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Review

The Extended Genotype: Microbially Mediated Olfactory Communication

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Microbes are now known to influence inter- and intraspecific olfactory signaling systems. They do so by producing metabolites that function as odorants. A unique attribute of such odorants is that they arise as a product of microbial–host interactions. These interactions need not be mutualistic, and indeed can be antagonistic. We develop an integrated ecoevolutionary model to explore microbially mediated olfactory communication and a process model that illustrates the various ways that microbial products might contribute to odorants. This novel approach generates testable predictions, including that selection to incorporate microbial products should be a common feature of infochemicals that communicate identity but not those that communicate fitness or quality. Microbes extend an individual's genotype, but also enhance vulnerability to environmental change.

Highlights

We have recently moved from viewing species, individuals, and their DNA as fundamental units of biological organization, to recognizing that the microbiome contributes to the macroorganism's genome and hence its phenotype; this collective of organisms is termed the holobiont.

There are complex bidirectional interactions, mediated by chemical signals, between hosts and their microbiota.

Olfactory communication influences animal behavior, odors might be produced or altered by bacteria, and bacteria themselves might also affect the brain and behavior of their hosts.

We develop a novel framework to understand these relationships for microbially mediated olfactory communication.

We explore the potential host–microbe interaction space, reveal insights from signal–receiver theory, and develop a new process model for how microbes might produce or contribute to host olfactory cues and signals.

Macro- and Microorganisms: The Holobiont

Our understanding of the role that microbes play in macroorganismal biology, behavior, and communication is in the midst of a revolution [3,4]. We now know that microbes create products that influence an individual's behavior by directly targeting receptors in the gastrointestinal tract and the brain [5]. Dysbiosis of the gut **microbiota** (see Glossary) is linked to anxiety, depression, and other behavioral syndromes in humans [6,7]. This should not be surprising, given that the human body contains at least as many microorganisms as human cells [8]. Evidence for microbial control of macroorganismal behavior abounds, from bacterial control of courtship, mating, and reproductive behavior, to kin and nest mate recognition. The majority of this work examines the gut microbiota of insects or humans [9–12] and demonstrates a bidirectional interaction between the macrobe host and its microbes, mediated by chemical communication [1,13].

This paradigm shift deeply affects our understanding of micro- and macroorganismal biology, raising questions about who is in charge of the organismal machinery and for what purpose [14]. It is novel to move from viewing species, individuals, and their DNA as fundamental units of biological organization to recognizing that the **microbiome** contributes to the macroorganism's genome and hence its phenotype. This collective of organisms is termed the **holobiont**, and the composite cytonuclear and microbial genome of the holobiont has been called the **hologenome** [15,16].

Microbial Influences on Macroorganismal Communication

One model of holobiont composition and function [17] proposes multilayered microbial assemblages associated with a host, where macroorganisms have a 'core' set of microbial symbionts that are usually mutualistic, and a 'flexible pool' of potential microbial symbionts that might or might not be acquired. Core microbial symbionts are most likely to be vertically transmitted and confer adaptive advantages associated with a stable environmental feature; for

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example, the bacterial species that enable herbivores to digest cellulose. Host organisms might coevolve with mutualistic core microbial species and develop specific physical structures to house them, such as the cecum in hindgut fermenters or the rumen in foregut fermenting herbivores [18]. There is evidence for phylosymbiosis between the flexible pool of potential microbial symbionts and potential hosts; that is, the phylogeny of the host is congruent with the ecological relationships of observed microbial communities [19]. In other words, microbiota do not assemble randomly across host species, but rather certain microbial communities and community members are more likely to be found in or on certain host species [19–21].

While recent microbiome research has focused on the gut microbiota and its ability to influence health, the brain, and behavior via the microbiome–gut–brain axis (MGBA) [22,23], the microbiota inhabiting different external body sites has been shown to vary more between sites on a single individual host animal than they do among hosts. Consequently, different external body sites provide different 'habitats' for bacterial communities and thus harbor different microbiota. Microbial community differences among these habitats are better explained by features of the habitat type, such as the number of sebaceous glands, than by the identity of the host organism [24]. Thus, certain microbes and microbial communities could be associated with certain host species (phylosymbiosis) [19] yet be specific to individuals within a host species, and also show relatively predictable patterns among body sites within a single host individual [24].

The 'fermentation hypothesis' of chemical communication [25] proposed that symbiotic bacteria might produce chemicals that are important to mammalian signaling. However, only recently have technical advances enabled this hypothesis to be confirmed in some species. For example, microbiota living in hyena scent glands appear to synthesize infochemicals used to communicate social information to other hyenas [26–28]. Other examples of microbially meditated olfactory communication are rapidly being discovered, not just in mammals but also in birds and other taxa [29–32]. The microbiota associated with the scent glands might produce infochemicals in return for a stable, nutrient-rich environment, but microbially mediated chemical **cues** can also emanate from the skin, with different microbial communities and hence different **odors** coming from different skin habitats on a single animal. Consequently, the external microbiota colonizing an animal might also influence, if not drive, olfactory communication among hosts and their conspecifics.

This insight requires a re-examination of olfactory communication in animals. Recognition of the key role that microbes play in animal behavior, in brain and gut functioning, and now in olfactory communication has led to suggestions that **olfaction** be considered a key component of the MGBA [1]. However, there is a large theoretical gap in our understanding of how these host–microbe interactions might form, develop, and function in the context of animal olfactory communication. We aim to fill this gap by developing novel ecoevolutionary and process models of microbially mediated olfactory signaling.

Olfactory Cues and Signals

Animals live in a chemosensory world: **semiochemicals** provide information to both con- and heterospecifics about health and breeding status, group membership, kinship, competitors, predators, and other potential interaction partners [2]. Cues and signals can be transmitted in visual, aural, or olfactory modalities, but microbes influence olfactory communication via the diverse set of secondary metabolites they produce [1]. Olfactory communication is evolutionarily conserved in a wide array of taxa and represented by the largest gene families in vertebrates, and even more so in mammals [1]. Yet our understanding of olfactory communication in vertebrates

Glossary

Chemical profile: all of the chemicals emitted by an organism (or a substance that an organism emits; e.g., urine, scent-marking material) that can potentially act as semiochemicals (i.e., cues or signals). Sources of chemicals in the profile include the individual organism's metabolic products, the environment, and chemicals created by other organisms, including those transferred from conspecifics and chemicals produced by microbes.
Chemosensory receptor: receptors for chemical compounds that can be expressed on many different surfaces of the body [1]; includes both olfactory and gustatory receptors. Most chemical cues and signals are detected by olfaction rather than gustation [2].
Cue: a semiochemical (or mix of semiochemicals) that conveys information to another organism but did not evolve for that function [2].
Holobiont: the combination of a host and its microbial symbionts, including transient and permanent members.
Hologenome: the combined genomes of the host and its microbial symbionts.
Infochemical: see 'Semiochemical'.
Microbiome: the sum total of the genes encoded by the microbiota.
Microbiota: those species of microorganisms living on or in a host organism.
Odor: the smell or scent interpreted by the brain once odorants have been detected and communicated to the brain via olfactory receptors and neurons.
Odorant: an odor molecule, which can be of almost any size [2]; can bind to multiple different olfactory receptors, triggering an electrical signal in the brain. In mammals, the perception of a particular odorant comes from the stimulation of a cluster of olfactory receptors on different neurons signaling to the brain in a combinatorial manner (summarized in [1]).
Olfaction: the process through which odorants bind to olfactory receptors and are converted into electrical signals in the brain [1,2].
Olfactory receptor: receptor protein expressed by sensory neurons in the brain, to detect odor chemicals

lags behind that for invertebrates, not least due to the diversity and complexity of chemicals produced in mammalian breath, saliva, secretions, and excretions [33].

Olfactory cues differ from visual and acoustic cues in that they are less tightly associated with the presence of the donor because they can persist in the environment [34]. **Odorant** molecules can also be leveraged to encode additional information via the cue or signal's spatial and temporal variation. That is, the pattern of deposition and the speed with which a substance deteriorates over time create extra information for the **receiver** to interpret, much as the brightness of a visual display or the frequency of an acoustic signal encodes information for receivers in those modalities [33–35].

The odor interpreted by an animal's brain is the result of a combinatorial map of stimulated **olfactory receptors** firing electrical signals to the brain in response to an odorant (summarized in [1]). **Chemosensory receptors** are distributed throughout the body and their role in detecting chemical cues and signals and influencing vertebrate behavior has, until recently, remained relatively underappreciated [1]. Even in humans, there is good evidence for olfactory recognition of kin [36,37] and of babies by their mothers (and vice versa [38]).

There is astounding complexity and diversity in the use of olfactory cues and signals by vertebrates (reviewed in [1,2]). However, the investigation of vertebrate olfactory communication must deal with the enormous and data-rich chemical diversity that animals could potentially use for communication and to assess ecologically important information [35,39,40]. We can quantify and compare color variation in animal displays [41,42] or transmission fidelity and temporal information in acoustic communication [43,44]. However, no such mechanism exists to identify the dimensions of olfactory communication, partly due to the complexity of olfactory chemosensing [33,35,40]. If microbes contribute to the diversity of cues and signals produced by mammalian glands, the problem becomes even more complex, and we should reconsider our approach.

The Extended Genotype

The diversity of genes and biochemical pathways encoded by the microbiota vastly exceeds the genes and biochemical pathways encoded by the host [45]. The genomes of multicellular organisms have a limited coding capacity (approximately 20 000 genes for humans and other mammals [46]). By comparison, almost 10 million genes have been catalogued in the human gut microbiota [47]. This means that the microbial components of the holobiont have the potential to produce a much more diverse suite of odorants than can be encoded by the host genome [48]. By coopting microbiota to produce odorants, multicellular hosts can vastly extend the repertoire of their genome and utilize a much broader variety of cues and signals than they would otherwise be capable of producing. By doing so, they extend their genotype.

A New Framework for Microbially Mediated Olfactory Communication

Interspecific host–microbe relationships are easily imagined to be mutualistic (e.g., bobtail squid–*Vibrio* symbiosis [49]), pathogenic, or exploitative (where parasites or pathogens modify host behaviour; e.g., [50]), but of course, the world is more complex than this. An ecoevolutionary perspective reveals the full spectrum of host–microbe interactions for an olfactory signaling context (as well as their potential fitness outcomes). Importantly, host–microbe interactions differ from classic one-on-one interactions in that they involve interaction dynamics between one host and many microbes – a population or a community of microbes. While hosts

(odorants) [2]; concentrated in the olfactory epithelium at the back of the nasal cavity but can also be found throughout the body, including internally in the gut and kidneys [1].
Receiver: an organism that can receive a signal from a particular signaler and/or detect and respond to a cue.
Semiochemical: also called an infochemical; a chemical involved in an interaction between organisms. Cues and signals are types of semiochemical [2].
Signal: Evolves from a cue to alter the behavior of another organism. Signals work because receivers evolve detection structures and responses (summarized in [2]).
Signature mix: a mix of odorants that an animal learns to associate with an experience. These might not be the same odorants each time; they are specific to each event of associative learning. For example, a prey animal might learn to associate a signature mix with predation risk or an individual might associate a signature mix with a non-kin conspecific group.

might mostly control their microbes, it is also possible that microbes could overwhelm such control, producing unintended and undesirable chemical compounds that subvert olfactory communication, affecting host fitness.

At the same time, in the context of signaling theory, a semiochemical must be reliable before it can be useful to another animal and become a cue or a signal [51]. Microbial contributions to semiochemicals must be considered in the context of the reliability of potential information for potential receivers. Importantly, what might the implications of microbial influence be for the reliability of semiochemicals that correlate with organismal state ('fitness' or 'quality') versus semiochemicals that indicate identity? Below we discuss the effects of semiochemical reliability on selection for senders and receivers to improve the production and detection of olfactory cues and signals in each category.

Finally, by what mechanisms might microbes contribute to the production of a multicomponent olfactory cue or signal? We explore the full potential interaction space for host–microbe interactions in the context of olfactory communication, discuss the implications in light of signaling theory, and, finally, propose a process model to understand the mechanisms by which semiochemicals might be produced by a host and its microbes.

An Ecoevolutionary, Multispecies Theoretical Approach

Hosts and their microbes are involved in an ecological interaction, albeit one with the unusual property of being an interaction between a single macroorganism host species and either a single species or a community of different microbial species. While many studies focus on mutualistic symbioses, theoretically any type of interaction might be possible. In Box 1 we describe the potential ecoevolutionary space in which host–microbe interactions might play out in the context of olfactory communication. Importantly, costs and benefits might accrue differently to individual species in a microbial community, and such benefits might change according to the life stage of the host or with different environmental circumstances (e.g., [52]). Populations of different microbial species are constantly interacting with one another. There are also benefits associated with belonging to a community. Microbes exhibit community dynamics paralleling ecological successions in multicellular species and microorganisms can occur as consortia of mutually dependent organisms [53,54]. We propose that the one-versus-many dynamics of host–microbe interactions present additional complexities that are best examined through this ecoevolutionary lens (Box 1).

Mutualistic and parasitic interactions are well studied. Potentially the most interesting cases to consider are whether commensalism in favor of microbes or exploitation in favor of the host occurs in the context of olfactory communication (Box 1). Commensalism in favor of microbes might occur where microbes influence host behavior in a way that alters the odor profile of the host to the benefit of the microbes, without cost to the host. An example might be dogs rolling in other dog scent marks, potentially acquiring new microbes and/or facilitating the acquisition of new habitat for microbes via transmission to new canine hosts. Host exploitation of microbes is another interesting possibility to consider: could hosts 'enslave' microbes that benefit the host, even if the microbes would have better fitness in an alternative environment? Could this represent a case of a microbial 'ecological trap'?

We acknowledge the lack of examples for many of these interaction types, particularly in the context of olfactory communication. However, we suggest this might be a case of understudy rather than a true absence. We hope that by explicitly considering the costs and benefits for each participant (within the peculiarities of a one-versus-many scenario), researchers will begin to look for them.

Box 1. Ecoevolutionary Perspective of Host–Microbe Interactions in the Context of Olfactory Communication

The costs and benefits of microbes occupying a host and influencing odorant production, for both the host (individual) and the microbes (population and community), are shown in Table I. The simplest of interactions is one in which all interactants benefit (++). For example, ruminants house cellulose-digesting microbes that allow them to digest plants [66]. An exploitative interaction might also be possible where microbes were unwilling tools, enslaved to the benefit of the host (+−). These microbial species would have higher fitness in a different environment. The observation that gut microbiota have enormously rapid growth potential is congruent with this idea, because they must be under some control either from the host or via competition with other microbial species in the gut for resources [67]. Commensal interactions should theoretically be possible (+0) if the host benefits from a microbial product but those microbes experience neither cost nor benefit. However, host colonization depends on specific attributes for transmission, colonization, and recruitment to specific body sites and the ability to deal with host defenses. These attributes are likely to be costly for microbes, so there must be associated benefits. Alternatively, host fitness might be negatively impacted when one or many pathogenic microbial species become virulent and create odorants that betray this reduced fitness to other animals (−+). Such exploitative interactions might also occur if microbial species modify host dietary choices to provide better opportunities for microbial growth, to the detriment of host fitness via altered odorants; there is evidence that some gut microbes do this [68]. Competitive or spiteful interactions (−−) could potentially occur, resulting in reduced fitness for host and microbes. This would constitute an ecological trap [69] from both the host and the microbes' perspectives. While plausible, we find this difficult to imagine in the context of microbially mediated olfactory communication. In principle, amensalism (−0) could occur, whereby host fitness is negatively impacted by microbes but microbial fitness is not affected. We do not envision this to be important in scent-mediated microbial communication, since it is difficult to imagine how such interactions might form. Where neither host nor microbe benefits or pays costs, we see neutralism (00). However, while this might occur in stochastic microbial colonization of hosts more generally, by definition this form of commensalism would not apply to microbially mediated olfactory communication. Commensalism (0+) could also potentially occur if the microbes improved their fitness by altering the host's odorants without affecting host fitness. For example, dogs often roll in other dogs' urine, which might support microbial communities that are spread to host conspecifics, producing new microbial habitats, and might not carry costs to the host. We do not envision amensalism being an important process between a macroorganism host and a community of microorganisms.

Table I. The Costs and Benefits of Microbes Occupying a Host

	Microbes (many)			
Host (one)		+	−	0
	+	Mutualism Cooperation	Exploitation Slavery Microbes as unwilling tools	Commensalism
	−	Exploitation Pathogen Parasite	Competition Spite	Amensalism
	0	Commensalism	Amensalism	Neutralism

Signal–Receiver Theory for Micro- and Macroorganisms

For microbially mediated olfactory communication among host animals, signal–receiver theory [51] suggests that qualitatively different processes are likely to occur when odorants are used for identity signaling as opposed to the assessment of an individual's 'quality', 'state', or 'fitness'. Importantly, quality or fitness can include both transient (e.g., health, diet) and stable (e.g., MHC diversity and heterozygosity) components.

For identity signaling, we generally expect symmetrical selection on the signaler and the receiver, because both benefit from successful identification [51]. Selection on the signaler enhances the production of diverse odorants, the identity of which can be arbitrary provided they are detectable to receivers. Importantly, a diverse community of microorganisms offers the opportunity to generate new odorants without the need for the host to develop new biochemical pathways. This allows the host to extend its genotype and create a potentially astoundingly diverse array of odorants. Highly diverse identity and group-membership signals should

facilitate the development of complex social structures in hosts because more individuals and groups can be discriminated.

However, to be useful as identity signals, odorants produced by communities of microbes must remain relatively stable over time [51]. If microbial odorants are sufficiently stable, there might be no cost but rather a benefit from using microbial odorants as an inexpensive source of olfactory variation. This favors selection on the host for the ability to house microbes under relatively stable conditions; for example, in complex fermentation glands. Such specific glands permit the host some control over microbial colonization and proliferation and over their biochemical function through the establishment of microenvironments with specific pH, temperature, and so on. Where identity signaling is important for a host (e.g., territorial species, species with complex social structures), these glands should be more likely and more numerous, and house more diverse microbes (and hence odorants) than can be sustained externally, allowing increased microbial diversity without additional genomic requirements. While scent glands are ubiquitous in mammals, complex olfactory communication occurs in other taxa (e.g., birds, [52], reptiles [55]). The search for microbially mediated olfactory signaling should continue to expand [29].

When individuals assess conspecific quality, we expect selection on the receiver to obtain all available salient information about health, diet, disease status, heterozygosity, and MHC diversity [51]. These aspects of an individual animal's 'quality' might create honest cues. A particular diet will preferentially support certain bacteria and a healthy animal might therefore host different microbes than an unhealthy one [56]. It is likely that some microbial products are honest indicators of disease state in host animals. We would not expect strong selection on the host to change its microbiota in association with health status – unless, of course, it is possible to acquire or encourage the growth of certain microbes to mask or cloak honest cues of low individual quality. There is always the risk, however, that microbes could take over and produce an undesirable mismatch between quality and signal and/or cue. Thus, there is a conflict between the host and its microbes in that this relationship would no longer be a mutually beneficial symbiosis (Box 1). While this vulnerability might be ever present in a world rich with bacteria, individual host animals might have limited options but to rely on microbial products for communication.

Any cue or signal involving microbial products might be potentially less informative than those that are solely host genotype-derived odorants. Because we imagine identity signals to be arbitrary (but highly diverse), this might be less important for identity signaling systems than systems based on quality assessment. Consequently, we expect asymmetrical selection on signalers and receivers where cues are used for quality assessment. Receivers will be under strong selection to detect cues of individual quality, but signalers will be under selection pressure to alter these cues only if it is possible for them to mask or cloak cues of undesirable quality. Thus, microbe involvement in quality assessment is likely to be incidental rather than selected for.

These are unique predictions that emerge from ecoevolutionary analysis. However, to further understand how these systems might work, we require a process model that outlines how microbes could contribute to, or alter, olfactory cues and signals.

The Process by Which Microbes Might Mediate Olfactory Communication

The process model in Figure 1 details potential mechanisms for microbial influences on olfactory communication among animals. Odorants might fall into one of three categories: those produced

by the host, those produced by the host and then modified by microbes, and those produced by microbes. Furthermore, due to the combinatorial way in which odors are detected and interpreted in the vertebrate brain, an odorant might comprise substances produced by multiple different microbial species in a particular mix or ratio (Figure 1). An individual host animal's odor profile might comprise odorants in all of these categories. These are holobiont odorants, and some will act as cues, some as signals, and some as **signature mixes**. A key question is how much control a host animal has over the makeup of its odor-producing microbiota through intentional acquisition of microbes and/or the encouragement or discouragement of particular microbial species (Figure 1). This could be a primary way in which microbially generated odorants could honestly signal the quality or condition of the host. Addressing this question would shed light on the potential

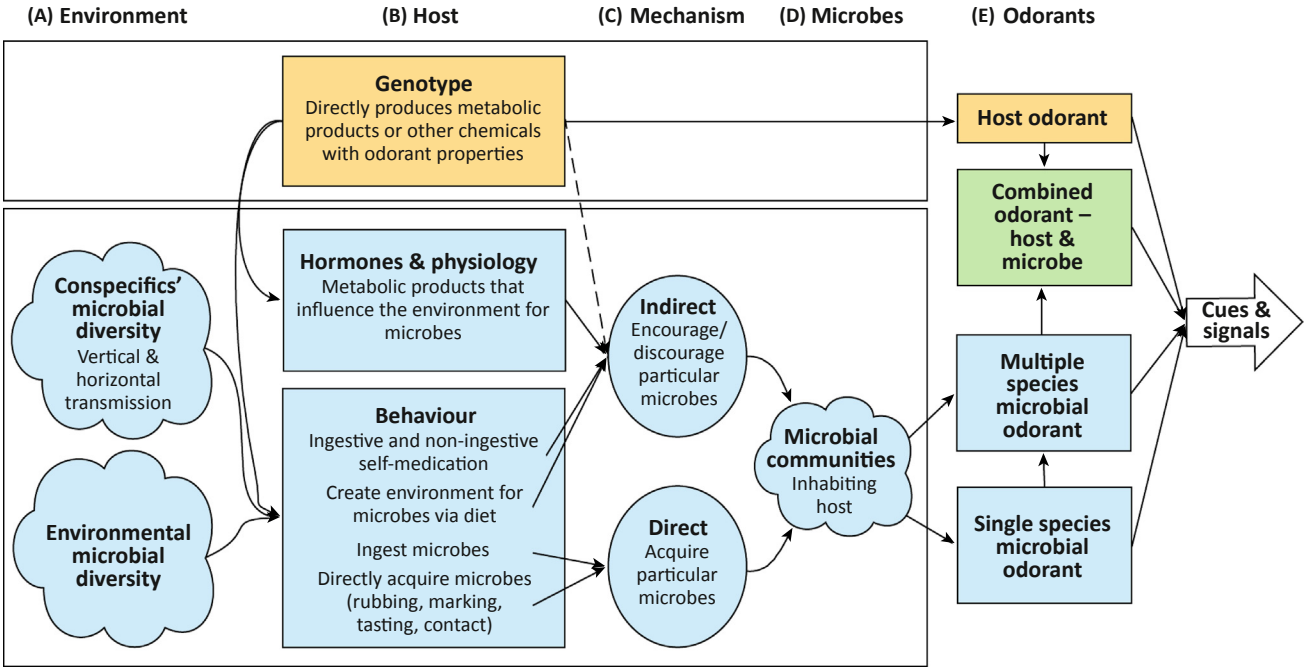

Figure 1. A Process Model for How Host Animals and Their Microbes Create Odorants. The host animal's genome encodes biochemical pathways that generate metabolic products that can be odorants (yellow pathway). The information conveyed by these semiochemicals is signals or cues that can modify the behavior of receivers [2]. The figure describes processes by which microbial diversity might be acquired and modified by a host and how their metabolites, in conjunction with host-produced metabolites, could potentially create four different odorant types (host, single microbe, multiple microbe, and combined), each of which might convey different information about the holobiont. (A) Environment. The host's microbiota is initially acquired through vertical transmission of microbes from parents, through horizontal transmission from other conspecifics, and from the environment via exposure and diet [70]. In this sense, microbial inputs into the host's system are influenced by standing environmental diversity and by species-specific host traits such as group size and level of social interaction. (B) Host. At the same time, the host animal's genotype will affect the host's internal physiological and hormonal environment. This influences which microbes can be successfully recruited, especially when the host produces metabolites and cell-surface molecules that influence microbial colonization and growth [71]. It might therefore be possible for the host to selectively cultivate or remove specific microbes or microbial communities via these physiological mechanisms. The host's genotype might also influence microbial community structure via traits such as the MHC (broken line). (C) Mechanism. Individual host animals might also modify their microbial communities via behavioral mechanisms. They might ingest dietary components that alter their internal environment in a way that indirectly encourages or discourages certain species of microbe [70]. This process could include ingestive [72] and non-ingestive self-medication. For example, capuchin monkeys (*Cebus* spp., *Sapajus* spp.) treat skin infections and insect infestations by rubbing themselves with microbicidal plants and invertebrates [73]. Other host behaviors, such as rubbing, marking, or tasting scents in the environment to acquire microbes and direct ingestion of propagules, all represent direct mechanisms by which hosts might influence the composition of their microbiome. (D) Microbes. Together, the host's genotype, hormones, physiology, and behavior act on the available microbial diversity in the host's environment to determine the host's microbial community. (E) Odorants. The microbial community then produces substances that combine with the host-produced substances that, if odorous, can act as semiochemicals (cues, signals, or signature mixes). Receivers might respond to a single microbial odorant or to complex mixtures of odorants generated by multiple microbial species. These might be mixed, or not mixed, with host encoded odorants to produce a **chemical profile** comprised of a suite of semiochemicals. We envision these as four separate channels of information from the perspective of receivers, because different types of information are likely to be encoded by each.

interaction spaces for hosts and their microbes, as discussed above. We describe the process model in the caption for Figure 1.

Microbially Mediated Signaling in the Anthropocene

Given the potential advantages conferred by the microbiota, such relationships should coevolve and exhibit a degree of codependence, or phylosymbiosis [19] This, in turn, raises questions about the potential for human influences on animal–microbe interactions in the Anthropocene – the new geological epoch precipitated by human activity [57]. What consequences might there be for domestic, agricultural, and wild animals if their microbially mediated olfactory cues and signals are also being perturbed? Because olfactory communication systems are in turn reliant on a complex interplay between environment and ecology, they could also be vulnerable to environmental perturbations.

Humans are now the largest evolutionary force on the planet [58]. We know that our own microbiota has been perturbed, particularly since the 1950s [59], and that this has potentially serious consequences [60,61]. These changes are thought to lie at the heart of the increasing frequency of many complex diseases in the modern world [62,63]. Given that these microbial perturbations are likely to be widespread, we need to consider the consequences of such changes for the macroorganisms that house these microbial communities.

Wild animals, and animals under human control, are likely to exhibit contractions in their microbial diversity. Declines in population sizes potentially lead to a stepwise decline in the microbial diversity available for transmission between individuals [60]. This phenomenon is exacerbated when species survive as small, fragmented populations, where an extinction vortex in host genetic diversity could be paralleled by a similar rapid decline in microbial diversity.

There is also a strong potential for loss of microbial diversity when animals are raised in captive breeding programs. Captive animals are removed from their natural ranges and, consequently, the microflora endemic to those regions. In particular, they cannot obtain microorganisms from their natural diet. This might be problematic for carnivores, whose feeding regimens in captivity do not normally include the gut contents of prey or meat in various stages of decomposition, as they would in the wild. The sanitized housing conditions for most zoo animals mean there is no opportunity to acquire microbiota from waterholes containing the feces of diverse fauna.

If a diverse microbiota is an essential component of interindividual signaling in animals, a decline in microbial diversity has serious implications for social cohesion and potentially even for reproduction. Such phenomena might be directly relevant to the group sizes of captive populations [64], since both host genetic diversity and the genetic diversity of the microbiota must be conserved. This is not an insignificant issue. There are over 26 billion animals from over 10 000 species kept in captivity around the world, and for those in zoos and aquaria reproductive problems are common [65]. We should investigate whether perturbations to the microbiome lie at the heart of these problems, and would predict that animals with complex microbial signaling systems will be overrepresented in these problem species.

Concluding Remarks

In sum, we have combined several novel approaches to develop a framework that will facilitate future research into microbially mediated olfactory communication in animals. We employed ecoevolutionary theory to understand the potential permutations of host–microbe interactions and their fitness benefits. A signaling theory perspective reveals how a host macroorganism

Outstanding Questions

Can this framework work as well for plants as it does for animals? How might microbes influence the search process and assessment of plant palatability for herbivores?

Are the cues arising from dietary and hormonally influenced nonmicrobial olfactory products unbluffable and therefore honest?

Do microbial products reveal honest information about host health, diet, and other indicators of fitness? If so, are hosts able to cloak or disguise undesirable odors?

What is the default effect of addition or subtraction of a microbe? We assume that the 'default' mutation is deleterious; is this true for the addition or deletion of a single microbe? Or are microbial metabolic pathways more important, such that loss of a microbe is critical only if it represents the loss of a metabolic pathway?

Can microbes alter a macroorganism's odorants in such a way that fitness is reduced? For example, can microbes 'take over' and produce socially undesirable odors that reduce feeding or mating opportunities, without being pathogenic?

Is being able to control your microbes in a chaotic world an honest signal of fitness?

What role do interactions between the microbiome and the virome play in these processes?

We know virtually nothing about olfactory products produced by Archaea.

Box 2. Key Predictions

This novel framework generates several testable predictions.

(1) Microbial products are more likely to be used for identity signaling than in quality and/or fitness assessment. Identifying a system where olfactory signals are demonstrably used for mate choice assessment as well as for identity signaling would permit us to test this prediction.

(2) Species for which identity signaling is important (e.g., territorial mammals, those living in large and/or hierarchical social groups) are most likely to have evolved elaborate scent glands to house odorant-producing bacteria.

(3) Species characterized by large group sizes and complex social systems should be more likely to have scent glands and microbially mediated olfactory communication.

(4) Being able to control your microbiome might be an honest signal of quality and/or fitness that could be signaled via the balance between microbial and host-produced odorants: a greater dominance of host-produced odorants would indicate greater fitness if this were true.

(5) Alternatively, animals may vary in their ability to host a large load of detrimental microbes that alter odor profiles in such a way that signals host fitness to potential mates via the handicap principle.

(6) Animals with complex microbial signaling systems and reliable vertical transfer of microbiota are expected to be overrepresented in species that are difficult to breed in captivity.

(7) The speed of anthropogenic changes to the environment suggest we have reason to be concerned about effects on olfactory communication systems: species may suffer from a mismatch between novel conditions and those that they evolved with.

might receive microbially mediated olfactory cues or signals. The process model clarifies how host–microbe interactions produce olfactory cues and signals. We considered the effects of humans on these signaling systems and how these interactions might be altered during the Anthropocene. Throughout, we recognize that animals use microbes as an 'extended genotype' to expand their communicatory repertoire at low cost to themselves. We have also made some key new predictions (Box 2 and see Outstanding Questions). We hope this fresh perspective and new framework for microbially mediated olfactory communication will enhance our ability to manage biodiversity in an increasingly anthropogenic world.

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