REVIEW ARTICLE

Social immunity in insects

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Abstract: Social insect colonies have developed a collective immune defence against parasites. These defense systems, known as "social immune systems," result from individual group members cooperating to combat the increased risk of disease transmission that comes with social living. Traits that reduce the intensity and transmission of pathogens and parasites within the colony are called "social immunity." While there are several ways in which social insects enhance the overall immunity of the colony to defend against invading pathogens or parasites, they can be broadly classified into different types: maintaining nest hygiene, providing sanitary care for infected individuals, eliminating pathogens from the colony, modifying social interaction networks, and reducing vertical and horizontal parasite transmission. Social immune actions progress from initially protecting individual members to safeguarding the entire colony by preventing disease transmission.

Keywords: Social immunity, defense, disease transmission, vertical parasite transmission, horizontal parasite transmission

Social insects are characterized by their ability to live together in colonies or communities, exhibiting a range of social behaviours such as communication, food sharing, and protection of offspring and eggs (Liu et al., 2019). However, living in social groups also poses challenges, particularly regarding the spread of infectious diseases among group members, which occurs more quickly than solitary individuals (Rosengaus et al., 2011). This is due to the high density and frequent social interactions within the group, and the close genetic relatedness among group members, making them susceptible to the same parasites. Consequently, social insect groups are highly susceptible to transmitting infectious diseases. However, these social groups are expected to have evolved various strategies to counteract this threat.

Immunity refers to the ability of an organism to resist or be protected against harmful agents, particularly pathogens or infectious diseases. Immunity may occur naturally or be produced by prior exposure or immunization. In insects, immunity is of two types 1) individual/ innate immunity and 2) group/ social immunity (Cremer et al., 2019). At individual/ innate immunity, social insects have evolved various mechanisms to combat parasites and pathogens. The first line of defense is the cuticle (exoskeleton of insects) which is a mechanical and biochemical barrier covered by antimicrobial compounds. As a second defense, insects have developed an innate immune system based on cellular and humoral responses. Hemocytes primarily mediate cellular defense and include phagocytosis, nodulation or encapsulation of pathogens such as bacteria, protozoa, or nematodes. Humoral defense is based on the secretion of antimicrobial peptides (e.g., defensin, abaecin or hymenoptaecin in honey bees), using reactive oxygen intermediates as killing molecules and activating enzymatic cascades that regulate melanisation. This immune response is costly to the hosts and can reduce their life span and impair their cognitive functions (Gómez-Moracho et al., 2017; Meyel et al., 2018).

Social insects have developed cooperative behaviours, known as "social immunity," in addition to individual defense, to combat infections. These behaviours aim to reduce exposure to parasites and colony's transmission rate. Honey bees, for instance, collect antimicrobial substances from plant resins, which they mix with wax to create a paste called propolis (Lavine and Strand, 2002). They spread this propolis within the nest to control infections and reduce pathogen loads, including bacteria like *Paenibacillus larvae* and fungi like *Ascosphaera apis*. Another critical strategy for preventing infections is spatial segregation within the hive. Bees with higher risks of exposure to parasites and pathogens, such as foragers, have reduced physical contact with in-hive bees like nurses. This segregation helps minimize the chances of transmission. In the event of infection, adult bees detect and sacrifice infested broods to prevent further

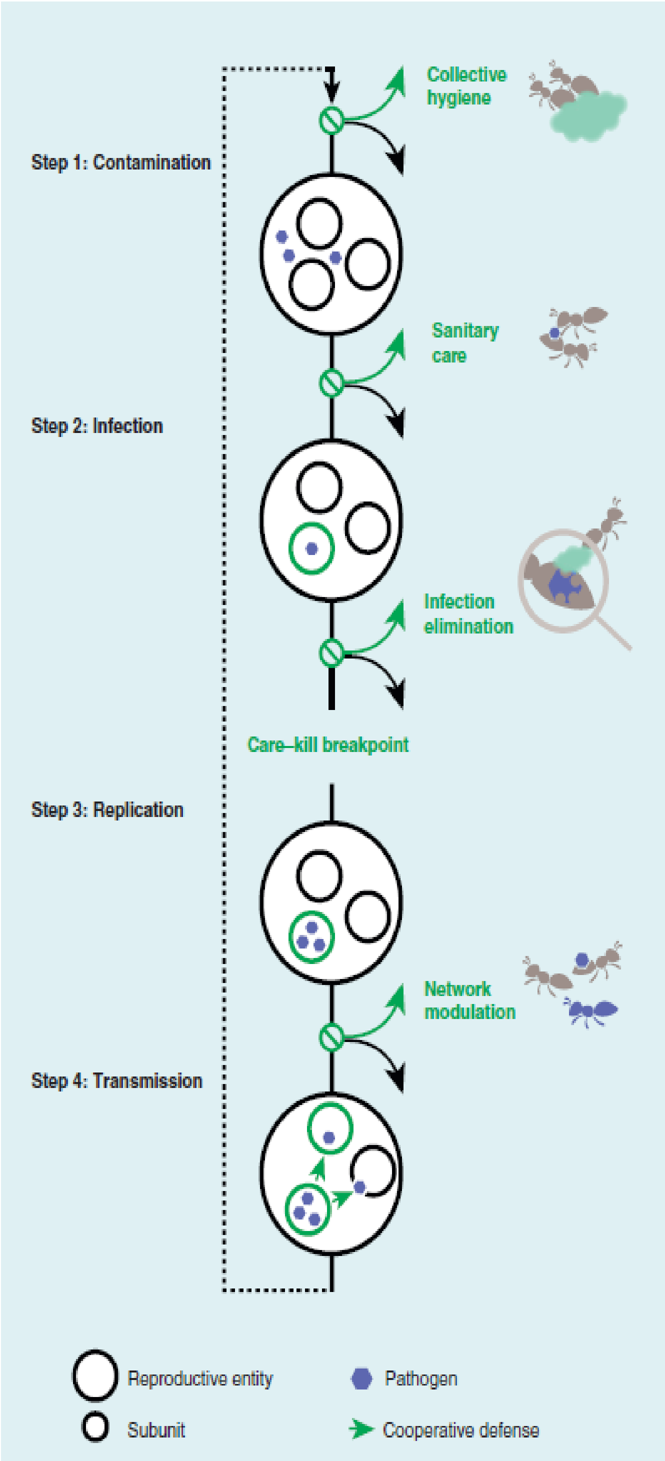

Fig. 1. Disease stage-dependent course of social immunity.

transmission. These hygienic behaviours serve as the central defensive mechanisms of honey bees against parasites.

Different kinds of parasites attacking the social insect colonies and their transmission:

Social insect colonies are susceptible to damage caused by a wide range of parasites, including macroparasites like helminth worms and arthropods, as well as microparasites such as fungi, bacteria, viruses, and certain protozoa (Cremer et al., 2018; Wilson et al., 2003). These parasites can enter the colony actively by searching for suitable hosts or be picked up and transported into the colony by individual members. The social nature of the group can contribute to the risk of an epidemic outbreak. This is because social insects often reside in environments with high microbial abundance, colonies are densely populated with closely related individuals, and there is frequent social contact among group members. As a result, parasite infections can be easily transmitted between individuals. In social insects, the distinction between "vertical" and "horizontal" transmission, referring to transmission from parent to offspring versus transmission among individuals of the same generation, becomes less clear. This is because parents and offspring live together permanently within colonies (Meyel et al., 2018; Patterson and Ruckstuhl, 2013). Therefore, in social insects, "horizontal" transmission refers to transmission within and between groups or colonies. In contrast, while "vertical" transmission refers to the transmission from a mother colony to a daughter colony in the next generation. Regardless of the type of transmission, the invasion of parasites into a colony involves multiple steps. The parasite must either actively approach or passively be transported to the colony. Once inside, it must establish itself within the nest environment and spread among group members. It may then exit the colony either horizontally or vertically, infecting new colonies in the process.

Components of social immunity / Mechanisms of defense in the colony

Social immunity in social insects operates at each stage of disease progression (steps 1-4). Cooperative defense mechanisms protect the reproductive entity, represented by green upward-bent arrows. However, if these defenses fail, the disease advances to the next step (black downward-bent arrows), allowing the pathogen (blue diamonds) to infect individuals (green circles), replicate (multiple diamonds), and transmit to new colony members (small green arrows). In response to disease progression, social immunity employs collective nest hygiene, elimination of infections, and modulation of the social interaction network through behavioral changes of colony members. Initially, the focus is on protecting individual members, but the aim shifts towards protecting the entire colony by preventing disease transmission (Cremer, 2019).

Avoidance strategy

The first and most effective line of defense in protecting insect colonies from infections is to prevent the entry of pathogens. Avoiding direct contact with pathogens is a vital aspect of this strategy. For instance, termites actively avoid areas where fungal pathogens are present. They employ vibratory warnings and seal off contaminated areas to prevent their nestmates from coming into contact with the pathogens. Similarly, while bringing nest mate's carcasses back to the colony for food, ants ensure that they do not come into contact with fungus-contaminated corpses (Liu et al., 2019).

Another important element of the avoidance strategy is careful handling materials brought into the colonies. Leaf-cutter ants exemplify this by having large foragers carry leaves into the colony. At the same time, while specialized workers known as hitchhikers are responsible for removing fungal contaminants from the leaves, akin to the skin immunity observed in vertebrates. Border defense is another significant component, where social insects incorporate antifungal materials into their nests. They collect these materials from the environment or produce them internally to enhance the nest's defense. For example, ants gather tree resin as nesting material to prevent fungal growth. Furthermore, termites, ants, and bees can add certain antifungal chemicals to the nesting materials. Termites and ants also utilize symbiotic microorganisms from their nesting structures to defend against fungal pathogens.

Collective nest hygiene

Nest hygiene serves as the initial step in the social immune response of a colony and is typically employed as a preventive measure in a non-specific manner. It involves the mechanical removal of potentially infectious materials and the application of broad-spectrum antimicrobials. Social insects maintain meticulous cleanliness within their nests, even in without pathogens, to eliminate any potential sources of infection. For instance, when garden ants establish a new nest, they treat the nest material, including the newly constructed brood chambers, with a self-produced disinfectant containing formic acid, which acts as a poison with antimicrobial properties (Evans et al., 2009). Termites incorporate their feces into their nests as they contain a rich microbial community that produces antimicrobial substances, ensuring a clean environment. Corpses and debris are carefully collected and relocated to specific areas called graveyards or middens, located within peripheral nest chambers or outside the nest., When faced with nest contamination, honeybees employ a strategy similar to our own bodies: they raise the temperature. This social fever is achieved by bees vibrating their flight muscles collectively, resulting in an overall increase in hive temperature. This behaviour has been observed to effectively eliminate heat-sensitive pathogens such as *Ascosphaera apis* (Starks et al., 2000).

Sanitary care

Sanitary care acts as the second step in the social immune response of a colony when an individual becomes contaminated with a pathogen, either due to inadequate nest hygiene or foraging outside. In a similar way, how monkeys groom each other to remove ectoparasites, honeybees groom their nestmates to eliminate pests like the *Varroa* mite (Evans et al., 2009). Ants and termites also engage in grooming behaviours to remove fungal spores from contaminated individuals, preventing them from penetrating the cuticle and causing internal infections. Allo-grooming, where individuals groom each other, is particularly effective in preventing infections compared to self-grooming because it allows for the grooming of body parts that may be difficult to reach individually, such as the thorax. Grooming is a common form of sanitary care observed in social insects and is highly effective (Land and Seeley, 2004). However, a question arises as to whether allo-grooming increases the risk of infection for the groomer. Grooming ants, for example, collect the infectious material they remove and store it in pouches within their mouth called infra buccal pockets. The material is then compacted and sterilized using antimicrobial gland compounds. Eventually, the compacted pellets are expelled and have a significantly reduced ability to germinate. Despite these measures, pathogen transfer can still occur from the contaminated individual to its nestmates, although it typically results in non-lethal, low-level infections. Such low-level infections can trigger a protective immunization response in ants and termites. In a study conducted by Neto et al. (2006), the researchers experimentally examined the three main hypotheses proposed to explain hitchhiking behavior in *Atta sexdens* and field colonies of *Atta laevigata*: a) defense against phorid flies, b) defense against fungal contaminants, and c) leaf sap obtention. The results of the study revealed the following findings: a) Limited evidence was found for an increase in hitchhiking in the presence of phorid flies. The presence of phorid flies only led to a slight increase

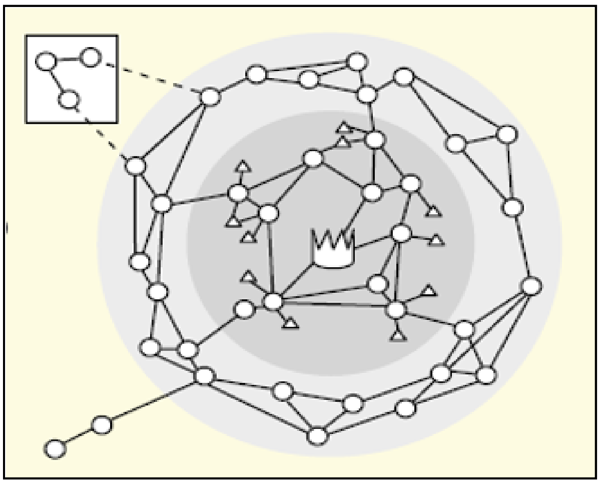

Fig. 2. Interaction network of a generalised social insect colony.

in the number of hitchhikers in *A. sexdens*, and no increase was observed in *A. laevigata*. b) In both species, the proportion of fragments with hitchhikers was significantly higher in fragments obtained from fresh leaves compared to those from dry leaves. This suggests that obtaining leaf sap may be one possible function of hitchhiking behavior in *A. sexdens* and *A. laevigata*. c) The primary function of hitchhiking behavior was found to be a defense against fungal contaminants. The proportion of fragments with hitchhikers was approximately 4 to 6 times greater in fragments experimentally inoculated with moldy bread compared to clean fragments or those inoculated with an inert substance. These findings indicate that hitchhiking behavior in these ant species serves as a defense mechanism against fungal contaminants, with obtaining leaf sap potentially playing a secondary role. Sanitary care, including grooming and removal of infectious material, likely contributes to the defense against fungal pathogens and may be involved in the hitchhiking behavior observed.

Infection elimination

Since the 1960s, it has been known that honeybees can detect infected broods within sealed comb cells and remove them by uncapping and dropping the brood outside the hive. This behaviour has also been observed in several ant species, although their brood is pooled in open piles instead of sealed cells. Unlike bees, ants risk reinfection if they drop infectious brood outside the nest as they forage in the same territories. Garden ants have developed a complex multicomponent behaviour to address this challenge to eliminate brood infections. The process begins with the ants slicing open the cocoon of an infected pupa and biting through its soft cuticle. They bend their abdomen over the pupae and release a poisonous spray containing formic acid. This behaviour allows the poison to enter the infected pupa, disinfecting it from the inside out. Doing so, prevent pathogen replication is before new transmissible stages can be produced. This process, known as "destructive disinfection," serves a function similar to eliminating infected cells in a body and operates mechanistically equivalently (Pull et al., 2018).

The "care-kill dichotomy" is an important aspect of social immunity. Nest hygiene and sanitary care are crucial in preventing pathogens from establishing within the colony. While many studies focus on these early defense mechanisms, they are only sometimes fully effective. When initial defense fails and infection takes hold, social immunity shifts its focus from prevention to combating disease replication and transmission. In such cases, the emphasis shifts to excluding or eliminating infected individuals. Once an infection reaches an irreversible stage, social immunity transitions from a "care" strategy to a "kill" strategy, aiming to minimize the spread of the disease within the colony.

Modulation of the social interaction network

Social insects exhibit structured interaction networks, which are shaped by the clustering of individuals according to their tasks and the spatial organization within the nest. In many social insect colonies, such as ants and termites, the contact rates between individuals are limited due to spatial and behavioural compartmentalization (Cremer et al., 2007; Ament et al., 2008). This compartmentalization is most evident in the division of labour based on age and caste. Young workers, known as nurses, are responsible for caring for the brood and queen in the central area of the nest, while older workers venture outside the nest to forage. This inherent network structure may have evolved, at least in part, as a mechanism to restrict the transmission of infectious diseases, as suggested by the "organizational immunity hypotheses." These compartments within the nest consist of groups of workers arranged in concentric circles, with individuals of the same age and/or caste performing similar tasks. The central region (a dark grey area) houses the queen (represented by a crown) and her brood (triangles), which are attended to by the young workers. On the periphery (a light grey area), older workers engage in nest maintenance and leave the nest for foraging purposes. The disposal of dead bodies and waste occurs in specific locations at the edge or outside of the nest (upper left corner, depicted as a rectangle for the garbage dump workers), with limited indirect contact with the main nest. In response to contamination from foragers, the nurses adjust their behaviour by bringing the brood even closer to the center of the nest. This further separates the foragers from the nurses, reducing the risk of infection for the nurses and queen. Consequently, pathogen transmission is primarily observed among individuals within the same age and task groups, particularly among foragers, while the nurses and queens typically receive only low amounts of pathogens. These lower pathogen levels often result in immunization rather than disease development.

Reducing vertical and horizontal parasite transmission

Once a parasite has established within a colony, it can spread to other groups, including neighbouring independent colonies or daughter colonies. Vertical transmission to daughter colonies can occur when reproducing queens lay infected brood or when the daughter queens or accompanying workers (in the case of nest budding) acquire an infection before leaving the parental colony, either through horizontal or vertical transmission (Wilson et al., 2003) While there may not be strong selection against avoiding horizontal infections between colonies, there is likely intense selection pressure to prevent vertical transmission to daughter colonies. This is because the colony's fitness heavily relies on the successful production of offspring colonies. To prevent vertical transmission, social insects have developed various strategies. Infected honeybee workers may stop tending to the queen, and wasps protect their juvenile stages by rearing them in brood cells impregnated with antimicrobial secretions. Ant queens, while laying eggs, sometimes coat them with venom, and workers can spray venom over the brood to reduce fungal infections. Protective substances, such as royalisin and other antimicrobial peptides, can also be directly fed to the brood, as seen in honeybees. Furthermore, social insects exhibit a "transgenerational transfer of immunity," similar to what is observed in other organisms, where immunity is passed down to the offspring. On the other hand, avoiding horizontal infection between neighbouring colonies is not commonly expected, except in cases where the neighbouring colonies are closely related and/or when it directly reduces the risk of re-infection for their colony.

Conclusions

Social immune systems in social insects serve as functional barriers at every stage of parasite invasion, providing a comprehensive defense mechanism. They have evolved to minimize the energy expenditure associated with individual immune responses by harnessing the power of collective action. Through social immunity, colonies gain significant resistance against generalist parasites, effectively reducing the risk of infection and transmission within the group. However, it is important to note that specialist parasites may have evolved strategies to overcome or circumvent social immunity defenses. Overall, the up-regulation of immunity at the colony level enhances the fitness and survival of the entire social insect colony.

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