

Review Article

Parasite defense mechanisms in bees: behavior, immunity, antimicrobials, and symbionts

 Alison E. Fowler¹, Rebecca E. Irwin² and Lynn S. Adler¹

¹Department of Biology, University of Massachusetts Amherst, Amherst, MA, U.S.A.; ²Department of Applied Ecology, North Carolina State University, Raleigh, NC, U.S.A.

Correspondence: Alison E. Fowler (aefowler@umass.edu)

Parasites are linked to the decline of some bee populations; thus, understanding defense mechanisms has important implications for bee health. Recent advances have improved our understanding of factors mediating bee health ranging from molecular to landscape scales, but often as disparate literatures. Here, we bring together these fields and summarize our current understanding of bee defense mechanisms including immunity, immunization, and transgenerational immune priming in social and solitary species. Additionally, the characterization of microbial diversity and function in some bee taxa has shed light on the importance of microbes for bee health, but we lack information that links microbial communities to parasite infection in most bee species. Studies are beginning to identify how bee defense mechanisms are affected by stressors such as poor-quality diets and pesticides, but further research on this topic is needed. We discuss how integrating research on host traits, microbial partners, and nutrition, as well as improving our knowledge base on wild and semi-social bees, will help inform future research, conservation efforts, and management.

Introduction

Bees are important pollinators in both natural and agricultural ecosystems, but populations of many species are at risk due to interacting stressors such as limited food resources, habitat loss, pesticides, and parasites [1]. Emergent parasites are becoming increasingly important as bees are transported for pollination services, thus spreading parasites to new geographic ranges and hosts [2]. The parasites of bees are taxonomically diverse, ranging from metazoans such as nematodes and mites, to microbes such as protists, fungi, bacteria, and viruses. Additionally, different parasites threaten bees during larval and adult life stages, and different factors affect outcomes at particular stages [3]. Recent reviews have synthesized current information on bee susceptibility to environmental stressors [4], viruses [5] and fungal parasites [6], medicinal diets [7], and the impacts of parasites on bee foraging behavior [8] and cognition [9]. Here, we evaluate recent literature on bee defense mechanisms against parasites and pathogens, such as behavioral, chemical, and immunological adaptations, as well as associations with beneficial microbial symbionts (see Table 1 for summary and definitions). We discuss how those mechanisms are negatively impacted by stressors such as low-quality diets and pesticide exposure. We use the term ‘parasite’ to refer to both macro-parasites such as parasitoids as well as microbes, although we focus in this review primarily on microbial parasites. While important, cleptoparasites were outside the scope of this review, although we address some relevant literature on the topic.

Bees have diverse life-history strategies that impact their risk of parasitism. While the order Hymenoptera is known for its eusocial insect clades, the majority of bee species are solitary or lack clearly defined castes [10]. There are also many gradations of sociality in between eusocial and solitary, including sub-social, semi-social, and facultatively social (collectively ‘presocial’ [11]; Table 2). Social behavior affects parasite transmission and thus has important consequences for the adoption of defense mechanisms, and so our review will highlight differences between host taxa that differ in

Received: 15 August 2019
Revised: 14 November 2019
Accepted: 26 November 2019

Version of Record published:
16 December 2019

Table 1. Summary of defense mechanisms against microbial parasites in bees, separated into three non-mutually exclusive categories: behavioral, immunological, and chemical.

Defense strategy	Definition	Bee species	References
Behavioral			
Allogrooming	Grooming between members of a colony; can remove parasites from grooming recipient but also transmit parasites to groomer	<i>Apis mellifera</i> <i>Bombus</i> spp.	[38,117]
Aggression towards potentially infected nestmates	Bees were stung and bitten more when coated with cuticular secretions from an immune-challenged nestmate	<i>Apis mellifera</i>	[38,118]
Removing/killing infected brood	Bees may remove larvae or fill cells with soil if they are infected with a parasite	<i>Apis mellifera</i> <i>Halictus</i> spp. <i>Lasioglossum</i> spp. <i>Evylaeus</i> spp. <i>Nomia melanderi</i>	[119] and references therein, [120]
Hive fever	Increased colony temperature via thorax vibration	<i>Apis mellifera</i>	[121]
Propolis production	Propolis is a mixture of wax and resin; seals off crevices, protects developing brood, and lowers bee immune gene expression	<i>Apis mellifera</i>	[122]
Immunological			
General insect immunity	Recognition and response to invader; production of AMPs; results in phagocytosis and encapsulation	All bees	[123]
Antimicrobial peptide (AMP) production	Synthesized in the fat bodies, transported via hemolymph, and degrades microbial invaders	All bees	[124]
Melanization and encapsulation	Melanin deposition around a foreign body	All bees	[125]
Immunization	Protection of the host against a parasite upon secondary exposure via a specific immune memory (i.e. immune priming) or nonspecific immune up-regulation	<i>Apis mellifera</i> <i>Bombus</i> spp.	[126]
Transgenerational immune priming (TGIP)	Mother's exposure to a parasite results in a protective phenotype in offspring	<i>Apis mellifera</i> <i>Bombus</i> spp.	[40]
Chemical			
Cuticular secretions	Compounds secreted by the cuticle, many of which have antimicrobial properties	All bees, but varies between species	[25]
Dufour's gland	Gland near the venom duct that produces chemicals	All bees, but function varies	[34]
Collection of exogenous materials (plant products)	Many bees collect plant leaves, resins, and oils, which protect their nest. This may also be considered a 'behavioral' defense.	Megachilidae (leaves and resins) Melittinae (oils) <i>Apis</i> (resins) <i>Austroplebeia</i> and <i>Tetragonula</i> (resins), <i>Anthidium</i> (trichomes)	[7,33,122,127–129]

sociality. While most studies on bee parasitism and immunity have been in eusocial honey bees (*Apis mellifera* [12]) and bumble bees (*Bombus* spp. [13]) due to their tractability as study organisms and their importance in agriculture, parasites have been identified in many solitary and presocial bee species [14,15]. Studies on the defense mechanisms such as immunity are scarce for non-eusocial species (Table 3), and deserve further attention, given that many of those species provide valuable pollination services [16] and could serve as parasite reservoirs for other host species [17].

Recent advances in genomics and transcriptomics have informed our perspective on bee health, but most of these studies have yet to be summarized or connected to related disciplines such as genetics, physiology, or landscape ecology. First, we summarize new ideas about whether the evolution of social behavior in insects

Table 2. Levels of social organization in bees. Note that some levels, such as communal and quasisocial, are not referenced in the text but are included here for completeness. Modified from reference [11].

Category	Subcategory	Definition
Eusocial	Highly eusocial	Multiple females co-operate in nesting, exhibit reproductive division of labor, and overlapping generations. Colonies are relatively large and long-lived, reproductive females (queens) are morphologically and developmentally distinct from non-reproductive females (workers)
	Primitively eusocial	Colonies are relatively small and short-lived, and morphological differences between queens and workers are minimal (i.e. body size) or non-existent
Presocial	Semi-social	Females exhibit social behavior beyond sexual interactions, yet short of eusociality. Some females lay most or all eggs. Other females are relegated to foraging, nest building, and caring for the young
	Quasisocial	Some females lay eggs, but all females co-operate in brood care and share a nest.
	Sub-social	Females nest alone but interact with their developing larvae by progressive provisioning and may interact with adult offspring
	Facultatively social	Exhibit social behavior in certain environmental contexts
Solitary	Socially polymorphic	Females from a single population can produce either social or solitary nests
	Communal	Individuals share a nest site with others of the same generation; no cooperative brood care
		Each female is reproductive, constructs and mass-provisions her own nest, and does not interact with offspring

impacts individual-level immunity. Second, we review our current understanding of immunization and immune priming in bees. Third, we discuss recent findings on the diversity and function of bee-associated microbes that play roles in defense against parasites. We end by proposing future directions.

Parasite defense and sociality

Organisms must evolve to avoid, resist, and/or tolerate parasite infections. These outcomes are achieved by various defense mechanisms, and different bee taxa have evolved different types and combinations of mechanisms (Table 1). Social behavior in bees has had large implications for the evolution of defense. Eusocial species, for example, generally have group-level as well as individual-level defenses, while solitary species have individual-level defenses only, although this varies with the level and evolutionary history of sociality in the species (Table 2). Group defenses are primarily behavioral, while individual defenses are chemical and immunological (Table 1). There are multiple hypotheses about how group-level defenses might affect selection for individual-level defenses, and recent analyses of whole genomes and gene expression profiles provide new insights into some of these hypotheses.

The social group hypothesis predicts that social insects have higher individual-level defenses than solitary insects due to increased contact and genetic relatedness among individuals and thus increased disease risk [18].

Table 3. Studies on the immune system of bees other than *Apis* and *Bombus*.

Bee species	Topic/immune metric	Reference
<i>Agapostemon virescens</i>	Encapsulation	[29]
<i>Halictus ligatus</i>	Encapsulation	[29]
<i>Xylocopa virginica</i>	Encapsulation	[29]
<i>Megachile rotundata</i>	Immune gene number	[31]
<i>Megachile rotundata</i>	Immune gene expression under fluctuating temperatures	[130]
<i>Megachile rotundata</i>	Immune gene expression under parasite challenge	[131]
<i>Osmia cornifrons</i>	Antimicrobial activity of a serine protease inhibitor	[132]
<i>Osmia bicornis</i>	Hemolymph and fat body antimicrobial activities differences between sexes and across ages	[133]

For example, some animals respond to crowding by up-regulating their immune responses, a process known as density-dependent prophylaxis [19]. This is documented in lepidopteran larvae [20], but social adult termites lack this plasticity [21]. Interestingly, more similar to the caterpillars than the termites, worker bumble bee adults increase immune activity in group settings compared with solitary settings [22,23] and with colony age and density [24]. Additionally, eusocial bee species (*Exoneurella tridentata* and *Trigona carbonaria*) and presocial species (*Exoneurella robusta* and *E. nigrescens*) exhibit stronger individual-level defenses in the form of cuticular antimicrobial compounds than solitary species (*Amegilla asserta* and *A. bombiformis* [25]). These studies support the social group hypothesis because individual bees living in social groups (both within and across species) exhibit higher individual defense mechanisms than those in isolation.

Alternatively, the relaxed selection hypothesis predicts that group-level behaviors such as allogrooming (Table 1) reduce parasite risk and thus reduce selection for individual-level defenses [18,26,27]. This hypothesis was inspired by documentation that termites that were inoculated with fungal spores had lower survivorship when isolated from the group [28]. Another study compared individual immunity (encapsulation response; Table 1) of multiple bee and non-bee insect taxa ranging in social behavior and colony size. While they found no significant difference between the binary categories of social and non-social groups, individuals from larger colonies had lower encapsulation responses, suggesting that insects living in large groups may not rely as heavily on individual-level immunity [29]. Taken together, these studies suggest that group size impacts selection for individual-level defenses, but the outcome varies with species. Perhaps insects with social plasticity invest more in individual defense plasticity, whereas eusocial insects with large colonies have lower constitutive individual defenses due to investment in group-level behaviors (Table 1).

Analysis of immune genes may shed light on how the adoption of social behavior has impacted selection for individual-level defenses. The honey bee was one of the first insects to have its genome sequenced and at the time (2006), there were few other insect genomes to compare it to (*Drosophila melanogaster*, *Anopheles gambiae*, and *Manduca sexta*). The honey bee had about one third the immune genes as these other insects, which are all solitary. The authors hypothesized that the evolution of sociality reduced the selection of immune genes and led to immune gene loss [18]. However, the rapid increase in the number of organisms with sequenced genomes, including other Hymenoptera, has informed our interpretation of this finding. The loss of immune genes likely predates the split between bees and ants since many ants and a solitary bee species (*Megachile rotundata*) also have few immune genes [30,31]. Fewer immune genes likely result in a simpler or reduced individual immune response and may have led to positive selection for group-level defense mechanisms via sociality, rather than sociality resulting in loss of immune genes [31]. Research on immunity in presocial bee species (and other social Hymenopterans) would shed light on how immunity correlates with social behavior.

If fewer immune genes result in reduced individual immune response, social bees have evolved a range of group behaviors that appear to compensate for this loss. For example, honey bees exhibit group-level behaviors such as propolis production and hive fever (Table 1). However, if all bees have fewer immune genes, what defensive adaptations have other bee species acquired to compensate for this deficiency? If solitary and presocial bees lack common immune genes and group-level defenses, they may rely disproportionately on exogenous materials for protection, such as plant metabolites or other plant products. For example, resins collected by leafcutter bees can produce a hydrophobic nest lining that protects against fungal growth [32], while other products such as leaf trichome secretions can defend against brood parasites, but not mold growth, in wool carder bee nests [33]. We speculate that leaf secondary compounds may provide protection to megachilid species that collect and line their nest with leaves, although this has not been explored yet. The reliance of these bee taxa on exogenous materials may make them more susceptible to parasites when such materials are unavailable. Interestingly, the Aculeates (ants, bees, and wasps) have the chemical-producing Dufour's gland (Table 1), which arose sometime before the loss of the immune genes and serves many different functions across these taxa [34]. In many ground-nesting bees, Dufour's gland secretes compounds that create a hydrophobic brood cell lining and thus protects developing brood and pollen provisions from microbial growth [34]. The use of exogenous materials and Dufour's gland may be adaptations that allow solitary and presocial bees to ward off parasites with fewer immune genes.

Future work examining intraspecific variation may inform how social behavior affects the immune response, such as studying differences between castes of the same species and individuals at different life stages. For example, male bumble bees are more 'solitary' than female queens and workers. Males typically leave the nest immediately to seek mates, like many adult solitary insects. Comparing male and female bumble bees may shed

light on the need for immune defense in social and solitary contexts within a single species. We might expect males to have lower immune function than females since males are not engaged in as much social contact in the nest. Additionally, queen bees undergo a solitary portion (mating, over-wintering, and foraging for brood provisions) and a social portion (maternal care of adult offspring) of their life cycle. Rehan et al. [35] compared brain transcriptomes of females of a sub-social bee species, *Ceratina calcarata*, which generally follows this life cycle pattern [35]. They found that some immune genes (*hymenoptaecin*) were up-regulated in spring, 'solitary' mothers, while others (*apidaecin*) were up-regulated in autumn, 'social' mothers. While *C. calcarata* does not have colonies as large or dense as eusocial species, it still exhibits parent-offspring and offspring-offspring interactions, making it an excellent study organism to further investigate the evolution of social behavior and immunity. Performing similar studies on immune function in bees across life stages would improve our understanding of how the immune function depends on social interactions and parasite risk. This could help us understand when female bees are most susceptible to stressors, which can improve strategies for mitigating population declines.

Immunization and transgenerational immune priming

Immunization is the protection of the host against a parasite upon secondary exposure via two mechanisms: a specific immune memory (i.e. immune priming) or nonspecific immune up-regulation (Table 1). Social insects may be more dependent on immunization than solitary insects because they are more prone to repeated exposure to microbial parasites established in a colony [36]. There is evidence for immunization in numerous social insects including bumble bees [36,37]. In bees, immunization may occur through allogrooming of nestmates. Honey bees increase allogrooming behaviors when nestmates' immune systems are challenged [38], which removes parasites but may also prime the groomers against potential infection. However, close contact via allogrooming could also increase parasite transmission.

Transgenerational immune priming (TGIP; Table 1) is thought to be particularly beneficial for social or multivoltine insects (those that produce two or more broods per season), where offspring are reared in the same environments as their mothers and likely face similar pressure from parasites [39,40]. For example, bumble bee queens directly secreted antibacterial factors onto their eggs after a non-pathogenic immune challenge [41]. Additionally, healthy bumble bee workers born from an immune-challenged queen expressed immune genes nearly identical to workers that were themselves challenged [42]. In honey bees, workers from an immune-challenged mother had lower mortality and three times more differentiated immune cells than workers from a naïve mother when challenged with a heat-killed bacteria (*Paenibacillus larvae* [43]). TGIP should be tested in non-eusocial, multivoltine bee species (such as some carpenter bees) to better understand the factors that select for this trait.

The ecological costs of TGIP are largely unexplored in bees [44]. Caterpillar (*M. sexta*) offspring from a primed mother, that were not themselves challenged, grew faster but laid fewer eggs than offspring from a naïve mother [45], highlighting the potential costs of TGIP in fluctuating environments. The movement of managed honey bees has introduced parasites to native bees [46,47]. Thus, it is crucial to consider immunization and TGIP to predict how native bee populations will fare in the face of new and variable parasite exposure.

Immunity is compromised by low-quality diets and pesticides

Diet

Stress compromises many physiological processes that can exacerbate infections. The immune system is costly and thus sensitive to stressors such as nutrient limitation. In adult bumble bees, an immune challenge increased sucrose consumption [48] and mortality when compensatory consumption was not allowed [49]. Worker bumble bees which had no access to pollen had up-regulated fewer and less specific immune genes when infected with a gut parasite (*Crithidia* sp.) than bees fed pollen [50], but food limitation did not affect the general encapsulation response in workers [51].

While access to enough food is essential for bees to mount an immune response [50], diet composition can also impact immune activity. In honey bees, immunocompetence and glucose oxidase activity (which disinfect the brood and hive environment) were improved with polyfloral compared with monofloral diets, but were not affected by protein content [52]. Similarly, another study found that protein content in different types of pollen

did not impact the immune response in adult bumble bees [53]. These findings are somewhat surprising because dietary protein increased immune activity in *Spodoptera littoralis* caterpillars [54]. The role of dietary protein may be different for immune function in larval and adult insects or in different insect taxa. Most research on bees is conducted with adults, whereas Lepidoptera immunity research often focuses on larvae; it would be helpful if research is carried on the immune response across different life stages, and compare it with other insect systems such as *Drosophila* where immunological research has been conducted on both larval and adult stages.

While monofloral diets and starvation may exacerbate infections through immune deficiency, other diets can reduce infections and increase immune activity. For example, honey bee immune gene expression was bolstered in bees fed certain monofloral pollens such as *Erica* and *Rubus* [55] and *p*-coumaric acid, a structural compound in pollen and honey [56]. In other cases, certain diets reduced infections. For example, *Crithidia* infections were reduced in bumble bees fed secondary compounds found in nectar [57–59], but whether this is due to direct inhibition or the host immune system is unclear. Monofloral pollens from sunflowers (*Helianthus annuus*) and goldenrod (*Solidago* spp.) also reduced *Crithidia* infection in bumble bees [60,61]. However, extracts from sunflower and other pollens increased parasite growth *in vitro*, suggesting that the host mediates parasite inhibition [62]. Additionally, *Nosema* (an intracellular spore-forming fungus) infection was reduced in honey bees fed sunflower honey or pollen [60,63], and *Rubus* pollen increased survivorship of honey bees infected with *Nosema* [55]. Interestingly, honey bees preferentially fed on sunflower honey and their preference for it increased with *Nosema* infection intensity [63]. These studies highlight the important role that diet quality plays in parasite resistance in bees.

We currently have little understanding of how dietary components such as protein and secondary chemistry affect parasite resistance in solitary bee species. Diet does, however, impact development and brood parasitism in the solitary bees that have been studied thus far. Several solitary bee species are Asteraceae specialists, despite the low protein content of the pollen. One explanation for this apparent paradox may be protection against brood parasites. *Osmia* that specialize on Asteraceae plants (*Osmia* (*Helicosmia*) *coloradensis*, *Osmia* (*Cephalosmia*) *montana*, and *Osmia* (*Cephalosmia*) *subaustralis*) experience less brood parasitism by wasps than Fabaceae-specialists (*Osmia* (*Hapsidosmia*) *iridis*) or generalists (*Osmia* (*Melanosmia*) *tristella* and *Osmia* (*Melanosmia*) *tersula*), potentially because the bees have acquired some adaptation to develop on this low-protein diet, while the wasps have not [64]. Diet composition and quality are important for bee growth, development, and immunity, and further studies on the impact of diet on solitary bee parasite infection in both adults and larvae are needed.

Pesticides

Chemical pesticides (insecticides, herbicides, and fungicides) have lethal, sub-lethal, and indirect negative effects (e.g., reducing available forage) on bees. One sub-lethal impact is via immunosuppression, which can lead to increased mortality when combined with parasite exposure or other stress. Bees are exposed to pesticides through pollen and nectar, as well as through the soil for ground-nesting bees [65]. Recent reviews have explored physiological mechanisms of immunosuppression by pesticides in bees, such as reducing hemocyte function [66], and the positive correlations between pesticides and viral occurrence in honey bees [67].

These reviews highlight links between pesticide exposure and susceptibility to parasites; however, relatively few studies have linked pesticide exposure directly to immune function. Work on honey bees has found that neonicotinoids and certain fungicides reduce immune and detoxification gene expression, with the most well-documented effects on viruses and *Nosema* [68–70]. Exposure to a common neonicotinoid in bumble bees reduced constitutive levels of the immune enzyme phenoloxidase, maintenance of antimicrobial activity in the hemolymph after an immune challenge, and survivorship, when combined with an immune challenge [71]. Additionally, field observations have identified trends between pesticide-treated landscapes, parasites, and bee populations. For example, among 17 landscape variables, fungicide use had the third highest correlation with *Nosema* prevalence in bumble bees (the two factors with higher correlations were the amount of area developed and latitude [72]). A few experimental studies have identified effects of pesticides on solitary bees [65,73,74], but no studies have yet examined the effects of pesticides on solitary bee immune systems. Impacts of pesticides on the solitary bee immune function and parasite loads are an open area for future research.

Host-specific microbes can protect against parasites and modulate immunity

Host-associated microbes play a large role in organism fitness by mediating interactions with other individuals, parasites, food, and the environment [75]. Bees are no exception; studies on social species have found residential microbes — bacteria being the most well studied — to aid in digestion [76,77], immune system regulation [78,79], and detoxification [80,81]. Associations between bee hosts and beneficial microbes vary with life-history strategies such as sociality. Recent reviews have covered the microbiomes of insects [82] and specifically bees [83–85]. Here, we synthesize the studies that shed new light on how microbes mediate bee–parasite interactions.

Many factors impact the bacterial communities found in bee guts and nests, including social interactions [86], diet and forage composition [87–89], urbanization [90], and pesticide exposure [91]. However, causal patterns are still difficult to identify due to high within-species and within-colony variation in these communities, which may be due to transient taxa. For example, there are changes in the gut microbiome between bumble bee larval and adult life stages [92] and before and after hibernation for queens [90]. Similarly, the gut microbiome varies with honey bee castes [93] and workers' behavioral tasks [94]. However, supplemental food and the immune challenge had no effect on wild bumble bee gut microbiomes [95]. The rearing environment has variable effects on bumble bee gut microbes; one study found that indoor-reared and wild bumble bees had similar microbial communities [96], while two others found that the transition from indoor to outdoor environments altered gut microbial communities [97,98]. Lastly, some parasites can alter the gut microbiome; the presence of American foulbrood (*P. larvae*) altered the honey bee adult gut microbiome [99], but experimental infection with *Nosema* did not [100]. This difference may be due to different life histories of the parasites and how they interact with host cells; *P. larvae* is passively transmitted in adult honey bees (and only infects larvae) and may, therefore, interact directly with gut bacteria [101], while *Nosema* spores invade adult gut epithelial cells [102].

Social interactions transmit microbes between bee hosts, and strongly influence short- and long-term gut microbiome associations. Early social contact dictates succession of the gut microbiome [86] and has facilitated long-term establishment of certain microbial taxa in eusocial corbiculate bee hosts (a monophyletic group including bumble bees, honey bees, and stingless bees that diverged from other bees 80 MYA [103]), resulting in relatively simple and highly specific gut microbial communities in those taxa [104,105]. Recent studies with bumble and honey bees have identified correlations between the gut microbiome and parasite incidence (Table 4). For example, in honey bees, disrupting the gut microbiome via antibiotics increased mortality when infected with *Nosema* [100]. Interestingly, gut bacteria in the genus *Gilliamella* are positively associated with *Nosema* infection in honey bees [106], but studies have found either positive [107] or negative [108] associations with *Crithidia* infection in bumble bees.

A handful of studies have demonstrated a causal relationship between microbiome composition and parasite resistance [107,109] (Table 4). For example, bumble bees reared in a sterile environment that were inoculated with feces had lower parasite loads after one week compared with those without a microbiome (via being maintained in isolation or by treatment with antibiotics [107]). Further studies have identified potentially causal links between the bee gut microbiome and parasites by studying the effects of the microbiome on immune response in honey bees. Honey bees with an unmanipulated gut microbial community up-regulated the expression of the antimicrobial peptides (AMPs) apidaecin and hymenoptaecin compared with bees lacking a gut microbiome, showing that gut microbes can activate the host immune system. However, cultured strains of five gut bacteria were more resistant to honey bee AMPs than *Escherichia coli*, suggesting the gut bacteria have evolved to tolerate the host immune system [78]. Additionally, the honey bee gut bacterium *Frischella perrara* induced melanization, a general immune response [110], and up-regulated expression of host immune genes (including pattern-recognition receptors, AMPs, and transporter genes [79]). The role of bacteria in activating, modulating, and/or tolerating the bee immune system is an area of active inquiry that merits further investigation to distinguish causal links between the gut microbiome and parasite infection.

While the corbiculate eusocial bees host a relatively small, highly specific group of gut bacteria, other bee species host more variable and less specific microbes that are acquired primarily from the environment [111] (Table 5). Because of this, these gut microbes are less coevolved with their hosts than corbiculate bee microbes. Interestingly, social behavior itself is not responsible for host specificity, as eusocial sweat bees (*Halictus ligatus*) and multiple species of ants do not host highly specific microbiomes [104,112,113].

Bees exhibit a gradient of specificity and evolutionary relationships with their gut microbes; some solitary bees may not harbor residential bacteria at all (their microbiomes may be purely transient [114]) or harbor

Table 4. Recent studies on the relationship between the microbiome and parasites in *Apis* and *Bombus* bees.

Parasite	Relationship between microbiome and infection	Reference
Host: <i>Apis mellifera</i>		
<i>Nosema ceranae</i>	Experimental <i>Nosema</i> infection positively correlated with <i>Gilliamella</i> gut bacteria and weakly associated with gut microbiome community structure	[106]
<i>Nosema ceranae</i>	Addition of <i>Parasaccharibacter apium</i> bacterium (found in food stores, larvae, queen, worker hypophorangeal glands, and worker jelly) to pollen reduced <i>Nosema</i> spore count	[134]
<i>Nosema ceranae</i>	Addition of <i>Bifidobacterium</i> and <i>Lactobacillus</i> bacteria to sugar syrup reduced <i>Nosema</i> spore count	[135]
<i>Nosema ceranae</i>	Bees treated with antibiotics had higher <i>Nosema</i> spore counts than bees with unmanipulated gut microbiomes	[100]
<i>Nosema ceranae</i>	<i>Nosema</i> infection negatively correlated with <i>Snodgrassella alvi</i> and positively correlated with <i>Frischella perrara</i> gut bacteria	[136]
Colony collapse	Gut bacterial community composition correlated with the incidence of colony collapse disorder	[137]
<i>Paenibacillus larvae</i>	Novel lactic acid bacteria inhibited <i>P. larvae</i> in honey bee larvae	[138]
<i>Melissococcus plutonius</i>	Lactic acid bacteria inhibited <i>M. plutonius</i> <i>in vitro</i> and <i>in vivo</i> , increasing survivorship of honey bee larvae when exposed to the parasite	[139]
Host: <i>Bombus</i>		
<i>Crithidia</i> and <i>Nosema</i>	No relationship between parasite prevalence and gut microbial diversity, but the prevalence of <i>Nosema</i> was negatively correlated with the relative abundance of <i>Snodgrassella</i> bacteria	[90]
<i>Crithidia</i>	Bees treated with antibiotics, no gut microbiome, or Gammaproteobacteria had higher <i>Crithidia</i> loads than bees with unmanipulated microbiomes; <i>Crithidia</i> negatively correlated with Betaproteobacteria and Gammaproteobacteria (although weaker in the latter) in wild-caught bees	[107]
<i>Crithidia</i>	Gut microbiota instead of host genotype determine parasite infection levels	[140]
<i>Crithidia</i>	<i>Crithidia</i> infection negatively correlated with <i>Gilliamella</i> and core bacterial taxa in wild-caught bees	[108]
<i>Crithidia</i>	<i>Crithidia</i> infection was higher in individuals with higher gut microbial community diversity. Host microbial community structure and diversity do not change from before and after parasite infection.	[141]
<i>Crithidia</i> , <i>Paenibacillus larvae</i> , <i>Melissococcus plutonius</i> , and <i>Ascosphaera apis</i>	Bacterial strains from bumble bee guts inhibited bumble bee and honey bee parasites <i>in vitro</i>	[142]
<i>Crithidia</i>	<i>Crithidia</i> infection negatively associated with high gut microbiome diversity and abundance, and the presence of <i>Apibacter</i> , <i>Lactobacillus</i> , and <i>Gilliamella</i> spp. Commercial and wild-caught bees had different gut microbiomes and wild bees were more susceptible to infection than commercially reared bees.	[109]
<i>Crithidia</i>	<i>Lactobacillus bombicola</i> gut bacteria had a higher optimal growth temperature than <i>Crithidia</i> and inhibited growth at high temperatures	[143]
<i>Crithidia</i>	<i>Lactobacillus bombicola</i> gut bacteria inhibited <i>Crithidia</i> by decreasing gut pH	[144]

bacteria that provide few or no functions that are important for host health. The consequences of few residential bacteria for host health are not yet explored in bees. We propose two hypotheses about the role of gut microbes in bee–parasite interactions: (1) bee reliance on microbes for parasite resistance is dependent on specificity and evolutionary history. Microbes that are not as highly coevolved with their host play less of a role in modulating the host immune system than microbes that are coevolved with and under selection to protect their host. This gradient in host-microbe specificity across bee taxa should result in differences in the role of microbes in host immunity and parasite resistance. (2) Bee species that do not have a highly specific microbiome may be more vulnerable to environmental change, opportunistic pathogens, and stressors than species with a highly specific and protective microbiome. There are thousands of non-corbiculate bee species and we

Table 5. Studies on microbes associated with bees other than *Apis* and *Bombus*. When several species in the same study have the same social level and family, labels are provided after the final species in that group. Part 1 of 3

Host species [sociality] (family)	Topic	Reference
<i>Ceratina australensis</i> [sub-social] (Apidae)	Nest pollen species richness and community composition were not correlated with richness and community composition of bacterial species.	[145]
<i>Ceratina australensis</i> [sub-social] (Apidae)	Nest pollen species composition was strongly correlated with fungal community composition in nests.	[146]
<i>Megachile rotundata</i> [solitary] (Megachilidae)	Leaf and flower forage affect the leafcutter bee nest bacterial and fungal communities, including bee and plant pathogens.	[147]
<i>Megachile rotundata</i> [solitary] (Megachilidae)	Chalkbrood fungus inhibits other fungi, and may affect and even facilitate bacterial communities in the larval gut.	[148]
<i>Megachile rotundata</i> [solitary] (Megachilidae)	The diversity of microbes in the larval gut and frass was lower than those in the crop, pollen from the abdomen, and nest provisions. Larvae infected with chalkbrood had higher abundance (in weight) of bacteria and filamentous fungi, but not yeasts, than healthy larvae.	[149]
<i>Megachile rotundata</i> [solitary] (Megachilidae)	Larvae fed pollen with natural microbiota had a higher incidence of chalkbrood compared with larvae fed microbe-free pollen.	[150]
<i>Megachile rotundata</i> [solitary] (Megachilidae)	Larval guts had inconsistent and small populations of bacteria.	[151]
<i>Megachile rotundata</i> [solitary] (Megachilidae)	Dominant fungal and bacterial taxa from adult bees, larvae, provisions, and nests were characterized.	[152]
<i>Megalopta</i> [polymorphic] (Halictidae) <i>Ceratina</i> [sub-social] (Apidae)	<i>Ceratina</i> adults and pollen provisions have some of the same microbes; <i>Megalopta</i> adults have more diverse microbial communities with little overlap between adults and pollen provisions.	[153]
<i>Hesperapis cockerelli</i> [solitary] (Dasypodidae), <i>Rediviva saetigera</i> [solitary] (Melittidae), <i>Calliopsis subalpinus</i> [solitary] (Andrenidae), <i>Halictus patellatus</i> , <i>Agapostemon virescens</i> [solitary] (Halictidae), <i>Colletes inaequalis</i> , <i>Caupolicana yarrowi</i> [solitary] (Colletidae), <i>Megachile odontostoma</i> , <i>Hoplitis biscutellae</i> [solitary] (Megachilidae), <i>Diadasia opuntia</i> , <i>Xylocopa californica</i> [solitary] (Apidae)	Gut microbes in eusocial corbiculate bees are less diverse and more consistent than in solitary, non-corbiculate bee species.	[104]
<i>Lithurgus gibbosus</i> , <i>L. littoralis</i> , <i>Megachile brevis</i> , <i>M. parallela</i> , <i>M. polycaris</i> , <i>Osmia chalybea</i> , <i>O. subfasciata</i> [solitary] (Megachilidae)	<i>Lactobacillus micheneri</i> was found in all adult and larval guts, pollen provisions, and host flowers, suggesting that flowers are transmission hubs of these bacteria between bees.	[154]
<i>Nomia melanderi</i> [solitary] (Halictidae), <i>Megachile rotundata</i> [solitary] (Megachilidae)	Yeast and bacteria were common and are pioneer colonizers during microbial succession in brood cells for both species.	[155]
<i>Osmia cornuta</i> [solitary] (Megachilidae)	Old pollen provisions had higher bacterial diversity than fresh ones, and adult gut samples had higher diversity than larval samples.	[156]

Continued

Table 5. Studies on microbes associated with bees other than *Apis* and *Bombus*. When several species in the same study have the same social level and family, labels are provided after the final species in that group. Part 2 of 3

Host species [sociality] (family)	Topic	Reference
<i>Osmia cornuta</i> [solitary] (Megachilidae), <i>Andrena vaga</i> [solitary] (Andrenidae)	<i>Crithidia bombi</i> and novel viruses detected in <i>O. cornuta</i> .	[157]
<i>Osmia bicornis</i> , <i>O. caerulescens</i> , <i>Megachile rotundata</i> , <i>M. versicolor</i> [solitary] (Megachilidae)	Microbe communities were similar within species, and not affected by landscape. Within species, larvae and stored pollen microbial communities were consistent, and microbes in pollen provisions shifted as larvae grew.	[158]
<i>Osmia bicornis</i> [solitary] (Megachilidae)	Nest and pupal microbes were highly diverse and contained potential parasites.	[159]
<i>Osmia ribifloris</i> [solitary] (Megachilidae)	Bees fed sterile pollen experienced reduced growth rates, mass, and survivorship compared with those fed unsterilized pollen.	[160]
<i>Osmia bicornis</i> [solitary] (Megachilidae)	High intraspecific variation in the gut microbiome structure compared with <i>Bombus</i> and <i>Apis</i> . Community differences were observed between years and between larvae and adult life stages.	[161]
<i>Andrena cineraria</i> , <i>Andrena fulva</i> , <i>Andrena haemorrhoa</i> [solitary] (Andrenidae), <i>Osmia bicornis</i> , <i>Osmia cornuta</i> [solitary] (Megachilidae)	Three novel parasites and yeasts of the genus <i>Metschnikowia</i> were detected in wild bees.	[162]
<i>Diadasina distincta</i> , <i>Ptilotrix plumata</i> [solitary] (Apidae)	New species of yeasts were identified in pollen provisions, larvae, pupae, and adults, which likely contribute to the fermentation of pollen provisions.	[163]
<i>Scaptotrigona depilis</i> [eusocial] (Apidae)	Larvae require the consumption of <i>Zygosaccharomyces</i> spp. fungus in brood provisions to properly develop.	[164]
<i>Megalopta centralis</i> , <i>M. genalis</i> [polymorphic] (Halictidae)	Host species and developmental stage, but not host social structure, affected the microbiome in socially polymorphic bees.	[165]
<i>Halictus maculatus</i> [eusocial], <i>H. scabiosae</i> [eusocial], <i>H. fulvipes</i> [eusocial], <i>H. tumulorum</i> [polymorphic], <i>H. rubicundus</i> [polymorphic], <i>H. pollinosus</i> [unknown], <i>Lasioglossum laticeps</i> [eusocial], <i>L. malachurum</i> [eusocial], <i>L. nigripes</i> [eusocial], <i>L. marginatum</i> [eusocial], <i>L. morio</i> [eusocial], <i>L. fulvicorne</i> [eusocial], <i>L. pauxillum</i> [eusocial], <i>L. interruptum</i> [eusocial], <i>L. albipes</i> [polymorphic], <i>L. calceatum</i> [polymorphic], <i>L. laevigatum</i> [solitary], <i>L. zonulum</i> [solitary], <i>L. leucozonium</i> [solitary], <i>L. villosulum</i> [solitary], <i>L. limbellum</i> [solitary], <i>L. mediterranium</i> [unknown] (Halictidae)	Social behavior did not affect bacterial communities, except for <i>Sodalis</i> bacteria, which was identified more commonly in solitary species and solitary polymorphs.	[166]
<i>Tetragonula</i> spp., <i>Austroplebeia</i> sp. [eusocial] (Apidae)	A new clade of <i>Lactobacillus</i> bacteria was found in guts of three species.	[167]
<i>Centris atripes</i> <i>Anthophora abrupta</i> [solitary] (Apidae), <i>Paratrigona subnuda</i> , <i>Partamona helleri</i> ,	Core bacterial taxa are found in the corbiculate bee clade including <i>Apis</i> , <i>Bombus</i> , and the stingless bees — all eusocial species listed here. These taxa are consistent within this host group and are absent in solitary bee outgroups.	[103]

Continued

Table 5. Studies on microbes associated with bees other than *Apis* and *Bombus*. When several species in the same study have the same social level and family, labels are provided after the final species in that group. Part 3 of 3

Host species [sociality] (family)	Topic	Reference
<i>Trigona</i> spp., <i>Plebeia droryana</i> , <i>Heterotrigona itama</i> , <i>Sundatrigona moorei</i> , <i>Geniotrigona thoracica</i> , <i>Tetragonula</i> spp. [eusocial] (Apidae)	Novel parasiteic viruses were identified associated with stingless bees.	[168]
<i>Melipona quadrifasciata</i> [eusocial] (Apidae)	<i>Bacillus</i> spp. bacteria were found in brood samples of both species.	[169]
<i>Centris pallida</i> and <i>Anthophora</i> sp. [solitary](Apidae)	<i>Bacillus</i> spp. bacteria were found in samples of all three solitary species, which include brood provisions from <i>C. flavofasciata</i> and <i>X. californica</i> and the gut from <i>C. luctosa</i> .	[170]
<i>Centris flavofasciata</i> , <i>Xylocopa californica</i> [solitary](Apidae), <i>Crawfordapis luctuosa</i> [solitary] (Colletidae)		

have only investigated the microbiomes associated with a handful of them (Table 5). Characterizing the communities, functions, and host dependence of microbes associated with non-corbiculate bees is an open area for future research.

Solitary bees are susceptible to parasites transmitted from social hosts via shared flowers [115], and these host transitions could become more frequent as non-native pollinators are transported long distances for agricultural pollination [116]. It is critical to understand how the solitary bee microbiome and health are impacted by interactions with non-native pollinator and parasite communities. Future work should prioritize identifying links between parasite prevalence and microbial communities for more bee species, particularly non-social species.

Conclusions and future directions

Bees are susceptible to a diverse group of parasites and have evolved multiple defense mechanisms, including innate immunity, immune priming, feeding on antimicrobial diets or collecting antimicrobial nest materials, and reliance on beneficial microbes. There are multiple avenues of future research in these topics, particularly characterizing differences between the relatively well-studied social bees and understudied presocial and solitary bees. We highlight four topics to address in future research:

(1) Intraspecific variation in immunity in varying ecological contexts.

We report that social behavior influences investment in individual immunity, but the level of investment likely depends on colony size and degree of sociality. Characterizing investment in immunity in facultatively social species such as *Megalopta*, *Halictus*, and *Euglossa* would shed light on how social interactions impact parasite risk and investment in defense. We also note that bees that do not rely on group-level defense mechanisms may rely more on plant products for protection against parasites.

(2) TGIP in multivoltine and presocial bee species.

TGIP is considered to be an important aspect of the social insect immune system, but its role in multivoltine and presocial species has not yet been explored. We report that multivoltine and presocial bee species also rely on TGIP, which may make those taxa more resilient to parasites than species that do not exhibit this adaptation. However, identifying the factors that influence immune plasticity and costs associated with it is critical to understand how their populations will respond to fluctuating parasite environments.

(3) Effects of low-quality forage and pesticides in presocial and solitary bee health.

Low-quality diets, starvation, and pesticide exposure have major consequences for bee susceptibility to parasites, but research linking this to immune function is lacking for most species. The diversity of life-history strategies and defense mechanisms in bees suggest that taxa should respond to these stressors differently. Understanding

which taxa may be most susceptible to environmental stressors — which may be solitary species — is critical to inform prioritization of conservation efforts, policy on pesticide use, and plantings that benefit a diversity of pollinators.

(4) The role of environmental factors on microbes associated with presocial and solitary bees and the role of microbes in parasite resistance.

Recent studies have identified correlations between gut microbial communities and parasites in some bee taxa, and that immune systems are activated by and coevolved with certain gut microbes. Solitary and presocial species may not have a highly specific microbiome, which may make their responses to stressors less predictable or consistent. Identifying links between microbes and host health in presocial and solitary bee species will inform conservation strategies for these taxa.

Environmental changes such as urbanization, landscape fragmentation and simplification, pesticide use, invasive species, and climate change impact interactions between plants, pollinators, and microbes. Current research approaches to the ‘pollination crisis’ are primarily through the lens of social model bee species. Social behavior impacts bee immune systems and microbiomes — two important traits that determine host susceptibility to parasites and resilience in the face of stress — and so insights from social species may not apply to all bee pollinators. Bees are critical to ecosystem function and agricultural pollination, and thus it is imperative that we continue to broaden our understanding of bee–parasite interactions to protect bee populations and conserve biodiversity.

Summary

- Bees are susceptible to a diverse group of parasites and have evolved diverse defensive adaptations, including behavioral, immunological, and chemical mechanisms. Many bee species, particularly the corbiculate bees, may also rely on bacterial symbionts for parasite defense.
- Social behavior impacts selection for defensive traits such as immune systems and microbiomes, and bee species with varying levels of sociality and colony sizes rely on different defensive strategies.
- Low-quality diets and pesticides can render bees more susceptible to parasites and exacerbate infections by impairing the immune system and altering the gut microbiome.
- Most research has been on eusocial bee species and relatively little is known about immunity and the role of beneficial microbes in non-eusocial bees. Future research should focus on defense strategies in non-eusocial bee species and the effects of environmental factors such as low-quality forage and pesticides on their susceptibility to parasites.

Abbreviations

AMPs, antimicrobial peptides; TGIP, transgenerational immune priming.

Author Contributions

A.E.F. wrote the first draft of the paper and incorporated insight from R.E.I. and L.S.A.

Funding

A.E.F. funded by Lotta Crabtree Agricultural Fellowship and the National Science Foundation Graduate Research Fellowship.

Acknowledgements

We thank Adam Porter, David Sela, Joan Milam, and two anonymous reviewers for the helpful comments.

Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

References

- Goulson, D., Nicholls, E., Botias, C. and Rotheray, E.L. (2015) Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science* **347**, 1255957–1255957 <https://doi.org/10.1126/science.1255957>
- Wilfert, L., Long G, Leggett HC, Schmid-Hempel P, Butlin R, Martin SJ et al. (2016) Deformed wing virus is a recent global epidemic in honeybees driven by Varroa mites. *Science* **351**, 594–597. <https://doi.org/10.1126/science.aac9976>
- Goulson, D., O'Connor, S. and Park, K.J. (2017) The impacts of predators and parasites on wild bumblebee colonies. *Ecol. Entomol.* **43**, 168–181 <https://doi.org/10.1111/een.12482>
- Klein, S., Cabiroi, A., Devaud, J., Barron, A.B. and Lihoreau, M. (2017) Why bees are so vulnerable to environmental stressors. *Trends Ecol. Evol.* **32**, 1–11 <https://doi.org/10.1016/j.tree.2016.12.009>
- Grozinger, C.M. and Flenniken, M.L. (2019) Bee viruses: ecology, pathogenicity, and impacts. *Annu. Rev. Entomol.* **64**, 205–226 <https://doi.org/10.1146/annurev-ento-011118-111942>
- Evison, S.E.F. and Jensen, A.B. (2018) The biology and prevalence of fungal diseases in managed and wild bees. *Curr. Opin. Insect Sci.* **26**, 105–113 <https://doi.org/10.1016/j.cois.2018.02.010>
- Tihelka, E. (2017) The immunological dependence of plant-feeding animals on their host's medical properties may explain part of honey bee colony losses. *Arthropod. Plant. Interact.* **12**, 1–8 <https://doi.org/10.1007/s11829-017-9553-1>
- Koch, H., Brown, M.J. and Stevenson, P.C. (2017) The role of disease in bee foraging ecology. *Curr. Opin. Insect Sci.* **21**, 60–67 <https://doi.org/10.1016/j.cois.2017.05.008>
- Gomez-Moracho, T., Heeb, P. and Lihoreau, M. (2017) Effects of parasites and pathogens on bee cognition. *Ecol. Entomol.* **42**, 51–64 <https://doi.org/10.1111/een.12434>
- Michener, C. (1974) *The Social Behavior of the Bees: A Comparative Study*, Harvard University Press, Cambridge
- Ross, K.G. and Matthews, R.W. (1991) *The Social Behavior of Wasps*, Cornell University Press, Ithaca
- Simone-Finstrom, M. (2017) Social immunity and the superorganism: behavioral defenses protecting honey bee colonies from pathogens and parasites. *Bee World* **94**, 21–29 <https://doi.org/10.1080/0005772X.2017.1307800>
- Cameron, S.A., Lim, H.C., Lozier, J.D., Duennes, M.A. and Thorp, R. (2016) Test of the invasive pathogen hypothesis of bumble bee decline in North America. *Proc. Natl Acad. Sci. U.S.A.* **113**, 4386–4391 <https://doi.org/10.1073/pnas.1525266113>
- Tian, T., Piot, N., Meeus, I. and Smaghe, G. (2018) Infection with the multi-host micro-parasite *Apicystis bombi* (Apicomplexa: Neogregarinorida) decreases survival of the solitary bee *Osmia bicornis*. *J. Invertebr. Pathol.* **158**, 43–45 <https://doi.org/10.1016/j.jip.2018.09.005>
- Moure-Oliveira, D., Hirotsu, C.M., Serrano, J.C. and Garofalo, C.A. (2019) Host-parasitoid interactions between the solitary bee *Centris analis* (Apidae: Centridini) and conopid flies (Diptera: Conopidae). *Sci. Nat.* **106**, 1–8 <https://doi.org/10.1007/s00114-019-1634-9>
- Garibaldi, L.A., Steffan-Dewenter, I., Winfree, R., Aizen, M.A., Bommarco, R., Cunningham, S.A. et al. (2013) Wild pollinators enhance fruit set of crops regardless of honey bee abundance. *Science* **339**, 1608–1611 <https://doi.org/10.1098/rspb.2006.3721>
- Müller, U., McMahon, D.P. and Rolff, J. (2019) Exposure of the wild bee *Osmia bicornis* to the honey bee pathogen *Nosema ceranae*. *Agric. For. Entomol.* **21**, 363–371 <https://doi.org/10.1111/afe.12338>
- Evans, J., Aronstein, K., Chen, Y.P., Hetru, C., Imler, J.L., Jiang, H. et al. (2006) Immune pathways and defence mechanisms in honey bees *Apis mellifera*. *Insect Mol. Biol.* **15**, 645–656 <https://doi.org/10.1111/j.1365-2583.2006.00682.x>
- Barnes, A.I. and Siva-Jothy, M.T. (2000) Density-dependent prophylaxis in the mealworm beetle *Tenebrio molitor* L. (Coleoptera: Tenebrionidae): cuticular melanization is an indicator of investment in immunity. *Proc. R. Soc. B Biol. Sci.* **267**, 177–182 <https://doi.org/10.1098/rspb.2000.0984>
- Wilson, K. and Reeson, A.F. (1998) Density-dependent prophylaxis: evidence from Lepidoptera–baculovirus interactions? *Ecol. Entomol.* **23**, 100–101 <https://doi.org/10.1046/j.1365-2311.1998.00107.x>
- Pie, M.R., Rosengaus, R.B., Calleri, D.V. and Traniello, J.F.A. (2005) Density and disease resistance in group-living insects: do eusocial species exhibit density-dependent prophylaxis? *Ethol. Ecol. Evol.* **17**, 41–50 <https://doi.org/10.1080/08927014.2005.9522614>
- Ruiz-Gonzalez, M.X., Moret, Y. and Brown, M.J.F. (2009) Rapid induction of immune density-dependent prophylaxis in adult social insects. *Biol. Lett.* **5**, 781–783 <https://doi.org/10.1098/rsbl.2009.0505>
- Richter, J., Helbing, S. and Erler, S. (2012) Social context-dependent immune gene expression in bumblebees (*Bombus terrestris*). *Behav. Ecol. Sociobiol.* **66**, 791–796 <https://doi.org/10.1007/s00265-012-1327-2>
- Moret, Y. and Schmid-Hempel, P. (2009) Immune responses of bumblebee workers as a function of individual and colony age: senescence versus plastic adjustment of the immune function. *Oikos* **118**, 371–378 <https://doi.org/10.1111/j.1600-0706.2008.17187.x>
- Stow, A., Briscoe, D., Gillings, M., Holley, M., Smith, S., Leys, R. et al. (2007) Antimicrobial defences increase with sociality in bees. *Biol. Lett.* **3**, 422–424 <https://doi.org/10.1098/rsbl.2007.0178>
- Viljakainen, L., Evans, J.D., Hasselmann, M., Rueppell, O., Tingek, S. and Pamilo, P. (2009) Rapid evolution of immune proteins in social insects. *Mol. Biol. Evol.* **26**, 1791–1801 <https://doi.org/10.1093/molbev/msp086>
- Harpur, B.A. and Zayed, A. (2013) Accelerated evolution of innate immunity proteins in social insects: adaptive evolution or relaxed constraint? *Mol. Biol. Evol.* **30**, 1665–1674 <https://doi.org/10.1093/molbev/mst061>
- Traniello, J.F.A., Rosengaus, R.B. and Savoie, K. (2002) The development of immunity in a social insect: evidence for the group facilitation of disease resistance. *Proc. Natl Acad. Sci. U.S.A.* **99**, 6838–6842 <https://doi.org/10.1073/pnas.102176599>
- López-Urbe, M.M., Sconiers, W.B., Frank, S.D., Dunn, R.R. and Tarpay, D.R. (2016) Reduced cellular immune response in social insect lineages. *Biol. Lett.* **12**, 20150984 <https://doi.org/10.1098/rsbl.2015.0984>

- 30 Gadau, J., Helmkamp, M., Nygaard, S., Roux, J., Simola, D.F., Smith, C.R. et al. (2012) The genomic impact of 100 million years of social evolution in seven ant species. *Trends Genet.* **28**, 14–21 <https://doi.org/10.1016/j.tig.2011.08.005>
- 31 Barribeau, S.M., Sadd, B.M., du Plessis, L., Brown, M.J., Buechel, S.D., Cappelle, K. et al. (2015) A depauperate immune repertoire precedes evolution of sociality in bees. *Genome Biol.* **16**, 83 <https://doi.org/10.1186/s13059-015-0628-y>
- 32 Messer, A.C. (1985) Fresh dipterocarp resins gathered by megachilid bees inhibit growth of pollen-associated fungi. *Biotropica* **17**, 175–176 <https://doi.org/10.2307/2388512>
- 33 Eltz, T., Küttner, J., Lunau, K. and Tollrian, R. (2015) Plant secretions prevent wasp parasitism in nests of wool-carder bees, with implications for the diversification of nesting materials in Megachilidae. *Front. Ecol. Evol.* **2**, 1–7 <https://doi.org/10.3389/fevo.2014.00086>
- 34 Mitra, A. (2013) Function of the Dufour's gland in solitary and social Hymenoptera. *J. Hymenopt. Res.* **35**, 33–58 <https://doi.org/10.3897/jhr.35.4783>
- 35 Rehan, S.M., Berens, A.J. and Toth, A.L. (2014) At the brink of eusociality: transcriptomic correlates of worker behaviour in a small carpenter bee. *BMC Evol. Biol.* **14**, 1–11 <https://doi.org/10.1186/s12862-014-0260-6>
- 36 Sadd, B.M. and Schmid-Hempel, P. (2006) Insect immunity shows specificity in protection upon secondary pathogen exposure. *Curr. Biol.* **16**, 1206–1210 <https://doi.org/10.1016/j.cub.2006.04.047>
- 37 Korner, P. and Schmid-Hempel, P. (2004) In vivo dynamics of an immune response in the bumble bee *Bombus terrestris*. *J. Invertebr. Pathol.* **87**, 59–66 <https://doi.org/10.1016/j.jip.2004.07.004>
- 38 Richard, F.J., Aubert, A. and Grozinger, C.M. (2008) Modulation of social interactions by immune stimulation in honey bee, *Apis mellifera*, workers. *BMC Biol.* **6**, 1–13 <https://doi.org/10.1186/1741-7007-6-50>
- 39 Sadd, B.M., Kleinlogel, Y., Schmid-Hempel, R. and Schmid-Hempel, P. (2005) Trans-generational immune priming in a social insect. *Biol. Lett.* **1**, 386–388 <https://doi.org/10.1098/rsbl.2005.0369>
- 40 Cooper, D. and Eleftherianos, I. (2017) Memory and specificity in the insect immune system: current perspectives and future challenges. *Front. Immunol.* **8**, 539. <https://doi.org/10.3389/fimmu.2017.00539>
- 41 Sadd, B.M. and Schmid-Hempel, P. (2007) Facultative but persistent trans-generational immunity via the mother's eggs in bumblebees. *Curr. Biol.* **17**, 1046–1047 <https://doi.org/10.1016/j.cub.2007.11.007>
- 42 Barribeau, S.M., Schmid-Hempel, P. and Sadd, B.M. (2016) Royal decree: gene expression in trans-generationally immune primed bumblebee workers mimics a primary immune response. *PLoS One* **11**, 1–13 <https://doi.org/10.1371/journal.pone.0159635>
- 43 López, J.H., Schuehly, W., Crailsheim, K. and Riessberger-Gallé, U. (2014) Trans-generational immune priming in honeybees. *Proc. R. Soc. B Biol. Sci.* **281**, 20140454 <https://doi.org/10.1098/rspb.2014.0454>
- 44 Sadd, B.M. and Schmid-Hempel, P. (2009) A distinct infection cost associated with trans-generational priming of antibacterial immunity in bumblebees. *Biol. Lett.* **5**, 798–801 <https://doi.org/10.1098/rsbl.2009.0458>
- 45 Trauer, U. and Hilker, M. (2013) Parental legacy in insects: variation of transgenerational immune priming during offspring development. *PLoS One* **8**, e63392 <https://doi.org/10.1371/journal.pone.0063392>
- 46 Graystock, P., Blane, E.J., McFrederick, Q.S., Goulson, D. and Hughes, W.O.H. (2016) Do managed bees drive parasite spread and emergence in wild bees? *Int. J. Parasitol. Parasites Wildl.* **5**, 64–75 <https://doi.org/10.1016/j.ijppaw.2015.10.001>
- 47 Alger, S.A., Burnham, P.A., Boncristiani, H.F. and Brody, A.K. (2019) RNA virus spillover from managed honeybees (*Apis mellifera*) to wild bumblebees (*Bombus* spp.). *PLoS One* **14**, e0217822 <https://doi.org/10.1371/journal.pone.0217822>
- 48 Tyler, E.R., Adams, S. and Mallon, E.B. (2006) An immune response in the bumblebee, *Bombus terrestris* leads to increased food consumption. *BMC Physiol.* **6**, 6 <https://doi.org/10.1186/1472-6793-6-6>
- 49 Moret, Y. and Schmid-Hempel, P. (2000) Survival for immunity: the price of immune system activation for bumblebee workers. *Science* **290**, 1166–1168 <https://doi.org/10.1126/science.290.5494.1166>
- 50 Brunner, F.S., Schmid-Hempel, P. and Barribeau, S.M. (2014) Protein-poor diet reduces host-specific immune gene expression in *Bombus terrestris*. *Proc. R. Soc. B Biol. Sci.* **281**, 20140128 <https://doi.org/10.1098/rspb.2014.0128>
- 51 Schmid-Hempel, R. and Schmid-Hempel, P. (1998) Colony performance and immunocompetence of a social insect, *Bombus terrestris*, in poor and variable environments. *Funct. Ecol.* **12**, 22–30 <https://doi.org/10.1046/j.1365-2435.1998.00153.x>
- 52 Alaux, C., Ducloz, F., Crauser, D. and Le Conte, Y. (2010) Diet effects on honeybee immunocompetence. *Biol. Lett.* **6**, 562–565 <https://doi.org/10.1098/rsbl.2009.0986>
- 53 Roger, N., Michez, D., Wattiez, R., Sheridan, C. and Vanderplanck, M. (2017) Diet effects on bumblebee health. *J. Insect Physiol.* **96**, 128–133 <https://doi.org/10.1016/j.jinsphys.2016.11.002>
- 54 Lee, K.P., Simpson, S.J. and Wilson, K. (2008) Dietary protein-quality influences melanization and immune function in an insect. *Funct. Ecol.* **22**, 1052–1061 <https://doi.org/10.1111/j.1365-2435.2008.01459.x>
- 55 Di Pasquale, G., Salignon, M., Le Conte, Y., Belzunces, L.P., Decourtye, A., Kretzschmar, A. et al. (2013) Influence of pollen nutrition on honey bee health: do pollen quality and diversity matter? *PLoS One* **8**, 1–13 <https://doi.org/10.1371/journal.pone.0072016>
- 56 Mao, W., Schuler, M.A. and Berenbaum, M.R. (2013) Honey constituents up-regulate detoxification and immunity genes in the western honey bee *Apis mellifera*. *Proc. Natl Acad. Sci. U.S.A.* **113**, E488–E488 <https://doi.org/10.1073/pnas.1303884110>
- 57 Richardson, L.L., Adler, L.S., Leonard, A.S., Andicoechea, J., Regan, K.H., Anthony, W.E. et al. (2015) Secondary metabolites in floral nectar reduce parasite infections in bumble bees. *Proc. R. Soc. B Biol. Sci.* **282**, 20142471 <https://doi.org/10.1098/rspb.2014.2471>
- 58 Manson, J.S., Otterstatter, M.C. and Thomson, J.D. (2010) Consumption of a nectar alkaloid reduces pathogen load in bumble bees. *Oecologia* **162**, 81–89 <https://doi.org/10.1007/s00442-009-1431-9>
- 59 Biller, O.M., Adler, L.S., Irwin, R.E., McAllister, C. and Palmer-Young, E.C. (2015) Possible synergistic effects of thymol and nicotine against *Crithidia bombi* parasitism in bumble bees. *PLoS One* **10**, 1–16 <https://doi.org/10.1371/journal.pone.0144668>
- 60 Giacomini, J.J., Leslie, J., Tarpy, D.R., Palmer-Young, E.C., Irwin, R.E. and Adler, L.S. (2018) Medicinal value of sunflower pollen against bee pathogens. *Sci. Rep.* **8**, 14394 <https://doi.org/10.1038/s41598-018-32681-y>
- 61 LoCasio, G.M., Aguirre, L., Irwin, R.E. and Adler, L.S. (2019) Pollen from multiple sunflower cultivars and species reduces a common bumblebee gut pathogen. *R. Soc. Open Sci.* **6**, 190279 <https://doi.org/10.1098/rsos.190279>

- 62 Palmer-Young, E.C. and Thursfield, L. (2017) Pollen extracts and constituent sugars increase growth of a trypanosomatid parasite of bumble bees. *PeerJ* **5**, e3297 <https://doi.org/10.7717/peerj.3297>
- 63 Gherman, B.I., Denner, A., Bobiş, O., Dezmirean, D.S., Mărghițaș, L.A., Schlüns, H. et al. (2014) Pathogen-associated self-medication behavior in the honeybee *Apis mellifera*. *Behav. Ecol. Sociobiol.* **68**, 1777–1784 <https://doi.org/10.1007/s00265-014-1786-8>
- 64 Spear, D.M., Silverman, S. and Forrest, J.R.K. (2016) Asteraceae pollen provisions protect *Osmia* mason bees (Hymenoptera: Megachilidae) from brood parasitism. *Am. Nat.* **187**, 797–803 <https://doi.org/10.1086/686241>
- 65 Anderson, N.L. and Harmon-Threatt, A.N. (2019) Chronic contact with realistic soil concentrations of imidacloprid affects the mass, immature development speed, and adult longevity of solitary bees. *Sci. Rep.* **9**, 1–9 <https://doi.org/10.1038/s41598-018-37186-2>
- 66 Pamminger, T., Botías, C., Goulson, D. and Hughes, W.O.H. (2018) A mechanistic framework to explain the immunosuppressive effects of neurotoxic pesticides on bees. *Funct. Ecol.* **32**, 1921–1930 <https://doi.org/10.1111/1365-2435.13119>
- 67 Sánchez-Bayo, F., Goulson, D., Pennacchio, F., Nazzi, F., Goka, K. and Desneux, N. (2016) Are bee diseases linked to pesticides? - a brief review. *Environ. Int.* **89–90**, 7–11 <https://doi.org/10.1016/j.envint.2016.01.009>
- 68 Boncristiani, H., Underwood, R., Schwarz, R., Evans, J.D., Pettis, J. and vanEngelsdorp, D. (2012) Direct effect of acaricides on pathogen loads and gene expression levels in honey bees *Apis mellifera*. *J. Insect Physiol.* **58**, 613–620 <https://doi.org/10.1016/j.jinsphys.2011.12.011>
- 69 Di Prisco, G., Cavaliere, V., Annoscia, D., Varricchio, P., Caprio, E., Nazzi, F. et al. (2013) Neonicotinoid clothianidin adversely affects insect immunity and promotes replication of a viral pathogen in honey bees. *Proc. Natl Acad. Sci.* **110**, 18466–18471 <https://doi.org/10.1073/pnas.1314923110>
- 70 Brandt, A., Gorenflo, A., Siede, R., Meixner, M. and Büchler, R. (2016) The neonicotinoids thiacloprid, imidacloprid, and clothianidin affect the immunocompetence of honey bees (*Apis mellifera* L.). *J. Insect Physiol.* **86**, 40–47 <https://doi.org/10.1016/j.jinsphys.2016.01.001>
- 71 Czerwinski, M.A. and Sadd, B.M. (2017) Detrimental interactions of neonicotinoid pesticide exposure and bumblebee immunity. *J. Exp. Zool. A Ecol. Integr. Physiol.* **327**, 273–283 <https://doi.org/10.1002/jez.2087>
- 72 McArt, S.H., Urbanowicz, C., McCoshum, S., Irwin, R.E. and Adler, L.S. (2017) Landscape predictors of pathogen prevalence and range contractions in US bumblebees. *Proc. R. Soc. B* **284**, 20172181 <https://doi.org/10.1098/rspb.2017.2181>
- 73 Abbott, V.A., Nadeau, J.L., Higo, H.A. and Winston, M.L. (2008) Lethal and sublethal effects of imidacloprid on *Osmia lignaria* and clothianidin on *Megachile rotundata* (Hymenoptera: Megachilidae). *J. Econ. Entomol.* **101**, 784–796 [https://doi.org/10.1603/0022-0493\(2008\)101\[784:LASEOJ\]2.0.CO;2](https://doi.org/10.1603/0022-0493(2008)101[784:LASEOJ]2.0.CO;2)
- 74 Sandrock, C., Tanadini, L.G., Pettis, J.S., Biesmeijer, J.C., Potts, S.G. and Neumann, P. (2014) Sublethal neonicotinoid insecticide exposure reduces solitary bee reproductive success. *Agric. For. Entomol.* **16**, 119–128 <https://doi.org/10.1111/afe.12041>
- 75 McFall-Ngai, M., Hadfield, M.G., Bosch, T.C., Carey, H.V., Domazet-Lošo, T., Douglas, A.E. et al. (2013) Animals in a bacterial world, a new imperative for the life sciences. *Proc. Natl Acad. Sci. U.S.A.* **110**, 3229–3236 <https://doi.org/10.1073/pnas.1218525110>
- 76 Engel, P., Martinson, V.G. and Moran, N.A. (2012) Functional diversity within the simple gut microbiota of the honey bee. *Proc. Natl Acad. Sci. U.S.A.* **109**, 11002–11007 <https://doi.org/10.1073/pnas.1202970109>
- 77 Zheng, H., Powell, J.E., Steele, M.I., Dietrich, C. and Moran, N.A. (2017) Honeybee gut microbiota promotes host weight gain via bacterial metabolism and hormonal signaling. *Proc. Natl Acad. Sci. U.S.A.* **114**, 4775–4780 <https://doi.org/10.1073/pnas.1701819114>
- 78 Kwong, W.K., Mancenido, A.L. and Moran, N.A. (2017) Immune system stimulation by the native gut microbiota of honey bees. *R. Soc. Open Sci.* **4**, 1–9 <https://doi.org/10.1098/rsos.170003>
- 79 Emery, O., Schmidt, K. and Engel, P. (2017) Immune system stimulation by the gut symbiont *Frischella perrara* in the honey bee (*Apis mellifera*). *Mol. Ecol.* **26**, 2576–2590 <https://doi.org/10.1111/mec.14058>
- 80 Rothman, J.A., Leger, L., Graystock, P., Russell, K. and McFrederick, Q.S. (2019) The bumble bee microbiome increases survival of bees exposed to selenate toxicity. *Environ. Microbiol.* **21**, 3417–3429 <https://doi.org/10.1111/1462-2920.14641>
- 81 Zheng, H., Nishida A, Kwong WK, Koch H, Engel P, Steele MI et al. (2016) Metabolism of toxic sugars by strains of the bee gut symbiont *Gilliamella apicola*. *MBio* **7**, 1–9. <https://doi.org/10.1128/mBio.01326-16>
- 82 Engel, P. and Moran, N.A. (2013) The gut microbiota of insects - diversity in structure and function. *Microbiol. Rev.* **37**, 699–735 <https://doi.org/10.1111/1574-6976.12025>
- 83 Engel, P., Kwong, W.K., McFrederick, Q., Anderson, K.E., Barribeau, S.M., Chandler, J.A. et al. (2016) The bee microbiome: impact on bee health and model for evolution and ecology of host–microbe interactions. *MBio* **7**, 1–9 <https://doi.org/10.3391/mbi.2016.7.1.01>
- 84 Kwong, W.K. and Moran, N.A. (2016) Gut microbial communities of social bees. *Nat. Rev. Microbiol.* **14**, 374–384 <https://doi.org/10.1038/nrmicro.2016.43>
- 85 Raymann, K. and Moran, N.A. (2018) The role of the gut microbiome in health and disease of adult honey bee workers. *Curr. Opin. Insect Sci.* **26**, 97–104 <https://doi.org/10.1016/j.cois.2018.02.012>
- 86 Powell, J.E., Martinson, V.G., Urban-Mead, K. and Moran, N.A. (2014) Routes of acquisition of the gut microbiota of the honey bee *Apis mellifera*. *Appl. Environ. Microbiol.* **80**, 7378–7387 <https://doi.org/10.1128/AEM.01861-14>
- 87 Billiet, A., Meeus, I., Van Nieuwerburgh, F., Deforce, D., Wäckers, F. and Smagghe, G. (2016) Impact of sugar syrup and pollen diet on the bacterial diversity in the gut of indoor-reared bumblebees (*Bombus terrestris*). *Apidologie* **47**, 548–560 <https://doi.org/10.1007/s13592-015-0399-1>
- 88 Donkersley, P., Rhodes, G., Pickup, R.W., Jones, K.C., Power, E.F., Wright, G.A., et al. (2017) Nutritional composition of honey bee food stores vary with floral composition. *Oecologia* **185**, 749–761 <https://doi.org/10.1007/s00442-017-3968-3>
- 89 Jones, J.C., Hildebrand, F., Al Toufalilia, H., Balfour, N.J., Bork, P., Engel, P. et al. (2018) Gut microbiota composition is associated with environmental landscape in honey bees. *Ecol. Evol.* **8**, 441–451 <https://doi.org/10.1002/ece3.3597>
- 90 Bosmans, L., Pozo, M.I., Verreth, C., Crauwels, S., Wilberts, L., Sobhy, I.S. et al. (2018) Habitat-specific variation in gut microbial communities and pathogen prevalence in bumblebee queens (*Bombus terrestris*). *PLoS One* **13**, e0204612 <https://doi.org/10.1371/journal.pone.0204612>
- 91 Motta, E.V.S., Raymann, K. and Moran, N.A. (2018) Glyphosate perturbs the gut microbiota of honey bees. *Proc. Natl Acad. Sci. U.S.A.* **115**, 10305–10310 <https://doi.org/10.1073/pnas.1803880115>
- 92 Parmentier, A., Meeus, I., Van Nieuwerburgh, F., Deforce, D., Vandamme, P. and Smagghe, G. (2018) A different gut microbial community between larvae and adults of a wild bumblebee nest (*Bombus pascuorum*). *Insect Sci.* **25**, 66–74. <https://doi.org/10.1111/1744-7917.12381>

- 93 Kapheim, K.M., Rao VD, Yeoman CJ, Wilson BA, White BA, Goldenfeld N et al. (2015) Caste-specific differences in hindgut microbial communities of honey bees (*Apis mellifera*). *PLoS One* **10**, 1–14 <https://doi.org/10.1371/journal.pone.0123911>
- 94 Jones, J.C., Fruciano, C., Marchant, J., Hildebrand, F., Forslund, S., Bork, P. et al. (2018) The gut microbiome is associated with behavioural task in honey bees. *Insectes Soc.* **65**, 419–429 <https://doi.org/10.1007/s00040-018-0624-9>
- 95 Koch, H., Cisarovsky, G. and Schmid-Hempel, P. (2012) Ecological effects on gut bacterial communities in wild bumblebee colonies. *J. Anim. Ecol.* **81**, 1202–1210 <https://doi.org/10.1111/j.1365-2656.2012.02004.x>
- 96 Meeus, I., Parmentier, L., Billiet, A., Maebe, K., Van Nieuwerburgh, F., Deforce, D. et al. (2015) 16S rRNA amplicon sequencing demonstrates that indoor-reared bumblebees (*Bombus terrestris*) harbor a core subset of bacteria normally associated with the wild host. *PLoS One* **10**, 1–15 <https://doi.org/10.1371/journal.pone.0125152>
- 97 Parmentier, L., Meeus, I., Mosallanejad, H., de Graaf, D.C. and Smagghe, G. (2016) Plasticity in the gut microbial community and uptake of Enterobacteriaceae (Gammaproteobacteria) in *Bombus terrestris* bumblebees' nests when reared indoors and moved to an outdoor environment. *Apidologie* **47**, 237–250 <https://doi.org/10.1007/s13592-015-0393-7>
- 98 Newbold, L.K., Oliver, A.E., Cuthbertson, L., Walkington, S.E., Gweon, H.S., Heard, M.S. et al. (2015) Rearing and foraging affects bumblebee (*Bombus terrestris*) gut microbiota. *Environ. Microbiol. Rep.* **7**, 634–641 <https://doi.org/10.1111/1758-2229.12299>
- 99 Erban, T., Ledvinka, O., Kamler, M., Nesvorna, M., Hortova, B., Tyl, J. et al. (2017) Honeybee (*Apis mellifera*)-associated bacterial community affected by American foulbrood: detection of *Paenibacillus larvae* via microbiome analysis. *Sci. Rep.* **7**, 5084. <https://doi.org/10.1038/s41598-017-05076-8>
- 100 Li, J.H., Evans, J.D., Li, W.F., Zhao, Y.Z., DeGrandi-Hoffman, G., Huang, S.K. et al. (2017) New evidence showing that the destruction of gut bacteria by antibiotic treatment could increase the honey bee's vulnerability to *Nosema* infection. *PLoS One* **12**, 1–18 <https://doi.org/10.1371/journal.pone.0187505>
- 101 Garrido-Bailón, E., Higes, M., Martínez-Salvador, A., Antúnez, K., Botías, C., Meana, A. et al. (2013) The prevalence of the honeybee brood pathogens *Ascosphaera apis*, *Paenibacillus larvae* and *Melissococcus plutonius* in Spanish apiaries determined with a new multiplex PCR assay. *Microb. Biotechnol.* **6**, 731–739 <https://doi.org/10.1111/1751-7915.12070>
- 102 Huang, W. and Solter, L.F. (2013) Comparative development and tissue tropism of *Nosema apis* and *Nosema ceranae*. *J. Invertebr. Pathol.* **113**, 35–41 <https://doi.org/10.1016/j.jip.2013.01.001>
- 103 Kwong, W.K., Medina, L.A., Koch, H., Sing, K.W., Soh, E.J.Y., Ascher, J.S. et al. (2017) Dynamic microbiome evolution in social bees. *Sci. Adv.* **3**, e1600513 <https://doi.org/10.1126/sciadv.1600513>
- 104 Martinson, V.G., Danforth, B.N., Minckley, R.L., Rueppell, O., Tingek, S. and Moran, N.A. (2011) A simple and distinctive microbiota associated with honey bees and bumble bees. *Mol. Ecol.* **20**, 619–628 <https://doi.org/10.1111/j.1365-294X.2010.04959.x>
- 105 Koch, H., Abrol, D.P., Li, J. and Schmid-Hempel, P. (2013) Diversity and evolutionary patterns of bacterial gut associates of corbiculate bees. *Mol. Ecol.* **22**, 2028–2044 <https://doi.org/10.1111/mec.12209>
- 106 Rubanov, A., Russell, K.A., Rothman, J.A., Nieh, J.C. and McFrederick, Q.S. (2019) Intensity of *Nosema ceranae* infection is associated with specific honey bee gut bacteria and weakly associated with gut microbiome structure. *Sci. Rep.* **9**, 3820. <https://doi.org/10.1038/s41598-019-40347-6>
- 107 Koch, H. and Schmid-Hempel, P. (2011) Socially transmitted gut microbiota protect bumble bees against an intestinal parasite. *Proc. Natl Acad. Sci. U. S. A.* **108**, 19288–19292 <https://doi.org/10.1073/pnas.1110474108>
- 108 Cariveau, D.P., Powell, J.E., Koch, H., Winfree, R. and Moran, N.A. (2014) Variation in gut microbial communities and its association with pathogen infection in wild bumble bees (*Bombus*). *ISME J.* **8**, 2369–2379 <https://doi.org/10.1038/ismej.2014.68>
- 109 Mockler, B.K., Kwong, W.K., Moran, N.A., Koch, H. and Drake, H.L. (2018) Microbiome structure influences infection by the parasite *Crithidia bombi* in bumble bees. *Appl. Environ. Microbiol.* **84**, 2335–2352 <https://doi.org/10.1128/AEM.02335-17>
- 110 Engel, P., Bartlett, K.D. and Moran, N.A. (2015) The bacterium *Frischella perrara* causes scab formation in the gut of its honeybee host. *MBio* **6**, 1255–1257 <https://doi.org/10.1128/mBio.00193-15>
- 111 McFrederick, Q.S.M.C., Wcislo, W.T., Taylor, D.R. and Heather, D. (2012) Environment or kin: whence do bees obtain acidophilic bacteria? *Mol. Ecol.* **21**, 1754–1768 <https://doi.org/10.1111/j.1365-294X.2012.05496.x>
- 112 McFrederick, Q.S., Cannone, J.J., Gutell, R.R., Kellner, K., Plowes, R.M. and Mueller, U.G. (2013) Specificity between lactobacilli and hymenopteran hosts is the exception rather than the rule. *Appl. Environ. Microbiol.* **79**, 1803–1812 <https://doi.org/10.1128/AEM.03681-12>
- 113 Sanders, J.G., Lukasik, P., Frederickson, M.E., Russell, J.A., Koga, R., Knight, R. et al. (2017) Dramatic differences in gut bacterial densities correlate with diet and habitat in rainforest ants. *Integr. Comp. Biol.* **57**, 705–722 <https://doi.org/10.1093/icb/ix088>
- 114 Hammer, T.J., Sanders, J.G. and Fierer, N. (2019) Not all animals need a microbiome. *FEMS Microbiol. Lett.* **366**, 1–11 <https://doi.org/10.1093/femsle/frz117>
- 115 Ravoet, J., De Smet, L., Meeus, I., Smagghe, G., Wenseleers, T. and de Graaf, D.C. (2014) Widespread occurrence of honey bee pathogens in solitary bees. *J. Invertebr. Pathol.* **122**, 55–58 <https://doi.org/10.1016/j.jip.2014.08.007>
- 116 Hedtko, S.M., Blitzer, E.J., Montgomery, G.A. and Danforth, B.N. (2015) Introduction of non-native pollinators can lead to trans-continental movement of bee-associated fungi. *PLoS One* **10**, 1–18 <https://doi.org/10.1371/journal.pone.0130560>
- 117 Richard, F., Holt, H.L. and Grozinger, C.M. (2012) Effects of immunostimulation on social behavior, chemical communication and genome-wide gene expression in honey bee workers (*Apis mellifera*). *BMC Genom.* **13**, 1–17 <https://doi.org/10.1186/1471-2164-13-558>
- 118 Waddington, K.D. and Rothenbuhler, W.C. (1976) Behaviour associated with hairless-black syndrome of adult honeybees. *J. Apic. Res.* **15**, 35–41 <https://doi.org/10.1080/00218839.1976.11099831>
- 119 Batra, S.W.T. and Bohart, G.E. (1969) Alkali bees: response of adults to pathogenic fungi in brood cells. *Science* **165**, 607–608 <https://doi.org/10.1126/science.165.3893.607>
- 120 Evans, J.D. and Spivak, M. (2010) Socialized medicine: individual and communal disease barriers in honey bees. *J. Invertebr. Pathol.* **103**, S62–S72 <https://doi.org/10.1016/j.jip.2009.06.019>
- 121 Starks, P.T., Blackie, C.A. and Seeley, T.D. (2000) Fever in honeybee colonies. *Naturwissenschaften* **87**, 229–231 <https://doi.org/10.1007/s001140050709>
- 122 Simone, M., Evans, J.D. and Spivak, M. (2009) Resin collection and social immunity in honey bees. *Evolution (N. Y.)* **63**, 3016–3022 <https://doi.org/10.1111/j.1558-5646.2009.00772.x>

- 123 Rolff, J. and Reynolds, S.E. (2009) *Insect Infection and Immunity: Evolution Ecology and Mechanisms*, Oxford University Press
- 124 Rolff, J. and Schmid-Hempel, P. (2016) Perspectives on the evolutionary ecology of arthropod antimicrobial peptides. *Philos. Trans. R. Soc. Chem. B.* **371**, 1–12 <https://doi.org/10.1098/rstb.2015.0297>
- 125 Gillespie, J.P., Kanost, M.R. and Trenczek, T. (1997) Biological mediators of insect immunity. *Annu. Rev. Entomol.* **42**, 611–643 <https://doi.org/10.1146/annurev.ento.42.1.611>
- 126 Masri, L. and Cremer, S. (2014) Individual and social immunisation in insects. *Trends Immunol.* **35**, 471–482 <https://doi.org/10.1016/j.it.2014.08.005>
- 127 Cane, J.H., Eickwort, G.C., Wesley, F.R. and Spielholz, J. (1983) Foraging, grooming and mate-seeking behaviors of *Macropis nuda* (Hymenoptera: Melittidae) and use of *Lysimachia ciliata* (Primulaceae) oils in larval provisions and cell linings. *Am. Midl. Nat.* **110**, 257–264 <https://doi.org/10.2307/2425267>
- 128 Kambli, S.S., Aiswarya, M.S., Manoj, K., Varma, S., Asha, G., Rajesh, T.P. et al. (2017) Leaf foraging sources of leafcutter bees in a tropical environment: implications for conservation. *Apidologie* **48**, 473–482 <https://doi.org/10.1007/s13592-016-0490-2>
- 129 Drescher, N., Wallace, H.M., Katouli, M., Massaro, C.F. and Leonhardt, S.D. (2014) Diversity matters: how bees benefit from different resin sources. *Oecologia* **176**, 943–953 <https://doi.org/10.1007/s00442-014-3070-z>
- 130 Torson, A.S., Yocum, G.D., Rinehart, J.P., Kemp, W.P. and Bowsher, J.H. (2015) Transcriptional responses to fluctuating thermal regimes underpinning differences in survival in the solitary bee *Megachile rotundata*. *J. Exp. Biol.* **218**, 1060–1068 <https://doi.org/10.1242/jeb.113829>
- 131 Xu, J. and James, R. (2009) Genes related to immunity, as expressed in the alfalfa leafcutting bee, *Megachile rotundata*, during pathogen challenge. *Insect Mol. Biol.* **18**, 785–794 <https://doi.org/10.1111/j.1365-2583.2009.00927.x>
- 132 Lee, K.Y., Kim, B.Y., Lee, K.S., Yoon, H.J. and Jin, B.R. (2015) A serine protease inhibitor from the hornfaced bee, *Osmia cornifrons*, exhibits antimicrobial activities. *J. Asia. Pac. Entomol.* **18**, 489–495 <https://doi.org/10.1016/j.aspen.2015.06.004>
- 133 Strachecka, A., Chobotow, J., Paleolog, J., Aleksandra, Ł., Schulz, M., Teper, D. et al. (2017) Insights into the biochemical defence and methylation of the solitary bee *Osmia rufa* L.: a foundation for examining eusociality development. *PLoS One* **12**, 1–24 <https://doi.org/10.1371/journal.pone.0176539>
- 134 Corby-Harris, V., Snyder, L., Meador, C.A., Naldo, R., Mott, B., Anderson, K.E. et al. (2016) *Parasaccharibacter apium*, gen. Nov., sp. Nov., improves honey bee (Hymenoptera: Apidae) resistance to *Nosema*. *J. Econ. Entomol.* **109**, 537–543 <https://doi.org/10.1093/jee/tow012>
- 135 Baffoni, L., Gaggia, F., Alberoni, D., Cabbri, R., Nanetti, A., Biavati, B. et al. (2016) Effect of dietary supplementation of Bifidobacterium and Lactobacillus strains in *Apis mellifera* L. against *Nosema ceranae*. *Benef. Microbes* **7**, 45–51 <https://doi.org/10.3920/BM2015.0085>
- 136 Maes, P.W., Rodrigues, P.A.P., Oliver, R., Mott, B.M. and Anderson, K.E. (2016) Diet-related gut bacterial dysbiosis correlates with impaired development, increased mortality and *Nosema* disease in the honeybee (*Apis mellifera*). *Mol. Ecol.* **25**, 5439–5450 <https://doi.org/10.1111/mec.13862>
- 137 Cornman, R.S., Tarpay, D.R., Chen, Y., Jeffreys, L., Lopez, D., Pettis, J.S. et al. (2012) Pathogen webs in collapsing honey bee colonies. *PLoS One* **7**, e43562 <https://doi.org/10.1371/journal.pone.0043562>
- 138 Forsgren, E., Olofsson, T.C., Vasquez, A. and Fries, I. (2010) Novel lactic acid bacteria inhibiting *Paenibacillus larvae* in honey bee larvae. *Apidologie* **41**, 99–108 <https://doi.org/10.1051/apido/2009065>
- 139 Vasquez, A., Forsgren, E., Fries, I., Paxton, R.J., Flaberg, E., Szekely, L. et al. (2012) Symbionts as major modulators of insect health: lactic acid bacteria and honeybees. *PLoS One* **7**, e33188 <https://doi.org/10.1371/annotation/3ac2b867-c013-4504-9e06-bebf3fa039d1>
- 140 Koch, H. and Schmid-Hempel, P. (2012) Gut microbiota instead of host genotype drive the specificity in the interaction of a natural host-parasite system. *Ecol. Lett.* **15**, 1095–1103 <https://doi.org/10.1111/j.1461-0248.2012.01831.x>
- 141 Näpflin, K. and Schmid-Hempel, P. (2018) High gut microbiota diversity provides lower resistance against infection by an intestinal parasite in bumblebees. *Am. Nat.* **192**, 131–141. <https://doi.org/10.1086/698013>
- 142 Praet, J., Parmentier, A., Schmid-Hempel, R., Meeus, I., Smaghe, G., Vandamme, P. et al. (2018) Large-scale cultivation of the bumblebee gut microbiota reveals an underestimated bacterial species diversity capable of pathogen inhibition. *Environ. Microbiol.* **20**, 214–227 <https://doi.org/10.1111/1462-2920.13973>
- 143 Palmer-Young, E.C., Raffel, T.R. and McFrederick, Q.S. (2018) Temperature-mediated inhibition of a bumblebee parasite by an intestinal symbiont. *Proc. R. Soc. B Biol. Sci.* **285**, 20182041 <https://doi.org/10.1098/rspb.2018.2041>
- 144 Palmer-Young, E.C., Raffel, T.R. and McFrederick, Q.S. (2019) pH-mediated inhibition of a bumblebee parasite by an intestinal symbiont. *Proc. R. Soc. B Biol. Sci.* **146**, 380–388 <https://doi.org/10.1017/S0031182018001555>
- 145 McFrederick, Q.S. and Rehan, S.M. (2016) Characterization of pollen and bacterial community composition in brood provisions of a small carpenter bee. *Mol. Ecol.* **25**, 2302–2311 <https://doi.org/10.1111/mec.13608>
- 146 McFrederick, Q.S. and Rehan, S.M. (2019) Wild bee pollen usage and microbial communities co-vary across landscapes. *Microb. Ecol.* **77**, 513–522 <https://doi.org/10.1007/s00248-018-1232-y>
- 147 Rothman, J.A., Andrikopoulos, C., Cox-Foster, D. and McFrederick, Q.S. (2018) Floral and foliar source affect the bee nest microbial community. *Microbial. Ecol.* **78**, 506–516 <https://doi.org/10.1007/s00248-018-1300-3>
- 148 McFrederick, Q.S., Mueller, U.G. and James, R.R. (2014) Interactions between fungi and bacteria influence microbial community structure in the *Megachile rotundata* larval gut. *Proc. R. Soc. B Biol. Sci.* **281**, 20132653. <https://doi.org/10.1098/rspb.2013.2653>
- 149 Inglis, G.D., Sigler, L. and Goette, M.S. (1993) Aerobic microorganisms associated with alfalfa leafcutter bees (*Megachile rotundata*). *Microb. Ecol.* **26**, 125–143 <https://doi.org/10.1007/BF00177048>
- 150 Inglis, G.D., Goettel, M.S. and Sigler, L. (1993) Influence of microorganisms on alfalfa leafcutter bee (*Megachile rotundata*) larval development and susceptibility to *Ascospaera aggregata*. *J. Invertebr. Pathol.* **61**, 236–243 <https://doi.org/10.1006/jipa.1993.1046>
- 151 Inglis, G.D., Yanke, L.J. and Goettel, M.S. (1998) Anaerobic bacteria isolated from the alimentary canals of alfalfa leafcutting bee larvae. *Apidologie* **29**, 327–332 <https://doi.org/10.1051/apido:19980403>
- 152 Goerzen, D.W. (1991) Microflora associated with the alfalfa leafcutting bee, *Megachile rotundata* (Fab) (Hymenoptera: Megachilidae) in Saskatchewan, Canada. *Apidologie* **22**, 553–561 <https://doi.org/10.1051/apido:19910508>
- 153 Graystock, P., Rehan, S.M. and McFrederick, Q.S. (2017) Hunting for healthy microbiomes: determining the core microbiomes of *Ceratina*, *Megalopta*, and *Apis* bees and how they associate with microbes in bee collected pollen. *Conserv. Genet.* **18**, 701–711 <https://doi.org/10.1007/s10592-017-0937-7>

- 154 McFrederick, Q.S., Thomas, J.M., Neff, J.L., Vuong, H.Q., Russell, K.A., Hale, A.R. et al. (2017) Flowers and wild megachilid bees share microbes. *Microb. Ecol.*, 188–200 <https://doi.org/10.1007/s00248-016-0838-1>
- 155 Batra, L.R., Batra, S.W.T. and Bohart, G.E. (1973) The mycoflora of domesticated and wild bees (Apoidea). *Mycopathol. Mycol. Appl.* **49**, 13–44 <https://doi.org/10.1007/BF02057445>
- 156 Lozo, J., Berić, T., Terzić-Vidojević, A., Stanković, S., Fira, D. and Stanislavljević, L. (2015) Microbiota associated with pollen, bee bread, larvae and adults of solitary bee *Osmia cornuta* (Hymenoptera: Megachilidae). *Bull. Entomol. Res.* **105**, 470–476 <https://doi.org/10.1017/S0007485315000292>
- 157 Schoonvaere, K., De Smet, L., Smagghe, G., Vierstraete, A., Braeckman, B.P. and de Graaf, D.C. (2016) Unbiased RNA shotgun metagenomics in social and solitary wild bees detects associations with eukaryote parasites and new viruses. *PLoS One* **11**, 1–19 <https://doi.org/10.1371/journal.pone.0168456>
- 158 Voulgari-Kokota, A., Grimmer, G., Steffan-Dewenter, I. and Keller, A. (2018) Bacterial community structure and succession in nests of two megachilid bee genera. *FEMS Microbiol. Ecol.* **95**, 1–11 <https://doi.org/10.1093/femsec/fiy218>
- 159 Keller, A., Grimmer, G. and Steffan-Dewenter, I. (2013) Diverse microbiota identified in whole intact nest chambers of the red mason bee *Osmia bicornis* (Linnaeus 1758). *PLoS One* **8**, e78296 <https://doi.org/10.1371/annotation/435abed9-4628-4699-b020-faa83a1ef3b7>
- 160 Dharampal, P.S., Carlson, C., Currie, C.R. and Steffan, S.A. (2019) Pollen-borne microbes shape bee fitness. *Proc. R. Soc. B Biol. Sci.* **286**, 20182894 <https://doi.org/10.1098/rspb.2018.2894>
- 161 Mohr, K.I. and Tebbe, C.C. (2006) Diversity and phylotype consistency of bacteria in the guts of three bee species (Apoidea) at an oilseed rape field **8**, 258–272 <https://doi.org/10.1111/j.1462-2920.2005.00893.x>
- 162 Schoonvaere, K., Smagghe, G., Francis, F. and de Graaf, D.C. (2018) Study of the metatranscriptome of eight social and solitary wild bee species reveals novel viruses and bee parasites. *Front. Microbiol.* **9**, 1–12 <https://doi.org/10.3389/fmicb.2018.00177>
- 163 Rosa, C.A., Lachance, M.A., Silva, J.O., Teixeira, A.C., Marini, M.M., Antonini, Y. et al. (2003) Yeast communities associated with stingless bees. *FEMS Yeast Res.* **4**, 271–275 [https://doi.org/10.1016/S1567-1356\(03\)00173-9](https://doi.org/10.1016/S1567-1356(03)00173-9)
- 164 Paludo, C.R., Menezes C, Silva-Junior EA, Vollet-Neto A, Andrade-Dominguez A, Pishchany G et al. (2018) Stingless bee larvae require fungal steroid to pupate. *Sci. Rep.* **8**, 1–10 <https://doi.org/10.1038/s41598-018-19583-9>
- 165 McFrederick, Q.S., Wcislo, W.T., Hout, M.C. and Mueller, U.G. (2014) Host species and developmental stage, but not host social structure, affects bacterial community structure in socially polymorphic bees. *FEMS Microbiol. Ecol.* **88**, 398–406 <https://doi.org/10.1111/1574-6941.12302>
- 166 Rubin, B.E., Sanders, J.G., Turner, K.M., Pierce, N.E. and Kocher, S.D. (2018) Social behaviour in bees influences the abundance of *Sodalis* (Enterobacteriaceae) symbionts. *R. Soc. Open Sci.* **11**, 180369 <https://doi.org/10.1098/rsos.180369>
- 167 Leonhardt, S.D. and Kaltenpoth, M. (2014) Microbial communities of three sympatric Australian stingless bee species. *PLoS One* **9**, 1–6 <https://doi.org/10.1371/journal.pone.0105718>
- 168 Caesar, L., Cibulski, S.P., Canal, C.W., Blochtein, B., Sattler, A., Haag, K.L. et al. (2019) The virome of an endangered stingless bee suffering from annual mortality in southern Brazil. *J. Gen. Virol.* **100**, 1153–1164 <https://doi.org/10.1099/jgv.0.001273>
- 169 Gilliam, M., Buchmann, S.L. and Lorenz, B.J. (1984) Microbial flora of the larval provisions of the solitary bees, *Centris pallida*. *Apidologie* **15**, 1–10 <https://doi.org/10.1051/apido:19840101>
- 170 Gilliam, M. (1990) Original article Bacteria belonging to the genus *Bacillus* associated with three species of solitary bees. *Apidologie* **21**, 99–105 <https://doi.org/10.1051/apido:19900202>