



(More than) Hitchhikers through the network: The shared microbiome of bees and flowers

Alexander Keller^{1,2,8}, Quinn S McFrederick^{3,8},
Prarthana Dharampal⁴, Shawn Steffan^{4,5}, Bryan N Danforth⁶ and
Sara D Leonhardt⁷

Growing evidence reveals strong overlap between microbiomes of flowers and bees, suggesting that flowers are hubs of microbial transmission. Whether floral transmission is the main driver of bee microbiome assembly, and whether functional importance of florally sourced microbes shapes bee foraging decisions are intriguing questions that remain unanswered. We suggest that interaction network properties, such as nestedness, connectedness, and modularity, as well as specialization patterns can predict potential transmission routes of microbes between hosts. Yet microbial filtering by plant and bee hosts determines realized microbial niches. Functionally, shared floral microbes can provide benefits for bees by enhancing nutritional quality, detoxification, and disintegration of pollen. Flower microbes can also alter the attractiveness of floral resources. Together, these mechanisms may affect the structure of the flower-bee interaction network.

Addresses

¹ Department of Bioinformatics, Biocenter, University of Würzburg, 97074, Würzburg, Germany

² Center for Computational and Theoretical Biology, Biocenter, University of Würzburg, 97074 Würzburg, Germany

³ Department of Entomology, University of California, Riverside, Riverside, CA 92501, USA

⁴ Department of Entomology, University of Wisconsin-Madison, Madison, WI 53706, USA

⁵ USDA-ARS, Vegetable Crops Research Unit, Madison, Wisconsin, USA

⁶ Department of Entomology, Cornell University, Ithaca, NY 14850, USA

⁷ Department of Life Science Systems, School of Life Sciences, Technical University of Munich, 85354 Freising, Germany

Corresponding author:

Keller, Alexander (a.keller@biozentrum.uni-wuerzburg.de)

⁸ Shared first authors.

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Introduction

Bee-plant associations are crucial for most terrestrial natural and agricultural systems as they maintain primary

production, biodiversity, and ecosystem functions. Therefore,

pollination is an intensively studied research field. One emerging facet is the interaction of bees and plants with microbes [1,2]. Microbes can contribute positively or negatively to host health, development and fecundity. Detrimental effects on bees and plants are caused by pathogens and competitors, while beneficial symbionts affect nutrition, detoxification, and pathogen defense [1,2]. Insights into the occurrence, nature, and implications of these associations strongly contribute to our understanding of the current risk factors threatening bee and plant populations.

Microbe-host associations in pollination systems are however not isolated, but embedded in complex and multi-layered interaction networks [3,4]. New insights propose a dynamic and variable system of microbial associations, where the structure of the pollination network may be a main determinant of microbial dispersal routes [5^{••}]. Flower microbes can contribute to large proportions of microbiomes in dietary provisions stored in nests and larval guts of bees, particularly in solitary bee species [6]. Bees can thus establish microbial associations from environmental, particularly floral, sources by foraging [7]. Also, microbes classified as bee-adapted were found in flowers [8], which can in turn serve as intermediary transmission hubs facilitating intra- and inter-specific microbial exchange [8]. Consequently, microbes are vectored between flowers by pollinators, and this give-and-take of microbes can also shape floral microbiomes. Microbial associations in the pollination system thus have an underlying meta-community structure [9^{••}], affected by interactions between their respective hosts.

On the other hand, it is unlikely that network structure is the sole driver of microbial community assembly, since hosts nevertheless uphold distinctive microbiome elements despite their connectivity in the overall network [2,5^{••},10]. Host traits can determine which microbes are transferred, establish and proliferate, not only between interacting bees and flowers, but also between individuals or species on the same side of the network. In this review, we highlight current literature on bee and flower microbiomes with special emphasis on the role of local network structure for microbe sharing, evolutionary predisposition and relevant microbe and host traits.

Multidirectional microbial transmission is central in plant-bee interaction networks

During prolonged floral visitation trips, foraging bees leave behind microbes passively by sloughing of propagules from the integument, or actively by feeding and defecation [11]. Recent studies investigating floral microbiomes reported microbes usually attributed to bees or other insects, such as *Apilactobacillus* and *Bifidobacterium* [8,12,13], although visitation by bees does not appear as the exclusive transmission pathway for these bacteria [8]. These microbes occurred in lower abundances in flowers than in bees, suggesting that they are better adapted to and more active in bee guts than in flowers, but do have characteristics that enable them to persist in both environments [8]. With bees eating pollen, drinking nectar or storing these resources in the nest, floral microbes become a central part of the nest environment and are taken up by adults and larvae [14,15]. The interaction between bees and plants is thus recognized as a general transmission route for microbes in both directions. In complex networks, this reciprocal exchange can in theory lead to a series of transmission events within and between species given visitation of a single flower by multiple bees and foraging of individual bees on multiple flowers [8,16,17]. Such microbiome homogenization may explain why pollen microbiomes of insect-pollinated plant species were more similar than those of wind-pollinated plants [16].

Local network architecture determines potential dispersal routes

While the architecture of mutualistic plant-pollinator networks is well studied, we hardly know how this architecture affects transmission of microbes. Pollination networks are mostly non-random with a predominantly nested architecture, i.e. rare (specialized) bees visit common (generalized) plants and rare (specialized) plants are visited by common (generalized) bees [18]. Modularity (exclusive network subsets with dense interactions) increases with species richness [19]. This typical structure of local pollination networks has direct implications on the transfer of microbes; without any filtering mechanisms preventing microbial transmission, the default prediction would be high microbiome overlap for bee-plant networks showing high connectance (all possible interactions are realized), low modularity (even distributions of interactions), and/or high nestedness (shared resource usage for specialists and generalists). On the other hand, highly modular networks with lower nestedness might generate more divergent microbiomes between hosts due to less spreading throughout entire bee and plant communities. Such network architecture and resulting potential microbial transmission pathways might or might not be independent of whether partners are generalists or specialists and should thus be considered separately.

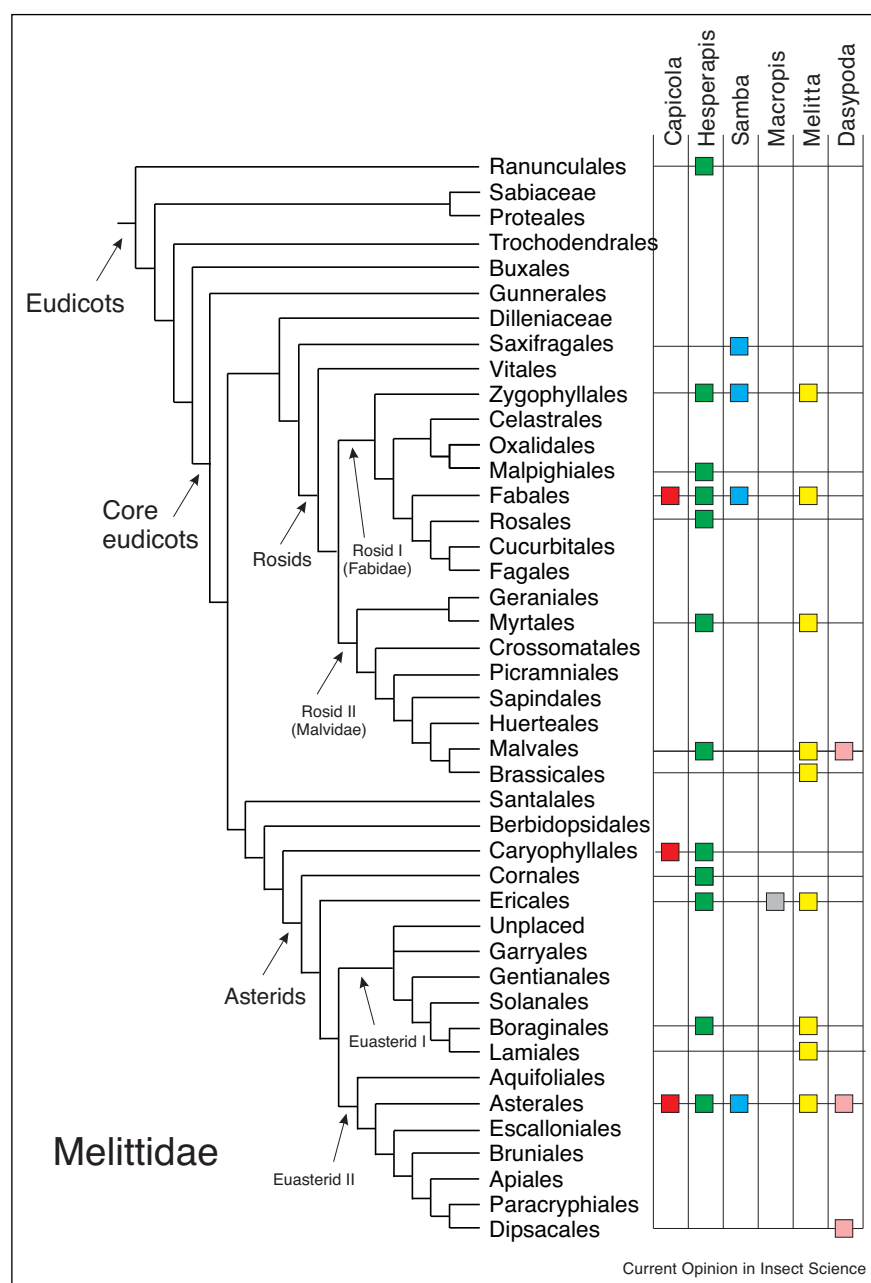
Plant-pollinator network characteristics, however, can vary within and between habitats. For example, while all communities in Andean plant-pollinator communities (Table S1) exhibited significant nestedness and modularity, nestedness decreased and modularity increased with altitude in Chile [20]. As a consequence of greater shared flower visitation, microbiomes across bee species may be more homogeneous at lower altitudes. At higher altitudes, increased network modularity may lead to greater modularity also in bee-related and floral microbiomes across specialized and generalized host species. In general, more emphasis should be given to how diversity and distribution of microbes across bees and flowering plants are related to modularity, nestedness and habitat-specific differences in interaction networks. Recently, Zemenick and colleagues [5**] used coupled plant-pollinator and plant-microbe networks to show that a plant's position in a pollination network influences, but does not fully explain, the composition of its floral microbiome. Introduced microbes may undergo a process of 'filtration' by the flower and resident microbes such that some microbes are enriched, and others are inhibited. This would ultimately impact transmission via subsequent visitors [5**].

The degree of specialization can shape microbial transmission networks

While it is tempting to assume that specialist (oligolectic) bees will have different (i.e. more specialized) microbes than generalist (polylectic) floral visitors, known pollination network structures do not entirely support this assumption. Depending on the degree of nestedness and the local network structure, specialized oligolectic bees may rather have microbiomes which represent a subset of those found in more generalized polylectic bees; and the same may hold true for specialist and generalist plants. Nonetheless, taxonomic specialization of bees on specific plant partners can narrow down potential transmission links for microbes, particularly by contributing to modularity in networks.

Narrow host-plant associations and constrained floral visitation patterns could in theory be a strategy to additionally ensure recruitment of beneficial microbes for each new generation of bees. Due to co-evolution, floral microbial communities of particular plant species or families are well adapted to the plants' inherent floral and pollen chemistry, morphology and other characteristics [21–23]. Microbes therefore have functional traits that match respective plant characteristics. Such microbes will thus thrive on provisions composed of few plant taxa only, as is typically the case for oligolectes, if not suppressed by other means. By that they carry over their functional repertoire to the bees' nests and their offspring. Potential functions which are also useful for bees include accession of nutrients, fermentative processes or detoxification of secondary plant metabolites. Interestingly, several plant families seem to host the vast majority of specialist bee lineages

Figure 1



Phylogeny of eudicot orders based on the Angiosperm Phylogeny Website. (Stevens P.F. 2017, Version 14, <http://www.mobot.org/MOBOT/research/APweb>, accessed July 2020). Host plant associations of some of the well-known, largely oligolectic genera in the bee family Melittidae are mapped onto the ordinal phylogeny of the Eudicots, which comprise 75% of angiosperm species. Host-plant association data from Michez et al. [66]. Note that several orders host a large number of host-plant specialist species across melittid genera, including Zygophyllales (Zygophyllaceae), Fabales (Fabaceae), Malvales (Malvaceae and Convolvulaceae), Ericales (Ericaceae, Polemoniaceae and Primulaceae), and Asterales (Asteraceae and Campanulaceae). The tendency for host-plant specialist bees to convergently evolve close associations with a limited number of plant families suggests that these plant families offer some advantage – perhaps nutritional or microbial – to these bee species.

(Figure 1). These include Malvaceae, Cactaceae, Asterales, Convolvulaceae, Boraginaceae, and Zygophyllaceae [24,25]. A bee's potential to source and host specific floral microbes in nest provisions for such lineages, i.e. microbiome compatibility, might be an important interaction

trait for specialists, likewise to tongue length to corolla tube length or body size to flower size.

Recent evidence suggests that the provision microbiome of an Ericaceae specialist solitary bee likely functions as

an 'external rumen' for developing larvae, which helps mobilizing nutrients from the substrate [15,26]. Such a partnership with a pollen sourced external microbiome may confer a strong adaptive advantage to oligoleges, allowing them to exploit pollen of relatively poor quality. While particularly important for oligoleges, this might also help other bees foraging on 'challenging' pollens. For example, Asteraceae pollen is hard to digest, has unsuitable protein to fat content and contains toxic substances. Therefore most polyleges typically collect only small quantities of aster pollen [27,28–30]. In various recent studies, Lactobacillales were found to be particularly associated with provisions and larval guts of megachilid bees foraging on Asteraceae (Figure 2), while comparable patterns could not be found for the closely related *Osmia bicornis* that entirely avoids Asteraceae pollens [6,8,14,17,31]. It is unclear whether this pattern simply results from transfer within the network or whether it is driven by microbe functional properties, such as pollen disintegration or detoxification that may allow bees to exploit Asteraceae pollen.

Species-specific microbial filtering limits realized niches

While pollen grain and nectar hitchhiking by microbes are likely important in microbial transmission, the quality of a bee as a pollinator may however not necessarily predict its importance in transferring microbes [5**]. For example, frequency and abundance of yeasts in floral nectar differs strongly between flowers visited by two effective pollinator groups, i.e. bumblebees and solitary bees [32,33]. Such species- or group-specific differences may be explained by cuticular hygiene, morphology, 'hairiness' and packing of pollen in corbicula or scopa. This and also how collected resources are stored in the nests (e.g. directly adjacent or separated from larvae) generates subsequent differences in osmotic, positional, and chemical properties of loads and provisions [34] and thereby has implications on microbial growth dynamics [6,35]. Microbial filtering by features of the nest can also be a prolonged process that persists even after the direct interaction itself, for example, plant nesting substrate affects provision microbiomes of leafcutting bees either by foliar chemistry or microbiomes long after nests are closed [36]. Many bees also add glandular secretions to brood cells which can contain high loads of lactones with antimicrobial properties [37,38]. Likewise, other nest building materials, like resin, waxes or composite propolis, may also play a role in repression of imported bacteria [35,39]. Furthermore, introduced microbes have to compete with already established associates, especially in bee species where social interactions support a controlled microbial transmission between nest mates [2,40,41]. Thus, the net effect of transmission and filtering, both of which lead to either persistence or elimination of a given microbial population, can make microbial sharing range from highly relevant (e.g. fitness benefits by florally

sourced bacteria for solitary bee species [15]) to presumably minor importance (e.g. honey bees [35]). This however does not imply that bee species with strong filters are not contributing to microbial sharing. Particularly given the high abundances of individuals and thereby frequency of flower visits that social bees can exhibit, they are likely significant sources for microbes on flowers.

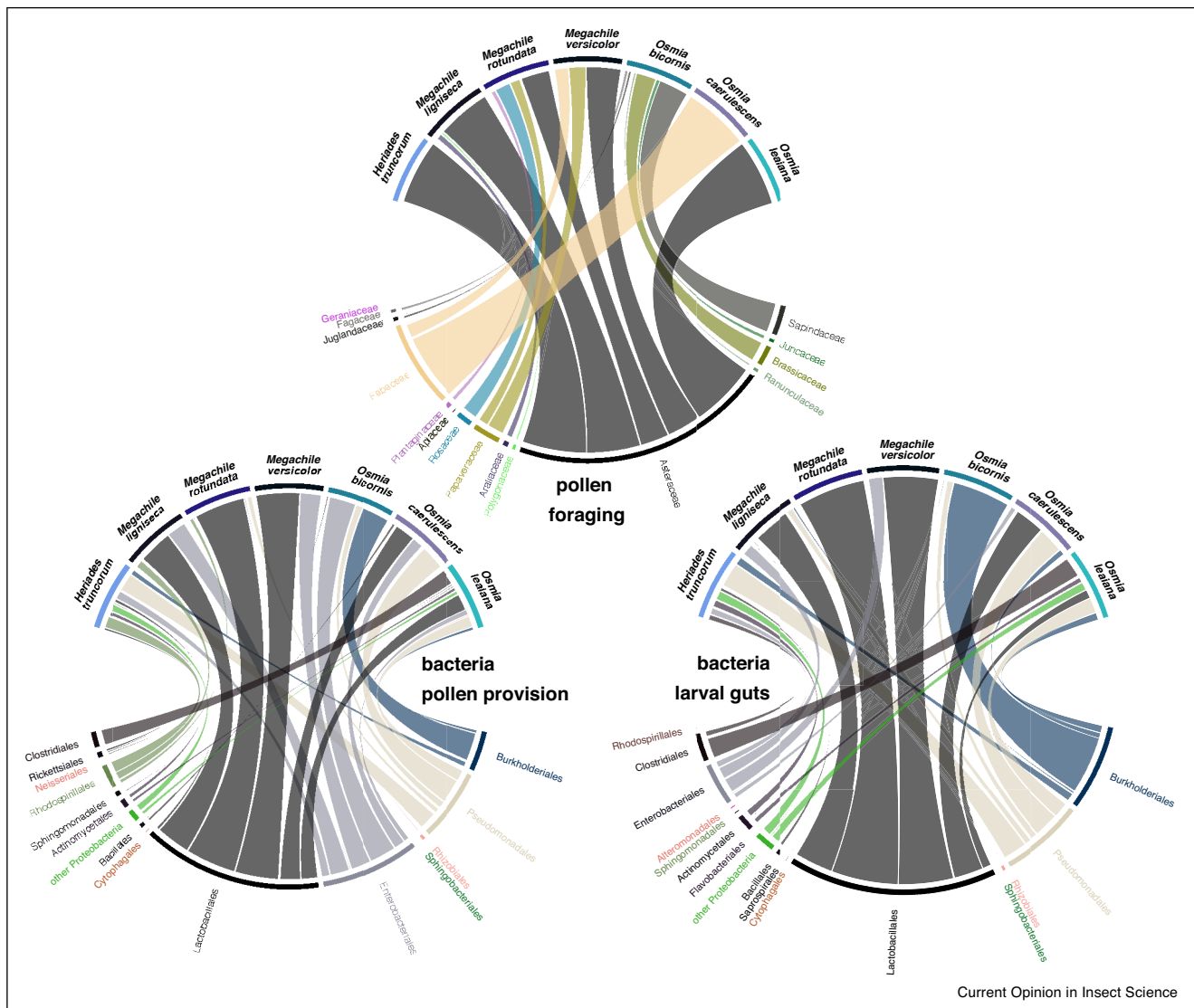
Similarly for flowers, floral chemistry and microenvironment may have immediate detrimental effects on bee-transmitted microbes and thus break transmission chains [42,43]. Species-specific filtering mechanisms may consequently decouple host-microbe networks from plant-pollinator networks, rendering not only the interaction but also the traits of bees and plants important [5**]. To add to this complexity, bees seek flowers to collect pollen and nectar, yet the spectrum of plant species visited for each resource type often shows little overlap. The individual resources are mixed afterwards in context- and species-specific concentrations [44,45]. This also has direct implications on microbial transmission. For example, moisture content may affect the provision microbiome, with more liquid provisions harboring high quantities of microbes [46,47] and potentially different ratios of yeasts and bacteria according to source proportions [46]. Also, abiotic properties (pH, texture, nutrient composition, osmotic pressure and consistency) of the newly composed microhabitat change over time.

Functional implications of microbe sharing

In many social bees, like *Apis mellifera*, above mentioned filtering mechanisms and social transmission of microbes promote the conservative transfer of symbionts through inheritance [2,40,41], whereas environmental contributions seem less pronounced [35]. By contrast, the microbiome of solitary bees, assuming the microbes are predominantly sourced from the environment (e.g. nest or flowers) each season, can be highly variable and heterogeneous [10], which may also affect microbiome functionality. The nest microbiome of solitary taxa, however, may serve as a means of vertical transmission between a female bee and her progeny. It is conceivable that the microbial propagules within a solitary bee nest would be effectively gathered on the setae of the adult when she emerges from her cocoon. Such 'hitchhiking' microbial communities might be distributed among the floral substrates visited by the solitary bee adult, and thus, would have a high likelihood of perpetuating among the progeny by colonizing the pollen-provisions that the adult bee prepares for her offspring.

While many flower- and nest provision-associated microbes have not yet undergone functional or genomic study, those that have suggest functions that support microbial existence in the different habitats associated with the two hosts. Vuong and McFrederick [48] found

Figure 2



Interaction networks of megachilid bees based on the study by Voulgari-Kokota et al. [17]. In the investigated Megachilidae, different foraging specialization patterns occur: *Osmia leaiana* and *Heriades truncorum* are Asteraceae specialists, *Osmia caerulea* is polylectic but with Fabaceae and Lamiaceae preference, while *Megachile ligniseca*, *Megachile rotundata*, *Megachile versicolor* and *Osmia bicornis* are generalists. The latter avoids Asteraceae almost entirely, while in the former three species Asteraceae contribute strongly to the diet. (Top) plant families found in larval provisions, (left) bacterial orders in provisions and (right) bacterial orders in larval guts. All different types of samples were taken from the same individual nests (100 samples each network). The mean of relative abundances per sample was taken and families/orders that contributed less than 5% were removed to improve the clarity of the networks. Notably, interactions of bees foraging with Asteraceae pollen and with Lactobacillales in provisions and guts show striking similarities, raising the question whether this is related to functional connections.

signatures of natural selection on *Apilactobacillus micheneri* genes involved in osmotolerance, detoxification, and oxidative stress, as signs of adaptation to fermentation of provisions but also to the floral niche. Such characteristics may allow florally sourced bacteria to exist in provisions long after harvesting, and *vice-versa* bee provision bacteria to endure flower or nectar conditions, as long as other filtering mechanism do not apply. Interestingly, these lactobacilli are closely related to the sourdough inhabiting *Fructilactobacillus sanfrancensis*, known to form

communities in synteny with yeasts that are difficult to invade [49]. Associations between bacteria and yeast might strengthen overall meta-community stability in a highly dynamic system [50,51], such as pollen or sourdough, and thereby support both hosts' immunity and spoilage inhibition.

Pollen is notoriously tough to digest, and our understanding of how bees utilize pollen is still rudimentary [52,53]. To overcome this digestive challenge, a variety of bees

across all major lineages seem to rely on nutritive functions of exo- or endosymbiotic microbes associated with their pollen stores or guts [15,41,52,54^{*}] (but see [35]). Specific microbial taxa possess the enzymatic machinery to degrade the recalcitrant ‘shell’ of pollen grains e.g. with pectate lyase genes [48]. This process liberates the nutrient-rich cytoplasm within, allowing both microbes as well as bee larvae to exploit the resource. Such heterotrophic microbes continue to proliferate within the fermenting pollen substrate by consuming and consolidating pollen nutrients within their own biomass. The pollen, then, appears to be transformed from a largely plant-based dietary resource into a blend of microbial and plant biomass [50,54^{*}]. While there is compelling evidence supporting such microbial functions for bees in general [15,41,54^{*}], we need to clarify to which degree and for which bee species florally sourced microbes are behind this mechanism [15,51]. Further studies that carefully measure microbial biomass, growth, and metabolic activity in nest provisions across the diversity of global bee fauna will be needed to better characterize the roles of microbes in pollen-provisions.

Consequences for flowers are mostly unclear, e.g. how such microbes might affect germination, growth of pollen tubes or floral phenotypes [23]. Yet, there is strong evidence that the presence of particular microbes can alter flower chemistry and by that also influence bee visitation and thereby weaken [55,56] or enhance [57,58] pollination interactions. Volatile compounds emitted or modified by microbes contribute to the scent bouquet of flowers and thereby pollinator attraction or repellence [43,59,60]. Evaluating floral scent associated with specific microbes might also help bees reduce risks of collecting detrimental bacteria when foraging on shared resources, which may also prevent a fast and uncontrolled spread of pathogens within a highly connected network [61–64]. Also, floral microbes can modify volume and nutritional quality (e.g. free amino acid concentration and composition) of nectar [65^{*}]. Given the adaptive behavior of bees [58], it is likely that not only the visitation network influences microbe transmission, but also that microbes contribute to structuring the network itself. Next steps should experimentally verify these hypotheses and reveal the underlying genetic mechanisms and resulting functional implications. Although microbes clearly represent rather inconspicuous ‘silent partners’ in bee-microbe-flower interactions, they may mediate or facilitate some of the patterns observed in the pollination ecology literature.

Conclusion

Microbial spill-over between bees and flowers has been identified as a factor contributing to the hosts’ microbiome assemblies. In theory, multiple subsequent microbial transmissions can lead to microbiome homogenization between host species in this system. Most microbiome studies, however, focus on individual hosts isolated

from such effects. In this review, we explore a multi-layered approach to the plant-bee-microbe ‘triangle,’ which can be helpful to disentangle effective microbial sharing. We suggest that (1) local pollination network architecture, (2) phylogenetic predisposition and specialization of bee foraging as well as (3) filtering of microbial species at the plant and bee level, may jointly determine how strongly meta-community dynamics drive host-microbiome associations. Given that each transfer and subsequent establishment of a microbial population may generate new, emergent properties within the microbial community, the functional impacts of such microbial assemblages can be exceedingly diverse. These changing microbial assemblages can set up new cost-benefit relationships among the players, with shared microbes emerging as considerable drivers, rather than passive hitchhikers within pollination networks. Particularly for solitary bee populations missing social symbiont transmission routes, and static flowers, microbial sharing may facilitate inter-generational transmission both for symbionts and pathogens. Importantly, this framework is not limited to bees, as we focus on in this review, but can be extended to other invertebrate (e.g. butterflies, wasps, flies, thrips, beetles) and vertebrate (e.g. birds and bats) flower visitors. Given the very different community assembly rules, network interaction traits and emergent, functional characteristics associated with different visitor groups, tripartite plant-pollinator-microbe interactions represent a complex, yet intriguing new research field.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.cois.2020.09.007>.

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