



SYMPOSIUM

Links between Natural Variation in the Microbiome and Host Fitness in Wild Mammals

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Synopsis Recent studies in model organisms have shown that compositional variation in the microbiome can affect a variety of host phenotypes including those related to digestion, development, immunity, and behavior. Natural variation in the microbiome within and between natural populations and species may also affect host phenotypes and thus fitness in the wild. Here, I review recent evidence that compositional variation in the microbiome may affect host phenotypes and fitness in wild mammals. Studies over the last decade indicate that natural variation in the mammalian microbiome may be important in the assistance of energy uptake from different diet types, detoxification of plant secondary compounds, protection from pathogens, chemical communication, and behavior. I discuss the importance of combining both field observations and manipulative experiments in a single system to fully characterize the functions and fitness effects of the microbiome. Finally, I discuss the evolutionary consequences of mammal–microbiome associations by proposing a framework to test how natural selection on hosts is mediated by the microbiome.

Introduction

Recent studies in animal models have shown that variation in the microbiome can affect the host phenotype in many ways, including through traits related to digestion, development, immunity, and behavior (McFall-Ngai et al. 2013). These findings suggest that compositional differences in symbiotic microbial communities may play a fundamental role in host ecology and evolution. Understanding the beneficial effects of the mammalian microbiome in natural populations is particularly important for understanding human health as well as for generalizing the findings from laboratory-reared models and domestic animals. Although the importance of microbes in host health has been well-established mainly using culturing methods, we know less about how natural variation in the microbiome can affect host fitness in wild mammals.

The idea that the microbes may play a beneficial role in host fitness is not new. For example, one of the best studied beneficial functions of the mammalian microbiome is its role in the digestion of plant materials, such as cellulose. In the late 1800s, microbial fermentation

was proposed as a mechanism of cellulose digestion (Zuntz 1879) and was supported empirically (Tappeiner 1884). In the 1940s, protozoa (Hungate 1942, 1943) and bacteria (Hungate 1947; Sijpesteijn 1948) with capabilities to digest cellulose were isolated from the stomach of ruminants. Fermentation products, such as volatile fatty acids, have been shown to be absorbed by the host from the rumen wall into the blood system as energy sources (Barcroft et al. 1944). The concentration of these fatty acids is highest in the fermentation chambers compared with the rest of the gastrointestinal tract in herbivores (Elsden et al. 1946). These findings led to classic reviews and books on gut microbiota and digestion (Hungate 1966; Van Soest 1994; Stevens and Hume 1995; Hume 1999).

In the last decade or so, the advancement of DNA sequencing technology has made it possible to investigate variation of the entire microbial community (which was impossible to study based on culturing methods alone). For example, the host-associated microbiome varies between species as well as within and between natural populations. Interspecific

variation in microbiomes exists both in captivity (Ley et al. 2008; Muegge et al. 2011) and in the wild (Ochman et al. 2010; Phillips et al. 2012). Between-population variation in the microbial composition has been observed in rodents (Linnenbrink et al. 2013; Kohl et al. 2014b; Kreisinger et al. 2015) and primates (Amato et al. 2013; Moeller et al. 2013a) including humans (Suzuki and Worobey 2014). Within-population variation in the microbiome has also been observed in humans (Arumugam et al. 2011), chimpanzees (Moeller et al. 2012), and house mice (Wang et al. 2014). Most of the work has focused on the gut microbiome, but studies have also described microbes associated with other body sites of the host (Costello et al. 2009; Huttenhower et al. 2012).

In principle, variation in the microbiome could have a positive effect, a negative effect, or no effect on host fitness. However, it appears unlikely that compositional differences in microbiome would have no impact on host fitness given the known role of microbes in digestion (Hungate 1966; Van Soest 1994; Stevens and Hume 1995; Hume 1999), the abundant biomass of symbiotic microbes associated with hosts (Sender et al. 2016), the known association between microbes and host immunity (Round and Mazmanian 2009), and the observations that even rare microbes are suggested to play a large role in microbial ecosystem functions (reviewed in Jousset et al. 2017). Nevertheless, it is possible that some rare and transient microbes have little or no effect on host fitness. The current examples of positive and negative effects of microbes on host fitness are sometimes restricted to studies of a single bacterial lineage. For example, the positive effects of bacteria in sap-sucking insects are well-established including the role of bacteria in providing essential nutrients (Baumann 2005), defense from pathogens (Oliver et al. 2003), and tolerance to heat (Dunbar et al. 2007). The negative effects of microbes on host fitness have also been studied, primarily in the context of microbial pathogens including protists (Allison 1964), bacteria (Lowy 1998), and viruses (Pierrney and Oliver 2006). However, fitness effects of variation in an entire microbial community are not well understood. Understanding the association between variation of the microbiome and variation of host fitness requires molecular tools to characterize differences in microbial community composition in natural populations.

Here, I review studies over the last decade investigating inter- and intraspecific variation in the mammalian microbiome that suggest a link between variation in the microbiome and variation in host phenotype and thus fitness (Table 1). Although most of the evidence is correlational, these studies suggest that natural

variation in host-associated microbiomes play a role in mammalian host biology including aspects of digestion, detoxification, immunity, and behavior. I then identify potential future research directions, including the need for manipulative experiments.

Assistance of energy uptake from diet

A series of studies by Gordon and colleagues using fecal transplant experiments in mouse models showed that the gut microbiome can affect fat storage and energy extraction from the diet (Bäckhed et al. 2004; Turnbaugh et al. 2006). Distantly related mammalian host species kept in zoos show convergence in the microbiome by diet types despite the significant effects of host phylogeny and numerous other factors affecting the composition of the microbial community (Ley et al. 2008; Muegge et al. 2011). Furthermore, differences in the microbiome may reflect functional differences in digestion. For example, the microbiomes of mammalian herbivores are enriched for microbial genes that synthesize amino acids compared with those of carnivores which are enriched for microbial genes that degrade amino acids (Muegge et al. 2011). Together, the convergence in microbiomes in distant mammalian groups that share a similar diet suggests that the microbiome may play a fundamental role in the ability of mammals to specialize on a particular source of food.

Similar patterns of convergence of microbiomes associated with dietary types have been observed in wild mammals. For example, convergence in microbiomes has been observed among myrmecophagous mammals such as anteaters, armadillos, and armadillos that feed on ants and termites (Delsuc et al. 2014), among bat species that share similar diet types (Phillips et al. 2012; Carrillo-Araujo et al. 2015), between chimpanzees and gorillas that share a large fraction of fruit diet (Moeller et al. 2013a), and between carnivorous marine mammals (Nelson et al. 2013a, 2013b; Soverini et al. 2016). Population-level differences in the microbial composition of humans were also associated with differences in the amount of dietary fiber (De Filippo et al. 2010; Ou et al. 2013; Schnorr et al. 2014; Angelakis et al. 2016). Although these studies are consistent with the idea that convergent microbiomes are involved in diet-specific digestion, there are alternative explanations. For example, some of the observed patterns might be explained by common microbes in the shared diet or co-variation with other aspects of the shared environment (Moeller et al. 2013a; Suzuki and Worobey 2014). Thus, convergence of microbiomes among distant mammalian lineages does not, by itself, provide definitive evidence of the beneficial effects of the

Table 1 Suggestive evidence of compositional and functional variation of the microbiome affecting the host phenotype in natural or semi-natural populations of mammals using culture-independent techniques

Host phenotype	Mammalian host species		Links between variations of the microbiome and the host phenotype	References
Energy uptake from diet	Plant fiber digestion			
	Humans	<i>Homo sapiens</i>	Population differences in fiber-rich diet were associated with differences in the microbiota in a way that is consistent with the findings in experimental studies.	De Filippo et al. 2010; Ou et al. 2013; Schnorr et al. 2014; Angelakis et al. 2016
	Tammar Wallaby ^a	<i>Macropus eugenii</i>	The foregut microbiome varied compositionally from other herbivores, but the microbiome converged functionally in plant fiber breakdown pathways using metagenomics.	Pope et al. 2010
	Giant panda ^a	<i>Ailuropoda melanoleuca</i>	Genes involved in cellulose metabolism and lignin oxidation were identified, but the microbiome differs compositionally from other herbivores and similar to carnivorous bears.	Zhu et al. 2011; Fang et al. 2012; Xue et al. 2015
	Flying squirrel	<i>Petaurista alborufus lena</i>	Convergence in microbiota of fermentation chambers along the gastrointestinal tract was observed (e.g., fermentation chambers tend to have greater alpha-diversity compared with the small intestines regardless of the presence or absence of the foregut chambers).	Lu et al. 2014
	White-throated woodrat	<i>Neotoma albigula</i>		Kohl et al. 2014a
	House mouse	<i>Mus musculus</i>		Suzuki and Nachman 2016
Chitin digestion	Baleen Whales	<i>Mysticeti</i> sp.	The microbial composition is distinct from other mammals and enriched in bacterial genes associated with chitin degradation using metagenomics.	Sanders et al. 2015
	Myrmecophagous mammals ^a	(e.g., echidna, aardvark, anteater, tamandua, armadillo, aardwolf, etc.)	Convergence of microbiota was observed across a wide range of myrmecophagous mammals.	Delsuc et al. 2014
Fish digestion	Marine mammals ^a	<i>Mirounga leonina</i> , <i>Hydrurga leptonyx</i> , <i>Dugong dugong</i>	Convergence of microbiota was observed among marine carnivorous, distinct from the rest of marine and terrestrial mammals.	Nelson et al. 2013a, 2013b
	Bottlenose dolphins ^a	<i>Tursiops truncatus</i>	Convergence of microbiota was observed between bottlenose dolphins and carnivores fish that share precocious diet.	Soverini et al. 2016
Fruit digestion	Chimpanzee and Gorilla	<i>Pan</i> and <i>Gorilla</i>	Convergence of microbiota was observed between chimpanzees and gorillas that share large fraction of a fruit diet.	Moeller et al. 2013a
Seasonal diet shifts	Lemurs	<i>Lemur catta</i> and <i>Propithecus verreauxi</i>	Seasonal shifts in diet were associated with the shifts in microbiota.	Fogel 2015
	Wood mouse	<i>Apodemus sylvaticus</i>		Maurice et al. 2015
	Reindeer ^a	<i>Rangifer tarandus tarandus</i>		Salgado-Flores et al. 2016
	Gorilla	<i>Gorilla gorilla</i> sp.	Seasonal shifts in diet were associated with shifts in the microbiota and the metabolome.	Gomez et al. 2015

	Black Howler Monkey	<i>Alouatta pigra</i>	Seasonal shifts in diet were associated with shifts in the microbiota and volatile fatty acids.	Amato et al. 2014
	Tibetan Macaques	<i>Macaca Thibetana</i>	Seasonal shifts in diet were associated with shifts in microbiota and predicted bacterial gene functions.	Sun et al. 2016
Mucin digestion during food limitation	Thirteen-lined ground squirrel	<i>Ictidomys tridecemlineatus</i>	Fasting was associated with changes in the microbiota. The increase in acetate (%) was associated with the increase in acetate producing bacteria (%) during fasting and hibernation.	Carey et al. 2013
	Arctic ground squirrel	<i>Urocitellus parryi</i>		Stevenson et al. 2014
	Syrian hamster ^a	<i>Mesocricetus auratus</i>	Fasting was associated with changes in the microbiota and the increases in acetate producing bacteria.	Sonoyama et al. 2009
Detoxification of plant dietary toxins	White-throated woodrat	<i>Neotoma albigula</i>	Bacterial communities that are associated with oxalate-degradation are most abundant in the foregut compared with the rest of the gastrointestinal tract.	Miller et al. 2014
Resin detoxification	Desert woodrat	<i>Neotoma lepida</i>	Population differences in microbial composition and functions were associated with creosote diet. Antibiotic treatment and transplant experiments demonstrated a causal link.	Kohl et al. 2014b
Tannin detoxification			Transferring the microbiome of woodrats to a laboratory rats revealed that differences in the microbial community structure can increase the ability of recipients to consume tannic acid.	Kohl et al. 2016b
Immunity and pathogen defense	Humans	<i>Homo sapiens</i>	Immunodeficiency due to viral infection was associated with shifts in the microbiota.	reviewed in Salas and Chang 2014, Williams et al. 2016
	Chimpanzee	<i>Pan troglodytes</i>		Moeller et al. 2013b
	Gorilla	<i>Gorilla gorilla gorilla</i>		Moeller et al. 2015
	House mice	<i>Mus musculus domesticus</i>	Antibody-based viral infection status was positively correlated with alpha-diversity of microbiota.	Weldon et al. 2015
Interaction with macroparasites	Yellow-necked mouse	<i>Apodemus flavicollis</i>	Infection statuses of nematodes and mites were negatively correlated with alpha-diversity of the microbiota.	Kreisinger et al. 2015
			A weak association was observed between microbial composition and helminth composition.	
Immune gene expression	House mice	<i>Mus musculus</i> spp.	Aberrant immune gene expression and intestinal histopathology in hybrids were associated with differences in the microbiota in relation to the parental species.	Wang et al. 2015
Chemical communication and behavior	Spotted and Striped hyenas	<i>Crocuta crocuta</i> and <i>Hyaena hyaena</i>	The microbiota including odor-producing bacteria in scent gland secretions differed among species, social groups, sex, and reproductive status using pyrosequencing.	Theis et al. 2012, 2013

(continued)

Table 1 Continued

Host phenotype	Mammalian host species	Links between variations of the microbiome and the host phenotype	References
Meerkat	<i>Suricata suricatta</i>	The microbiota (based on ARISA) in scent secretion differed among sex, age, and social groups.	Leclaire et al. 2014
Musk deer ^a	<i>Moschus moschiferus</i>	The chemical and microbial composition of musk secretion differed between mated and unmated males using pyrosequencing.	Li et al. 2016
European Badger	<i>Meles meles</i>	The chemical and microbial composition (based on T-RFLP) of subcaudal gland secretion were significantly correlated and varied by age and female reproductive status.	Sin et al. 2012

Note: See text for culture-based studies and functional studies for further evidence on microbiome functions.

^aCaptive individuals in semi-natural environment were used mostly or entirely.

microbiome on the host. In fact, microbes can compete for resources with the host when the nutrients, such as simple sugars and iron, can be utilized by both hosts and microbes ([Wasieleski et al. 2016](#)).

The best evidence of the beneficial role of microbes in energy extraction comes from components of the diet that cannot be utilized directly or easily by the host, such as cellulose. Mammalian herbivores have evolved two major mechanisms for breaking down plant polysaccharides; foregut fermentation (as in ruminants) and hindgut fermentation ([Stevens and Hume 1995](#); [Feldhamer et al. 2007](#)). In the case of foregut fermenters, the stomach and/or esophagus are modified and enlarged, while in hindgut fermenters, the main site of fermentation can be either the large intestine or the enlarged cecum. Foregut and hindgut fermenters each have distinct microbial communities that contain high densities of microbes which aid in digestion ([Ley et al. 2008](#); [Muegge et al. 2011](#)). As mentioned briefly above, the role of microbes in ruminant digestion has been studied extensively in large part due to its economic and agricultural importance ([Hungate 1966](#); [Van Soest 1994](#); [Stevens and Hume 1995](#); [Hume 1999](#)). This is now being followed up using molecular techniques ([McCann et al. 2014](#)). Culture-based studies have suggested a role for protozoa, bacteria, and fungi in plant digestion not only in domestic animals, but also in various wild herbivores ([Sahu and Kamra 2002](#)). Shotgun metagenomic studies have revealed that microbiomes of herbivorous mammals contain bacterial genes involved in fiber fermentation ([Pope et al. 2010](#); [Zhu et al. 2011](#); [Fang et al. 2012](#)).

Species-rich microbial communities may be important for digestion of plant fibers. From interspecific comparisons, the gut microbial communities of herbivores often have the highest species richness (i.e., alpha-diversity) compared with those of other dietary types ([Ley et al. 2008](#); [Muegge et al. 2011](#); [Phillips et al. 2012](#)). An exception to this pattern is the giant panda which has one of the lowest alpha-diversity measures across mammals ([Ley et al. 2008](#); [Xue et al. 2015](#)). Although microbial genes involved in cellulose degradation and lignin oxidation were identified in panda feces ([Zhu et al. 2011](#); [Fang et al. 2012](#)), their poor digestion rate may be due to deficiencies in cellulose-digesting bacteria and low alpha-diversity ([Xue et al. 2015](#)). Interestingly, even within an individual's gastrointestinal tract, there appears to be an association between alpha-diversity and plant fermentation. The foregut and hindgut fermentation chambers have greater alpha-diversity compared with the small intestine in multiple species of wild-caught rodents ([Kohl et al. 2014a](#); [Lu et al. 2014](#); [Suzuki and Nachman 2016](#)). The stability–diversity relationship ([Tilman and](#)

Downing 1994), an observation that species-rich communities are more stable, resilient, and recover faster from disturbance, has been suggested as an explanation for the species-rich communities observed in herbivores' fermentation chambers (Lu et al. 2014; Suzuki and Nachman 2016).

Chitin is the second most abundant biopolymer on the planet and common in the cell walls of fungi and the exoskeletons of arthropods. Although chitin can be broken down by both mammalian and microbial enzymes, microbes may play a particularly important role in chitin degradation (Herwig et al. 1984; Simunek et al. 2001; Delsuc et al. 2014; Sanders et al. 2015). Chitinase-producing bacteria have been isolated in insectivorous bats (Whitaker et al. 2004) as well as in herbivorous and omnivorous mammals that are not adapted uniquely to a chitin-rich diet (Simunek et al. 2001). Chitinase activity has also been detected in the stomach of nine-banded armadillos (Smith et al. 1998) and the intestines of insectivorous bats (Whitaker et al. 2004). The involvement of microbes in chitin degradation has been suggested in myrmecophagous mammals that show convergence in their microbial composition (Delsuc et al. 2014). Baleen whales also feed on prey that is rich in chitin, and their foregut stomach has been suggested to play a role in the microbial degradation of chitin (Herwig et al. 1984). In fact, a recent metagenomic study found an enrichment of bacterial genes associated with chitin degradation in baleen whales (Sanders et al. 2015).

Seasonal changes in diet and gut microbial composition in wild mammals also support the involvement of the microbiome in energy extraction. In multiple species of primates (Amato et al. 2014; Fogel 2015; Gomez et al. 2015; Sun et al. 2016), wood mice (Maurice et al. 2015), and reindeer (Salgado-Flores et al. 2016), microbial composition was found to vary seasonally. The seasonal change in the microbial composition was correlated with the changes in the metabolic profiles in gorilla (Gomez et al. 2015) and howler monkeys (Amato et al. 2014). Hibernation and torpor are perhaps the most extreme cases of seasonal shifts in diet, requiring both hyperphagia and fasting (Carey et al. 2003). The microbiota is known to differ between active seasons and hibernation periods in 13-lined ground squirrels (Carey et al. 2013; Dill-McFarland et al. 2014) and arctic ground squirrels (Stevenson et al. 2014). Microbially-provided energy sources such as short-chain fatty acids (SCFAs) have been suggested to serve as a source of fuel during hibernation by bacteria degrading host-derived polysaccharides such as mucins (Carey et al. 2013). Although hibernation reduces the total amount of cecal SCFAs, the relative proportion of acetate (a specific SCFA) increases

during hibernation compared with active seasons in the two species of ground squirrels (Carey et al. 2013; Stevenson et al. 2014). The increase in acetate-producing bacteria such as *Akkermansia muciniphila* that degrades mucins (Derrien et al. 2008) was observed in fasting and hibernating ground squirrels (Carey et al. 2013; Stevenson et al. 2014) and fasting Syrian hamsters (Sonoyama et al. 2009).

Together, these studies are consistent with the hypothesis that variation in the gut microbiome is associated with the variation in energy extraction from diverse diets in mammalian hosts.

Detoxification of plant dietary toxins

Plants defend themselves from herbivores by producing plant secondary compounds or dietary toxins. Thus, detoxifying dietary toxins is a critical challenge for herbivores specializing on plant diets. Mammalian hosts often rely on microbes to detoxify plant toxins. For example, culture-based studies have isolated dietary toxin-degrading bacteria from a wide range of mammalian hosts including various domestic species (Osawa and Sly 1992; Nemoto et al. 1995), marsupials (Osawa 1990; Osawa and Sly 1992; Nemoto et al. 1995), Ethiopian ruminants (Ephraim et al. 2005), and rodents (Sasaki et al. 2005; Dai et al. 2014; Miller et al. 2014; Kohl et al. 2016b). The occurrence of toxin degrading bacteria was associated with mammals that consume tannin rich diet (Osawa and Sly 1992), but not in others (Nemoto et al. 1995). Although detoxification enzymes can be produced both by the host tissue and the microbes, microbial enzyme activity per unit protein greatly exceeds that of the host in spiny mice (Kohl et al. 2016a) consistent with the findings in laboratory rats (Nakano and Gregory 1995). Two species of woodrats that consume dietary toxins in the wild converged on microbial community composition when they were fed dietary toxins in a common environment (Kohl et al. 2012). Dietary toxin feeding experiments in Japanese large wood mice demonstrated that individuals that have prior experience to dietary toxins were associated with a greater abundance of toxin-degrading bacteria and have better performance measured by weight change over time (Shimada et al. 2006).

A series of elegant papers by Kohl and Dearing used manipulative experiments to show that microbes aid woodrats in detoxifying plant secondary compounds (reviewed in Kohl and Dearing 2016). Microbes cultured from woodrat guts can degrade plant secondary compounds (Miller et al. 2014; Kohl et al. 2016b), and the ability of woodrats to consume dietary toxins is impaired when rats are given antibiotics (Kohl et al. 2014b). When feces of woodrat donors that regularly

consumed dietary toxins were fed to woodrat recipients that were naive to the dietary toxin, the recipients showed an increased ability to feed on the dietary toxin (Kohl et al. 2014b). Similarly, microbial transplant experiments from woodrats to laboratory rats increased the ability of laboratory rats to consume tannic acids (Kohl et al. 2016b). Together, these studies provide strong evidence that differences in the gut microbiome in natural populations are involved in detoxification of dietary toxins of herbivores.

Pathogen defense and development of immune system

While the microbiome clearly plays an important role in digestion and detoxification, another well-studied role is its effect on host immunity (Round and Mazmanian 2009). In model organisms, the microbiota has been shown to benefit hosts by occupying the niche space of pathogens or by priming the development of the immune system. Recently, viruses in the gut mucus layer were suggested to play a role in protection against bacterial pathogens (Barr et al. 2013). Understanding the role of the microbiome in host immunity in wild mammals is another growing research area.

The microbiome of a host may help provide protection from lethal pathogens in natural populations. For example, white-nose syndrome is an emerging infectious disease in North American hibernating bats caused by the fungus, *Pseudogymnoascus destructans* (Warnecke et al. 2012). Recent studies isolated bacteria from the skin of healthy bats and showed anti-fungal effects against *P. destructans*, suggesting a beneficial role of skin microbes in pathogen defense (Hoyt et al. 2015; Hamm et al. 2017). A similar link has been identified in chytrid fungus infections in amphibians (reviewed in Rollins-Smith et al. 2011; Colombo et al. 2015). Thus, natural variation in the non-pathogenic microbial community may affect the susceptibility and transmission of fungal diseases in wild mammals.

Infections of viruses and macroparasites are also associated with differences in the gut microbiota in wild mammals. Immunodeficiency virus infection (e.g., HIV in case of humans) was associated with changes in the microbiota of humans (reviewed in Salas and Chang 2014; Williams et al. 2016), chimpanzees (Moeller et al. 2013b), and domestic cats (Weese et al. 2015), but not in gorillas (Moeller et al. 2015). After HIV infection in humans, gut microbiomes may affect the progression of the disease (Vujkovic-Cvijin et al. 2013). Microbial communities seem to respond differently towards different infectious agents. For example, in wild-caught house mice, the degree of viral infection was positively correlated with alpha-diversity of the gut

microbial community, whereas the degree of nematode and mite infection was negatively correlated with the alpha-diversity (Weldon et al. 2015). Aberrant immune gene expression and intestinal histopathology in hybrid house mice were associated with changes in the microbial community composition compared with the two parental species (Wang et al. 2015). Associations between helminth infection and the microbial composition were observed in yellow-necked mice, although the effect size was very low (Kreisinger et al. 2015). These correlations between host immunity and the microbial composition in wild mammals are interesting, but the correlations alone cannot disentangle the cause and the consequence of the association. Nor is it always clear whether these associations reflect positive or negative effects on host fitness. Manipulative experiments including infection trials are necessary to understand the role of microbial communities in host immunity.

Modification of chemical communication and host behavior

Emerging evidence suggests that the microbiome can alter the behavior of mammalian hosts in two ways; (1) changes in olfactory signaling by microbial communities in the scent glands (Ezenwa and Williams 2014), and (2) changes to the central nervous system by microbial communities in the gut (Sampson and Mazmanian 2015). The relationship between the host behavior and the microbiome can be reciprocal in the sense that social interactions between hosts can affect variation in the microbiome (Archie and Tung 2015; Tung et al. 2015; Moeller et al. 2016a). Using computer models, Lewin-Epstein et al. (2017) suggested that microbes might play a role in the evolution of host altruism. The implications of the microbiome in behavioral ecology have been reviewed elsewhere (Archie and Theis 2011; Archie and Tung 2015).

In mammals, olfactory communication plays an important role in various behaviors, including mate preference and individual and species recognition (Ezenwa and Williams 2014). The fermentation hypothesis of chemical recognition is the idea that microbial communities in mammalian scent glands play an important role in the production of volatile odors (Albone et al. 1974; Albone and Perry 1976; Gorman 1976; Albone 1984). There is now evidence for microbe-produced signals in a variety of animals (reviewed in Ezenwa and Williams 2014). In wild mammals, the microbiomes in scent glands are known to differ among closely related species (Theis et al. 2013), social groups (Theis et al. 2012, 2013; Leclaire et al. 2014), individuals (Gorman 1976; Merritt et al. 1982) and individuals with different sex (Gasset et al. 2000; Alexy et al.

2003; Voigt et al. 2005; Theis et al. 2013; Leclaire et al. 2014), reproductive status (Sin et al. 2012; Li et al. 2016), and age (Sin et al. 2012; Leclaire et al. 2014). Furthermore, bacteria-mediated odor significantly altered the outcomes of choice tests in Indian mongooses (Gorman 1976) and Brazilian guinea pigs (Zechman et al. 1984), consistent with findings in laboratory mice (Li et al. 2012).

Involvement of microbes in olfactory signaling has been observed in humans as well. Humans exhibit MHC-dependent mate preference and kin recognition based on odors (Wedekind et al. 1995; Wedekind and Furi 1997; Weisfeld et al. 2003). Axillary odors are known to be associated with microbial composition (Leyden et al. 1981) and there is evidence that microbes transform odorless host axillary secretions into volatile odors (James et al. 2013). Bacteria-produced odorant was more similar between monozygotic twins compared with unrelated individuals (Kuhn and Natsch 2009) supporting the potential involvement of axillary microbes in inbreeding avoidance and kin recognition in humans. The behavioral, hormonal, and neurochemical effects of gut microbiomes through the gut–brain axis has been studied in humans and laboratory mice including the role of the microbiota on anxiety-like behavior, depression-like behavior, Parkinson's disease, Alzheimer's disease, and autism spectrum disorder (reviewed in Sampson and Mazmanian 2015). However, outside of humans and laboratory mice, the implications of the gut–brain axis in the ecology and evolution of wild mammals are largely unexplored.

Manipulative experiments are needed

Overall, the studies presented above are consistent with the idea that natural variation in the microbiome can affect differences in host fitness. One of the advantages of omics-approaches (metagenomics, transcriptomics, proteomics, and metabolomics) is the ability to connect the compositional variation in the microbiome to functional variation in the microbiome, and eventually to the differences in host phenotypes and fitness. However, most of the current evidence is correlative (Table 1). Manipulative experiments in controlled settings are needed to identify causal relationships.

The effects of the microbiome on host phenotype have commonly been tested in model organisms mainly using two approaches; (1) measuring the phenotype of the host without the microbiome by knocking-down the microbial community with antibiotics or by using germ-free animals, or (2) measuring the phenotype of the host with a modified microbiome through transplant experiments, either using bacterial isolates or a whole bacterial

community. Although the use of antibiotics has been criticized by having many confounding effects (reviewed in Lundberg et al. 2016), there are several advantages over germ-free models such as studying the depletion of microbiome at different developmental stages and the ability to target bacterial groups by using different classes of antibiotics. While germ-free models also have their own limitations, germ-free models still seem to be the best system for microbial transplant experiments (Lundberg et al. 2016). For example, germ-free laboratory mice exhibit a variety of abnormalities including the development of the gut, immune system, and brain (Martín et al. 2016). Fecal transplant experiments have demonstrated a causal role of the microbiome in fat storage (Turnbaugh et al. 2006), anxiety-like behavior (Heijtz et al. 2011), and priming the immune system (Olszak et al. 2012). Conducting manipulative experiments to test hypotheses generated by field observations would greatly increase our understanding of the functions of the compositional variation of the microbiome in wild mammals (Kohl et al. 2014b, 2016b; Brooks et al. 2016).

A particularly powerful approach would be to combine field observations and manipulative experiments in a single species. In human microbiome research, laboratory mice are often used as a stand-in for human subjects because conducting manipulative experiments in humans can be difficult or impossible (Nguyen et al. 2015). However, results from mice may not translate easily to humans due to differences in anatomy, physiology, and genetics (Nguyen et al. 2015) as well due to the existence of species-specific bacterial lineages (Moeller et al. 2016b) and communities (Ochman et al. 2010; Phillips et al. 2012; Brooks et al. 2016). A system that can combine both observations in natural populations and manipulations in the laboratory would be useful for assessing the role of microbiome variations on host fitness. Rodents are a particularly tractable group for combining these two approaches for microbiome research in general (Kohl et al. 2014b, 2016b; Brooks et al. 2016).

Evolutionary consequence of microbiome functions

Evolutionary change in a host that is driven by natural selection could be mediated by microbiome in cases where microbes are faithfully transmitted from one generation to the next. The implications of the fitness effects of the microbiome for host evolution have been widely discussed in the literature (Margulis and Fester 1991; Rosenberg et al. 2007; Zilber-Rosenberg and Rosenberg 2008; McKnite

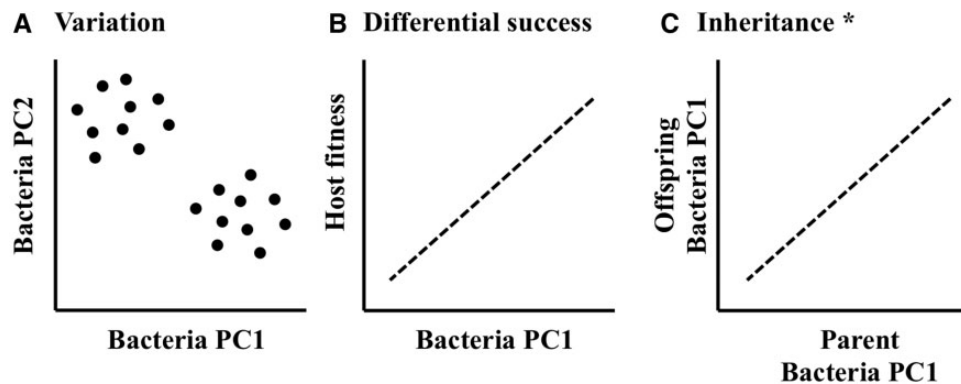


Fig. 1 Three components of natural selection applied to the host-associated microbiome. **(A)** Evidence of variation in the microbiome represented by hypothetical PCA plot of microbial taxa or genes. **(B)** Evidence of the differential success of the host based on the differences in the microbiome. **(C)** Evidence of inheritance of the microbiome, where offspring microbiome resembles the parent microbiome. All three components are required for the host to evolve in response to the natural selection on the microbiome across multiple generations. *See the text for genetic and non-genetic mechanisms of microbiome inheritance.

et al. 2012; Bordenstein and Theis 2015; Moran and Sloan 2015). Here, I propose a framework for testing for evidence of host evolution mediated by the microbiome.

The three components of natural selection can be applied to the microbiome; variation, differential success, and inheritance (Fig. 1). First, in order for natural selection to act on the microbiome, variation in the microbiome is required. Second, some of the variation in the microbiome must affect the host fitness. Lastly, inheritance of the microbiome associated with the host fitness is also required, where the offspring microbiome resembles the parent microbiome. Providing evidence of each of the three components in a single system will support the idea that natural selection on hosts is mediated by the microbiome. However, resemblance of the microbiome among related individuals can be generated by a variety of processes and the mechanism is largely unexplored especially in natural populations.

Resemblance of the microbiome between parent and offspring can occur through genetic and non-genetic mechanisms. For example, reciprocal transplant experiments between zebrafish and mice have demonstrated that the host genotype can assemble species-specific microbial communities (Rawls et al. 2006). Genes with immune and behavioral functions are known to affect the microbiome from gene knockout mouse strains (Spor et al. 2011). In contrast, the mammalian offspring can acquire beneficial microbes either vertically (e.g., mother's vaginal canal, milk, skin) or horizontally (e.g., environment) without host genotype effects. For example, rodents and lagomorphs often acquire microbes through coprophagy (e.g., eat mother's feces), whereas young ungulates commonly consume soil to acquire microbes (Feldhamer et al. 2007). Some insect hosts

can acquire beneficial bacteria that confer insecticide resistance from the soil every generation (Kikuchi et al. 2012). In laboratory mice, exposure to a different temperature resulted in different assemblages of microbiome that improves host energy metabolism by plasticity, without any genotypic differences (Chevalier et al. 2015; Ziętak et al. 2016). Therefore, studying the degree and the stability of the inheritance of the beneficial microbiome including genetic and non-genetic mechanisms is important to understand the evolutionary outcomes of the host.

Finally, a major goal in evolutionary genetics is to link genotypes to phenotypes that affect fitness. Identifying the genetic basis of host phenotypes (e.g., immunity, behavior) that interact with the microbiome is important for understanding how the host genome might regulate the functions of the microbiome (Spor et al. 2011). Host genomic regions that associate with the microbial composition have been identified in genome-wide association studies in humans (reviewed in Goodrich et al. 2016b) and quantitative trait locus mapping studies in mice (Benson et al. 2010; McKnite et al. 2012; Srinivas et al. 2013; Leamy et al. 2014; Wang et al. 2015). Conversely, "heritable" microbial taxa have been identified and can have significant effects on host phenotype such as weight gain (Goodrich et al. 2014, 2016a). Linking host genotype to microbiome variation that affects host fitness will help to understand the evolution of host–microbial interactions.

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