Genetic, Individual, and Group Facilitation of Disease Resistance in Insect Societies

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Abstract

In this review, we provide a current reference on disease resistance in insect societies. We start with the genetics of immunity in the context of behavioral and physiological processes and scale up levels of biological organization until we reach populations. A significant component of this review focuses on *Apis mellifera* and its role as a model system for studies on social immunity. We additionally review the models that have been applied to disease transmission in social insects and elucidate areas for future study in the field of social immunity.

INTRODUCTION

Living in groups may increase the fitness of individuals by decreasing the costs associated with important life-history activities, including foraging efficiency, cooperative brood care, colonizing and competitive abilities, defense from enemies, and the ability to adaptively modify the environment (88, 128). Living in groups also poses unique fitness constraints on individuals, including increased competition and increased risk of disease transmission due to close living quarters with closely related individuals (95, 116). Evidence from the global success of social animals suggests that the benefits of social living may outweigh its costs (34, 47, 59, 70, 108, 128).

The success of social insects remains enigmatic in regard to their ability to resist disease. The high level of cohesion in eusocial animal colonies may increase the risk of disease outbreak as a result of close living quarters, high genetic relatedness among individuals, and continuous physical interactions between individuals both within and across generations (30, 35, 95, 126). In response, eusocial insects in particular have evolved novel physiological, behavioral, and organizational adaptations to combat the increased risk of disease (3, 27, 49, 87, 88, 109, 118, 130, 131). These collective defenses against parasites and pathogens are examples of social immunity. As one would expect, disease resistance traits are under the influence of genetic and environmental constraints (15, 26, 92). The ability to mount an immune response can be quantified empirically by using measurements of immunocompetence (IC). We define IC as the ability of an organism to mount an immune response. Multiple forms of IC are seen throughout the biological world, as well as in behavioral, cellular, and humoral (noncellular) and behavioral processes (84). Eusocial insects are a unique system for studying the relationship between IC and behavioral ecology because of their behavioral processes, many of which mitigate susceptibility and thus bolster immunity. The term antiseptic behavior is introduced in this review to describe behaviors of individuals within a social insect colony that, analogous to the cellular and humoral processes within an individual, provide defenses against pathogens to decrease transmission and increase resistance to diseases. Some examples of antiseptic behavior include grooming, hygienic behavior, undertaking, avoidance, glandular secretions, and use of resins in the nest. Antiseptic behavior in social insects may provide another level of defense in addition to cellular and humoral IC. At the physiological level, eusocial insects are similar to nonsocial invertebrates with regard to their cellular and humoral immune physiology. Unfortunately, there have been remarkably few descriptive studies comparing the two, even though distinctions between social and nonsocial insects abound at the organismal and behavioral levels. In fact, examples of immune behavior exclusive to eusocial insects have been well described in the honey bee with regard to its mechanisms of antiseptic behaviors, which decrease disease transmission and susceptibility. Analogies between physiological and behavioral levels may also be drawn, for example, between cellular encapsulation of a foreign body and social aggregation of intruders by individuals within a social insect colony.

Here, we aim to synthesize some of the literature relating to disease resistance in eusocial animals, with special emphasis on the honey bee, *Apis mellifera*. The collective immune defense (or social immunity *sensu* 26) by social insects against parasites was recently reviewed by Cremer and colleagues (27). As such, we do not focus solely on the group response to disease. Instead, we provide a multilevel approach, from gene to population, for a detailed understanding of the underlying mechanisms upon which natural selection may act (**Figure 1**). Although we organize this review from gene to population, the distinction across levels with respect to genetics is somewhat artificial. While acknowledging that phenotypes and extended phenotypes do not exist without genotypes, we believe the heuristic value of organizing the review from gene to population outweighs any disadvantages associated with our categorization.
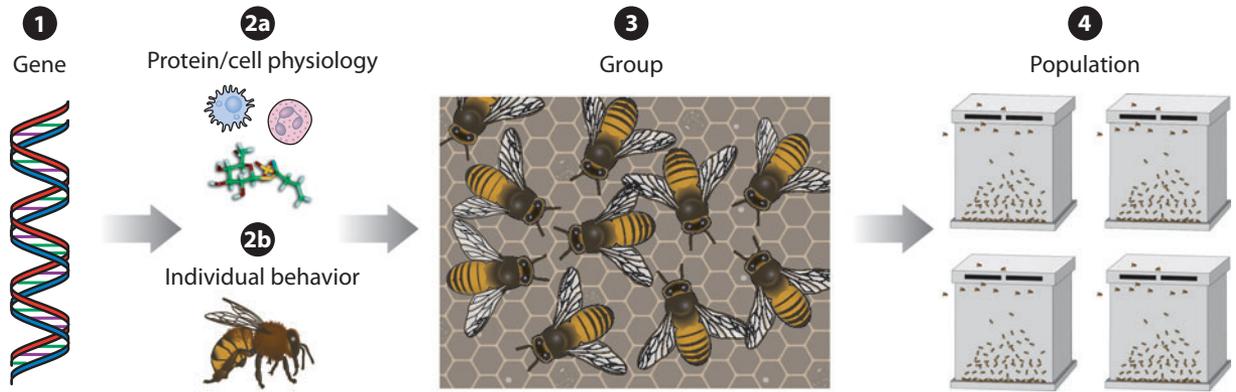
Figure 1

Graphical representation of the approach to this review. Social insects resist disease at multiple levels. Panel 1: Selection promotes genes that are most efficient in their ability to produce disease resistance phenotypes. Panel 2: Gene products include proteins and cells, which play vital roles in physiological immunity. Individual organisms engage in antiseptic behavior. Panel 3: Groups of individuals mount collective defenses, also known as social immunity. Panel 4: Populations provide important insight into large-scale disease dynamics, which can be studied using mathematical models.

WHAT ARE GENE-LEVEL DEFENSES AGAINST DISEASES?

Variation in pathogen intensity likely results in selection on disease resistance alleles. Solitary animals are limited in their ability to access social immunity and so should rely on physiological immune defenses more so than group-living organisms. Indeed, recent evidence from the honey bee genome has shown that the genetic variation underlying the ability of honey bees to mount an immune response is likely to be lower compared to that of solitary insects (29), although additional research is necessary to fully support this. How do individuals within a densely populated society such as a honey bee colony compensate for this reduced immune ability? One way is through the evolution of defenses that emerge at the colony level through the collective behaviors of individuals. One defense is hygienic behavior, in which individuals detect and remove diseased brood from the nest, resulting in colony-level resistance to pathogens and parasites. We review recent studies on honey bee genomics as they relate to disease resistance, the genetic basis of honey bee hygienic behavior, and allelic diversity (haploid susceptibility) to provide a model for similar research on the genetic basis of social immunity.

Genome-Level Studies: Honey Bee Genomics

Bioinformatics provides useful tools for answering questions related to allelic function and phylogenetic relatedness among genomes. The honey bee genome was recently sequenced by The Honeybee Genome Sequencing Consortium (125). This accomplishment provides the first genomic insight into the genetic makeup of a eusocial species.

Genetic diversity contributes to parasite resistance in ants (96), bumble bees (6, 7, 8, 62), and honey bees (99, 116). Honey bee queens mate with an average of 7–17 drones (132). Many hypotheses have been posited to explain this extreme polyandry in the mating system of the honey bee, including improved division of labor within a colony (86), heightened probability for sperm acquisition (25), and decreased disease susceptibility via increased genetic diversity at disease resistance loci (39, 95, 99, 100, 116). The latter hypothesis, termed the 'polyandry versus parasitism hypothesis' (100), posits that polyandry is a defense mechanism against pathogens and parasites. Tarpy (116) and Seeley and Tarpy (99) found that colonies headed by queens artificially inseminated with multiple males have decreased variation in their

Hygienic behavior: the ability to detect and remove diseased and parasitized brood in the nest

ability to resist infection to the diseases chalk brood and American foulbrood, respectively. This decrease in variation appears to guard against broad-scale disease susceptibility within a relatively genetically diverse colony.

In regard to disease resistance, honey bees possess fewer immune genes than fruit flies (*Drosophila melanogaster*) and mosquitoes (*Anopheles gambiae*), which affects every step of the immune response, from pathogen recognition to the production of immune proteins (125). This finding suggests a reduced flexibility in the abilities of honey bees to recognize and resist pathogens (125). Similarly, honey bees possess decreased variability in prophenoloxidase (proPO) genes compared with other insects, whereby honey bees have only one proPO gene compared to three in *D. melanogaster* and nine in *A. gambiae* (29). proPO is the inactive zymogen precursor to phenoloxidase (PO), an important enzyme to innate immune function that is responsible for oxidation of tyrosine derivatives to toxic quinones and downstream polymerization into melanin. Although honey bees have intact pathways implicated in immunity (Toll, Imd, JAK/STAT, and JNK), these pathways seem to lack some of the flexibility seen in other insects for responding to and targeting pathogens. As one example, honey bees have half as many peptidoglycan recognition proteins as do *D. melanogaster*, *A. gambiae*, and *Tribolium castaneum* (flour beetles) (125) and fewer plausible exons for splice-site variation in peptidoglycan transcripts (29, 125).

The honey bee genome also indicates differential expression of disease resistance peptides across castes and between developmental stages that are motile (adults) and those that are nonmotile (brood), suggesting another important link between physiological and behavioral IC. The proPO gene in honey bees is expressed more strongly in adults and older pupae than in younger pupae and larvae (63). An additional proteomic study of honey bee hemolymph showed the proPO zymogen was 50-fold more prevalent in the hemolymph of adult honey bee workers compared with larvae (22), and levels of the antibacterial peptide hymenoptaecin are also higher in adults than in larvae (22). Expression of peptidases, defensins, and transferrin homologues is preferentially upregulated in queens over workers (38), each of which is likely affected by differences in longevity and behavioral role. Together, these results suggest a relationship between behavioral capacity and the expression of disease resistance genes.

Honey bee sociality and nesting ecology likely play an important role in compensating for the limited number of immune genes and differential gene expression between castes. The high level of cooperative brood care and nest hygiene (defined as behaviors that increase sanitation of an individual or the nest) performed by adult honey bees likely augments hive disease resistance. Larvae and pupae are confined within the brood comb and adult bees perform nest hygiene (132). Larvae are offered protection by the antibacterial properties of royal jelly (14, 53). Natural products stored in the colony (honey and pollen) are protected from bacterial decay by enzymatic secretion of glucose oxidase and the physical properties of honey (124). The inner hive is further protected from pathogens by deposition of propolis, which has antimicrobial properties (11, 12).

Genetics of Honey Bee Hygienic Behavior

Hygienic behavior is defined as the ability to detect and remove diseased brood from the nest. Hygienic behavior is an antiseptic behavior and differs from undertaking (the removal of dead adult nestmates) and grooming [the removal of foreign objects and pathogens from oneself (autogrooming) or from another adult in the nest (allogrooming)]. Sumana & Starks (114) showed grooming occurs largely for cleaning purposes and not only to spread secretions such as cuticular hydrocarbons. Hygienic behavior in honey bees was first described in the 1930s when researchers sought to determine the mechanism by which some honey bee colonies were resistant to the highly infectious brood disease American foulbrood, caused by
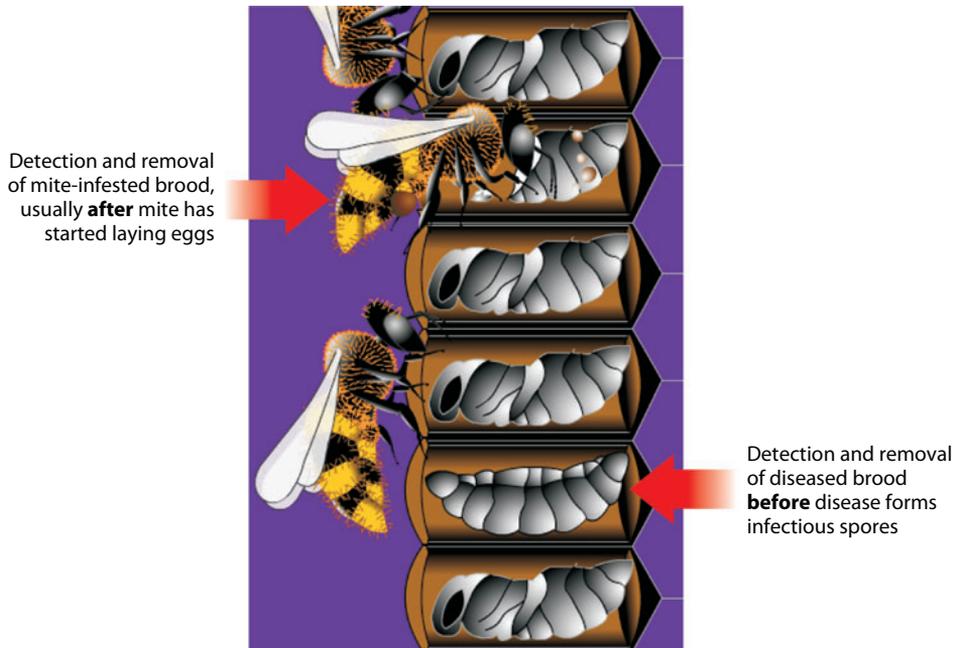
Detection and removal of mite-infested brood, usually *after* mite has started laying eggs

Detection and removal of diseased brood *before* disease forms infectious spores

Figure 2

Graphical representation of honey bee hygienic behavior.

the bacterium *Paenibacillus larvae* (**Figure 2**) (78, 79). Park and colleagues (80) observed that, ". . . the bees sometimes remove and dispose of [diseased] larvae very soon after they die, thus eliminating the evidence." Their observations were confirmed by Woodrow and Holst (133), who concluded, "The data show that resistance to American foulbrood in the honey bee colony consists in its ability to detect and remove diseased brood before the causative organism, *P. larvae*, reaches the infectious spore stage in the diseased larvae"; and that "The early removal of diseased larvae while they contain only the noninfectious rods of *P. larvae* prevents dissemination of disease in the colony, whereas removal of infected brood containing the highly infectious spores results in spread of disease to other larvae."

In 1964, Rothenbuhler and his students published a six-part series of articles on the behavioral genetics of hygienic behavior in honey bees (52, 71, 89, 90, 117, 119). He developed a two-locus model of inheritance for hygienic behavior, which was recognized as a classic ex-

ample of the effects of Mendelian inherited genes on behavior (1). The process of uncapping a cell containing dead brood and removing

HYGIENIC BEHAVIOR

In 1956, W.C. Rothenbuhler first used the term hygienic behavior to describe a specific trait of honey bees: the ability to uncap and remove diseased brood from the nest. Currently, the term hygienic behavior is sometimes used to refer to general nest hygiene of social insects, such as trash removal and removal of dead adults (undertaking). Because of the extensive amount of research on the genetics, neuroethology, and applied ecology of honey bee hygienic behavior, we prefer to retain the usage of the term hygienic behavior to refer to the removal of diseased and parasitized brood from the nest by honey bees, dampwood termites, and other social insects that might perform this specific task. In our framework, hygienic behavior is an antiseptic behavior and a form of nest hygiene. The term nest hygiene refers to the broader collection of behaviors used by social insects to remove pathogens and parasites from the nest. In this way, hygienic behavior, undertaking, and trash removal are examples (subcategories) of nest hygiene.

the contents was thought to be dependent on homozygosity for two recessive genes (*u* and *r*). Workers heterozygous at both loci should not be hygienic. Homozygosity at one of the two loci should result in workers that either uncap (*uu*) or remove (*rr*).

Other researchers have proposed that a three-locus model [*u*, r_1, and r_2 (73) or u_1, u_2, and *r* (36)] may better fit the original data set. More recently, using molecular techniques and quantitative trait loci (QTL) linkage mapping, Lapidge et al. (57) associated seven suggestive QTLs with hygienic behavior. Each putative QTL controlled only 9%–15% of the observed phenotypic variance in the character.

Honey bee hygienic behavior is also a mechanism of defense against the parasitic mite *Varroa destructor*. *A. cerana* (81) and some *A. mellifera* colonies are able to detect and remove pupae that are parasitized with these mites, particularly Africanized bees (reviewed in 16, but see 72) as well as commercial lines available in the United States, for example, a line bred for Varroa Sensitive Hygiene (42) and the MN Hygienic line (102). The removal process interrupts the mite reproductive cycle, thereby lowering the mite population (106, 107). It is unclear, however, if the detection of diseased brood by honey bees is influenced by the same loci as is the detection of mite-infested brood (50).

One consideration for these and future studies on the genetics of hygienic behavior is that all worker honey bees are able to, and do, perform the motor tasks of uncapping and removing diseased brood at some point in their adult life. The genetic difference among colonies lies in how quickly individual bees within a colony detect the presence of diseased brood within the nest. Individual bees with low-threshold responses to the cues from the diseased brood rapidly initiate the removal process. Future research would benefit from studies on the genetic differences in the detection of diseased brood, and more studies are needed on the neuromodulation of olfactory sensitivity and responsiveness among bees within lines bred for hygienic behavior.

Allelic Diversity: The Haploid Susceptibility Hypothesis

Haplo-diploid systems are ideal for scaling up from single gene effects to collective impacts because these systems have adults that are either haploid or diploid. Comparing across haploid and diploid conspecifics may thus shed light on the collective impacts of some genetic traits. And indeed, data do suggest that males of some eusocial hymenopterans are more susceptible to certain pathogens, for example *Varroa* mites in honey bees (94), although the multiple hypotheses explaining increased male susceptibility remain to be tested, e.g., developmental time, size, or location in the brood comb.

This susceptibility may drive other observable characteristics. For example, isolation of hymenopteran males has been observed in honey bees and paper wasps. Honey bee drones accompany swarms at a lower rate than expected given the number of males in the colony (83), and paper wasp males in *Polistes dominulus* are isolated from returning foragers (112). The haploid susceptibility hypothesis suggests that haploid males exhibit increased disease susceptibility, and that this vulnerability may have been a factor in the evolution of behavioral interactions in social animals (76). This hypothesis assumes that decreased diversity at disease resistance loci negatively influences the survival of haploid organisms (as haploids and homozygous diploids have one type of defense loci, whereas heterozygotes have two and thus possess a heterozygote advantage).

The theoretical implications behind the haploid susceptibility hypothesis were elaborated upon by O'Donnell and Beshers (76) in the context of male behavioral roles in eusocial Hymenoptera. Empirical support for this hypothesis includes lower IC in haploid male eusocial insects, including the wood ant *Formica exsecta*, (122), the leaf-cutting ant *Acromyrmex echinatior* (8), and the bumble bee, *Bombus terrestris* (32). However, other studies have shown that haploid males are not more susceptible to disease than diploid females (for *B. terrestris*, see Reference 91). Alternative explanations may

elucidate why these males have lower IC than diploid females, including differences in life history (5), behavioral role (122), and coevolution between parasites and the predominantly female physiology of eusocial colonies (91). One method of addressing embedded confounding factors when comparing haploid males with diploid females would be to include diploid males—a common genetic misfit in *Polistes* wasps (60, 61)—thereby controlling for morphological differences between males and females.

WHAT ARE INDIVIDUAL SYSTEM-LEVEL DEFENSES AGAINST DISEASE?

Scaling up another level of organization, individuals within some social insect colonies are able to detect diseased nestmates, which stimulates the expression of antiseptic behavior. Antiseptic behavior includes grooming, hygienic behavior of honey bees, undertaking, avoidance behavior, and metapleural gland secretion spreading.

Antiseptic behavior displayed by eusocial insects is likely to influence the selective pressure on other modes of IC (i.e., cellular and humoral), assuming each mode of IC is costly. As such, physiological immune strength in eusocial insects likely differs from that of nonsocial insects, whereas the qualitative aspects appear similar. Cellular and humoral immune cascades prevent infection within the hemocoel of insects. One method of investigating differences in cellular and humoral IC between insects of varying degrees of sociality is to assay IC within a phylogenetic lineage, thereby controlling for differences relating to evolutionary history. A similar approach by Stow and colleagues (113) provided support for this hypothesis by documenting more antimicrobial compounds on the cuticle of eusocial bees compared with subsocial bees. More antimicrobial cuticular compounds might alleviate the heightened risk of disease transmission facilitated by colonies of closely related individuals.

Cellular Immunity

Cellular immunity is common to all animals possessing mobile blood cells, including social insects. The cellular immune processes of social insects are similar to those of nonsocial invertebrates. Invertebrates possess many types of blood cells, termed hemocytes. Three types of hemocytes are common to all insects: prohemocytes, granulocytes (also called granular hemocytes), and plasmatocytes (23). Recently, Manfredini and colleagues (66) confirmed that these three types of hemocytes constitute the cellular component of hemolymph in the eusocial paper wasp, *P. dominulus* (Hymenoptera). Remarkably few descriptive hemocyte studies have been reported in other eusocial species. Prohemocytes are the stem cells of the circulatory system and may differentiate into other types of hemocytes (58). Granulocytes release chemotactic factors into the hemolymph to attract plasmatocytes and play an important role in clotting, healing wounds, and immune processes such as nodulation and encapsulation (84). These cells are likely the first hemocytes to recognize a foreign body (FB) (58). Plasmatocytes are homologous in function to vertebrate macrophages in that they may phagocytose small FBs or mark larger ones as nonself for subsequent isolation. Nodulation occurs when plasmatocytes aggregate onto one another to form a nodule on the FB, thereby marking it for isolation and/or excretion (84). A nodule typically occurs when the FB is too large to be encapsulated. Encapsulation occurs when plasmatocytes recognize a FB and differentiate into flattened cells called lamellocytes. These cells attach to the FB, deactivate it with toxic quinones, and encapsulate it by producing a hard layer of melanin around it.

Humoral Immunity

As with cellular immunity, humoral immune processes in eusocial insects are similar to those in nonsocial invertebrates. Humoral immunity is defined as the noncellular antimicrobial component of the hemolymph. PO is an important enzyme in the invertebrate immune response,

which catalyzes the oxidation of dopamine precursors to toxic quinones. Reactive quinones are toxic to microbes and directly contribute to pathogen neutralization (64). PO also catalyzes the polymerization of quinones to melanin, a protein that hardens and darkens around a FB to further isolate it within host hemolymph. The precursor to PO, proPO, is constitutively expressed in the hemolymph (21).

Social insects may possess a unique humoral immunity advantage against pathogens. Dampwood termites (*Zootermopsis angusticollis*) constitutively express proteins in the hemolymph with some degree of antimicrobial activity; some of these proteins are inducible and may be transferred between individuals within a colony (88). Antimicrobial proteins are produced by hemocytes and fat bodies in response to recognition of broad classes of microbes (e.g., gram-negative and gram-positive bacteria and fungi) (46). Over 170 insect immune proteins have been identified (93). Some antimicrobial proteins are less selective in their activity and are effective against all bacteria (e.g., cecropins) (77). These proteins are produced when pathogen-recognition receptors on host hemocytes bind to pathogen-associated membrane patterns. Transcriptional activation of peptides effective against gram-positive bacteria (e.g., defensins) and fungi (e.g., drosomycins and metchnikowins) occurs through the Toll signal transduction pathway, whereas the IMD pathway produced peptides effective against gram-negative bacteria (e.g., drosocin, diptericins, attacins, and cecropins) (46, 77, 121).

Limitations to Individual Defense

The foraging and nesting ecology of eusocial insects exposes them to a diversity of pathogens, which are defended against through multiple modes of immunity. Immunity does not come without costs (28, 33), and these costs should confer a selective advantage to those with the most efficient defenses. Each mode of immunity (e.g., behavioral, cellular, and humoral) may be activated exclusively or concomitantly. Energy should be preferentially invested into the most effective type(s) of immunity, especially when resources are limited. The favored mode should be the one most efficient at reducing pathogen virulence while augmenting host survival and reproduction.

The costs of mounting an immune response have seldom been assessed, but when investigated they are shown to be high (48). For example, bats spend considerable time grooming accompanied by a significant increase in oxygen consumption (33). Fly-swatting behavior in howler monkeys consumes 24% of their total metabolic budget (28). Our understanding of the energetic cost of immunity in eusocial insects remains remarkably limited. Immune solicitation in bumble bees has been associated with increased food consumption (120; but see 97) and memory loss (65, 85). Foraging activity is associated with decreased encapsulation response (56). Because immunity is multimodal, mixed results relating to the costs of immunity do not necessarily refute one another if different immune pathways were measured; modes of immunity likely differ in energetic requirements. Furthermore, because immune pathways do not necessarily correlate with one another, accurate conclusions of IC parameters are likely to result from studies that investigate multiple immune processes (130).

WHAT ARE COLLECTIVE DEFENSES AGAINST DISEASES?

Naturally, the costs of immunity may be alleviated through group facilitation (e.g., nest hygiene and antiseptic behavior). Social immunity refers to the collective disease defense mechanisms of a collaborative group (27). Many different physiological and behavioral mechanisms can contribute to these social immune defenses. Some behaviors effect protection against pathogens only at the group level, whereas others may be individually protective as well (hence antiseptic). Behavioral structuring (age and caste) and spatial nest

compartmentalization are excellent examples of collective pathogen defenses (95). Behavior related to social immunity is commonly observed in animal societies and is most easily explored in those societies that rely on both social interactions and societal organization to survive, i.e., those societies that lend themselves well to the term superorganism (127).

Antiseptic behavior is a vital component of behavioral defenses enabled by sociality. Some additional examples include construction of nests from antimicrobial materials in wood ants (20, 24), social transfer of antipathogenic proteins in dampwood termites (118), and social fever in response to disease in honey bees (109). Behavioral fever is a mechanism of behavioral IC that occurs when poikilothermic individuals manually increase their body temperature in response to pathogen exposure. In this process, body temperature is increased beyond the optimal range of pathogen development and is noted to occur both by individual movement to warmer areas and through group facilitation (i.e., huddling). Behavioral fever has been observed in solitary, gregarious, and eusocial organisms [e.g., cockroaches (18), lizards (123), locusts (129), and honey bees (109)]. Fever is considered an adaptive trait, as it increases host survival and fitness (55, 75).

In honey bees, behavioral fever is induced by adults positioned over brood comb in response to infestation by *Ascosphaera apis* (109). Fungal spores are introduced into the colony by foraging adults, who vector the spores to larvae via feeding regurgitated nutrients. *A. apis* germinates in the larval gut when colony temperature falls below 32°C for more than two hours (9). Although the process of temperature upregulation in this system is known, the mechanism by which *A. apis* infests colonies is just beginning to be understood. Indeed, given the energetic costs associated with the production of fever, one might hypothesize a benefit in localizing that response.

Thermoregulation in general is used by the honey bee to defend against disease. Develop-

ing brood is highly vulnerable to changes in temperature (132). Honey bee workers participate in complex behaviors that limit both the magnitude and frequency of temperature fluctuations away from the ideal conditions (43, 44, 101, 109–111). The optimal temperature for honey bee brood development is 32°C–36°C (19, 43, 44, 98, 132), and prolonged exposure to temperatures outside this range can cause developmental abnormalities, disease, and even death (9, 19, 31, 51, 132).

Maintaining optimal hive temperatures requires significant energy and coordination of adult workers. Cooling the hive is achieved by wing fanning, which may be performed in conjunction with spreading water to induce evaporation, and heating the hive is done by isometrically contracting thoracic muscles (43, 44). Fine-tuned local heating is achieved by individual bees heating their thoraces and placing them close to cells to increase the temperature of specific brood cells (19, 54). Honey bees can thwart temperature fluctuations by congregating in response to localized temperature stress (hot or cold), a behavior termed shielding (101, 110, 111). In this stereotyped behavior, bees perch and are stationary on the hive wall with their ventral side facing the heat stress (110, 111).

Case Study: Honey Bee Hygienic Behavior

Hygienic behavior is defined specifically as the removal of diseased and parasitized brood from the nest and is one model system of social immunity. Studies of this antiseptic behavior in honey bees scale levels of biological organization ranging from its genetic basis, to its neuromodulation, which facilitates the detection of pathogens by individual bees, to the assembly of individual-level responses, to colony-level social immunity.

Based on the premise that the genetic basis of honey bee hygienic behavior lies in how quickly individual bees within the colony detect the presence of diseased brood within the nest,

Spivak and students conducted a series of experiments to test the hypothesis that some individual honey bees are particularly responsive to olfactory-based stimuli associated with diseased brood. Bees with the greatest olfactory sensitivity to diseased brood odors might first detect the problem and initiate the removal response. To test this hypothesis, Spivak & Gilliam (103, 104) bred a line of bees derived from Italian strains of *A. mellifera* L. for rapid-hygienic behavior and a complementary line for nonhygienic or slow-hygienic behavior. Although Spivak used the terms hygienic behavior and nonhygienic behavior for the bred lines in all her publications, the terms rapid-hygienic behavior and slow-hygienic behavior are better descriptors of the differences between the lines, and we recommend they be used in all future research. The rapid-hygienic line, called the MN Hygienic line, is currently sold commercially throughout the United States.

Individual bees from the rapid-hygienic line exhibited significantly increased sensitivity to the odor of chalkbrood disease at lower concentrations compared with bees from the slow-hygienic line, based on electrophysiological recordings of nerve impulses from the antennae [electroantennogram recordings (67)]. Proboscis-extension response conditioning showed that bees from the rapid-hygienic line discriminated between the odors associated with healthy brood and brood infected with chalkbrood at a significantly lower stimulus level compared with bees from the slow-hygienic line (67, 68). The combined results provide supportive evidence for differential detection and behavioral response thresholds between the two lines of bees. Even within the line bred for rapid-hygienic behavior, there was significant variation in olfactory sensitivity and responsiveness among bees that tend to uncap dead brood and bees that tend to remove dead brood, which may lead to partitioning of the uncapping and removal tasks (37).

Spivak, Mesce, and colleagues (105) further hypothesized that heightened olfactory sensitivity of these hygienic bees may be mediated by the sensitizing effects of particular biogenic amines in the bee brain. Because the neuromodulator octopamine (OA) enhances the response of bees to olfactory stimuli (45, 69) and plays a pivotal role in olfactory-based behavior (40, 41), they examined whether bees from the rapid-hygienic and slow-hygienic lines differed with regard to their OA expression. The staining intensity of octopamine-immunoreactive (OA-ir) neurons in the deutocerebral region of the brain, medial to the antennal lobes, was compared in the brains of rapid-hygienic and slow-hygienic bees collected while performing hygienic behavior and in the brains of same-age bees not performing the behavior at the time of collection. The probability of having highly expressed OA-ir neurons was significantly greater in bees collected while performing hygienic behavior than in same-age bees not performing the behavior, independent of genotype, indicating that OA may play a part in modulating the behavior (105).

Oral administration of OA increased olfactory sensitivity in individual bees selected for slow-hygienic behavior based on electroantennogram recordings but had no effect on bees selected for rapid-hygienic behavior (105). In turn, oral administration of epinastine, a highly specific OA antagonist, reduced the sensitivity of bees selected for rapid-hygienic behavior but had no effect on bees selected for slow-hygienic behavior. Combined, these results suggest that the two lines may differ in the distribution and responsiveness of their OA receptors, which is most consistent with the previous electrophysiological and anatomical studies (105).

The chemosensory and neuroethological data provided mechanistic underpinnings for behavioral studies of individual bees within colonies in the field. Bees performing hygienic behavior are middle-aged, on average 15.7 ± 6.9 days (2); they are significantly younger than foragers from the same colony. If bees that detect and remove diseased brood are older than the majority of bees that feed larvae, it would suggest that the age-based division of labor

among hygienic and nurse bees within a colony may help reduce disease transmission.

The performance of hygienic behavior depended on the proportion of bees in the colony from the rapid-hygienic line. When colonies were composed solely of bees from the rapid-hygienic line, some bees performed the task of uncapping cells at higher frequencies than the task of removing cell contents, and another group performed both tasks to the same extent (2, 4). An individual bee's persistence (defined as the number of times an individual was observed performing uncapping or removal tasks) was significantly lower in colonies composed of bees from the rapid-hygienic line than in a colony with a minority of bees from that line. Only 18% of the bees, on average, in the rapid-hygienic colonies were observed performing any component of hygienic behavior at one time. Despite the lack of persistence and the low number of bees engaged in the behavior, the rapid-hygienic colonies were significantly more efficient in achieving the task (removing 100% of the dead brood within a specified time) compared with colonies with fewer bees from this line. When the bees from the rapid-hygienic line were in the minority, they were observed uncapping and removing the freeze-killed brood well beyond middle age and tended not to partition the hygienic behavior components into subtasks (4).

Bees from the slow-hygienic line were significantly less likely to perform hygienic behavior in the presence of rapid-hygienic bees. They also tended to recap cells that had been uncapped by bees from the rapid-hygienic line, contributing to colony-level inefficiency of the mixed genotype colonies (3). An explanation backed by the chemosensory data is that slow-hygienic bees recap cells containing diseased brood because they have reduced sensitivity to olfactory cues associated with diseased brood. Thus, slow-hygienic bees may perceive a hole in the pupal cap but may not necessarily detect that the brood within the cell is dead or diseased, and respond by resealing the hole with wax instead of continuing the process of uncapping. The delay in removing diseased brood allows the pathogen to reach the infectious stage (133), facilitating disease transmission. This hypothesis remains to be tested experimentally.

Studies of the chemosensory, neural, and behavioral profiles of bees from the rapid- and slow-hygienic lines provided the framework for explaining hygienic behavior on the basis of a response-threshold model. This model, used to explain aspects of the division of labor within a social insect colony, suggests that individuals encounter different stimuli and that those with lower response thresholds perform tasks specifically associated with those stimuli (13, 17). All bees can perform uncapping and removal behaviors, but bees that detect abnormal brood odors at a low stimulus level may rapidly initiate uncapping behavior, resulting in the removal of diseased brood before it becomes infectious.

Slow-hygienic bees, with less olfactory sensitivity, detect and discriminate abnormal from normal brood only when the stimulus level is higher and thus tend to recap brood that has been uncapped, and proceed with the full process of uncapping and removal only after the brood is dead or infectious, leading to disease transmission. Colonies composed of a majority of rapid-hygienic bees have a larger proportion of bees with relatively high olfactory sensitivity for diseased and dead brood (37) and tend to partition tasks between uncapping and removal (2, 4, 37). Task partitioning, and the tendency not to recap brood that has previously been uncapped (3), leads to greater efficiency at the colony level. In contrast, a colony with a high proportion of slow-hygienic bees tends to take longer to detect infected brood and may then proceed to uncap, recap, and uncap these cells multiple times, and remove the diseased brood much later, if at all. This repetitive performance of the initial subtask of uncapping cells increases the probability that these bees will make repeated contact with the pathogen, resulting in an increased probability that the pathogen is transmitted throughout the colony.

POPULATION-LEVEL ANALYSIS: MODELING OF ANTISEPTIC BEHAVIOR IN SOCIAL INSECTS

Mathematical and computational modeling provides a method for exploration of aspects of disease-defensive behavior inaccessible to direct empirical manipulation. These models are used to examine multiple scales of effect, providing a quantitative, controlled, manipulable framework. These types of investigations into the efficacy of a broadly defined set of individual- and colony-level behaviors have already provided great theoretical insight into the social processes of disease defense, although only a few such examinations currently exist. Exploiting the known structure of social insect behaviors, disease-specific etiological rates, and nesting ecologies, the techniques employed have used empirically determined measurements to create individual-, cellular automata–, and differential equation-based models. Each of these methods makes different assumptions and therefore provides a different mathematical perspective of the problem. Together, and in conjunction with empirical studies, these techniques can provide a quantitative understanding of the effects of social immunity on social insect disease dynamics.

Individual-based models focus on mobile individual actors, who interact with each other according to a set of predetermined rules. These rules can apply to interactions with other individuals and can also extend to the consideration of location within an explicit spatial structure. By definition, these models apply best to the examination of how individual behaviors lead to organized, colony-wide differences in disease load and mortality costs. Cellular automata–based models examine spatially explicit processes, in which the state of each location (or cell) is determined by a function of the current state of all neighboring locations. Naturally, these models are well suited to study the spatiotemporal propagation of disease throughout social insect colonies (74, 82), considering the state of a cell to be defined by the state of individuals occupying the physical space represented. In the examination of the impact of social immunity, cellular automata models focus at the colony level by incorporating effects among local interactions (e.g., transmission of disease between individuals in adjacent cells or among individuals within the same cell) and then by measuring the colony-wide differences in either the number of infected individuals or the disease-related mortality over time achieved by incorporation of those local effects.

Fundamentally, both cellular automata– and individual-based models can be considered mathematically based frameworks for empirical experimentation. The formulation of neither provides any theoretically meaningful result. Just as with laboratory-based experimentation, the results must be analyzed (frequently with the use of statistics) and interpreted within the context of the manipulations and alterations included in the individual behaviors examined and their assumed effects.

In contrast, models of disease spread composed of systems of differential equations provide theoretically meaningful results. By assuming average rates of transmission and contact, and removing the individual levels of effect from consideration, these models yield important insights into threshold values for colony-level assumed behavioral effects. They assume the average colony-level effect of incorporating the behaviors and then examine the resulting disease outcomes: colony death; epidemic outbreaks; low, constant endemic disease presence; or clearing pathogen presence from the population entirely. Models using these more analytical epidemiological formulations can be used in conjunction with the cellular automata– and individual-based models to provide a complete understanding of the local (among small groups of individuals) and global (colony-wide) effects of individual behaviors.

Fefferman et al. (30) used a set of individual-based models to perform behavioral knock-out experiments of behaviors hypothesized to enhance social immunity and found that the early removal of infected brood in a colony of dampwood termites (hygienic behavior) and

social-contact-generated immune protection offered protective benefits against disease risks, although each was found to confer maximal protection under different timescales of effect. Allogrooming also acts as an effective antiseptic behavior, despite the potential to cause increased disease transmission. Behavioral structuring was also examined to determine whether it effectively contributed to social immunity: Colony demography and the spatial segregation of etiologically distinct subgroups did not confer substantial protective benefits.

Naug & Camazine (74) employed a set of cellular automata models to examine the social immune contribution of division of labor, colony demography, and nest architecture. By experimentally altering the density of infective individuals and varying the behavioral rates of interactions among different subgroups, they determined that these behavioral partial segregations were effective social immune defenses only when combined with assumed differential replacement rates of individuals within each subgroup.

Pie et al. (82) constructed both a set of cellular automata models and a differential equation model to examine behavioral structuring aspects of social immunity. The theoretical model demonstrated that the likelihood of epidemics was significantly increased by increasing the density of individuals in the colony, and the experimental models demonstrated that nest architecture was increasingly protective against the spread of disease as the spatial segregation among nest chambers increased. These results showed that increasing homogeneous contact rates among nestmates increased the disease risks for the colony, implying that any behaviorally segregating structuring (e.g., division of labor, caste, or developmentally dependent segregation) could be considered an element of social immunity. Although some of these results seem to contradict those of the empirical models in Naug & Camazine (74), it is important to recall the important influences of the different model assumptions employed: Pie et al. (82) did not assume that individuals dying from the disease were automatically replaced by new, susceptible individuals. This difference can be interpreted as comparing a timescale for the duration of infection with that for the egg laying/brood rearing rate of the colony, and the differences in the results, rather than contradicting each other, therefore only provide a more complete understanding of the sensitivity of the host-pathogen dynamic to the behavioral and ecological conditions of the entire system.

Sumpter & Martin (115) used purely theoretical modeling to examine the dynamics of viral epidemics in mite-infested honey bee colonies. (For a review of coinfection by mites and viruses in honey bee colonies, see Reference 10.) By incorporating specific variables to represent bee behaviors, they isolated the theoretical thresholds of impact caused by certain behaviors (e.g., honey bee hygienic behavior) to the epidemic spread of both mite and viral presence. They found that, taken in isolation, no collective behavioral responses provided any stable protective effects against either macro- or microparasites. However, they did determine that honey bee hygienic behaviors that succeeded in reducing the mite infestation to less than 15% of the initial number of mites per bee would be effective at reducing the viral transmission below a critical threshold level, thereby preventing viral epidemics.

Each of these models examined aspects of the effect of individual- and colony-level behaviors on the spread of infectious disease within a social insect colony. The results from these studies of a broadly defined set of behaviors contributing to collectively protective social immunity provide, however, only limited understanding of the possible effects of these responses. Further work, involving both theoretical and experimental models, will continue to provide insight into the protective effects of social immunity (composed collectively from cellular, humoral, and behavioral immunity, and from antiseptic and nest hygienic behavior) in ways that empirical investigations could not achieve directly.

Behavioral immunity: any behavioral act that decreases susceptibility to infection

CONCLUSIONS

Organisms are in a continuous coevolutionary arms race with some pathogens and parasites. Because disease is both a variable and a constant selective force, animals have evolved a myriad of methods of combating infection. Examining disease from an evolutionary perspective is becoming increasingly common. In no animal models can we more precisely examine disease-prevention techniques, and their colony- and population-level implications, than in social insects. The ability to breed for increased nest hygiene and antiseptic behavior attests to the genetic diversity underlying disease resistance behaviors, and the ability to examine colonies in the wild allows for within-colony, within-population, and across-population studies. A multilevel review of disease resistance behavior and physiology in insect societies provides a theoretical, evolutionarily sound, and biologically relevant foundation for examining disease resistance in other systems—systems unlikely to be as tractable.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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