Trends in Microbiology

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REVIEWS

Review

Drivers, Diversity, and Functions of the Solitary-Bee Microbiota

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Accumulating reports of global bee declines have drawn much attention to the bee microbiota and its importance. Most research has focused on social bees, while solitary species have received scant attention despite their enormous biodiversity, ecological importance, and agro-economic value. We review insights from several recent studies on diversity, function, and drivers of the solitary-bee microbiota, and compare these factors with those relevant to the social-bee microbiota. Despite basic similarities, the social-bee model, with host-specific core microbiota and social transmission, is not representative of the vast majority of bee species. The solitary-bee microbiota exhibits greater variability and biodiversity, with a strong impact of environmental acquisition routes. Our synthesis identifies outstanding questions that will build understanding of these interactions, responses to environmental threats, and consequences for health.

Bee Biodiversity and the Importance of the Bee Microbiota

Bees form a large and diverse group, with more than 17 500 described species [1]. While the western honey bee (*Apis mellifera*) is the most studied and recognizable bee for the public, it may not always serve as a model for the rest of bees (e.g., [2]), and the number of solitary species of bees far outweigh the social species. Honey bees (*Apis* spp.), bumble bees, and stingless bees are the three main corbiculate bee tribes constituting a monophyletic clade [3]. Corbiculate bees, along with some species from the Allodapini and Halictidae, are the only bee taxa which demonstrate social behavior, meaning the vast majority of the bee species (>90%) are solitary [4]. Given their high species diversity, solitary bees vary strongly in phenology, geographic distribution ranges, nesting habitats, and foraging preferences [5,6]. Moreover, solitary bees provide indispensable pollination services even where managed honey bee populations exist (e.g., [7,8]).

The global decline of bee populations in general has set off the alarm both for ecological and economic reasons [9,10], mainly due to the importance of bees as the main pollinators in most ecosystems with flowering plants [11,12]. For many insect species, the microbiota is a proven element of host fitness and overall health [13–15] (but see [16]). At the same time, microbes have an important role in many insect–environment interactions [17–20]. The research on the bee microbiota is constantly increasing with respect to drivers of diversity and temporal dynamics, as well as consequences for bee health, development, and offspring recruitment. However, only a few studies have dealt with the solitary bees' natural microbiota, while the majority of studies concentrated on social bees, foremost honey bees, and their gut symbionts (reviewed in [21]). Indeed, the honey bee is considered to be a model for gut microbiota research [22]. The main objective of the present review is to provide an overview of studies on the solitary-bee microbiota, including current knowledge on its drivers, function, and dynamics. We further contrast this to knowledge about model social bee microbiota to identify where transferability is applicable and which future research questions need to be addressed, particularly for solitary bees.

Different Microbiota Transmission Routes between Social and Solitary Bees

Eusociality, defined as family groups living in colonies with an overlap of generations and a division of labor [23], is central in the study of microbiota transmission. It has been shown that the evolution of intimate associations between the host and the microbiota is favored in social hosts [24]. In general, close social contact of individuals is likely to aid transmission of beneficial symbionts both from parent

Highlights

Social bees are tightly linked to host-specific microbes which are actively transmitted within and between generations through social contact. For solitary bees, the more direct exposure to the environment and lack of active offspring care, are factors leading to a more diverse and environmentally associated microbiota. This limits the applicability of the social-bee model.

Known social-bee-associated bacteria defend the hive against microbial pathogens, activate the host's immune system, and ferment complex carbohydrates. In solitary bees, bacteria may assist with pollen fermentation, nutrition, spoilage inhibition, and defending the nest against harmful microbes.

Honey bee guts are colonized during the first days of adult life and have a relatively stable microbiota. In solitary bee species, likely environmental or nest bacteria drive bacterial community shifts during development.

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to offspring and also between colony members, assisting the emergence and evolution of mutualistic interactions [25,26]. The main microbial transmission routes in the social honey bee hive include direct fecal–oral connectivity, oral trophallaxis, and contact with the hive material [27,28]. Nontypical bacteria, which are not considered part of the core microbiota, are established when workers are exposed to hive components, such as honeycomb, honey, and bee bread (pollen and nectar) [26]. Sociality by itself, however, does not always drive host specificity. A comparison of lactobacilli associated with several ant species, honey bees, bumble bees, and other bees found that only honey bees and bumble bees harbor host-specific lactobacilli [29]. Studies of socially polymorphic bees have shown either no difference in microbial communities in social versus solitary nests from the same environment [30], or consistent symbiosis occurring only in the solitary form of socially polymorphic bees [31]. Social interactions in honey bees and bumble bees are clearly important in maintaining the distinct 'core microbiota' that these bees harbor, but why sociality does not always result in such host specificity remains an open question.

In contrast, the solitary insect lifestyle is thought to prevent transfer of bacterial symbionts between concurrent generations or between individuals of the same generation [15,24], since there is no direct contact between larvae and adults, and the larvae grow in separate nest chambers. However, mixing of the pollen provision with nectar and salivary gland secretions by the mother bee, prior to egg laying, might inoculate the offspring's diet with beneficial bacteria for the developing larvae, assisting with the pollen digestion and the defense against environmental pathogens [32]. Indeed, the same bacteria have been reported from mothers, pollen provisions, and larvae in solitary and primitively eusocial bees [30,33–35]. Females of several solitary insect species inoculate their eggs with specific bacteria, preserving a stable host–microbe relationship [36].

As for environmental acquisition routes, the effect that imported materials, such as pollen, might have on honey bees has not been extensively studied, but existing work suggests that the honey bee microbiota is mostly robust to changes in diet. A recent study used honey bee colonies reared under identical conditions in order to investigate whether different landscapes along with their accompanying plant diversity and availability would influence the bee microbiota [37]. The characterized gut bacterial communities of adult bees from the colonies did not show significant differences; however, the overall results suggested that the broad environment can have some influence on the relative abundance of some members of the honey bee microbiota [37]. Another recent study showed that the bee-bread bacteria varied significantly with hive location, and it was suggested that the reduced floral diversity in grasslands leads to a reduction in bacterial diversity [38]. Supplemental winter forage compared with typical artificial pollen supplements had only subtle effects on the honey bee microbiota [39]. In comparison with honey bees, bumble bees and stingless bees are more likely to undergo microbial composition shifts due to their different regions of origin and food availability [40,41]. In the case of solitary bees, diverse foraging preferences, as well as different nesting habits and materials (e.g., soil, leaves, resin) [6] of different species, have been studied as a candidate way of forming the microbiota. Several studies have shown that foraging preferences can establish conserved routes for bacterial colonization in the nest [34,42,43]. These findings together indicate that the various discussed acquisition routes gain different weights of importance in driving bee microbiota for different bee species, particularly in comparison with honey bees (Figure 1, Key Figure).

Main Members of the Microbiota in Social and Solitary Bees

The western honey bee is reported to have distinctive and recurring gut microbiota, harboring a set of less than ten major bacterial phylotypes [40,44]. These bacteria are transmitted through social contact [26] and are specialized to the hive environment [45]. Only a few bacterial taxa, such as Enterobacteriaceae, *Lactobacillus kunkeei,* and *Parasaccharibacter apium* have been found in the honey bee hive environment [46]. Most members of the *A. mellifera* core microbiota have been found also in other *Apis* spp. [47,48]. Also, bumble bees and stingless bees harbor microbiota similar to that of the western honey bee [25,45,49,50].

Key Figure

Microbial Acquisition Routes Relevant for Solitary and Social Bees

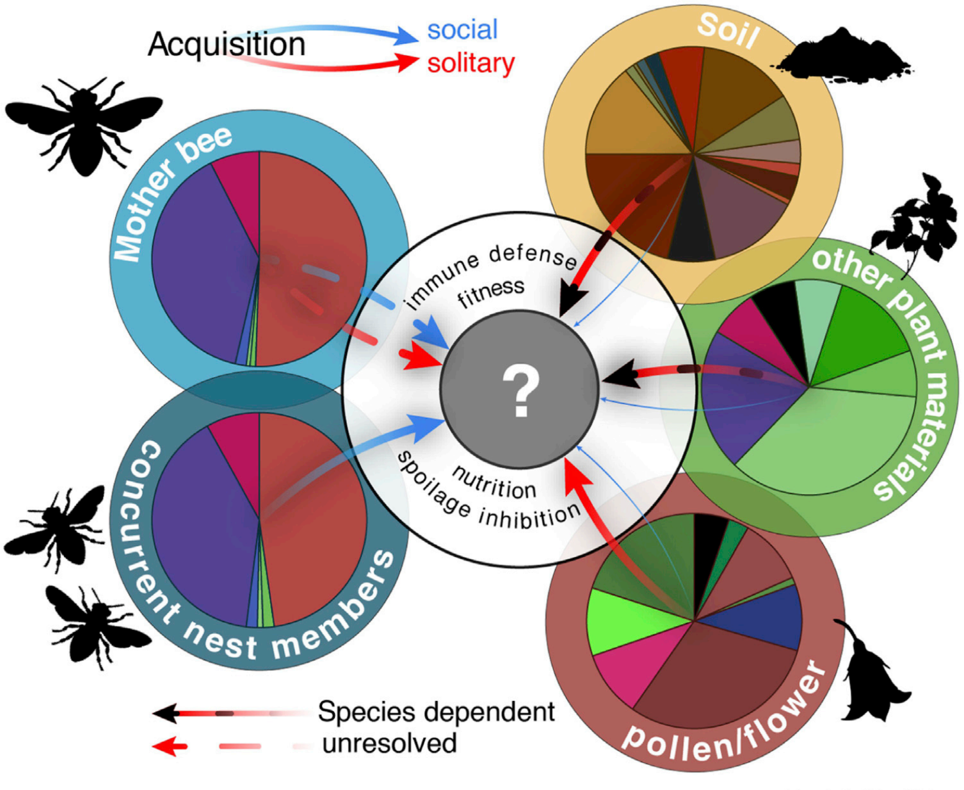

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Figure 1. Known (solid lines) or potential (dashed lines) transmission routes are illustrated for solitary bees (red lines) and social bees (blue lines). The thickness of the lines reflects the currently identified strength of these associations. Variation in contributions of the environmental routes (red-black dashed lines) are species-dependent in solitary bees, mostly due to different life histories and foraging preferences as well as nesting habits and materials. Inner pie charts serve as simplified and conceptual proxies to illustrate microbial compositions and diversity associated with these origins. In most cases, the functional consequences and susceptibility of environmental threats for solitary bees are currently not well resolved.

Solitary bee species, on the other hand, comprise highly diverse and fluctuating bacterial communities. Culture-independent studies have linked several solitary bee species with numerous bacterial families, such as Acetobacteraceae, Bacillaceae, Burkholderiaceae, Clostridiaceae, Comamonadaceae, Enterobacteriaceae, Lachnospiraceae, Lactobacillaceae, Methylobacteriaceae, Moraxellaceae, Sphingomonadaceae, and Oxalobacteraceae [34,51–53]. It has been argued that the composition of the solitary-bee microbiota is species-specific, yet individual bacteria are not necessarily strictly bee-associated and may be acquired via environmental transmission pathways [33,34,42,51,54,55]. Studies on quantities of microbes associated with solitary bees are scarce; however, one recent study identified great variability in cell numbers of *Lactobacillus* for adult and larval guts, as well as pollen provisions (estimated by qPCR) ranging from below 1000 cells to more than a million [34]. Further, reduction of the density of bacteria in pollen provisions resulted in higher mortality and slower growth of the larvae [56]. These studies support the notion that

Table 1. Studies and Main Research Findings on Solitary-Bee Microbiota in Chronological Order

Solitary bee species	Method	Major findings	Year/Refs
Larval provisions from the soil-nesting *Centris pallida* and *Anthophora* sp.	Culture dependent	Isolated *Bacillus* spp. strains with suggested role in the metabolic conversion, and/or preservation of the larval provisions	1984 [32]
Alimentary canal from female *Crawfordapis luctosa* adults, larval provisions from the soil-nesting *Centris flavofascia* and the plant-nesting *Xylocopa californica arizonensis*	Culture dependent	Isolated *Bacillus* spp. strains with enzymatic activity involved in protein, lipid, and carbohydrate catabolism	1990 [54]
Nectar, pollen provisions, larval guts, and frass from the leaf-cutter bee *Megachile rotundata*	Culture dependent	Varied aerobic microflora, including yeasts from nectar and pollen, and several bacterial strains assigned to *Bacillus* spp., *Corynebacterium* sp., *Micrococcus* sp. and *Streptomyces* sp.	1993 [102]
Alimentary canals from third-instar leaf-cutter *Megachile rotundata* larvae	Culture dependent	Bacterial strains primarily assigned to *Paenibacillus macerans*, *Bacillus licheniformis*, *B. brevis*, *B. mycoides*, and *P. polymyxa*	1998 [55]
Larvae from the red mason bee *Osmia bicornis*	Genetic profiling with single-strand conformation polymorphism (SSCP) of partial 16S rRNA genes	Bacterial phylotypes mainly assigned to *Sphingomonas* sp., *Ralstonia* sp., *Burkholderia* sp., and *Acinetobacter* sp. Existence of structurally different bacterial communities for each developmental stage	2006 [80]
Besides social bees, digestive tracts of *Agapostemon virescens, Calliopsis subalpinus, Caupolicana yarrowi, Colletes inaequalis, Halictus patellatus, Hesperapis cockerelli, Megachile odontostoma, Rediviva saetigera, Xylocopa californica*	16S cloning and diagnostic PCR of honey bee phylotypes	Profiles of *Apis* and *Bombus* microbiota differ from other bee species. Phylotypes associated with *Apis* are not recovered by diagnostic PCRs in solitary bees	2011 [45]
Nest contents from the sweat bees *Augochlora pura*, *Halictus ligatus*, and *Megalopta genalis* in solitary life state	16S rDNA amplicon 454 pyrosequencing	Discovery of lactobacilli and other acidophilic bacteria also associated with flowers, presumably able to suppress mold growth inside the nest	2012 [33]
Nest contents from the red mason bee *Osmia bicornis*	16S rDNA amplicon 454 pyrosequencing	High bacterial biodiversity including Burkholderiales, *Bacillus* spp., *Paenibacillus* spp., Clostridiaceae, Enterobacteriaceae, and Acetobacteraceae	2013 [51]
Bees and nest contents from solitary *Megalopta centralis* and *M. genalis*	16S rDNA amplicon 454 pyrosequencing	Different prevalence of *Wolbachia* between the two host species, and different prevalence of the environmentally acquired *Lactobacillus kunkeei* according to the developmental stage.	2014 [30]
Nest contents from the mason bee *Osmia cornuta*	Culture dependent	Isolation of *Bacillus* spp., *Lactobacillus kunkeei*, *Paenibacillus polymyxa*, *Clostridium baratii*, *Serratia marcescens*, *Pantoea agglomerans*, and *Curtobacterium flaccumfaciens*	2015 [73]

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Table 1. ***Continued***

Solitary bee species	Method	Major findings	Year/Refs
Provisions from the stem-nesting bee *Ceratina calcarata*	Illumina 16S rDNA (bacteria) and RBCL (plants) amplicon sequencing	Discovery of OTUs assigned to the genera *Lactobacillus*, *Wolbachia*, *Acinetobacter*, *Sodalis*, *Erwinia*, and *Pantoea*, some of which are flower associated	2016 [52]
Adults, larvae and pollen provisions from the megachilid bee species *Lithurgus gibbosus*, *L. littoralis*, *Megachile brevis*, *M. parallela*, *M. policaris*, *Osmia chalybea*, and *O. subfasciata*	Illumina 16S rDNA amplicon sequencing	Monophyletic *Lactobacillus* clade, phylogenetically closer with bee-associated bacteria, dominating in flower- and megachilid-associated microbiomes	2017 [34]
Adult guts from *Agapostemon* spp., *Augochlora pomoniella*, *Dialictus* spp., *Halictus ligatus*, *H. tripartitus*, *Megachile policaris*	Culture dependent, Illumina genome sequence	Novel lactobacilli isolated from adult bee guts and flowers	2018 [74]
Leaf-cutter *Megachile rotundata* adults and pollen provisions	Illumina 16S (bacteria) and ITS[a] (fungi) rDNA amplicon sequencing	Diverse bacterial and fungal communities influenced by the available floral sources	2018 [43]
Guts and surfaces of *Osmia bicornis*, *O. brevicornis*, *O. caerulescens*, *Stelis punctulatissima*, *Halictus* spp., *Bombus* spp., *Apis mellifera*, *Anthophora aestivalis*, and nest materials from *Heriades truncorum*	Illumina 16S amplicon sequencing and isolate genome sequencing	Prevalence of *Paenibacillus polymyxa* in various bee species, and description of antimicrobial properties in a strain from *Heriades truncorum*	2018 [35]
Pollen provisions from the stem-nesting bee *Ceratina australensis*	Illumina 16S (bacteria), ITS (fungi), and RBCL (plants) rDNA amplicon sequencing	Pollen usage and microbial patterns covariance across landscapes	2019 [42]
Larvae and pollen provisions from the leaf-cutter bee *Megachile rotundata*, *Megachile versicolor*, and the mason bees *Osmia bicornis* and *O. caerulescens*	Illumina 16S rDNA amplicon sequencing	Mason bees characterized by Enterobacteriaceae and Acetobacteraceae, and leaf-cutter bees characterized by Lactobacillaceae. Bacterial succession in the pollen provisions in line with larval development	2019 [53]
Various body tissues *of Calliopsis andreniformis*, *Andrena nivali*, *Peponapis pruinosa*, *Colletes compactus*, *Ptiliglossa arizonensis*, *Megachile latimus*, *Osmia pumila*, *Macropis nuda*, and *Melitta eickworti*	Compound-specific isotopic analysis	Bee-assimilated proteins indicate bacterial origin. Digestion of bacteria is a food source for various bee species, particularly bacteria that are pollen-associated	2019 [76]
Osmia ribifloris	Pollen provision manipulation by adding sterilized pollens	Microbial density reduction in pollen provision leads to increased mortality and slower growth	2019 [56]
Megachile rotundata pollen provisions	Isolate genome sequencing	Comparative genomics of wild bee and flower-isolated *Lactobacillus*	2019 [75]
Larvae and pollen provisions from *Heriades truncorum*, *Megachile ligniseca*, *M. rotundata*, *M. versicolor*, *Osmia bicornis*, *O. caerulescens* and *O. leaiana*	Illumina 16S (bacteria) and ITS2 (plants) amplicon sequencing	Associations between the composition of pollen and that of nest microbiota	2019 [103]

[a] Abbreviation: ITS, internal transcribed spacer.

associations are important, yet less stable than in social bees, and that environmental transmission pathways play a strong role in the acquisition of beneficial bacteria. Studies on the microbiota of solitary bees, along with the bee species that have been studied, and major insights, are summarized in Table 1.

Functional Properties of the Social and Solitary Bee's Microbiota

Microbial associates are important players in the health of social bees. Larval bioassays have demonstrated that a number of honey bee-associated bacteria, such as *Lactobacillus* and *Bifidobacterium*, inhibit the causative agents of American and European foulbrood [57–59]. Lactic acid bacteria, in general, are valuable symbionts of the honey bee [58,60]. Recently, a study showed that the bacterial composition of the honey bee surface and gut was different between thriving and nonthriving hives, presumably indicating different host needs for pathogen defense and pollen degradation [61]. In bumble bees, deprivation of their gut microbiota led to high susceptibility to parasites [49,62], and acidification of the gut by lactobacilli appears to be the mechanism for suppression of gut parasites [63]. Indirectly, the gut microbiota might also be able to activate the host's immune system [64]. Furthermore, introduced agricultural chemicals often have lethal or fitness-reducing effects in bees – by disturbing their microbiota [65]. The solitary bee nest is very susceptible to the environment; here, the larvae need microbial associates to resist diseases caused by microbial pathogens. Acidophilic bacteria in solitary bee nests have been suggested to aid defense against mold growth inside the nest [33]. Other bacterial strains isolated from solitary bee nests have shown strong antifungal and antibacterial activity in designed bioassays [35], and it has been suggested that their origin is environmentally dependent [66].

Further important roles of the honey bee gut bacteria are the fermentation of complex carbohydrates, help with digestion, and the biosynthesis of necessary nutrients [67,68]. Bumble bee symbionts can also ferment carbohydrates [69], and others may form syntrophic interactions for partitioning of metabolic resources [70]. In both honey bees and bumble bees, maternally inherited, bee-specific lactobacilli encode various genes for carbohydrate utilization and phosphotransferase systems involved in the uptake of sugars [70,71]. In honey bees, bee bread is stored in different cells from those in which the larvae develop, allowing a clear separation of microhabitats and perhaps conditions for microbial establishment and activity. Honey bee bread appears to be mostly devoid of microbes [72]. In contrast, solitary bee larvae develop in chambers called brood cells which contain sufficient nectar and pollen for larval development and are typically sealed by the mother after the egg is laid. The larvae feed directly on the provided pollen on which they are firmly attached. Beneficial microbes may assist with pollen fermentation and digestion within the solitary bee brood cell while the larvae are already feeding. *Bacillus* spp. strains with proven catabolic ability [32], and *Lactobacillus* strains with proven fermentative ability, have been isolated from solitary bee nests on multiple occasions [73,74]. The solitary-bee-associated novel *Lactobacillus* species described in [74] possesses a wide range of lytic enzymes, including pectate lyases that could help digest the pollen intine and are probably acquired from flowers acting as hubs of transmission. These were not present in closely related strains of *Lactobacillus* as inferred by a comparative genomics approach [75]. A recent study demonstrated even further that microbes, and particularly those of pollen origin, are ingested in the diet and that a significant proportion of assimilated amino acids are of microbial origin (Box 1) [76].

Microbiota Shift with the Bee Developmental Stage

Honey bee larvae acquire bacterial symbionts via nursing by worker honey bees [26]. The number of the bacteria in the honey bee larval gut increases drastically during the initial days [50]. The most prevalent bacteria of the initial community are lactobacilli and acetobacteria [28]. Directly after metamorphosis, in which the larval gut is broken down and a new gut develops from imaginal disc cells, the callow adult bee gut has few to no bacteria. It has been proposed that the newly emerged bees acquire their first gut symbionts by chewing their way out of their cells and thus ingesting remnant gut bacteria [70]. Adult honey bee guts are fully recolonized during the first few days of adult life by the normal honey bee microbiota, long before the adult workers are

Box 1. Microbes as a Heterotrophic Food Source for Bees

A recent study by Steffan *et al.* [76] investigated a potential novel pathway of nutritional support for bees by microbes. The authors examined isotopic compositions of amino acids from a variety of bee species from six different families, including solitary and social taxa. They were able to identify bee-assimilated amino acids originating from microbial sources throughout all investigated species. With that, the authors suggest that bees should be considered as omnivorous, rather than strictly herbivorous. Pollen-borne microbes seemed to be particularly important by contributing significantly to this amino acid assimilation. Given the different routes of microbe acquisition between solitary and social bees, this finding is of major relevance, particularly for solitary bees and their environmentally mediated microbiota. Especially for aged pollens in this study, approximately half of the original plant proteins had been converted into heterotrophic proteins, making microbes directly a significant and primary source of nutrients. A second case study by Menezes *et al.* [104] corroborates these findings, where the authors showed that larvae of the stingless bee *Scaptotrigona depilis* fed predominantly on fungal mycelia within brood cells, and that fungal proteins represented the primary protein source.

ready to leave the hive as foragers [77]. The adult gut communities comprise the same bacterial taxa even through transition of the worker bees through various states of temporal labor division [78]. These are also more stable than those harbored by male bees or queens [78]. However, despite the stability of the bacterial taxa that are present, there are shifts in relative abundances that are observed with time and are associated with the age of the individuals [77] and with their behavioral task [79].

In the case of solitary bees, environmental factors are likely important drivers of bacterial community structure and dynamics in the nest and larvae. The existence of different bacterial communities for different developmental stages has been also shown for *Osmia bicornis* larvae [80]. Furthermore, in the pollen provisions of *O. bicornis*, *Osmia caerulescens*, and *Megachile rotundata*, a bacterial community shift has been described, corresponding to a reduction in floral bacteria with progressing larval development [53]. To our knowledge, no published study has so far investigated how metamorphosis affects the solitary bee microbiota, but shared physiology with honey bees suggests that solitary bees may emerge as mostly germ-free adults as well. If this is the case, future studies should address how solitary-bee microbial associates are reobtained. Current data suggest that bacteria may be acquired by adult solitary bees either in the nest environment or via shared floral resources [33,34,53,74].

Microbiota-Related Diseases

Studies on honey bees have revealed bacterial agents which can disrupt their healthy microbiota and cause mortality in the hive. *Paenibacillus larvae* [81,82] and *Melissococcus plutonius* [83], for instance, are widely accepted as the main causative pathogens for American and European Foulbrood in honey bees, respectively. Although *Paenibacillus* is a notorious genus in bee microbiota studies, not all *Paenibacillus* spp. should be considered as harmful, since there are species important in environmental biocontrol [84,85] and, as already discussed, beneficial for bee pathogen defense [35,86]. Furthermore, the honey bee pathogens *Spiroplasma apis* and *Spiroplasma melliferum* [87,88] have been proven to reduce adult bee longevity. Nevertheless, the precise impacts of some pathogens on honey bee health and fitness are mostly unclear [89] or even controversial [90,91].

Apart from the described bacterial pathogens, there are other bacterial taxa which are opportunistic pathogens. Such environmental bacteria which affect the honey bee colonies include Enterobacteriaceae and, more specifically, genera such as *Enterobacter*, *Hafnia*, *Klebsiella*, *Pantoea*, and *Serratia*, as well as several Gammaproteobacteria [44,92]. These opportunists are often involved with microbial shifts observed in individual worker honey bees, which is referred to as gut dysbiosis. Bumble bees appear to be more susceptible than honey bees to such shifts [62,93]. Furthermore, introduced agricultural chemicals are often indirectly lethal to bees by making them more susceptible to

Outstanding Questions

Solitary bee species diversity and distribution

- What are the host and geographic ranges of microbes that associate with wild and solitary bees?
- Do bee species with a wide global distribution acquire different bacterial communities according to the available plant flora?
- How important is environmental versus vertical transmission of these microbes?

Functional roles

- What role do microbes play in pollen digestion? By what mechanism?
- How important are pollen-provision microbes for inhibiting the growth of spoilage organisms?
- Do gut microbes stimulate the immune system of wild and solitary bees as they do in honey bees?
- Do gut microbes inhibit gut pathogens in solitary bees as they do in social bees?
- Which taxonomic groups (bacteria, yeast, other fungi, protists) are the major drivers of bee health?

Human-mediated impacts

- Do temperature/land use changes/habitat fragmentation affect the microbiota of the same bee populations over numerous seasons?
- Do the chemical and nutritional properties of pollen of monoculture crops affect members of the natural solitary-bee microbiota as bees develop in the nest?
- How are crop-specific and other pesticides affecting solitary bee–microbial interactions?

Evolutionary pathways

- Do bacteria coevolve with bees, but also flowers, to establish conserved transmission routes?

opportunistic infections [94–96]. Apart from bacteria, a wide range of viruses, protozoans, and fungi harm the honey bee [89,97,98].

When it comes to solitary bees, studies investigating potential microbial pathogens have focused on viral/fungal infections, which are common between honey bees and solitary bees [99]. Also, several *S. melliferum* strains were isolated from honey bees, bumble bees, digger bees, and the mason bee *Osmia cornifrons* [100]. Due to lack of active nursing in the nests, solitary bees are considered more susceptible to opportunistic pathogens, and it has also been shown that they can transfer plant pathogens into their nests [43]. Whether gut dysbiosis leads to enrichment in pathogens in the solitary bee gut remains unstudied. Regardless, the presence of bee pathogens on flowers suggests that pathogens shared between different pollinator species are transmitted through the use of common floral sources [101].

- Which pathogens affect solitary bees? How are they distributed within the bacterial tree of life? Are they species-specific or do they affect broader groups of bees?

Concluding Remarks and Future Perspectives

Research on solitary bee–microbial interactions is in a very early stage. The higher environmental exposure and the lack of active brood care of the offspring in the nests are factors leading to types of microbiota which are diverse and environmentally driven. These can be associated with the pollen composition, and they reflect, at least in the beginning of the larvae's development, floral microbiota, suggesting possible plant-mediated bee–microbe relationships. Several identified lactobacilli and other acidophilic bacteria may assist with dietary nutrition, even though they are not strictly host specific. Also, other bacteria have been associated with defense of bee nests against harmful microbes. Beside these insights, still significant knowledge gaps exist (see Outstanding Questions for more details). We recommend that future research should address: (i) the diversity and composition of microbiota in bee species, given the broad diversity in life history traits and geographical distribution, (ii) the functional roles of microbial components of the microbiota as symbionts supporting food processing and immune defense, or as pathogens, (iii) the interactions among environmental drivers, including human-mediated impacts of intensive agriculture, invasive species, habitat fragmentation, and climate change, and (iv) evolutionary pathways to specific host–microbiota associations. The applicability of the social-bee model of the microbiota is limited for solitary bees, and thus dedicated research is necessary to understand these complex interactions.

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