

Review



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# Ecological and evolutionary mechanisms underlying patterns of phylosymbiosis in host-associated microbial communities

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Phylosymbiosis, where similarities in host-associated microbial communities recapitulate the phylogeny of their hosts, is a newly recognized yet pervasive pattern in the field of host–microbe interactions. While phylosymbiosis has been documented across many systems, we still have a poor understanding of the mechanisms that underlie this emergent pattern. Host selection of the microbiome is a widely cited mechanism, yet other basic ecological and evolutionary processes (dispersal, drift and diversification) may also be at play. This paper discusses the roles that each of these processes and their interactions may play in yielding phylosymbiotic signals across hosts. Finally, this paper will identify open questions and methods that are required to better understand the relative contributions of these basic processes to phylosymbiosis. Given that phylosymbiosis has been shown to relate to functional components of host fitness, understanding the processes that contribute to these patterns will be important for our understanding of the ecology and evolution of host–microbe interactions.

This article is part of the theme issue 'Conceptual challenges in microbial community ecology'.

## 1. Introduction

Recent advances in the field of host–microbe interactions have demonstrated that macroorganisms are largely colonized by communities of microbes that have the potential to influence host phenotypes [1]. Understanding the forces that drive the structure of these communities is of great interest to microbial ecologists and evolutionary biologists. Studies in numerous systems show that the compositions of host-associated microbial communities are shaped by a complex set of host and environmental factors, including host genotype, species and ontogeny, as well as diet, habitat, geographical location and anthropogenic disturbance [2–6]. However, a thorough understanding of the forces that shape host-associated microbial communities is still needed.

One rather consistent driver of microbial community structuring across numerous host clades is evolutionary history. Specifically, phylosymbiosis, or the congruence between the evolutionary history of various host species and the community structures of their associated microbiomes, has been observed in numerous animal clades, as well as in some plant-associated communities [7–9]. In other words, as host genetic differences increase over time, differences in the structure of host-associated microbial communities will also increase [7]. Detailed descriptions of phylosymbiosis and how it is measured have been reviewed elsewhere [10]. Importantly, phylosymbiosis is different from co-diversification in that the microbiome dendrogram is of microbial community structures, not of specific microbes (though this can be the case and will be discussed more below). A handful of studies also demonstrate that patterns of phylosymbiosis correlate with functional effects on the hosts, such that hosts inoculated with microbial communities from different host species exhibit decreased performance and fitness, suggesting that these relationships may be acted upon by natural selection. For example, *Peromyscus* mice inoculated

with the microbial communities from more distantly related hosts suffer decreases in their ability to digest food material [7], and *Nasonia* wasps inoculated with interspecific microbiomes exhibit lower survival than those inoculated with intraspecific microbial communities [11]. Moreover, hybridization of host species leads to a breakdown in phylosymbiotic communities, which may lead to negative consequences for host health and survival [12,13]. Despite the ecological and evolutionary importance of phylosymbiosis, we still have an extremely limited understanding of the mechanisms that underlie this pattern.

To date, it is largely assumed that differential selection of microbes by the host drives variation in microbial communities [8]. That is, hosts may be exposed to the same pool of potential microbial colonizers, but then filter and select certain microbes to persist as symbionts. However, theoretical studies have suggested that for some host-associated habitats, such as the vertebrate gut, host selection may not fully explain the observed patterns [8]. Therefore, other forces may contribute to the resulting patterns of phylosymbiosis across host species. This paper will discuss other basic ecological and evolutionary processes that underlie community assembly: dispersal, selection, drift and diversification [14,15], and how each may contribute to patterns of phylosymbiosis. Potential interactions between these processes, such as priority effects, will also be discussed. Finally, this paper will address future directions needed to enhance our understanding of phylosymbiosis, and how ecological and evolutionary theories of community assembly may need to be adapted to understand these processes. For the purposes of this paper, I will largely focus on gut microbial communities, as these systems have been best studied mechanistically and experimentally. Similar processes may occur on other body sites or in plants, and will be discussed briefly throughout the paper.

## 2. Microbial community ecology

Insights into the structure of microbial communities, including host-associated communities, were significantly advanced with the development of 16S rRNA gene sequencing technologies. These inventories provide thorough snapshots into the structure of a microbial community in a given sample. With these data, researchers have begun to apply classical ecological theory to host-associated microbial communities [16]. For example, one of community ecology's few 'laws' is the species–area relationship, such that larger islands should contain more species of macroorganisms [17]. Similar relationships have been found in the gut microbiome, where larger animals harbour more diverse microbial communities [18]. It is possible that ecological and evolutionary processes such as dispersal, selection, drift and diversification may underlie observed patterns of phylosymbiosis. These processes will first be discussed in isolation, but often these processes will occur simultaneously and have the potential to interact with one another. Therefore, interactions between processes (such as priority effects) will also be discussed.

### (a) Dispersal

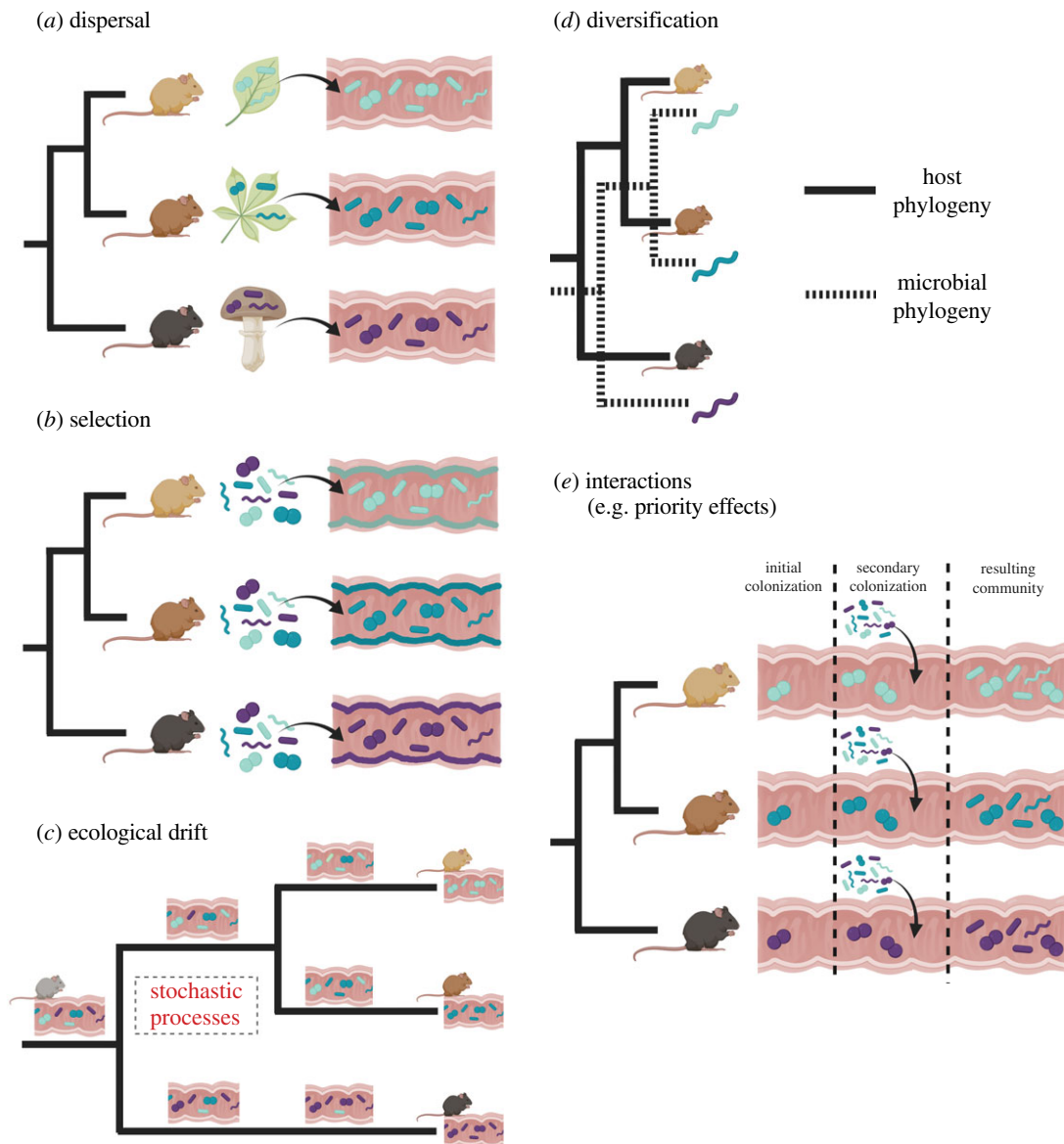
Dispersal, or the movement of microbes between environments, may underlie patterns of phylosymbiosis if species

differentially encounter microbes as part of their natural history (figure 1a). For example, if dietary strategies have diverged across species, they may be inoculated with differential communities from food items. Indeed, controlled experimental studies have demonstrated that environmental sources of microbes can be extremely important for inoculation and maintenance of host-associated microbial communities. For example, stinkbugs [19] and bobtail squid [20] acquire specific symbionts from the environment every generation. Moreover, salamanders housed in sterile containers lose a significant proportion of their skin microbiome compared with those housed in containers with soil [21]. Additionally, feeding *Drosophila* flies on sterile media causes them to lose members of their gut microbial community [22]. Such dispersal effects have also been suggested to exist in the wild, where allopatric animal populations exhibit lower overlap in gut microbial community structure compared with sympatric animal populations, and this overlap decreases with geographical distance [23]. Intraspecific phylosymbiosis has been reported across populations of pikas (*Ochotona princeps*), which exhibit distinct host genetic structure over geographical scales [24]. While host genetics could play a role (discussed more below), it could be that dispersal limitations across geographical scales underlie the phylosymbiotic pattern observed across these populations.

Dispersal may not be such a passive process as environmental or trophic acquisition. There may be microbial and host adaptations to facilitate dispersal-based symbiosis. For example, environmental microbes that have been experimentally evolved to be associated with the zebrafish gut exhibit increased motility and host colonization rates [25]. Moreover, transmission between conspecifics would represent a type of dispersal. Many animals exhibit distinct adaptations to promote vertical microbial transmission between generations [26]. For example, female stinkbugs (*Megacopta punctatissima*) place 'symbiont capsules' near their eggs, and nymphs engage in a wandering behaviour to increase acquisition of these microbial symbionts [27]. Further, animals may acquire microbes horizontally through social interactions between conspecifics [28]. In mice, gut microbial taxa that are aerotolerant are more likely to be transmitted horizontally across individuals [29]. These microbial and host adaptations may help to promote phylosymbiotic patterns across host species if hosts only acquire microbes through vertical and horizontal transmission from conspecifics. However, it should be noted that in the absence of other interacting forces (selection, drift or diversification), it seems unlikely that dispersal by transmission alone would yield phylosymbiotic patterns across organisms. When clades of animal hosts are reared under common environments (presumably removing dispersal barriers), they still harbour host-specific microbial communities [7,30], suggesting that other factors are at play.

### (b) Selection

Selection, or differential filtering of a microbial pool, is currently the leading hypothesis to explain patterns of phylosymbiosis (figure 1b; [8]). Studies have been conducted on several clades of animals reared under controlled experimental settings to explicitly remove potential environmental, sex and endosymbiont factors that could underlie or obscure patterns of phylosymbiosis [7]. Indeed, significant degrees of phylosymbiosis were detected in all animal clades,



**Figure 1.** Ecological and evolutionary processes that may contribute to observed patterns of phylosymbiosis. Here, colours of microbes represent different species, and shapes represent different functional guilds. (a) Dispersal—host species may come into contact with distinct microbial communities as part of their natural history or transmission from conspecifics. (b) Selection—hosts may exert selective forces that allow certain microbial members to colonize, while others are excluded from associating with the host. (c) Ecological drift—stochastic processes may result in the gain or loss of microbial members over evolutionary time. (d) Diversification—microbes may co-speciate with their host species. (e) Interactions—here, priority effects are depicted. Here, assume that there is no host selection occurring (hence the lack of coloured epithelial layers as depicted in (b)). Hosts may be initially colonized with a vertically transmitted microbe that has co-speciated with the host. These initial colonizing microbes may then exert subsequent selective forces through habitat modification or microbe–microbe interactions to only allow certain microbes to colonize, yielding phylosymbiotic patterns. (Online version in colour.)

suggesting that there are genetic underpinnings that structure host-associated microbial communities [7]. Other studies have inoculated numerous germ-free animals of different genotypes or species with the same pool of microbes and found that these host genotypes or species select for distinct microbial communities [31,32]. Together, these studies demonstrate that there are selective processes occurring in the animal gut.

What, then, might be the mechanisms of selection to filter and sculpt microbial communities? A likely candidate seems to be the immune system, as these processes recognize self from non-self and filter out pathogenic microbes. In *Hydra*, species-specific expression profiles of antimicrobial peptides (AMPs) contribute significantly to phylosymbiotic community structures, such that transgenic *Hydra* that are deficient in AMPs fail to select for particular microbial members

[33]. The adaptive immune system contributes to the filtering of environmental microbes to determine the community of the zebrafish gut, though dispersal seems to overwhelm this effect [34–36]. Numerous intraspecific genome-wide association studies between animal genomes and gut microbial community structure have identified immune genes as likely candidates [37–39]. Even components of the plant immune system (e.g. salicylic acid) have been shown to be important in determining the community structure of the root microbiome [40].

There may also be other physiological mechanisms by which hosts select for certain microbial members. For example, the gut is lined with mucins and glycans that can vary in their structure across species [41]. Mice that lack the  $\alpha 1-2$  fucosyltransferase gene, which codes for an enzyme that fucosylates glycans on the intestinal epithelium, harbour

microbial communities distinct from those of wild-type mice [42]. Host-encoded digestive enzymes can also alter the nutritional environment of the gut, and act to select for certain microbes in the gut [43,44]. Even host-encoded microRNAs, which normally regulate host gene expression, can influence bacterial gene expression and act to sculpt community structure [45,46]. Importantly, these studies have all been conducted within a single host species, and so we still lack an understanding of whether these mechanisms could underlie variation in microbial community structure across host species.

Much research on the selection of the microbiome is rather host-centric, and it should be recognized that microbes may also play a role in 'choosing' which hosts to colonize. Microbes may select hosts where their replication rates may be higher, and thus they gain a colonization (and/or transmission) advantage over other microbes [11]. Many pathogenic microbes recognize specific host receptors to determine host specificity [47], and similar processes may occur for non-pathogenic bacteria. Though, some specificity may be combinations of host and microbial factors. For example, the specificity of plant–*Rhizobium* mutualisms is driven by coordination of host-specific secretion of chemical signals and microbial-specific recognition of these chemical signals [48]. Similarly, it may be challenging to determine whether microbes capable of binding to specific glycans on the gut lining would represent selection by the host or by the microbe. Disentangling the directions and interactions associated with selection (hosts selecting microbes, microbes choosing hosts or a combination) represents a challenging but important gap in our understanding of phyllosymbiosis and host–microbe interactions.

### (c) Ecological drift

Stochastic or random processes may cause some microbes (especially ones with low abundances) to become extinct. The term 'ecological drift' is used to describe the random fluctuations in species abundances over time, and is somewhat analogous to the concept of genetic drift, or random variation in allele frequencies across generations. Processes of ecological drift could result in distinct microbial communities across host species, as these communities have been separated from one another over evolutionary time (figure 1c). Ecological drift generally occurs in communities where diversity and the strength of deterministic processes are low [49]. Given that the gut is typically densely populated and diverse, it seems unlikely that ecological drift in isolation would contribute largely to interspecies differences across hosts that are phyllosymbiotic. Indeed, theoretical studies suggest that stochastic processes are unlikely to contribute to microbiome community structure given that selective forces by the host or competitive forces between microbes are typically considerable and suppress the effects of ecological drift [50]. Some experimental studies suggest that stochastic processes may contribute to determining the structure of the phyllosphere microbiome [51], and underlie the well-known 'cage-effects' that exist in studies of the mouse gut microbiome [52]. A recent study also suggests that ecological drift likely occurs in gut communities, but after initial environmental selection of microbes [53]. Thus, ecological drift does likely occur in host-associated microbial communities, but is unlikely to be the sole determinant of phyllosymbiosis across

host species. Instead, interactions between ecological drift and other processes (dispersal, diversification and selection) may be important, and will be discussed below. Overall, our understanding of ecological drift in microbial communities is limited within a host species, and even more so for how these processes could play out over evolution and host speciation.

### (d) Diversification

As hosts speciate over evolutionary time, single or multiple symbionts may also evolve into new species in a manner that is congruent with host phylogeny (figure 1d). Such co-phylogenies are often observed in endosymbionts of invertebrates, which are often transmitted with high fidelity from generation to generation [54]. As mentioned earlier, animals exhibit a wide array of mechanisms to promote vertical transmission of the microbiome from one generation to another [26], and plants can transmit microbial symbionts in seeds [55]. With stable relationships over evolutionary time, it is possible that hosts and microbial species may exhibit co-speciation or co-phylogeny. Though, co-phylogenies can also occur as a result of diversification of microbes onto an existing host clade, a process known as host-shift speciation [56]. As mentioned above, there are many environmental sources of microbial symbionts which may obscure these patterns across microbial taxa, and often only a subset of the microbiome is transmitted across generations [57]. Thus, disentangling the relative importance of diversification in complex and diverse communities, and whether co-phylogenies represent co-speciation or host-shift speciation, remains poorly understood.

Only limited studies have addressed this question using members of complex host-associated microbiomes. One study used a novel sensitivity analysis to demonstrate that patterns of phyllosymbiosis observed in ants and apes may be due to recent bacterial evolution, even if the evolving microbial groups that contribute to this pattern make up only a small portion of the overall microbiota [58]. Indeed, detailed phylogenies of individual microbial taxa do exhibit co-phylogeny with their hosts [59,60]. However, as described above, there are numerous examples of horizontal transmission both within and across host species [23,28], suggesting that diversification is unlikely to contribute entirely to observed patterns of phyllosymbiosis.

### (e) Interactions

The processes described above are unlikely to act in complete isolation to yield phyllosymbiosis. Rather, there may be interactions between these processes that result in distinct microbial communities across host species. One example might be priority effects, where dispersal history can change later aspects of selection, drift and diversification [61]. For example, the first microbial colonizers of the gut may alter the gut environment by activating the host immune system or through the production of bioactive compounds that inhibit the growth of other microbial species (figure 1e). Indeed, one study elegantly demonstrated priority effects by inoculating germ-free mice in sequence with two contrasting microbial communities, and showing that the resulting communities inventoried six weeks later were distinct depending on colonization history [62]. Additionally, birds may transmit some microbial members *in ovo* [63],



and experimental administration of bacteria *in ovo* can alter the community structure of the chicken gut microbiome at 10 days of age [64]. More evidence supporting the hypothesis that priority effects act in the assembly of the gut microbiome, as well as a discussion of the mechanisms that could underlie these processes, are excellently reviewed elsewhere [61].

Related to the idea of priority effects is the notion of hub taxa, or microbial species that may have a large number of interactions with other microbes to either promote or prevent colonization, and thus alter overall community structure [65]. Hub taxa are often predicted through network analysis, though the experimental demonstration of their effects should be conducted. Experimental inoculations of *Arabidopsis thaliana* with putative hub taxa accounted for 15–20% of the variation in the community structure of the phyllosphere microbiome. Further, inoculation of mice with just four isolates of tannin-degrading bacteria significantly altered the community structure and function of the gut microbiome [66].

To date, studies on priority effects and hub taxa have been conducted within a single host species. How might priority effects or hub taxa then drive observed patterns of phylosymbiosis across host species? Again, both plants and animals have mechanisms to transmit microbial symbionts between generations [26,55]. However, it is usually a subset of the microbiota that is transmitted rather than the entire community. It could be that important hub taxa are transmitted early, which then exert priority effects to determine the resulting host-species-specific microbiome. With regards to hub taxa, it could be that hosts exert selection to favour a particular hub microbe, which then sculpts the rest of the microbial community through microbe–microbe interactions. These proposed mechanisms are purely speculative at this point, and require experimental validation in a diversity of systems.

### 3. Future directions

To date, most studies on phylosymbiosis have investigated concordance between host phylogeny and dendrograms of microbial communities, and potential functional effects of host-specific microbial communities [7–9]. These studies demonstrate the presence of phylosymbiotic patterns and their association with functional effects, but do not elucidate the processes that yield these patterns. Understanding the ecological and evolutionary processes that contribute to microbial community structures across host species will require numerous advances in theory and methodology. The processes discussed above are largely drawn from our understanding of community ecology in macroorganisms and free-living microbes. However, host-associated microbiomes present unique challenges in our understanding of community ecology, such as the high prevalence of horizontal gene transfer among organisms, which may alter aspects of selection, and the difficulty of disentangling host control from other environmental factors such as diet or geography [67]. Therefore, methodological advances and creative experimental design will be required to elucidate the processes that contribute to phylosymbiosis and their relative contributions.

Germ-free systems will likely be imperative to testing how ecological and evolutionary processes contribute to phylosymbiosis. In the past, germ-free techniques have largely been developed for model systems such as mice [68], fruit flies [69] and zebrafish [70]. However, new methods have been developed to generate germ-free *Nasonia* jewel wasps

[71] and sticklebacks [72], allowing host–microbe interactions to be studied in an evolutionary context [11,73]. The generation of additional germ-free systems that can be applied to several species within a host clade will be important for testing mechanisms underlying phylosymbiosis. For example, reciprocal transplant experiments of microbial communities across various germ-free host species could be used to test aspects of selection [32]. Additionally, previous experiments regarding priority effects in the gut have only been conducted on single host species [62]. Thus, establishing germ-free rearing techniques that can be used in several species of closely related host species will open more opportunities to test the processes that drive patterns of phylosymbiosis.

Experimental evolution experiments may also provide powerful systems in which to test the effects of community processes on phylosymbiosis [74]. For example, bank voles (*Myodes glareolus*) that have undergone repeated artificial selection for the ability to feed on high fibre diets exhibit distinct gut microbial communities compared with randomly bred control lines [75]. Under tightly controlled laboratory settings, the rodent gut microbiome is primarily inherited vertically [29], and so follow up experiments could be conducted to understand how host selection, microbial diversification or ecological drift may have contributed to these results in bank voles. Experimental evolution approaches have also been applied to host-associated microbes [25,76]. One could experimentally evolve microbes to specific hosts and then investigate their performance or success in another host species.

There has also been a call to use cultivated microbes to understand aspects of community assembly [77]. For example, microcosm experiments can help to disentangle aspects of dispersal, priority effects and microbial competition [78,79]. While a longstanding paradigm has been that less than 1% of microbes are culturable, a recent analysis found that approximately 75% of human-associated microbial taxa have close relatives that are culturable (as determined by 16S rRNA similarity; [80]). Further, studies that use a variety of culturing techniques have successfully isolated 50–95% of microbial taxa from human samples [81,82] and 23% of taxa from laboratory rodent samples [83]. Cultured microbes from diverse clades of host species, along with the development of microcosms that replicate the gut environment or experiments in germ-free model systems will help to understand the role of ecological processes in phylosymbiosis.

To truly understand community-level variation, it may be important to track individual strains of microbes, rather than relying solely on the 16S rRNA gene. Further, using the 16S rRNA gene may obscure functional differences between host-specific microbes. Two species of nematodes (*Caenorhabditis elegans* and *C. briggsae*) both host strains of *Enterobacter cloacae* that are indistinguishable at the 16S rRNA gene, yet confer different functional phenotypes to their specific hosts [30]. Strain tracking, or the use of genomic or metagenomic sequencing to track transmission of individual microbial strains, has been used to demonstrate the vertical transmission of some members of the human gut microbiota [84] and horizontal transmission across wild giraffes through social contact [85]. This powerful technique will help to better understand community composition and the processes that underlie phylosymbiosis across host species.

Finally, I argue that these questions should be addressed in a diversity of systems. The field of comparative physiology

subscribes to an idea known as Krogh's principle, which states that 'for such a large number of problems there will be some animal of choice, or a few such animals, on which it can be most conveniently studied' [86, p. 247]. Such a principle can easily be applied to the field of host–microbe interactions [87]. The strength of phylosymbiotic patterns varies across host clades; patterns are strong and consistent in mammals [24,60,88], unclear in *Drosophila* flies [7,89], and rare on plant surfaces [8]. Disentangling the ecological processes that contribute to the presence or absence of phylosymbiosis across organisms will greatly inform our understanding of the mechanisms behind these patterns.

## 4. Conclusion

While host selection or host filtering of microbial communities has been largely assumed to be a dominant contributor to patterns of phylosymbiosis, other ecological

and evolutionary processes may also contribute. Aspects of dispersal, drift, diversification and interactions between these processes may also contribute. While these processes have been demonstrated within a single or a few host species, they have rarely been studied in the context of phylosymbiosis across several host species. With advances in sequencing technologies, the development of more experimental systems to investigate host–microbe interactions, including many more germ-free systems, and creative experimental design, the time is ripe to investigate these processes and how they contribute to aspects of ecology and evolution of hosts and host-associated microbial communities.

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