al., 2016; Tung et al., 2015), among other factors.

Microbiomes shape host physiology

The effects of the microbiota on host physiology are largely driven by bacterial metabolites, and the development and maintenance of host–microbiome relationships involve host sensing of these metabolites (Fig. 1). The principal bacterial metabolites that affect host function are the short-chain fatty acids (SCFAs) acetate, propionate and butyrate (Koh et al., 2016), which are byproducts of anaerobic fermentation of carbohydrates. Microbes also produce other metabolites that influence host physiology, including secondary bile acids, amino acid derivatives and vitamins. Further, bacterial cell wall compounds (e.g. peptidoglycans and lipopolysaccharides) can serve as signals between bacteria and their hosts, promoting mutualistic relationships (Koropatnick et al., 2004). When absorbed and incorporated into the host metabolome (see Glossary), bacterial metabolites provide fuel and act as signaling molecules both locally within the gut and at distant sites in the body (Koh et al., 2016). Given the long association between metazoans and their microbial partners, it is not surprising that many host cells express transporters and receptors that are responsive to these molecules (Layden et al., 2013; Natarajan and Pluznick, 2014).

The short-chain fatty acid butyrate is favored by hindgut epithelial cells as their primary fuel source. It stimulates cell proliferation and signaling, affecting diverse functions including intestinal barrier integrity, anti-inflammatory pathways, release of gut hormones and gut motility (Donohoe et al., 2011; Hamer et al., 2008; Roediger, 1982). Because most of the bacterially derived butyrate is used by the gut, relatively low concentrations are found in systemic circulation. However, some propionate and acetate are

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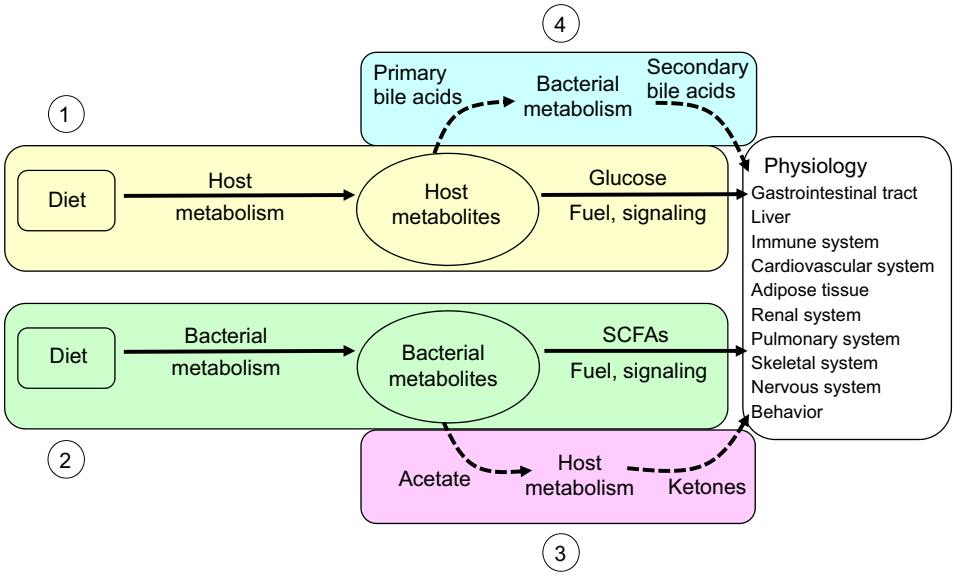

Fig. 1. Pathways by which gut microbiota link host diet to physiology and behavior. (1) Host digestion and metabolism of dietary components produce metabolites that provide fuel (e.g. glucose) and ligands for cell signaling that influence physiological processes across organ systems (examples in white box). (2) Bacterial metabolism of dietary substrates yields metabolites that are absorbed and provide fuel (e.g. SCFAs) and signaling molecules that affect host physiology. (3) Bacterially derived metabolites (e.g. acetate) can be further metabolized in host cells to produce molecules (e.g. ketones) that affect physiology. (4) Host-derived metabolites (e.g. primary bile acids) can be modified by gut bacteria to form other molecules (e.g. secondary bile acids) that affect physiology.

absorbed and circulate to host tissues, with the liver consuming propionate for gluconeogenesis. Acetate can directly affect adipose tissue, brain and liver, and has been implicated in neural signaling pathways that regulate appetite and the secretion of hormones, including insulin and ghrelin (Perry et al., 2016). During states of nutrient deprivation, bacterially derived acetate taken up by the liver can be metabolized to ketone bodies, providing an alternative to glucose for fueling organs such as the heart and brain (Crawford et al., 2009). Many of the regulatory effects that SCFAs exert on host cells are mediated by their inhibition of histone deacetylases and/or stimulation of membrane-bound G protein-coupled receptors, thereby altering cellular physiology and gene expression through transcriptional and epigenetic processes in ways that are not fully understood and are being actively explored (Koh et al., 2016; Layden et al., 2013; Natarajan and Pluznick, 2014).

Gut microbes can also contribute to a host's nitrogen balance. Several members of the gut microbiota produce urease or uricase enzymes that can degrade host nitrogenous wastes such as urea or uric acid, providing nitrogen to support bacterial amino acid and protein synthesis. Ruminant animals digest foregut bacteria in the abomasum (final stomach) and small intestine, releasing microbial amino acids that are absorbed and used for protein synthesis. The extent to which bacterial synthesis of new amino acids derived from urea nitrogen contributes to host nitrogen balance in non-ruminants is not clear, in part because the expression of amino acid and peptide transporters in hindgut segments – the site of greatest microbial density – is relatively low. However, there is evidence that nitrogen released from microbial urea hydrolysis can be incorporated into host tissues in non-ruminants, and that the contribution to host nitrogen balance varies with species, age, diet composition and host nutritional state (Metges et al., 2006; Millward et al., 2000; Singer, 2003).

The gut microbiota can influence a wide array of cell types and organ systems (Figs 1 and 2). Given the close contact of microbes with the gastrointestinal tract, it is not surprising that this organ is highly affected by host–microbial relationships. Colonization with gut microbes can alter the gene expression profiles of intestinal cells (Hooper et al., 2001). Further, gut bacteria regulate digestive and neuroendocrine functions of the gut and play a dominant role in priming the immune system. Some of the effects of gut microbes extend to non-intestinal tissues through the generation of molecules that originate as absorbed SCFAs and are subsequently modified biochemically by intestinal epithelial cells; the conversion of acetate to ketone bodies is an example of this (Fig. 1). Bacterial metabolites can affect renal and cardiovascular systems (Peti-Peterdi et al., 2016), musculoskeletal (Charles et al., 2015) and neuroendocrine function (Neuman et al., 2015; Yano et al., 2015), adipose tissue (Velagapudi et al., 2010), and cellular and whole-body metabolism (Bauer et al., 2016; Mika and Fleshner, 2016; Wong et al., 2014). Liver-derived bile acids within the gut lumen are modified by bacterial enzymes, which alters their rates of absorption into the systemic circulation. Bile acids can stimulate receptors on a variety of cell types, including hepatocytes and brown and white adipocytes, thus influencing aspects of host thermal, metabolic and endocrine physiology in addition to their effects on intestinal lipid absorption and mucosal defense (Wahlstrom et al., 2016). As mentioned above, the gut microbiota is affected by host exercise level, and changes in the microbiota appear to mediate some of the beneficial effects of exercise on host physiology (Mika and Fleshner, 2016). Microbially derived metabolites can also regulate central and hepatic circadian clocks of the host (Leone et al., 2015).

One of the more intriguing aspects of host–microbe associations is the effect on central and peripheral nervous systems. For example, recent work suggests that metabolites released from the gut microbiota early in the life of the host influence the development of the brain and the blood–brain barrier (Braniste et al., 2014; Goyal et al., 2015), and there is increasing evidence for bacterial metabolites modulating the gut–brain axis (Perry et al., 2016). The microbiota have been implicated in a variety of animal behaviors, including stress responses, sociality, fear and even mate choice (Arentsen et al., 2015; Buffington et al., 2016; Gacias et al., 2016; Mika and Fleshner, 2016; Sharon et al., 2010).

The gut microbiota can also affect an animal's thermal physiology. Reduction of microbiota by oral antibiotics in rodents, lagomorphs and ruminants reduces body temperature, presumably by reducing the heat generated by bacterial metabolism and/or host oxidation of bacterially derived metabolites such as SCFA (reviewed in Rosenberg and Zilber-Rosenberg, 2016). Experimental cold exposure shifts microbiota composition in lab mice, and transplantation of the 'cold microbiota' into warm-adapted mice increases insulin sensitivity, browning of white adipose tissue, gut size and absorptive capacity, with the changes

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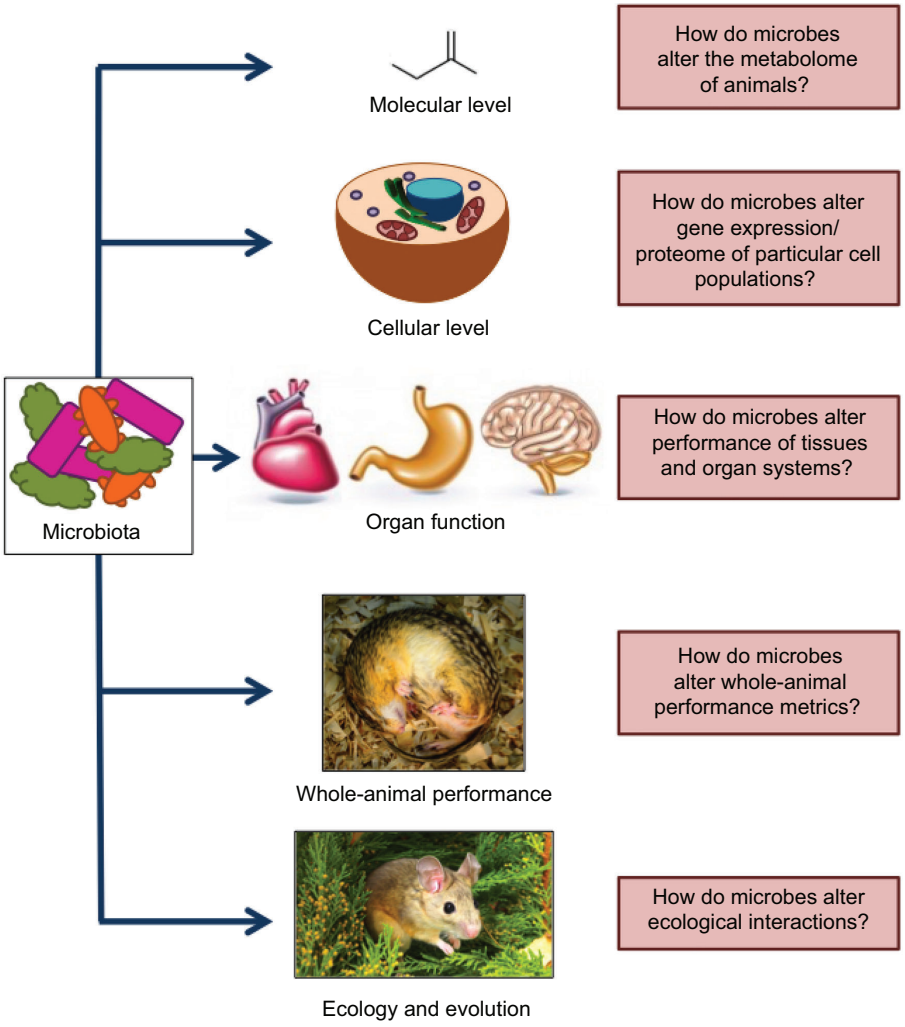

Fig. 2. Numerous levels of biological organization can be affected by animal–microbe symbioses. Addressing questions at each of these levels and the integration across them will greatly enhance our understanding of animal–microbe symbioses.

apparently enhancing adaptation to the cold (Chevalier et al., 2015). Changes in the microbiota that occur when mice are maintained at a cool ambient temperature (12°C) alter the metabolism of bile acids, with subsequent effects on gene expression and thermogenesis in brown fat (Ziętak et al., 2016). Interestingly, ingestion of a high-fat diet exacerbates the effects of cold exposure on microbiota-related host responses, suggesting a functional link between microbiota, diet and thermogenesis.

Animal–microbe symbioses contribute to host ecology and evolution

Changes in physiological performance largely underlie adaptations to novel food sources, temperature regimes and other environmental variables. Given that host–microbe interactions can influence host physiology, it could be hypothesized that some 'physiological adaptations' to environmental conditions may actually be driven by host-associated microbes. In turn, if specific host–microbe interactions are critical to the survival and reproduction of animals in a given environment, there may be selection to preserve the fidelity of these relationships.

As mentioned above, one well-known example of how microbes influence animal ecology and evolution is the digestion of high-fiber plant material. The evolution of the rumen is considered a 'key innovation' that has largely contributed to the success of ruminants over evolutionary time (Mackie, 2002). Similarly, the transmission of gut symbionts between generations is important for herbivorous iguanas to consume fibrous plant material (Troyer, 1982). Yet, consuming plant material that is often laden with defensive plant secondary compounds (PSCs) can present toxic challenges to herbivores. Recent work in herbivorous rodents (woodrats) has demonstrated that the gut microbiota is instrumental in allowing the animals to safely ingest toxin-rich plants (Kohl and Dearing, 2016). For example, reduction of the gut microbiota with antibiotics significantly impairs woodrats' abilities to consume PSCs (Kohl et al., 2014c). Remarkably, the toxin-degrading functions bestowed upon hosts by the gut microbiota can be readily transferred across populations and host species through microbial transplants (Kohl et al., 2016b,c; Miller et al., 2016a). There is evidence that similar detoxifying abilities also occur in the guts of avian herbivores, such as the hoatzin (Garcia-Amado et al., 2007) and sage-grouse (Kohl et al., 2016a), as well as in insect hosts (Ceja-Navarro et al., 2015; Hammer and Bowers, 2015). Together, the presence of fiber-degrading and/or toxin-degrading microbes in animal guts may determine the breadth of plant resources that an animal can consume.

Gut microbes may act as a nutritional buffer for animals experiencing seasonal fluctuations in food quality and availability. For example, seasonal switching from the consumption of primarily young leaves and fruit in wet seasons to mature plants higher in fiber in dry seasons reduces the intake of foods that are digestible by

mammalian enzymes. Changes in microbiota composition induced by seasonal changes in diet (Bergmann et al., 2015; Fon and Nsahlai, 2012; Sun et al., 2016) can help to compensate for such a reduction by increasing the production of SCFAs from less-digestible foods, thus providing much-needed energy and nutrients (Amato et al., 2015). Further, when some animals are faced with periods of low food availability they can undergo prolonged periods of fasting. Gut microbes aid in the supply of alternative energy sources (e.g. SCFAs and ketone bodies) and contribute to nitrogen recycling when hosts are faced with low food availability (Barboza et al., 1997; Crawford et al., 2009), which may contribute to host survival. Indeed, germ-free mice and rats (which lack a gut microbiota; see Glossary) die sooner when faced with starvation than do conventional mice and rats, despite similar body mass loss (Einheber and Carter, 1966; Tennant et al., 1968). Thus, one could speculate that animals that are adapted to periods of low food availability are able to overcome these challenges in part through the aid of their gut microbes. This idea, however, requires testing in ecologically relevant systems.

Many animals that have evolved in highly seasonal environments possess strong biological rhythms in thermal and other physiological parameters that can affect their microbial symbionts, which in turn influences the way in which indigenous microbes affect host physiology (Carey and Duddleston, 2014). For example, the circannual rhythms in food intake and metabolism that are hallmarks of mammalian hibernation alter microbiota structure and function (Carey et al., 2013; Dill-McFarland et al., 2014; Stevenson et al., 2014). Although more research is needed to determine whether seasonal remodeling of the gut microbiota contributes to the hibernation phenotype, a recent study in brown bears suggests that gut microbes may provide physiological benefits to their hosts. Transplantation of the gut microbiota of summer bears into germ-free mice induced greater adiposity than did microbiota from hibernating bears, suggesting that gut microbes may contribute to pre-hibernation fattening (Sommer et al., 2016).

Although the majority of these examples relate to feeding and nutrition, animal–microbe associations can influence a suite of other physiological traits. For example, symbiotic bacteria have been implicated in determining the social cues of hyenas (Theis et al., 2013), the evolution of the nervous system (Eisthen and Theis, 2016) and resistance to infection by pathogens in insects (Dillon et al., 2005; Koch and Schmid-Hempel, 2011). Thus, the time is ripe for physiological ecologists to conduct similar studies in relation to numerous physiological adaptations or processes (Fig. 2). Might inter-population or inter-species differences in ecoimmunological traits be driven by commensal microbes? An animal's metabolic rate scales to influence many aspects of its biology and ecology. What role do gut microbes play in determining metabolic rates across animals? These and other questions require the attention of comparative and ecological physiologists.

Host–microbe associations can have evolutionary implications if microbes impart physiological adaptations to their hosts that confer fitness benefits. It has been posited that natural selection may act not on animals in isolation, but on the host organism and its collective microbiota, which together form a 'holobiont' (Bordenstein and Theis, 2015; Shapira, 2016; Theis et al., 2016). This concept has been debated recently in the literature (Douglas and Werren, 2016; Moran and Sloan, 2015). Regardless of the level at which natural selection is acting, from the perspective of comparative physiologists it is clear that host-associated microbes can play substantive roles in the ecology and evolution of their hosts.

Incorporating host–microbe symbioses into physiological studies

How might animal physiologists incorporate host–microbe interactions into their research? For laboratories that are not set up to analyze microbes or their metabolites, collaborative or fee-for-service arrangements with scientists from other disciplines – including microbiology, virology, bioinformatics, metabolomics and others – can be formed. An increasing number of academic and research institutions have core facilities to support these analyses (for internal and sometimes external clients), and commercial vendors are available to analyze samples with simple or more sophisticated data analysis. For any of these arrangements, it is imperative that initial discussions take place in order to best design the studies so that the multiple variables that can influence experimental outcomes in host–microbiome studies are considered (Stappenbeck and Virgin, 2016). These considerations include best practices for initial collection and storage of samples, types of samples (e.g. fecal, gut contents, host tissue), time of sampling (e.g. point during experimental time course or within circadian cycle) and the analytical methods that will be used. Additionally, it is important to collect and sequence blank samples since DNA extraction kits and other reagents contain microbial DNA that can obscure microbial inventories, especially in low biomass samples (Salter et al., 2014). To the greatest extent possible, relevant metadata should be collected for individual animals (e.g. sex, age, body mass, body temperature), as well as information on environmental and dietary conditions under which animals are studied in the field or in captivity. For captive studies, ambient temperature, light/dark cycle, feeding regimen, bedding type and single versus group housing are important variables, among others. Recent studies have examined the impact of captivity on gut microbial communities (Kohl and Dearing, 2014; Kohl et al., 2014b; Nelson et al., 2013), but studies on additional animal taxa are needed.

The use of fecal samples is the most common means of assessing gut microbial communities and their relationship with features of host physiology. Fecal sampling allows repeated sampling within individuals and may be the only option for some field studies and for work with endangered populations. However, reliance on fecal sampling alone to answer some experimental questions has limitations. Fecal sampling cannot detect differences in microbial communities that are spatially separated along the length of the gut (Kohl et al., 2014a), and it may not distinguish between luminal and mucosa-associated bacterial communities (Galley et al., 2014; Ringel et al., 2015; Yasuda et al., 2015).

With regard to microbiota analysis, the most widespread method currently used for taxonomic assignment is sequencing regions of the 16S ribosomal RNA gene. Although studies sequencing the bacterial 16S rRNA gene are widely referred to as studying the 'gut microbiota', it is important to note that many other types of organisms (in addition to bacteria) are present in these samples. Alternative primer sets and sequencing approaches are necessary to produce inventories of the archaeal (Gruninger et al., 2016), fungal (Miller et al., 2016b), protozoan (Parfrey et al., 2014) and viral (Minot et al., 2011) communities. Sequencing techniques vary, as can the pipelines used to process the resulting 'operational taxonomic units' (see Glossary) that loosely represent a microbial species (Caporaso et al., 2010; Schloss et al., 2009). Although the choice of methodology in microbial community analysis may rest with the collaborators or core services used, the approaches should be clearly identified in the methods or supplemental material sections of any resulting publications. The same holds for methods

used to assess the genetic potential of a given microbial community (metagenomics), the genes expressed by the community at the time of sampling (metatranscriptomics) and the collection of metabolites produced by the microbiome (metabolomics). Increasingly, efforts are being made to interrogate these measures of microbiome functionality simultaneously with the expression of host genes from relevant tissues projected to be affected by the microbiome.

The cataloging of descriptive, association-based data is often a necessary first step along the investigative path for analysis of host–microbe effects on physiological functions. Ultimately, though, investigators should strive to uncover the mechanistic and functional linkages between a host and its microbial symbionts – e.g. through intervention studies. Examples are studies that remove host-associated bacteria wholly, or in part, from the process under study before or after a physiological intervention or in separate groups of control and treated animals. Common techniques are the use of animals engineered to be germ-free or the administration of antibiotics to conventionally raised animals to fully or selectively deplete their microbiotas. These techniques have been successfully employed in a number of fish and invertebrate hosts (Charnley et al., 1985; Marques et al., 2006; Milligan-Myhre et al., 2016). Unfortunately, generating germ-free animals can be highly challenging for studies with non-laboratory terrestrial vertebrates, especially if they are difficult to maintain or manipulate in captivity, or the question of interest must be asked and studied in the field. That said, microbial transplants have been performed between wild-caught rodents (Kohl et al., 2014c) and antibiotic treatments have been administered to penguins in the field (Potti et al., 2002). Creative solutions tailored to specific study systems will be required to advance our knowledge of how host–microbiome symbioses affect the physiology, ecology and evolution of individual animals and their populations.

Conclusions

The study of animal physiology is evolving as our understanding of animal–microbial symbioses grows. It is clear that physiologists must now recognize that an animal's phenotype may be shaped not only by its own genes, but also by those encoded in its associated microbiota. We are still largely at the descriptive stage in characterizing many of the physiological consequences that result from host–microbe interactions, particularly for free-living species. The integration of host-microbiota symbioses into animal population and community dynamics (e.g. Li et al., 2016) is an area ripe for exploration by collaborative groups of animal physiologists, plant ecologists, microbial ecologists and ecological modelers. Ecophysiologists who consider the potential impacts of the microbiota on physiological responses to changing environments should be participants in multidisciplinary teams that are working to understand the impacts of climate warming and other rapid anthropomorphic changes on animal populations and ecosystems. This is particularly important because ecological adaptations conferred by microbial systems typically happen at a much more rapid pace than evolutionary processes can in many animal hosts (Alberdi et al., 2016). The ability to fully integrate microbiomes into the ecology of free-living animal species will aid conservation biology efforts, particularly for animals that experience environmental changes that affect food resources (Bahrndorff et al., 2016; Barelli et al., 2015). It is our hope that this integration of biology across kingdoms will produce comparative and ecological physiologists who routinely consider whether the adaptations they are studying may be linked to the activity of microbes associated with the animal's body. Such approaches may also bring valuable insights to host–microbiome interactions in humans, similar to the contributions that eco-immunology has brought to biomedicine (Beura et al., 2016; Maizels and Nussey, 2013; Pedersen and Babayan, 2011). For instance, understanding how the bidirectional interactions between a host and its gut microbiota are altered when food quantity or quality fluctuate seasonally in the wild may lead to new therapeutic avenues that promote a healthy gut ecosystem when humans are exposed to nutritional challenges due to disease or other perturbations.

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Competing interests

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