The gut microbiota of bumblebees

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Abstract

Bumblebees (*Bombus*) are charismatic and important pollinators. They are one of the best studied insect groups, especially in terms of ecology, behavior, and social structure. As many species are declining, there is a clear need to understand more about them. Microbial symbionts, which can influence many dimensions of animal life, likely have an outsized role in bumblebee biology. Recent research has shown that a conserved set of beneficial gut bacterial symbionts is ubiquitous across bumblebees. These bacteria are related to gut symbionts of honeybees, but have not been studied as intensively. Here we synthesize studies of bumblebee gut microbiota, highlight major knowledge gaps, and suggest future directions. Several patterns emerge, such as symbiont-host specificity maintained by sociality, frequent symbiont loss from individual bees, symbiont-conferred protection from trypanosomatid parasites, and divergence between bumblebee and honeybee microbiota in several key traits. For many facets of bumblebee-microbe interactions, however, underlying mechanisms and ecological functions remain unclear. Such information is important if we are to understand how bumblebees shape, and are shaped by, their gut microbiota. Bumblebees may provide a useful system for microbiome scientists, providing insights into general principles of host-microbe interactions. We also note how microbiota could influence bumblebee traits and responses to stressors. Finally, we propose that tinkering with the microbiota could be one way to aid bumblebee resilience in the face of global change.

Keywords

symbiosis; *Bombus* ; *Apis* ; microbiome; bacteria

Introduction

Bumblebees (Hymenoptera: Apidae: *Bombus*) are among the most widely appreciated and best-studied insect groups. They are important pollinators in agricultural and natural ecosystems [1,2], and have served as models in research on social evolution, plant-pollinator

interactions, thermal biology, chemical ecology, insect immunity, mimicry, and cognition (e.g., [3–9]). Because many of the >250 *Bombus* species are declining, with some approaching extinction [10,11], there is an urgent need to conserve bumblebees and the pollination services they provide.

Recently, it has become clear that bumblebees are home to characteristic and specialized gut microbial communities, known as the microbiota. Typically, only a few host-specific bacterial symbiont species dominate these communities, though nonspecific microbes—commensals, transients, and opportunistic pathogens—are also present in the gut (Fig. 1, [12–16]). The gut microbiota has been shown to protect bumblebees from infection by the common trypanosomatid parasite *Crithidia bombi* [14,17], and may provide other benefits.

These studies have established that bumblebees, along with their social corbiculate cousins, the honeybees (*Apis*) and stingless bees (Meliponini), are distinctive in harboring host-specific and beneficial gut bacteria [13,16,18,19]. Such communities are not universal among bees, much less insects in general. Unlike social bees, some insects—including certain solitary bees, ants, and butterflies—have gut microbiota that are low-density, not host-specific, and of as-yet unclear importance to their hosts [16,20–24]. In several respects, gut microbiota of social bees show closer parallels with those of mammals than those of many insects [19,25]. One common element between these two groups is sociality, which provides microbes with an intergenerational transmission route, thus facilitating host specialization.

What is known about social bee gut microbiota comes predominantly from research on *Apis mellifera*, the Western honeybee, and honeybee microbiota have been the focus of several literature reviews (e.g., [19,25,26]). But studies of bumblebee gut microbiota are increasing (Table S1), and we argue that findings from honeybees are useful, but not sufficient, for understanding bumblebees. Our review contrasts microbiota of these two groups, providing an example of how divergent life history and ecology of insects can affect symbiont diversity and function.

Our main goals are to highlight emerging patterns in the bumblebee microbiota literature, point out gaps in knowledge and recommend ways to address them, and suggest applications of bumblebee microbiota research in other fields and in conservation. We focus on the specialized bacterial communities of adult-stage bumblebee guts. Based on sampling to date, these communities appear to be a universal feature of *Bombus*. Other types of microbes that interact with bumblebees, such as parasites, pathogens, and flower microbes, have been reviewed previously [27–29] and are only briefly discussed here.

We suggest that research on other aspects of bumblebee biology could benefit from information about the gut microbiota. Microbes can influence a range of insect phenotypes [23,30,31], and they may prove useful for functional genetic studies in bumblebees [32]. Furthermore, researchers studying microbiota of other hosts might find elements relevant to their own systems, including dysbiosis, colonization resistance, and strain-level dynamics. Finally, given the importance of microbiota to parasite resistance and possibly other aspects of bee health, microbiota research has potential to benefit bumblebee conservation.

The core bumblebee gut symbionts

Mature adult bumblebees have large numbers of gut-dwelling bacteria, as shown by quantitative PCR. Estimates of the number of bacterial 16S rRNA gene copies per bumblebee gut are consistently close to ~10^8 [16,33–39]. This number equates to ~30 million cells per gut [33], or ~800 million per gram gut tissue. The density of gut bacteria in bumblebees is similar to or slightly less than that in honeybees; however, it exceeds levels in some species of stingless bees and solitary bees, as well as other insects, by orders of magnitude [16,22,40,41].

Studies based on culture-independent 16S rRNA gene sequencing have shown that the bumblebee gut microbiota is also distinctive in its composition, as compared with solitary bees and other insects. Just a handful of bacterial taxa dominate the community: *Snodgrassella*, *Gilliamella*, *Schmidhempelia*, Bifidobacteriaceae (particularly *Bifidobacterium* and *Bombiscardovia*), and two clusters within *Lactobacilliaceae* [42]: *Bombilactobacillus*, previously known as *Lactobacillus* Firm4, and *Lactobacillus* cluster near *melliventris*, previously known as *Lactobacillus* Firm5 (Fig. 1). These core symbionts are found across bumblebee individuals and species, including lab-reared bees as well as wild bees collected in Europe, Asia, and the Americas (Fig. 1). With the exception of *Schmidhempelia*, all of the core bacteria have been cultured [43–47].

It is likely that all of these core bacterial symbionts live, or at least replicate, exclusively within the bumblebee gut. Members of the genera *Schmidhempelia* and *Bombiscardovia* have been found only in bumblebees [16]; the other core bacterial genera (e.g., *Snodgrassella* and *Gilliamella*) occur in other social corbiculate bees too, but distinct host-specific lineages are associated with each bee group. There are two lines of evidence for host specificity of the core bumblebee gut bacteria. First, they form clades that are exclusively composed of taxa sampled from bumblebees [16,18,48,49]. Second, laboratory trials have shown that bumblebee-derived symbiont strains have either no ability or a very limited ability to colonize *A. mellifera* [16,50,51]. (However, colonization has not been tested in other bees).

Although not shown in Fig. 1, each core bumblebee gut bacterial taxon contains multiple sublineages, or strains. Strains are generally lumped together by the standard approach to characterizing microbiota, which sequences only short regions of 16S rRNA genes. To resolve strain diversity, one can instead sequence full-length 16S rRNA genes, faster-evolving protein-coding genes, metagenomes, or genomes of cultured isolates (e.g., [18,49,50,52]). These methods have revealed details of strain-level evolution and functional potential. For example, strain phylogenies are significantly, though not perfectly, congruent with the *Bombus* phylogeny; this suggests that the core symbionts are not only restricted to bumblebees, but have also co-diversified with them [18,49,53,54]. *Snodgrassella* strains vary in how widely distributed they are across *Bombus* species, indicating variation in dispersal or colonization capabilities [49]. Strains of both *Snodgrassella* and *Gilliamella* vary in gene sets, reflecting horizontal gene transfer and gene loss [50,53]. Some of these differences may affect host ecology; for example, strains vary widely in the presence of genes for sugar metabolism [53,55] and interbacterial antagonism [54].

Where and when symbionts inhabit bumblebees

The core symbionts appear to form large, stable populations only within guts of adult bumblebees. 16S rRNA gene sequencing of larvae has shown that they have very different bacterial communities from adults [12,56] with a high proportion of Enterobacteriaceae and a low proportion of core symbiont taxa [56]. However, this method does not distinguish between sparse and highly dense communities, nor does it determine whether bacteria are active, dormant or dead; hence, it cannot rule out the possibility that the detected taxa are only transiently present [20]. Imaging has shown that bacteria do not colonize the gut of honeybee larvae [57].

Even if present in larvae, most bacteria are eliminated by gut purging, sterilization, and reorganization during metamorphosis [58]. Indeed, if bumblebee pupae are aseptically removed from their cocoons and the resulting adults are kept in sterile conditions, they are typically devoid of culturable bacteria [50,59,60]. Molecular methods, including quantitative PCR, have also indicated that bacterial biomass in these adults is very low [14,17,37,61,62].

Symbionts are further confined in terms of where they grow within the gut. Bumblebees are probably similar to humans and some other insects in which the vast majority of microbes are contained in the distal gut [22]. In honeybees, imaging and quantitative PCR show that bacterial colonization of the crop and midgut is limited; 99% of gut bacteria are in the hindgut [57]. We lack comparable data on within-gut symbiont distribution in bumblebees, though there are some clues. Studies that include only the midgut and hindgut report similar bacterial communities to those that also include the crop, suggesting that the crop—the "social stomach"—contains relatively few bacteria. Furthermore, imaging has demonstrated hindgut colonization, at least in the proximal region, the ileum ([63], Fig. 2A,B). Symbionts are also spatially organized across the width of the gut. As in honeybees [19], *Snodgrassella* and *Gilliamella* form a biofilm coating the ileum wall (Fig. 2A).

Within-gut spatial distributions are likely governed by chemical, nutritional, and structural features of different gut compartments ([23], Fig. 2C). The bee midgut is lined by the peritrophic matrix, which is impermeable to bacteria and continually replenished [64], presumably hindering stable colonization. The midgut is also where insects digest and absorb most simple nutrients [65], leaving hindgut inhabitants with mainly undigested leftovers and nitrogenous waste entering from the Malpighian tubules (Fig. 2C). Based on data from honeybees, it is likely that gradients of oxygen and pH further regulate symbiont distribution within the bumblebee gut; conversely, such gradients are expected to be influenced by symbiont metabolism [66].

From the bumblebee's perspective, the microbes' localization to the hindgut affects what kinds of services they are capable of providing. For example, nutrients produced by symbionts in the hindgut will benefit bumblebees only if they can be absorbed. Short-chain fatty acids, typical byproducts of gut bacterial fermentation that can be used by animals as an energy source, can be absorbed in the hindguts of some insects (including honeybees) [66,67]. However, it is not clear whether the same is true for other nutrients [65]. Likewise, symbiont-mediated detoxification could be a useful function for herbivorous insects like bees [30], which have to contend with naturally occurring plant secondary metabolites [68]

as well as anthropogenic chemicals [37,69,70]. In bumblebees however, the core symbionts would only be able to perform this function if toxins are not absorbed or endogenously metabolized in the midgut (see [6]). Furthermore, even if they do reach the hindgut and its associated microbes, toxins may already have done most of their damage.

How symbionts are transmitted

The core gut symbionts of social bees are predominantly transmitted from parent colony to offspring colony. Colony-level transmission can therefore be considered as vertical, and it has resulted in symbiont specialization and co-diversification with bees over tens of millions of years [16,18]. But because symbionts do not infect eggs and are restricted to the adult stage, vertical transmission between colonies requires horizontal transmission among adults within colonies [19].

Exchange among nestmates plays out in different ways across the bumblebee life cycle (Fig. 3). In new colonies, the foundress queen inoculates the first batch of workers with her gut microbes. Afterwards, newly emerging workers and reproductives are inoculated by older workers and/or the foundress queen (the relative importance of each source to transmission is not yet clear). The colony's microbiota ultimately gets funnelled into the new queens, produced toward the end of the colony life cycle; these are the sole vectors of symbionts to the next year's colonies. At higher latitudes, where most *Bombus* species live, diapausing queens carry symbionts through the winter (Fig. 3).

Within a social bee colony, symbionts disperse from a colonized adult to a newly emerged, microbe-free adult via a fecal-oral route, but we do not know the exact path they take. In bumblebees, physical contact seems to be important for transmission of core bacterial symbionts [71], as well as *C. bombi* parasites—which are also spread through feces [72]. However, it is not clear if an active behavior is involved, such as proctodeal (anal-oral) trophallaxis in termites. As neither proctodeal nor oral trophallaxis between bumblebee adults has been observed [29,72], occupying a shared nest is likely to be sufficient for passive transmission. For example, bumblebees may ingest microbes while grooming fecal residues off the body or consuming contaminated food sources [18].

While symbiont transmission between bumblebee colonies is mostly vertical, some horizontal transmission is evident. Closely related symbiont strains are often shared between different *Bombus* species and subgenera [18,49], and *Snodgrassella* strains can be experimentally transferred between *Bombus* species [50,59]. In nature, foraging likely provides a route for symbiont exchange between colonies and species (Fig. 3). Most bumblebees are generalists, and sympatric species often overlap in the flowers they visit [73]. Bumblebees frequently defecate on flowers, depositing parasites like *Crithidia* in the process [74]. Core gut symbionts must be deposited too, but it is unclear whether they can survive on flowers and then infect new bees as effectively as the parasites [74]. Queens regularly usurp each other's nests [73], and workers often 'drift' into non-natal colonies [75]; these interactions might also facilitate horizontal transmission.

Other microbes associated with bumblebees

In addition to the core symbionts, 16S rRNA gene sequencing (Fig. 1) and culturing (e.g. [46,76,77]) have shown that bumblebee guts normally harbor a low level of non-core bacteria. Some of them, such as *Commensalibacter*, *Apibacter*, *Arsenophonus*, and more generalist species in the *Lactobacillus* cluster such as *Apilactobacillus kunkeei*, also occur in the guts of solitary bees and adult butterflies [21,78]. Unlike the core symbionts, these do not appear to have diverged into clades restricted to social bees. Non-core bacteria are more prevalent in bees exposed to the environment as compared with bees contained in the laboratory (Fig. 1, [79,80]). This observation, together with the lack of host specialization, suggests that bumblebees repeatedly acquire non-core bacteria from flowers or other environmental sources, rather than through sustained vertical transmission (Fig. 3).

Although most of the non-core bacteria are probably transient or commensal members of the gut microbiota, there are also likely opportunistic pathogens, especially certain Enterobacteriaceae (*Serratia*, *Hafnia* and others) (Fig. 1). As discussed in the next section, Enterobacteriaceae and other taxa like *Fructobacillus* often become highly abundant in conjunction with diminished levels of core bacterial symbionts (e.g., [33]).

Bumblebee guts can also harbor fungi, especially nectar-derived yeasts [46,63,81–83]. It has been suggested that many nectar yeasts are bee-specialized, depending on bees for transmission between flowers and for overwintering [81–83]. However, this specificity and dependency may be one-sided. Bumblebees sometimes lack detectable levels of gut-associated fungi [36,83]. In laboratory experiments, yeasts do not consistently colonize the gut and persist in overwintering queens [83], and they have inconsistent or negligible effects on bumblebee performance [84,85]. Some effects are similar between live and heat-killed yeasts [85], suggesting that gut colonization is not necessary to explain them. On the whole, gut-associated fungi appear to be either transients or opportunistic pathogens in bumblebees [27,36], as is the case in honeybees [81,86]. However, more work using imaging and quantitative molecular methods is needed to elucidate the role of fungi in bumblebee gut microbiota.

Besides bacteria and fungi, other microorganisms, as well as viruses, infect bumblebees. Eukaryotic parasites such as *Crithidia*, *Nosema*, and *Apicystis* often occur in the gut [27], and bacteriophages are probably abundant, as observed for honeybees [87,88]. The prevalence of these groups will become clearer once bumblebee gut metagenomes become available, as metagenomes are not limited to certain taxa (as are 16S rRNA gene profiles) nor to culturable microbes.

Microbes outside the gut may also influence bumblebees. For example, bacteria and yeasts often colonize nectar and can alter floral chemistry and attractiveness to bees [28]. Within the nest environment, microbes have long been recognized to cause spoilage of food stores [27,81]. More recently, microbial growth in food has also been hypothesized to be beneficial for bees [89]. When cultures are supplemented to the diet, some microbial species accelerate bumblebee colony development [90]—though this effect may or may not translate to increased lifetime fitness. Nectar and pollen collected by bumblebees harbor microbes [28,91,92]; however, it is not yet known whether these microbes are normally (i.e., excepting

cases of spoilage) abundant and metabolically active enough in food stores to substantially change nutritional content. As mentioned earlier, the standard method used to characterize microbial community composition—marker gene sequencing—does not provide information on total abundance, nor on activity. Other methods have shown that microbes are actually quite sparse in honeybee honey and pollen provisions, and they do not appear to pre-digest or otherwise upgrade the diet [93–95].

Symbiont loss and replacement

Although core symbionts usually dominate bumblebee gut microbiota (Fig. 1), they are frequently diminished, or lost altogether from some individuals. Given the ancient and specialized nature of the symbiosis, this is a remarkable phenomenon. It appears to be much more common in bumblebees than honeybees [19], though we do not yet know why.

Bumblebee queens seem to be particularly liable to losing core symbionts both as they enter and as they exit diapause [18,35,36,39]. For example, Bosmans et al. [36] found that some spring queens of *B. terrestris* had few or no core symbionts and instead had high levels of other bacteria and fungi. Pre-diapause changes in immunity and physiology [96,97], cold stress during overwintering, and resource limitation in the early spring may contribute to microbiota turnover in queens.

Workers also experience major shifts in their gut microbiota. Two microbial community states have been observed: the typical one in which core symbionts dominate, and another in which they are largely or entirely replaced by an erratic mix of non-core bacteria including *Fructobacillus*, Enterobacteriaceae, and others [15,33,55,80,98,99]. These alternative community states have been found in multiple *Bombus* species in Asia, Europe, and North America. We do not know what destabilizes worker bumblebee gut microbiota, but age [70,99,100] and exposure to stressors and pathogens in the environment [79,80,99] are potential drivers.

Losses of core symbionts and their replacement by non-core microbes may represent a form of "dysbiosis"—a perturbed microbiota state linked to disease or poor health outcomes in hosts [101]. At least some of the non-core microbes, like *Serratia*, belong to taxa that are common opportunistic pathogens of insects, including honeybees [102,103]. And as discussed below, the core symbionts are beneficial to bumblebees at least in terms of protection from *C. bombi*, so their loss is expected to be harmful. However, it has been suggested that the atypical enterotype could instead represent an adaptive form of plasticity, perhaps helping bumblebees cope with changing conditions [39,99]. Testing the fitness consequences of symbiont turnover, in both queens and workers, should be a priority for future work.

Life and death in the bumblebee gut

What substrates support bacterial growth in bumblebee guts? Although data are limited, we can make inferences based on gene repertoires of cultured bumblebee gut symbionts and on evidence from honeybees. As explained earlier, what the symbionts consume is different from what their hosts consume: insects digest and absorb much of the nutritional content of food in the midgut [65], leaving a limited range of metabolites for hindgut microbes

(Fig. 2C). Moreover, as discussed in more detail elsewhere [19,50,104], different symbiont species have distinct metabolic requirements and capabilities. For example, *Gilliamella* require carbohydrates, whereas *Snodgrassella* require carboxylates; these two groups live side by side (Fig. 2A) and likely engage in cross-feeding [50].

To survive in this resource-limited environment, most bumblebee-associated *Gilliamella* and *Snodgrassella* strains can synthesize all of the essential amino acids [104]; in honeybees, this capability was shown to be necessary for *Snodgrassella* to colonize the gut [105]. Amino acid biosynthesis is likely enabled by host-derived nitrogenous waste products (Fig. 2C, [50]). Complex polysaccharides from pollen walls, which are indigestible to hosts, may support *Gilliamella* and Bifidobacteriaceae to some degree. Interestingly, though, bumblebee strains seem to be less capable than honeybee strains of digesting pectin and hemicellulose and using the resulting sugars [46,51,53,104].

Gut microbes face additional threats beyond starvation. To live within the bee hindgut, a microbe must cope with the risk of being expelled (washout), temperature swings, ingested toxins, and attacks by other microbes, phage, and the host immune system. Combined with resource limitation, these challenges may contribute to the exclusive nature of the bumblebee gut microbiota. Collectively, the core symbionts of honeybees and bumblebees exhibit several traits that likely help them persist in guts, such as biofilm formation to prevent washout [105]; interbacterial toxin/antitoxin systems [54]; and resistance to heat stress [59], xenobiotic chemicals [37,69], *C. bombi* infection [106], and host immune defenses [100,107]. This robustness is presumably advantageous to bees; in some other insects, symbionts are fragile and constrain their host's ability to handle stressors (e.g., [108]).

How do symbionts help bumblebees?

Clearly, symbiont capabilities evolve to support symbiont fitness. To what degree do symbiont activities also benefit bumblebees—and what are the mechanisms behind these benefits? These questions are key to understanding the adaptive significance of microbial symbiosis to bumblebees, and to predicting how perturbing symbionts will affect bumblebee health. In theory, the microbiota could have a variety of beneficial effects, from gut-centric functions like digestion, detoxification, and defense from pathogens, to more peripheral processes like behavior [22,23,30,31]. In bumblebees, however, most of these roles remain speculative.

In terms of general nutrition, gut symbionts do not appear to be obligate for bumblebees, unlike certain other insects [23]. Microbiota-free bumblebees do not have lower survival rates than microbiota-colonized bees, at least for *B. terrestris* and *B. impatiens* under conditions with plentiful food and no parasites [14,37,109]. This outcome would be expected if bumblebees fulfill basic nutritional needs themselves. In line with this possibility, there is a hint that bumblebee gut microbiota have reduced digestive capabilities as compared with the honeybee gut microbiota. Honeybee gut symbionts break down pollen polysaccharides and are thought to supply their host with metabolic end products, like short-chain fatty acids [26,50,66]; in contrast, bumblebee symbiont strains have a much more limited repertoire of polysaccharide-degrading enzymes [46,51,53,104]. However, additional experiments on bumblebees are needed, especially under natural conditions and

on other *Bombus* species—which may differ substantially in feeding and nutrition-related traits [110].

Resistance to *C. bombi*, a prevalent and well-studied trypanosomatid parasite of bumblebees [29], is the only bumblebee gut microbiota function that has been clearly and repeatedly demonstrated. Bumblebees colonized by the microbiota are more resistant to *C. bombi* than microbiota-free bees, and experimentally varying microbiota composition changes infection outcomes [14,17,34,106]. Indeed, protection from microbial enemies (colonization resistance) is one of the most widespread functions of gut microbiota [22], and it may have been the primary selective advantage driving the evolution of symbioses between social bees and gut microbes. One bee colony collectively visits huge numbers of flowers [73], increasing its exposure to foodborne parasites and pathogens. As a supplemental immune defense, gut microbes might thus be particularly useful for social bees—and especially so in bumblebees, which are usually monandrous [73,111] and therefore have low genetic diversity within colonies. It should be noted, however, that microbiota effects on other bumblebee parasites and pathogens [27,29] have not yet been tested.

Even in the well-studied case of microbiota–*C. bombi* interactions, we do not know how symbionts confer colonization resistance. There are several possible mechanisms. First, the symbionts may form a physical barrier to *C. bombi* colonization [106]. The hindgut wall is normally coated in a bacterial biofilm (Fig. 2A), and this is where *C. bombi* needs to attach in order to persist [6]. Second, *C. bombi* is sensitive to low pH, so acids produced from bacterial fermentation might play a role [112]. Third, symbionts may outcompete parasites for nutrients. A fourth and somewhat distinct potential mechanism is symbiont induction of bee immunity [107,113]. Put another way, regulation of immune defenses goes awry in the absence of signals or nutritional inputs from the gut microbiota. This outcome might result from evolutionary "addiction" [22], where endogenous host processes become dependent on the presence of microbiota for normal functioning.

Beyond nutrition and defense, the gut microbiota may interact with behavior, presenting a potential example of the "gut-brain axis" in insects [114]. Social bees could be a good model in this regard, given their sophisticated cognitive capabilities and the well-established protocols available to study them (e.g., [3,115]). In bumblebees, behaviors such as social interactions, thermoregulation, and foraging [59,73,116] are likely to influence the acquisition and maintenance of gut microbes. But do gut microbes influence behavior? There is some precedent in honeybees, for which the gut microbiota can alter sucrose sensitivity (and hence motivation to feed) [66], as well as nestmate recognition involving cuticular hydrocarbon profiles [117]. In bumblebees, one study did not find a difference between microbiota-free and colonized individuals in performance on an associative learning assay [109], but more work is needed.

A number of features of the bumblebee microbiota make it possible to experimentally test what symbionts do and how they do it. A key advantage is the ability to create microbiota-free bees semi-naturally—without the need for antibiotics—and then inoculate them with cultured symbiont strains [50,59,60], feces, or gut homogenate (e.g. [14,34,37]). (Antibiotics rarely remove all microbes and may also be toxic to the bees themselves

[14,61,118]). Gnotobiotic bees, those colonized with defined mixtures of symbionts, will be particularly valuable in allowing us to test the specific microbial taxa underlying a given bumblebee phenotype. The aforementioned functional experiments manipulated whole microbial communities, but symbiont species and strains may differ in function. For example, in honeybees, *Snodgrassella* alone may increase susceptibility to *Lotmaria*, a trypanosomatid parasite related to *Crithidia* [119]. Genetic engineering of bumblebee gut symbionts [120] will also be an important tool, providing information about the molecular mechanisms underlying microbiota function.

Comparative evolution of bumblebee microbiota

To fill in knowledge gaps about bumblebee microbiota, it is useful to temporarily substitute findings from the better-studied microbiota of Western honeybees, as we have occasionally done here. After all, *Bombus* and *Apis mellifera* are both corbiculate bees with some shared ecological traits (e.g. generalist foraging, eusociality) [73] and core gut microbial taxa related by common descent [16]. But there is evidence that the two bee groups' gut symbionts have diverged in several ways (Table 1). Most notably, as compared with *A. mellifera*, bumblebees have lower strain-level diversity within individuals [49] and their symbionts have a smaller enzymatic repertoire for degrading plant polysaccharides and metabolizing diverse sugars [51,53,55,104].

We suggest that differences in host biology (Table 1) explain why these microbiota differences evolved. For example, in the annual life cycle of most bumblebee species, the microbiota is funnelled through the bottleneck of a single queen (Fig. 3), constraining the size of gut symbiont populations that can be transmitted and thereby limiting symbiont strain diversity [49]. In contrast, honeybees are perennial and reproduce by swarming [121]. Thousands of workers collectively seed a new colony's microbiota, thus permitting larger and more diverse symbiont populations to be transmitted. Honeybee queens play no part, as they lack the core gut symbionts of workers [122].

Other life history differences between honeybees and bumblebees may contribute to the observed differences in digestive capabilities of the gut microbiota (Table 1). One hypothesis pertains to foraging. Honeybees (unlike bumblebees) collect a large surplus of food [121], enabling storage for colony survival during winter or other periods of nutrient scarcity, but imposing high energetic demands. Honeybees may be more dependent on gut symbiont strains that can efficiently convert recalcitrant plant components into a supplemental energy source (e.g., short-chain fatty acids). A second hypothesis pertains to larval feeding strategies. Bumblebee larvae are primarily fed honey and pollen, but honeybee larvae are fed large amounts of nutrient-rich glandular secretions by nurse bees [121,123]. As compared with foragers, nurse honeybees harbor more polysaccharide-degrading and fermentative gut bacteria [124]. Digesting not only for one's self, but also for one's siblings, may require extra assistance from microbes.

Testing these and other hypotheses about the causes of cross-host taxon microbiota differences will benefit from including a broader diversity of bee species, from *Bombus* and other groups. To date, the literature on bumblebee microbiota is skewed towards *B. terrestris* and *B. impatiens* (Table S1). Cuckoos (brood parasites) and species in tropical,

arid, and polar biomes are particularly understudied (Table S1). Such species are distinct from typical temperate *Bombus* in terms of their ecology and life history [73,110,125,126]; characterizing their gut symbionts more thoroughly might reveal new associations between host and microbiota traits. Stingless bees, which are more closely related to bumblebees than are honeybees [127], are also understudied. Their microbiota appear to be quite variable, as compared with those of *Apis* and *Bombus* [16]; for example, some species have permanently lost *Snodgrassella* and *Gilliamella* [128]. Further exploration of bumblebees and stingless bees will help us better understand the origins of social bee microbiota and consequences for bee biology.

Applications for basic research and conservation

Gut symbionts could be employed as a tool for bumblebee genetics. Tests of gene function in social bees largely rely on RNA interference (RNAi), whereby double-stranded RNA (dsRNA), with a sequence matching the gene of interest, is fed or injected. However, feeding or injecting dsRNA can give erratic or ephemeral results. A new approach for bee RNAi, using genetically engineered *Snodgrassella* to synthesize dsRNA, has been validated in honeybees [32]. A transgenic *Snodgrassella* strain induced RNAi, knocking down expression of target genes throughout the honeybee body and also in varroa mites. Symbiont-mediated RNAi could be extended to bumblebees and perhaps even to their parasites.

The gut microbiota is also relevant to bumblebee conservation. It would be useful to know when symbionts do or do not mediate the effect of a given stressor on bees; if they do, microbiota-based interventions could be effective. For some stressors, such as heat, insecticides, and land-use change, the gut microbiota does not seem to be a major intermediary through which bumblebee populations are impacted [59,70,98]. However, parasites are another driver of bumblebee declines [129], and the gut microbiota has a well-established role in providing parasite resistance (at least against *C. bombi*). Given that some microbiota compositions provide more resistance than others [17,34,106], select symbiont strains could be used as probiotics to help defend at-risk bumblebee populations from parasitism. Microbes should also be considered in the planting of 'medicinal' flowers for bumblebee forage, as they might exert their beneficial effects through changes to the gut microbiota [130].

Bumblebees as a system for host-microbiota research

The gut microbiota of social bees have a number of convergent traits with other systems, including humans, some other mammals, and termites. These include social transmission, localization to the distal gut, degradation of plant polysaccharides with fermentation products absorbed by hosts, and, in some cases, colonization resistance against pathogens [19,25]. With bees, we can create gnotobiotic hosts (Fig. 2B) relatively easily and in large numbers, automatically track individual behavior [116], experimentally perturb the microbiota *in vivo*, and conduct other manipulations that would be impractical in many hosts.

Bumblebees have some advantages as a study system. In many countries, bumblebee colonies can be purchased and used for experiments year-round, while work with honeybees

is more seasonally limited. Bumblebee colonies can also be kept fully contained indoors; this is not only convenient but also allows diet to be controlled, and genetically engineered microbes to be introduced (with required containment measures are in place). Unlike honeybees, bumblebees are a diverse group (>250 species [73]), enabling large-scale comparative research. Bumblebees are also particularly well suited as a model for studies of mammalian microbiota phenomena such as dysbiosis and hibernation effects.

Outlook

We have an increasingly detailed picture of which microbial species inhabit bumblebee guts, but major gaps remain. The majority of gut microbiota research is on *B. terrestris* and *B. impatiens* (Table S1)—common, commercially reared, temperate-zone species. Species in different biomes and those with different feeding traits (e.g., oligolectic and pocket-making species [110]) need to be included. Declining *Bombus* species should be a particular priority, and could be studied using non-destructive fecal sampling. There is great interest in the factors that make these species vulnerable (e.g. [11,131]); the gut microbiota could be one such factor.

Even in *B. terrestris* and *B. impatiens*, we know very little about gut microbiota function beyond protection from the parasite *C. bombi*. This information would help explain why bumblebees acquired gut symbionts and retained them for tens of millions of years. It would also reveal whether the loss of core symbionts, as observed widely in members of many bumblebee species, is an indicator of stress or population decline. To support bumblebee health, we may be able to deploy probiotics derived from carefully selected and naturally occurring gut symbionts. Ultimately, these ancient denizens of the gut will help us better understand, and perhaps conserve, bumblebees.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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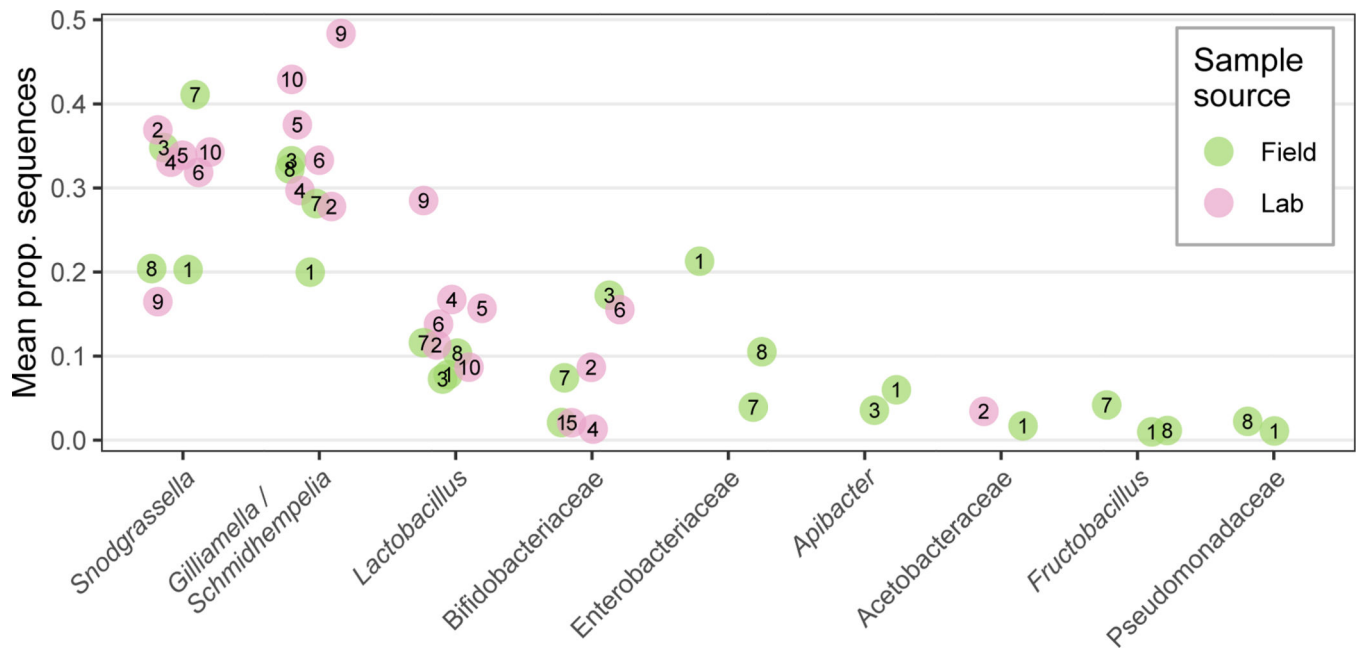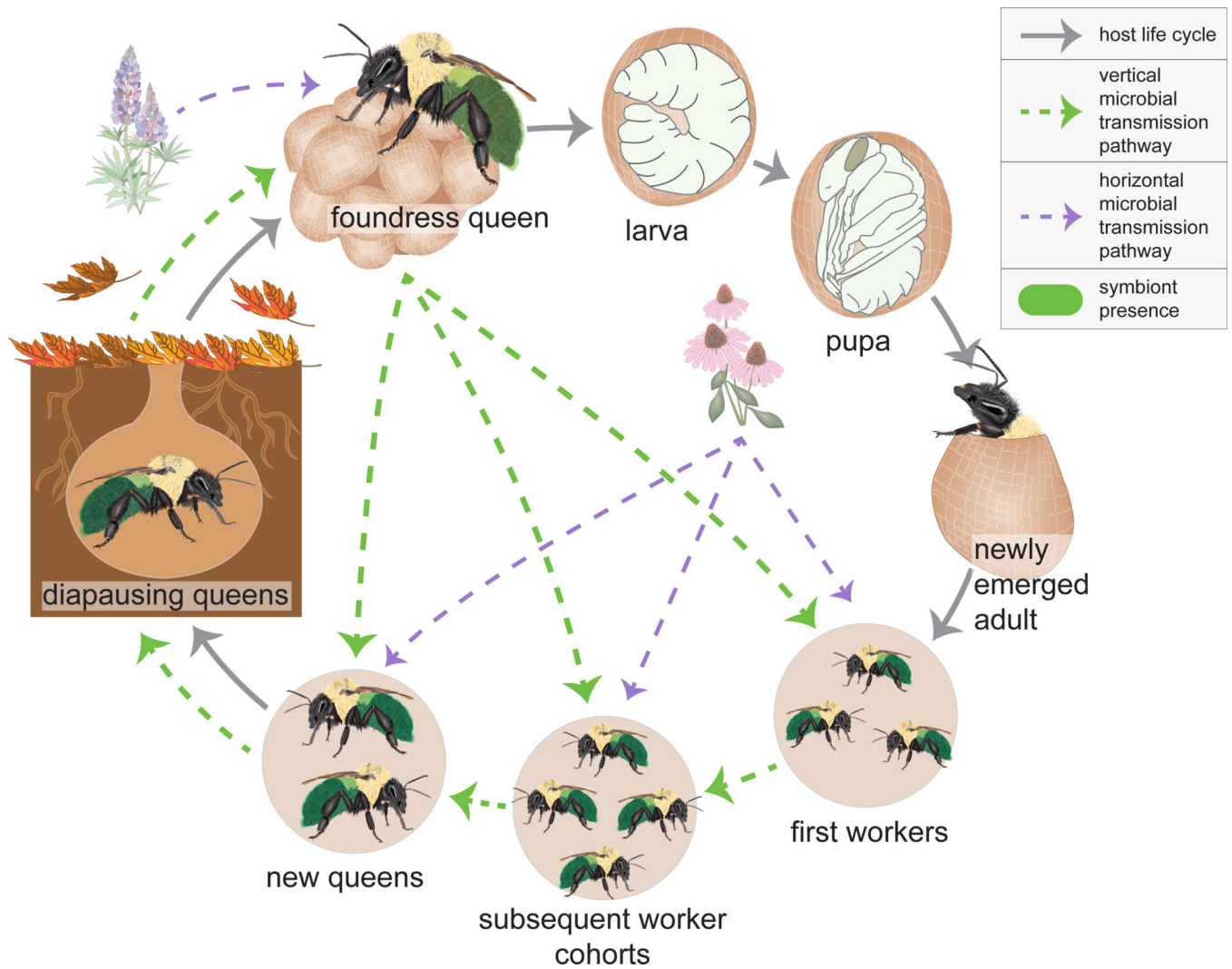
Figure 1.
Common bacteria in bumblebee gut microbiota. Dots represent the mean proportion of the microbiota (16S rRNA gene sequences) for each taxon across individuals. Numbers indicate datasets referenced in Table S1. Field samples refer to bees that were either wild-caught or reared with access to the field. Lab samples refer to indoor-restricted bees. Note that the *Lactobacillus* cluster contains both non-core species (e.g., *Apilactobacillus kunkeei*) as well as core lineages (*Bombilactobacillus* and *Lactobacillus* Firm5) [42]; these are not always distinguishable in sequencing datasets, but the latter usually predominate in bumblebee guts. See Supplementary Methods for details.
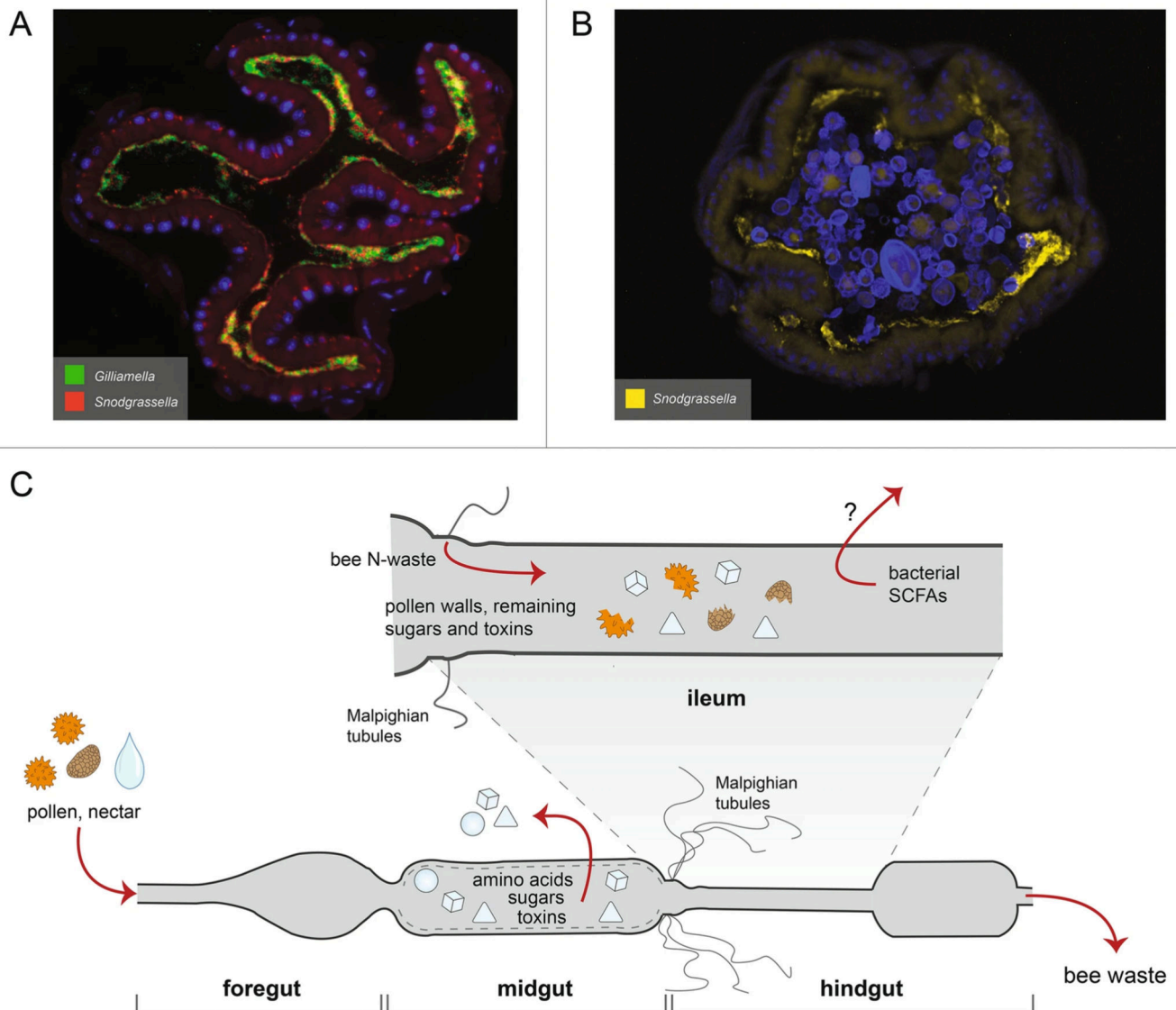
Figure 2.

Distribution of symbionts and metabolic processes across and along the bumblebee gut. A) Fluorescence *in situ* hybridization of a cross-section of the ileum from a conventional *Bombus impatiens* worker (see Supplementary Methods). Host nuclei are shown in blue. B) Same as A), but this image is from a gnotobiotic worker colonized with the core symbiont *Snodgrassella*. Round objects in the gut lumen are autofluorescing pollen. C) Digestion and nutrient transport in a simplified depiction of the bumblebee gut. Transport of short-chain fatty acids (SCFAs) across the hindgut wall is tentative in bumblebees. Note that the hindgut likely contains the large majority of bacterial cells in the bumblebee gut (see text).

Figure 3.
Transmission routes of core gut symbionts across the life cycle of a typical temperate-zone bumblebee species. (Tropical and arctic *Bombus* life cycles can differ in some respects [125]). Vertical transmission predominates, but horizontal transmission of core symbionts also occurs. Non-core microbes may also follow these routes but are not shown.

Table 1.

Some features of bumblebees and their gut microbiota that differ from features of honeybees. We suggest that divergent host traits shape, and may be shaped by, microbiota differences.

Host traits of *Bombus* relative to *Apis*

- collect more protein-rich pollen [132]
- annual life cycle with solitary founding queens [73]
- less genetic diversity within colonies [111]
- less defined division of labor among workers [133]
- limited production of surplus food reserves [5]
- origin and highest diversity in cold areas [134]

Microbiota traits of *Bombus* relative to *Apis*

- more prone to loss of core symbionts [19]
- less prevalent type VI secretion systems [54]
- less tolerant of high heat exposure [59]
- fewer genes for monosaccharide utilization [53]
- fewer genes for polysaccharide breakdown [51,104]
- lower species-level diversity [16]
- lower strain-level diversity [49]