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# Socialized medicine: Individual and communal disease barriers in honey bees

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ABSTRACT

Honey bees are attacked by numerous parasites and pathogens toward which they present a variety of individual and group-level defenses. In this review, we briefly introduce the many pathogens and parasites afflicting honey bees, highlighting the biology of specific taxonomic groups mainly as they relate to virulence and possible defenses. Second, we describe physiological, immunological, and behavioral responses of individual bees toward pathogens and parasites. Third, bees also show behavioral mechanisms for reducing the disease risk of their nestmates. Accordingly, we discuss the dynamics of hygienic behavior and other group-level behaviors that can limit disease. Finally, we conclude with several avenues of research that seem especially promising for understanding host–parasite relationships in bees and for developing breeding or management strategies for enhancing honey bee health. We discuss how human efforts to maintain healthy colonies intersect with similar efforts by the bees, and how bee management and breeding protocols can affect disease traits in the short and long term.

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## 1. Introduction

Honey bees (*Apis mellifera*) provide important pollination services in agricultural settings worldwide and in many natural ecosystems. Honey bees and other pollinating insects are under threat from a variety of natural and anthropogenic causes (Committee on the Status of Pollinators in North America, 2007), ranging from viruses and bacteria to other insects and even mammals (Morse and Flottum, 1997). Thanks to the cultural importance of honey bees during much of modern human history the study of honey bee disease is an ancient topic, discussed in the literature since the ancient Greeks. The advent of modern microbiology and methods for culturing and observing microbes led to the first formal confirmation of several honey bee pathogens. As one example, the causative agent for American foulbrood was identified as a Gram-positive, rod-shaped, spore-forming bacterium labeled *Bacillus larvae* (White, 1906) and since renamed several times, ending with a recent reclassification as *Paenibacillus larvae* (Genersch et al., 2006).

Bee pathology has grown substantially in the past 50 years, with the identification of additional bacterial, fungal, and viral disease agents (Bailey, 1976), and the more recent application of molecular-genetic techniques to track both pathogens (Govan et al., 2000; McKee et al., 2003; Bakonyi et al., 2003; Genersch, 2005, as examples for viruses and bacteria) and bee responses toward those pathogens (Evans, 2006). Research efforts to understand honey bee resistance mechanisms are motivated by desires to breed and manage bees that are naturally resistant to parasites and, more generally, to better understand how an insect host interacts with a diverse set of pathogens. As an example of the former, beekeepers and researchers have long tried to develop lineages of bees with traits that enable colonies to survive attacks from their pathogens and parasites (e.g., Harbo and Hoopingarner, 1997; Spivak and Gilliam, 1998b; Szabo, 1999; De Guzman et al., 2001; Büchler, 2000; Kefuss et al., 2004).

In this review, we will briefly introduce the many pathogens and parasites afflicting honey bees, highlighting the biologies of specific taxonomic groups mainly as they relate to virulence and possible defenses. Second, we will describe physiological, immunological, and behavioral responses of individual bees toward parasites. Honey bees have evolved diverse methods to control the impacts of their many parasites and pathogens. Like all animals, individual honey bees enlist mechanical, physiological, and immunological defenses against disease agents (Evans et al., 2006; Schmid et al., 2008; Wilson-Rich et al., 2008). Third, bees also show behavioral mechanisms for reducing the disease risk of their nestmates (Starks et al., 2000; Spivak and Reuter, 2001a). Accordingly, we discuss the dynamics of hygienic behavior and other group-level behaviors that can limit disease. These group-level dynamics, labeled 'social immunity' (Cremer and Sixt, 2009), provide an underappreciated benefit of living in crowded social groups with respect to reduction of disease. We will contrast the costs and benefits of individual versus social defenses and will address the

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enigma that honey bees show great genetic variation for the expression of their various defenses. Finally, we conclude with several avenues of research that seem especially promising for understanding host–parasite relationships in bees and for developing breeding or management strategies for enhancing honey bee defenses. We will discuss how human efforts to maintain healthy colonies intersect with similar efforts by the bees, and how bee management and breeding protocols can affect disease traits in the short and long term.

## 2. Parasites and pathogens

Domesticated and free-living honey bees are challenged by viruses, bacteria, fungi, mites and beetles, among others. Particularly enigmatic are the viral diseases of honey bees, most of which have been placed into two lineages of positive-strand RNA viruses, the Dicistroviridae and the Iflaviridae. The iflaviruses contain the agent responsible for one of the first recognized bee maladies (sacbrood virus) along with Deformed wing virus (DWV), a subject for numerous studies on bee pathology and epidemiology (Chen et al., 2005; Martin, 2001; Sumpter and Martin, 2004; de Miranda and Genersch, 2010). DWV is transmitted both vertically (by queens and their mates; Chen et al., 2006; de Miranda and Fries, 2008; Yue et al., 2007) and horizontally, especially via the ectoparasitic mite, *Varroa destructor* (Bowen-Walker et al., 1999; Chen et al., 2004; Shen et al., 2005; Yang and Cox-Foster, 2007; Yue and Genersch, 2005; Gisder et al., 2009). Recent evidence indicates that distantly related mites in the genus *Tropilaelaps* are also likely to be DWV vectors for *A. mellifera* (Dainat et al., 2009; Forsgren et al., 2009). DWV infections at high doses can lead to their definitive pathology and appear to generate negative effects on behavior and learning at lower doses (Iqbal and Mueller, 2007). There appears to be considerable variation among DWV relatives in their ability to cause behavioral changes among infected individuals (Fujiyuki et al., 2004; Rortais et al., 2006). In the Dicistroviridae, the genus *Cripavirus* contains several widespread bee viral pathogens, from Kashmir bee virus (KBV) to Acute bee paralysis virus (ABPV) and Israeli acute paralysis virus (IAPV), all of which can be found across multiple continents (Chen and Siede, 2007; de Miranda et al., 2010). IAPV was unrecognized outside of its type population in Israel until serendipitously discovered by metagenomic sequencing in bee colonies from parts of the United States (Cox-Foster et al., 2007).

Important bacterial diseases include American foulbrood disease (causative agent *Paenibacillus larvae*; Genersch et al., 2006; Genersch, 2010) and European foulbrood disease (causative agent *Melissococcus plutonius*; Bailey, 1983; Forsgren, 2010). The primarily fungal pathogens are *Ascosphaera apis* (cause of chalkbrood disease, Qin et al., 2006; Aronstein and Murray, 2010), *Aspergillus* sp. (stone brood disease, Morse and Flottum, 1997), and two members of the basal fungal lineage the Microsporidia (*Nosema apis* and *Nosema ceranae*: Zander, 1909; Fries et al., 1996; Fries, 2010). Along with their recognized pathogens, bees carry a diverse set of fungi and bacteria with poorly understood health impacts, with likely impacts on their bee hosts that range from pathogenic to benign or beneficial (Gilliam, 1997). Honey bees also harbor scattered parasites ranging from parasitic flies to trypanosomes and amoebae. One method now in use to document the 'neglected' parasites of honey bees and other organisms involves using modern high-throughput sequencing techniques to describe would-be pathogens on the basis of their chromosomes or expressed genes.

## 3. Mechanical, physiological, and immune defenses

Like all animals, *individual* honey bees of all ages and castes have evolved mechanisms to limit the impacts of their pathogens (Fig. 1a). These mechanisms involve *resisting* pathogens, by building barriers to infection or mounting defense responses once infection has occurred, or *tolerating* pathogens, by compensating for the energetic costs or tissue damage caused by either these pathogens or the bee's own immune responses. Mechanical, physiological, and immune defenses provide the classic route for resisting pathogens. Mechanical barriers include the insect cuticle and epithelial layers, which in many cases prevent microbes from adhering to or entering the body. Physiological inhibitors to microbial invasion can include changes in the pH and other chemical conditions of the insect gut (Crailsheim and Riessberger-Galle, 2001).

Honey bees are known to mount an induced immune response to wounding or pathogen exposure (Evans et al., 2006). Honey bees and other insects possess four major and interconnected routes for responding to parasite exposure; the Toll, Imd, Jak/STAT, and Jnk pathways (Theopold and Dushay, 2007). These pathways consist of proteins to recognize signals from invading parasites, proteins to modulate and amplify this recognition signal, and effector proteins or metabolites directly involved with parasite inhibition (Lemaitre and Hoffmann, 2007). Among the recognition proteins,

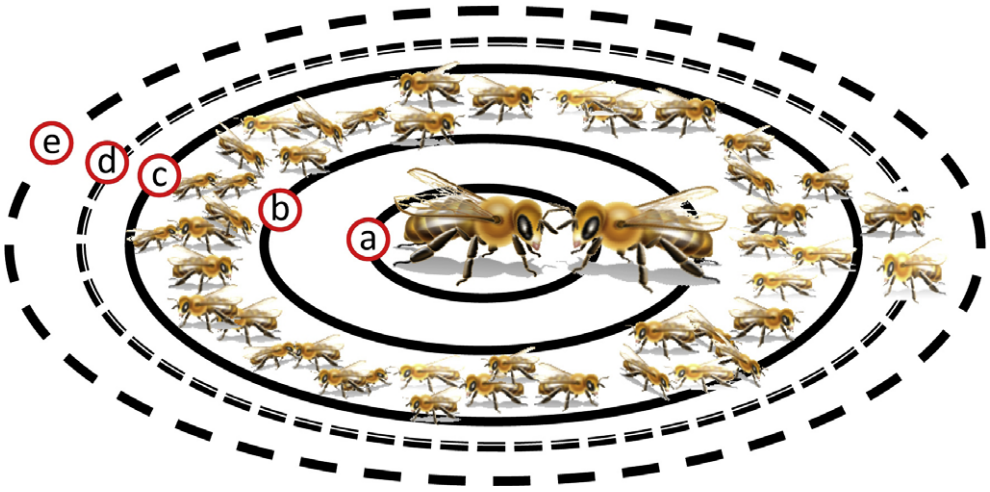


**Fig. 1.** Levels of defense in honey bee colonies from: (a) individual defenses, (b) pairwise defenses including grooming, (c) colony defenses such as task differentiation, (d) minimizing the entry of infectious agents, and (e) use of resins and other environmentals in colony shielding.

specificity toward pathogens can be achieved via differential binding properties to Microbe Associated Molecular Patterns (MAMP's). Mechanistically, recognition proteins bind differentially to invader moieties, such as the different classes of peptidoglycan presented by many bacterial invaders (peptidoglycan recognition proteins, PGRP's), to the β-glucan residues often found on the surfaces of eukaryotic parasites such as fungi (Gram-negative binding proteins, GNBP's, also called 1,3 β-glucan recognition proteins). Many recognition proteins appear to be related to enzymes found at the deepest branches of living organisms, suggesting that these abilities to pair with microbial proteins or other molecules are similarly ancient (Ferrandon et al., 2007).

Having met their microbial targets, recognition proteins generally intersect with proteases and other cytokines and the signals arising from these interactions eventually impact transmembrane proteins (e.g., Toll for the Toll pathway and Domeless for the Jak/STAT pathway), which themselves initiate a cascade of cytosolic processes involving proteins that can interact with proteins from one or more different pathways (cross-talk). An exception to this process is PGRP-LC, which is itself a transmembrane protein and therefore interacts directly with cytoplasmic proteins (ultimately IMD in the pathway of that name). Eventually, transcription factors released via immune pathways enter the nucleus and spur transcription of genes encoding antimicrobial peptides or other effectors, such as those involved with melanization. The core systemic immune pathway components appear to be conserved among the higher insects sequenced to date, and some dozens of proteins are known to be important for the known systemic immune responses. Nevertheless, there is substantial gain and loss of family members involved in immune responses over evolutionary time scales. Thus, it is difficult, if not impossible, to assign truly shared evolution of many immune-proteins across insect orders (orthology) versus simple family level homology that is muddled by gene duplications within lineages (paralogy). Logistically, this complicates the use of inference from extensive work completed in fruit flies and other insect models to predict immune function of related bee proteins. A determination of important immunity roles played by bee proteins therefore depends on a gene-by-gene experimental approach, as done recently to predict roles of proteins in the IMD immune pathway (Schluns and Crozier, 2007).

Responses by insect circulating cells (hemocytes) also can be mounted to reduce parasite loads. For example, phagocytic hemocytes are recruited to engulf bacteria in flies, mosquitoes, wasps and, presumably, honey bees. There are three primary classes of immune-related hemocytes in insects; the plasmatocytes, crystal cells, and lamellocytes (Williams, 2007); and these cell types or analogs are all likely to be present in honey bees (De Graaf et al., 2002). Bees also potentially carry granulocytes, a class of hemocytes that appears to be essential for phagocytosis in wasps. (Strand et al., 2006). In the fruit fly *Drosophila melanogaster*, genes implicated in phagocytosis have been found to be important for deterring bacteria (Kurucz et al., 2007), fungi (Li et al., 2008), and possibly viruses (Zambon et al., 2005). As with the humoral immune response, current efforts in *Drosophila* are utilizing genome-wide RNAi knockdown approaches to identify hundreds of candidate proteins involved with cellular immunity (Stroschein-Stevenson et al., 2009).

Honey bees show several proteins thought to be of general importance for cellular immunity, including the Down-syndrome cellular adhesion-molecule DSCAM, (Graveley et al., 2004; Kurtz and Armitage, 2006), which is also critical in neuronal differentiation, and EGF-family proteins such as Eater (Somogyi et al., 2008). Little is known about the importance of phagocytosis or other hemocyte-mediated immune processes in honey bees. Descriptive work by (Gilliam and Shimanuki, 1967) indicates that phagocytosis of spores from the microsporidian parasite, *N. apis*, occurs in honey bees, and might be an effective defense. Conversely, this observation might instead reflect an invasion strategy of microsporidia like *N. apis*, whereby they accept endocytosis by cells and then simply extrude themselves into a preferred host environment, the cytoplasm (Franzen, 2005). Several studies have attempted to document changes in hemocyte counts across worker lifespans, with conflicting results (Amdam et al., 2005; Schmid et al., 2008; Wilson-Rich et al., 2008), perhaps indicating the importance of nutritional status or other environmental factors for determining standing hemocyte density. Given the widespread evidence for the importance of cellular immunity in other insects, further studies aimed at documenting, and perhaps enhancing, the ability of bees to combat microbes by this mechanism are critical.

Not surprisingly, honey bees show a robust immune response following pathogen exposure (Evans et al., 2006) or exposure to surrogate signals of infection (Casteels-Josson et al., 1994; Casteels et al., 1993, 1990; Evans and Lopez, 2004). Most research on honey bee immune traits has focused on responses toward bacterial threats, toward which both larval and adult bees respond (Evans et al., 2006). Recent work has explored the changes in immune function across honey bee life stages. Wilson-Rich and colleagues (2009) found through biochemical and immune assays that phenoloxidase activity increased as adult bees age, as did the antibacterial properties of honey bee hemolymph. Curiously, these apparent increases in immune function run counter to physiological measures across the same time period, which showed a decrease in mass of one immune-protein source, the fat body, and little change in total hemocyte counts.

Honey bees do show one curious trait with respect to the established insect immune pathways. While the skeleton of each pathway appears to be intact, the numbers of family members (paralogs) at points throughout those pathways are lower relative to other insects with comparable data (Evans et al., 2006). This phenomenon could reflect many factors, including that honey bee immune sequences are much diverged from other studied insects and are therefore missed in current searches, or that honey bees rely on altogether different pathways or pathway components to regulate immune responses, or that honey bees face a taxonomically more narrow set of would-be pathogens. It is also conceivable that group-level mechanisms for decreasing disease pressure (discussed below) have somewhat decreased the selective pressure on bees to maintain a strong individual immune response.

Resistance mechanisms by honey bees toward viruses are less certain, given that these interactions are only now receiving significant attention. Nevertheless, honey bees, like other insects, are presumed to invoke both 'conventional' effectors including cellular responses and melanization, and targeted responses such as RNA interference when battling their viral threats (Chen and Siede, 2007). Recent work in *Drosophila* fruit flies shows a diversity of immune pathways that are regulated after exposure to viruses (Tsai et al., 2008; Zambon et al., 2005; Dostert et al., 2005). Several *Drosophila* studies also point toward the importance of RNAi in reducing infections of RNA viruses such as Drosophila C virus (DCV) and Flock House virus (FHV) (Aliyari and Ding, 2009; Saleh et al., 2009; Van Rij et al., 2006; Zambon et al., 2006). DCV is a close relative of several bee viruses in the Dicistroviridae, including ABPV, KBV, and IAPV. On the basis that honey bees actually possess more RNAi pathway components relative to flies (Honey Bee Genome Sequencing Consortium, 2006), and because bees appear to more readily mount a systemic RNAi response than do flies (Aronstein and Saldivar, 2005; Kim et al., 2006; Marco Antonio et al., 2008), it follows that bees should be quite capable of battling viruses and arguably other pathogens through knockdowns based on double-stranded RNAs of pathogen expressed genes. Indeed, an apparent RNAi mechanism was recently suggested by studies of honey bees inoculated with dsRNA matching a section of the genome of

the discistrovirus IAPV (Maori et al., 2009). More research is needed to resolve the effectiveness, and efficiency of RNAi mechanisms in reducing impacts of viruses and other bee disease agents.

## 4. Behavioral defenses

The immune responses within an individual bee are enhanced when coordinated behavioral interactions among nestmates result in colony-level immune responses. The collective defense against parasites arising from the behavioral cooperation among individuals is termed "social immunity" (Cremer et al., 2007). Even a relatively simple interaction between two individuals, such as during allo-grooming (Fig. 1b), can have far-reaching implications at the colony-level for parasite transmission and resistance. When hundreds or thousands of individuals within a honey bee colony interact, the social immune responses at the colony-level have analogous properties to the complex humoral and cellular immune systems within a multicellular organism (Cremer and Sixt, 2009). Social immune responses have been described recently for a number of social insects (Cremer et al., 2007; Cremer and Sixt, 2009; Wilson-Rich et al., 2009). Below we review honey bee social immune responses, paying particular attention to how this information could be incorporated in beekeeping practices to improve bee health.

### 4.1. Grooming

An individual bee may groom herself (auto-grooming) or one bee may groom another bee (allo-grooming). Auto-grooming is used to remove foreign particles and pollen from the body (Jander, 1976), but is also an important mechanism of defense against the tracheal mite, *Acarapis woodi* (Danka and Villa, 1998, 2000). Bees that display genetic resistance to these microscopic mites groom themselves with their mesothoracic legs as the mites exit the prothoracic spiracle thus preventing the mites from dispersing to a nestmate.

During allo-grooming, adult bees remove foreign particles and parasites from each other (Boecking and Spivak, 1999). One bee may elicit grooming by a "grooming dance" (Haydak, 1945; Milium, 1955) that involves jerking movements causing other bees to groom particularly at the base of the wings. Allo-grooming was described as an important mechanism of defense against phoretic *Varroa* mites by the original host, *Apis cerana* (Büchler et al., 1992; Peng et al., 1987a; Rath, 1999), and by Africanized *A. mellifera* in the tropics (Moretto et al., 1993). Subsequently a number of studies explored whether *A. mellifera* displayed allo-grooming in response to *V. destructor* (reviewed in Boecking and Spivak, 1999), and most concluded that grooming was not as pronounced as in *A. cerana* but was still worthy of further investigation. More recent work by Arechavaleta-Velasco and Guzman-Novoa (2001) argued that grooming is a heritable and important component of resistance. For grooming to be an effective defense against the mites, the bees must not only dislodge the phoretic mite from an adult nestmate, they must damage the mite by biting its idiosoma or legs (Rosenkranz et al., 1997; Aumeier et al. 2000). Current studies in the US, Canada, and Mexico, involve selecting for high and low grooming colonies to determine the number of quantitative trait loci involved in this trait, and developing lab and field bioassays for grooming to more accurately correlate the number of damaged mites with changes in mite infestation at the colony-level (Currie and Tahmasbi, 2008; G. Hunt, E. Guzman, M. Arechavaleta, personal communication). This research could have important practical potential in breeding programs to increase the ability of honey bees to resist *V. destructor*.

Grooming may be a double-edge sword if, as pointed out by Schmid-Hempel (1998), it increases parasite transmission. For example, honey bees infected with Chronic bee paralysis virus may be groomed or attacked by nestmates (Waddington and Rothenbuhler, 1976). If grooming involves licking and chewing then this could also be a viral strategy to increase transmission. Therefore, when honey bees are artificially selected for grooming behavior against *Varroa*, tests should also be conducted to determine if increased selection for grooming has a fitness cost by increasing transmission of different parasites such as viruses.

### 4.2. Hygienic behavior

Hygienic behavior is a specific type of general nest hygiene, and the two terms are not necessarily interchangeable. In the case of honey bees, hygienic behavior is a collective response by adult bees to the presence of diseased and parasitized worker brood (larvae and pupae; Rothenbuhler and Thompson, 1956; and recent review in Wilson-Rich et al., 2009). The behavior was originally defined as the ability of honey bees to detect and remove brood infected by American foulbrood from the nest (Park et al., 1937; Woodrow and Holst, 1942; Rothenbuhler, 1964). Later it was found that hygienic behavior was also an important behavioral defense against chalkbrood disease (Gilliam et al., 1988). Bees selected for rapid-hygienic behavior have high olfactory sensitivity to the odor of diseased brood (Masterman et al., 2001; Spivak et al., 2003; Swanson et al., in press). These neuroethological studies coupled with direct observations of individual bees (Arathi et al., 2000; Arathi et al., 2006; Arathi and Spivak, 2001; Gramacho and Spivak, 2003) have shown that early detection of diseased brood is critical for resistance; the bees must be able to detect and remove the brood before the pathogen reaches the infectious stage within the bee host. Colonies selected for rapid-hygienic behavior demonstrate resistance to American foulbrood and chalkbrood in the field (reviewed in Spivak and Gilliam, 1998a,b; Spivak and Reuter, 2001a). Like grooming behavior however, the process of removing diseased brood through handling or ingesting may facilitate pathogen transmission if the bees remove the diseased brood after the pathogen has reached the transmissible stage. This may happen in colonies comprised of slow-hygienic bees with lower olfactory sensitivity to diseased brood such that they detect and begin to remove it only after the stimulus level is high and the brood is highly infectious. However, the bees that handle diseased brood are, on average, 15–18 days old (older than typical nurse bees; Arathi et al. 2000), so the likelihood of the same bees returning to feed larvae and potentially transmitting spores into larval food is reduced. Thus, the effects of temporal polyethism (Fig. 1c and d) may help reduce the movement of pathogens from carriers to those most susceptible.

Hygienic behavior also provides an important mechanism of defense against *Varroa* when bees detect and remove pupae that are infested by mites. *A. cerana*, the original host of *Varroa*, detects mite-infested pupae, and may either make a hole in the wax capping covering an individual pupa, thereby releasing the mite, or may remove the wax capping entirely and remove the pupa (Peng et al., 1987b; reviewed in Boecking and Spivak, 1999). If the mites are released from the cell, they presumably become vulnerable to allo-grooming between adult bees. Thus, hygienic behavior explains some of the resistance by *A. cerana* to mites, relative to the heavily parasitized host *A. mellifera.* Nevertheless, in contrast to *A. mellifera,* successful reproduction of mites in *A. cerana* colonies is limited to drone pupae. It is possible that colony-level resistance of *A. cerana* to *Varroa* may largely reflect the seasonal production of drones and thus limited opportunity for mite reproduction (Fries et al., 1994; Rosenkranz et al., 2010).

Based on reports that *A. cerana* displays hygienic behavior toward mite-infested brood, interest in this behavioral trait arose in Europe and later in the US as a possible mechanism of defense

against *V. destructor* on *A. mellifera* (Boecking and Spivak, 1999). *V. destructor* reproduces on both drone and worker pupae of *A. mellifera*, leading to rapid population increase while brood is present in the nest. The removal of mite-infested worker brood through hygienic behavior would be a highly desirable trait because it would interrupt the reproductive cycle of the mite, killing any mite offspring, which could have cumulative negative effects on the mite population dynamics. It was found that some *A. mellifera* colonies detect and remove mite-infested worker pupae (Boecking and Drescher, 1992), and in colonies selected for hygienic behavior, up to 60% of the experimentally infested pupae were removed (Spivak, 1996; Spivak and Boecking, 2001).

A common field assay for hygienic behavior involves freezing a comb section containing pupae and recording the time it takes the colony to remove the freeze-killed brood (Spivak and Gilliam, 1998b; Spivak and Downey, 1998; Spivak and Reuter, 2005). Colonies that remove dead brood within 24–48 h also tend to remove higher proportions of diseased and mite-infested brood compared to colonies that take longer than 48 h to remove dead brood. Colonies selected for rapid-hygienic behavior based on the freeze-killed brood assay had significantly lower mite loads relative to unselected stocks of bees in three different large-scale field trials (Ibrahim et al., 2007; Spivak and Reuter, 1998, 2001b). However, presenting colonies with freeze-killed brood is not the most sensitive assay for hygienic behavior, and an alternate assay used by some researchers, in which individual pupae are killed by piercing them through the cell capping with a fine pin, is even less sensitive (Gramacho and Spivak, 2003; Spivak and Downey, 1998). Knowing that the detection of parasitized and diseased brood is based on olfactory stimuli (Masterman et al., 2001; Spivak et al., 2003), it would be best to identify the volatile compound(s) specifically associated with mite-infested pupae and present them to the bees in a low concentration such that only the bees in colonies with the highest olfactory sensitivity would respond to them. Such a study was recently conducted with the volatile compounds associated with chalkbrood-infected larvae. Bees had positive electroantennogram response to three specific volatiles collected from infected larvae, but only one, phenethyl acetate, elicited a strong hygienic response in very low concentrations ($10^{-9}$/ml) by colonies selected for rapid-hygienic behavior (Swanson et al., in press). Colonies with slower hygienic responses (e.g., those that did not remove freeze-killed brood within 24 h) removed fewer larvae treated with phenethyl acetate at that concentration. Such a study should be conducted to identify the volatile compounds associated with *Varroa*-infested pupae.

It is likely that the olfactory stimuli that hygienic bees use to detect *Varroa*-infested pupae are associated with the bee's wound response to mite feeding, although the mite's offspring or feces accumulation may also be important. This speculation is based on findings from an interesting line of bees bred by Harbo and Harris (1999) and Harbo and Hoopingarner (1997), now named VSH for *Varroa* sensitive hygiene (Harris, 2007). Originally, colonies were included in this selection program for mite resistance only if they displayed a reduction in mite levels over specified periods of time (Harbo and Hoopingarner, 1997). It was thought that the mites in the selected colonies had reduced reproductive success (Harbo and Harris, 1999, 2000, 2001). Later, two different experimental approaches revealed that in fact the line of bees was displaying hygienic behavior; VSH colonies were able to detect and remove mite-infested pupae (Harbo and Harris, 2005), and they removed more infested pupae compared to colonies from the hygienic line bred based on the freeze-killed brood assay (Ibrahim and Spivak, 2006). It was subsequently found that they remove the mite-infested brood only after the mite has initiated oviposition, indicating the stimulus must reach a critical intensity at that time (Harris, 2007). Considering the mite maintains an open feeding site for her teneral offspring, it could be that this site produces volatiles that the bees perceive as abnormal. Continued studies on the VSH line have shown that the bees do not always remove the mite-infested pupae; many times the cell capping is opened, allowing the mite to escape, then is resealed (Harris, 2008), reminiscent of the behavior of *A. cerana* colonies in Asia. There is considerable potential for understanding mechanisms of mite resistance through continued studies of the VSH line.

### 4.3. Undertaking

Undertaking, or necrophoric behavior, refers to the removal of dead adults from the nest. This collective behavior favors colony health by reducing contact with potential pathogens. Necrophory as a form of social immunity is pronounced in ants (e.g., Hart and Ratnieks, 2001; Howard and Tschinkel, 1976), and is effective because the garbage heap where dead ants are piled is a physically separated chamber within the nest, attended to by a group of specialized workers who have little contact with other nestmates. Undertaking is also pronounced in honey bees (Visscher, 1983), yet most studies have focused on the behavior in relation to genetic determinants of task specialization (e.g., Breed et al., 2002; Robinson and Page, 1988, 1995; Trumbo et al., 1997) and not in relation to social immunity. Honey bees do not have specific garbage heaps, and bees that die within the nest are carried outside the nest. However, most bees presumably die outside of the nest including those carrying potential parasites. Foragers carrying *V. destructor* mites or *Nosema* infections have compromised flight and homing abilities and often die before returning to the nest (Kralj and Fuchs, 2006, in press; Naug and Gibbs, 2009), which may reduce pathogen transmission by curtailing contact and handling by undertakers and other nestmates. Future research might focus on the relative importance of undertaking versus dying outside the nest as mechanisms of social immunity, particularly in relation to viral infections (Visscher, 1980; Waddington and Rothenbuhler, 1976 and references within).

## 5. Modifying the nest environment

### 5.1. Behavioral fever

Honey bees have evolved strategies to closely regulate the internal environment of their nest cavities through heating, cooling, and ventilation (Seeley and Visscher, 1985). When there is brood in the nest, they maintain the temperature of the combs around 32–34 °C and make efforts to dampen fluctuations in humidity. This ability to thermoregulate, in particular, has been co-opted in several ways as a defense against biological threats. Individual honey bees can group together in a ball, collectively raising the temperature to at least 45 °C around a foreign queen or predator such as a wasp (Esch, 1960; Ono et al., 1987). One study has investigated the possibility that bees generate a fever in response to pathogen challenge. Starks et al. (2000) found slight but significantly elevated brood comb temperatures when colonies housed in observation hives were challenged with the chalkbrood pathogen, *Ascosphaera apis*. This fungal pathogen tends to develop in challenged larvae when they are chilled to 30 °C (Bailey and Ball, 1981), so the authors speculated that the observed 0.56 °C average increase in brood comb temperature during pathogen challenge might prevent disease development. These results are intriguing and future studies should determine if elevated temperature actually reduces or prevents chalkbrood development in the challenged larvae. The development of *V. destructor* may also be hindered by heat (Garadew et al., 2003) so further investigations into colony fever as a form of social immunity are warranted.

### 5.2. Nest architecture and resin collection

In nature, honey bees nest in hollow trees (Seeley and Morse, 1976). Before building comb, they scrape loose, rotten wood off the walls of the tree cavity, which serves to remove fungal mycelia and to expose hard wood. They then coat the walls with a layer of resins (complex plant secretions called propolis when found inside the nest), eventually creating a propolis envelope surrounding the entire cavity (Seeley and Morse, 1976). This envelope acts as a waterproof barrier, and seals cracks and crevices to prevent the entry of air currents and sunlight into the nest (reviewed in Visscher, 1980). This also prevents further fungal decay of the tree cavity due to the antifungal properties of propolis (Lavie, 1968). The addition of small amounts of propolis to wax combs could provide additional antibiotic properties (Ribbands, 1953), although this has not been experimentally confirmed.

Some studies have explored the efficacy of propolis against the bacterial pathogen *P. larvae* that causes American foulbrood disease (Antunez et al., 2008; Bastos et al., 2008; Lindenfelser, 1968), against wax moths (Johnson et al., 1994), and even the mite *V. destructor* (Garadew et al., 2002). In all cases, propolis has been shown to be active against these bee threats. However, current studies are revealing a more subtle but evolutionarily important function of propolis as a form of social immunity: resins within the nest decrease investment in immune function of adult bees (Simone et al., in press). Colonies exposed to extracts from two sources of honey bee propolis led to significantly lowered expression of two honey bee immune-related genes (hymenoptaecin and AmEater in Brazilian and Minnesota propolis, respectively) in 7-day old bees, and to lowered bacterial loads in the MN-propolis treated colonies. Because elevated immune function carries a cost for honey bees (Evans and Pettis, 2005), the presence of resin in the nest may have important fitness benefits. This is the first direct evidence that a component of the honey bee nest environment affects immune-gene expression.

Additionally, bees use resins to encapsulate nest intruders, which illustrates a fascinating analogy between individual cellular immunity and a colony-level immunity. Honey bees entomb dead mice or large insects that are too large for bees to remove from the nest in propolis (reviewed in (Visscher, 1980). *A. m. capensis* fully encapsulates the parasitic small hive beetle *Aethina tumida* in "propolis prisons" which prevent the beetles from reproducing (Neumann et al., 2001).

### 5.3. Social organization

The sociogenetics, ergonomics and physiological underpinnings of the division of labor have been studied extensively in honey bee colonies (Ament et al., 2008; Johnson, 2008; Nelson et al., 2007; Rueppell et al., 2004). Less studied is how parasite transmission has shaped the division of labor and social organization within the nest (Fig. 1d). Using a far-sighted approach, Naug and Camazine (2002) modeled how the combined effects of division of labor, interaction networks, and colony demography interact and influence parasite dynamics in complex ways.

Temporal polyethism, one type of division of labor in which the performance of different tasks is based on age, within a honey bee colony can increase pathogen vulnerability within a specialized task group of individuals, but can also present a barrier to pathogen transmission. For example, foragers are usually considered, in terms of ergonomics, performing the risky task of collecting resources for the colony. But older foragers also present increased risk of pathogen transmission within the nest (Naug and Camazine, 2002) and leaving the nest to forage reduces this risk. Bees infected with *Nosema* and sacbrood virus are known to forage precociously (Bailey and Fernando, 1972; Hassanein, 1953), which may be an adaptation to reduce the rate of disease transmission, particularly if they die outside the nest. Together with temporal polyethism, the interaction network among individuals, particularly through trophallaxis, also influences the rate and frequency of transmission. Middle-aged bees that perform hygienic behavior (Arathi et al., 2000) and undertaking (Visscher, 1983) may not subsequently interact or share food with nurse bees and larvae, again reducing the risk of disease transmission. The interaction network is in turn influenced by colony demography (the size and density of the colony). Large, dense colonies although ergonomically more efficient, have higher contact and infection rates with pathogens. Increased persistence and transmission may be countered by temporal polyethism and differential rates of birth, development and death of individuals. Naug and Smith (2007) used the term "organizational immunity" to describe how the social organization within the nest interacts with epidemiological variables to create different risk categories of pathogen transmission within a social group. This avenue of thought has clearly opened up novel and important directions for future studies. Recently, Richard et al. (2008) showed changes in worker behaviors when exposed to nestmates that had experienced an immune challenge. Exposed workers received significantly more grooming behavior, arguably increasing the risk for horizontal disease transmission but decreasing disease loads through allo-grooming.

Finally, environmental factors such as the flow of resources (pollen and nectar) into the colony influence pathogen dynamics and disease transmission in ways that are little studied (Naug and Camazine, 2002). The relationship between nutrition (protein consumption through pollen and the amount of nectar available for trophallaxis) and the ability to withstand pathogen and parasite pressure is not well studied in honey bees. In other species the physiological costs to resisting pathogens are well documented, as are dietary steps taken to partially offset these costs (e.g., in the caterpillar *Spodoptera*; Lee et al., 2006; Povey et al., 2009).

### 5.4. Symbiotic bacteria

Honey bees maintain a stereotypical population of bacteria and other microbes in their bodies (Gilliam, 1997; Olofsson and Vasquez, 2008). These bacteria might play either positive or negative roles on bee health and are almost certainly important in nutritional health (Fig. 1e). Recently, endogenous bee bacteria have been shown to inhibit growth of the chalkbrood fungus (Reynaldi et al., 2004) and the bacterium responsible for American foulbrood disease (Evans and Armstrong, 2005, 2006; Sabate et al., 2009). While it remains unclear whether these bacterial species can be effective in reducing disease *in vivo*, it seems plausible that honey bees could have evolved means to maintain populations of microbial associates that act as shields, in part, from pathogens. If so, it should be possible to identify behaviors in bees, and perhaps morphological or physiological traits that favor the acquisition and maintenance of beneficial microbes. There is likely to be an interesting tradeoff between favoring the growth of beneficial microbes and avoiding conditions that allow more rampant growth by pathogens (Evans and Armstrong, 2006). Regardless, in bees, as in other insects, an understanding of disease is complicated by a need to understand interactive affects between various host microbes.

## 6. Conclusions and future directions

Honey bees have natural defenses that are relevant for their health in managed and free-living colonies. These defenses can be better enabled by both management and breeding decisions by the beekeeping industry. Sizable variation exists in honey bee

resistance traits ranging from hygienic behavior (Spivak and Reuter, 2001a) to antibacterial immune responses (Decanini et al., 2007). Below are several emerging directions for research aimed at a better understanding of the impacts of disease agents on honey bees and possible directions for reducing these impacts.

### 6.1. Interactions among multiple parasites

While it has long been appreciated that multiple parasites can co-infect honey bees (e.g., Bailey, 1983), studies showing how partners might act synergistically are scarce. Two major advances now allow the study of roles played by interactions between pathogens. First, prior surveys of honey bee disease candidates have focused on one taxonomic group at a time. This 'discovery' phase of determining potential causes of disease can now be carried out at ease to test for dozens of possible parasites (e.g., Cox-Foster et al., 2007). With recent advances, these techniques will be capable of screening many samples simultaneously, truly generating a fingerprint of the microbes associated with disease in the field or under controlled conditions. As another tool for better studying the interactions among honey bee parasites, it is now possible to precisely assess the genome copy numbers of each of the major bee parasites, and to thereby determine competitive or synergistic interactions among co-infecting parasites (e.g., Chen et al., 2009).

### 6.2. Parasite differences in virulence and the threats of parasite movement

Parasitology in honey bees has often treated potential disease agents as having fixed traits. In fact, there is ample evidence that microbes (Genersch et al., 2005; Palacios et al., 2008; Fünfhaus et al., 2009) and even mites (Solignac et al., 2005) differ substantially in their virulence traits toward bees. As one example, the RNA virus IAPV is associated with severe and distinctive pathologies in some Israeli populations, expressed as paralyzed bees and bees showing irregular behaviors (Maori et al., 2007). This virus has also been associated with bees from populations in severe decline (Cox-Foster et al., 2007), yet without showing these diagnostic traits. Similarly, isolates of the bacterium *P. larvae* differ greatly in both virulence and range across bee populations (Genersch et al., 2005; Rauch et al., 2009).

An understanding of variation across pathogen strains in impacts on honey bee hosts has practical importance for determining management strategies and for assessing the risks involved with possible pathogen movement into naïve populations. Scientists need to develop robust, repeatable, exposure assays for bacteria, viruses, and fungi in order to evaluate the virulence of a particular parasite or pathogen lineage. This information could improve the development of measures to limit the negative impacts on bee health, ranging from quarantines to chemotherapy of colonies. An attractive alternative to such bioassays is the development and implementation of diagnostic tools that enable the identification of genes determining differences in pathogen and parasite virulence (Fünfhaus et al., 2009). Once optimized, such tools could be applied across field colonies in order to assess disease risk levels. Given the current uncertainty over how virulence differs within and across pathogen species, the movement of colonies across regions or countries should be carried out with great caution, and only when critical for the honey bee industry.

### 6.3. Breeding for resistant or tolerant honey bees

There is considerable evidence that honey bees differ in survivorship when exposed to pathogens (Palmer and Oldroyd, 2003; Rothenbuhler and Thompson, 1956) and that this fact reflects in part variation across individuals in their individual immune response levels (Decanini et al., 2007). This variation is puzzling, given the apparently high disease threats faced by honey bees. Such variation in how individuals respond to disease has been proposed to reflect two distinct classes of selection. First, high energetic and life-history tradeoffs in the ability to fend off disease could lead to a mixed strategy, whereby some individuals might opt for a low-cost but risky strategy of maintaining weak immune defenses while others invest more heavily in defense. There is some evidence that heightened immune function carries a cost for honey bees (Evans and Pettis, 2005), and such costs of an induced response are widely supported in fruit flies (Vijendravarma et al., 2009) and aphids (Gwynn et al., 2005), among other insects. Second, variation across individuals in how well they respond to a specific pathogen strain could reflect a general fact of host–parasite coevolution, namely the unceasing evolutionary arms race between hosts and their parasites and pathogens. Bee hosts are unlikely to be able to effectively recognize and combat each strain or species of pathogen they might be exposed to and, when they key in on one such strain it might be at the expense of their abilities to respond to another. Knowledge of variation across bees in their immune function is needed before using this trait as a breeding tool for developing resistant or tolerant bees. As one example of immune traits suitable for breeding consideration, honey bee larvae are known to differ in their resistance to the widespread bacterial disease American foulbrood (*P. larvae*) under controlled and natural conditions (Evans and Pettis, 2005; Palmer and Oldroyd, 2003; Rothenbuhler and Thompson, 1956). Larval bees upregulate an expected subset of antimicrobial peptides in response to natural exposure to *P. larvae* (Evans, 2004). These responses appear to be moderately heritable ($h^2$ = ca. 0.25), are controlled by several genes, and are only capable of explaining a fraction of the observed variation in larval survivorship (Decanini et al., 2007). The relatively weak correlation between humoral immune responses and larval survival indicates that other heritable or environmental processes are involved with resisting or tolerating *P. larvae* infections. Among these other factors, individual bees might escape disease by speeding their development, because the first instar is a point of especially high vulnerability to infection (Sutter et al., 1968). In addition, these measures were focused on the humoral response and defenses at the cellular level in honey bees are almost certainly involved with disease. Finally, there is persistent evidence that diet or other environmental traits of individual bees could affect their disease risk. While it is certainly desirable to find traits in honey bee populations that confer resistance to disease, choosing specific traits for study and directed breeding will depend both on their effectiveness in resisting or tolerating disease, and on their potential costs when bees are not facing disease. Similarly, it is likely that immunity and other individual resistance traits will act synergistically with behavioral traits such as the hygienic removal of diseased larvae, so breeding programs might have more impact by simultaneously targeting individual and 'social' traits for selection.

Interestingly, the great diversity of patrilines in the typical honey bee colony predicts that individuals in colonies can expect to be raised by, and to care for, siblings with dramatically different disease-related phenotypes. In fact, if there is a synergy in resistance function (e.g., if having only some individuals focus on hygienic behavior is sufficient and less costly than having an entire colony of highly vigilant individuals), then having multiple patrilines with varied investment across disease-resistance mechanisms would be desirable at the colony-level. Tarpy and Seeley (2006) and Seeley and Tarpy (2007) have used artificial insemination schemes to provide compelling evidence that patriline diversity, *per se*, is important for reducing disease loads.

### 6.4. Mitigating disease risks through changes in honey bee management

We firmly believe that selective breeding programs have had strong impacts on reducing honey bee disease and offer the most potential for the long term health of the industry, and commend the many bee breeders who are focused on improving the stock they distribute. New tools ranging from standardized field tests for hygienics to genome-enabled screens for identifying, enabling, and maintaining resistance traits should help these efforts. Nevertheless, management changes could also reduce some of the costs of disease. The most important implication of research on social immunity in honey bees is that they have amazing capabilities to defend themselves as individuals and at the colony-level. Honey bees are faced with a multitude of pathogen challenges that are compromising their health in unprecedented ways. Many beekeeping practices rely on using antibiotic and pesticide treatments to control pathogens and parasites. This approach is not sustainable and leads to contamination of hive equipment (Tremolada et al., 2004) or hive products (Karazafiris et al., 2008; Waliszewski et al., 2003), off-target impacts on bees themselves (Burley et al., 2008; Collins et al., 2004), and the evolution of resistance by parasites and pathogens (Evans, 2003; Sammataro et al., 2005).

While beekeepers engage in many management tactics to reduce disease in their colonies, recent research and emerging risks indicate three management tools that are underutilized in beekeeping and are perhaps especially relevant. First, the design of modern bee boxes could be modified to allow the construction of a propolis envelope, an important antimicrobial layer with direct effects on individual and social immunity. Most beekeepers dislike the presence of propolis in beekeeping equipment because it forms bonds across wooden frames and boxes, making it difficult to manipulate and remove combs for inspection. The smooth inner walls of the hive boxes also eliminate the need for the bees to create a propolis envelope. The value of propolis in the nest as a colony-level defense and form of social immunity, directly affecting immune-gene expression and arguably microbial loads, should be taken into serious consideration in modern beekeeping practices and perhaps in future hive designs. Second, it would be beneficial to allow colonies to construct new wax brood combs yearly to prevent the build up of pathogens and pesticides in the wax, which present a chronic immune challenge to bees. Finally, rates of horizontal transmission of pathogens are likely to be at an all-time high in modern beekeeping, presenting great challenges. Lucrative pollination contracts have in many ways sustained beekeeping in North America and elsewhere. Nevertheless, the resulting wide-ranging transport of bees, even across international boundaries and often to high-density meeting grounds, favors the sharing of parasites and pathogens. This unnaturally high pressure of horizontal transmission, coupled with the use of antibiotics and pesticides to control pathogens, can lead to both short-term impacts on beekeepers and long-term effects on the ability of bees to evolve resistance toward their pathogens. It can also favor the spread and de novo evolution of more virulent pathogen strains (Read, 1994), which thrive in a world that provides them a steady supply of new and vulnerable hosts. Changing management strategies to reflect these factors, where feasible, can be complimentary to efforts to breed and maintain bee lineages showing natural resistance.

## Conflicts of interest

There are no conflicts of interest to be declared.

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