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Molecular and chemical immune defenses in ants (Hymenoptera: Formicidae)

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Abstract

Like all other organisms, ants can be afflicted by a large number of microbial parasites and pathogens. In response, ants have evolved a range of immune defenses. Here we review current knowledge of ant immune defenses, focusing on the genes, proteins and chemicals involved in ant innate immunity. Some pathogen recognition, signaling and effector molecules (such as antimicrobial peptides) have been described from a range of ant species in different subfamilies, but a very large number of innate immunity components remain to be identified. Secretions of the metapleural glands have special significance as an ant-specific immunity component. Chemically diverse substances from these glands are known to have antimicrobial properties and hence to play an important part in ant life. In addition to the molecular and chemical defenses of individual physiology, individual and group behavior patterns make substantial contributions to fighting disease. Examples of behavioral mechanisms contributing to disease resistance include parasite avoidance, active inclusion of antimicrobial plant resins into nest materials, and increased colony genetic diversity through polyandry, polygyny, or both. We propose that future research links immunity at the molecular level with the ecology of ants and their pathogens, and studies evolutionary mechanisms to yield a comprehensive understanding of ant immune defense mechanisms.

Key words: Formicidae, innate immunity, metapleural gland, antimicrobial peptides, review.

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"Disease is not the prerogative of man and the domestic animals, so it was quite natural to see if the lower animals, with very simple organizations, showed pathological phenomena, and if so, infection, cure and immunity could be observed among them."

from the Nobel Prize lecture of Ilya Metchnikoff (Илья Ильич Мечников),
the Nobel Prize winner in Physiology or Medicine 1908

Introduction

It is more than 100 years ago that Ilya Metchnikoff discovered phagocytosis as a defense mechanism against fungal spores in an arthropod (KAUFMANN 2008). A vast body of immunological knowledge has been accumulated since the first Nobel Prizes were awarded to Robert Koch (1905), Ilya Metchnikoff and Paul Ehrlich (1908) for their immunological work. Research on acquired immunity has progressed to a remarkable degree, especially that on birds and mammals. As pointed out by Metchnikoff, however, so called lower animals can also acquire infectious diseases and mount immune defense responses – ants are no exception. Here we apply a broad definition of immunity. The Latin word *immunis* means free from a burden (KLEIN & HOREJSI 1997). In the biological sense we call living organisms immune if they don't become diseased upon exposure with potential parasites or pathogens (Box 1). It is misleading to restrict the term to a protection against re-infections (as some immunologists prefer) and thus to exclude any form of innate immunity. The importance of the innate immune system has become obvious by knocking out parts of it and demonstrating detrimental effects of subsequent infections to host organisms (e.g., LEMAITRE & al. 1996). Interest in ultimate, negative consequences of parasitic attacks for the host's evolutionary fitness has grown substantially over the past two decades and has given rise to the scientific fields of ecological and evolutionary immunology (for reviews see SCHMID-HEMPEL 2005, SCHULENBURG & al. 2009). There has been some recent debate about the merits of studying immunity at the whole organism level (HAUTON & SMITH 2007, LITTLE & al. 2008). While the value of "mechanistic" research (i.e., genetics, biochemistry, physiology) is uncontroversial and obvious, organismic approaches are just as worthwhile in our opinion since they can uncover new phenomena (SCHMID-HEMPEL 2005). Moreover, host-parasite interactions are intrinsically ecological and evolutionary mechanisms have ultimately shaped immune systems to combat pathogens and are still doing so. Therefore, essential components of the entire range of immune defenses will remain undetected if immunity is studied without regard to the host's ecology and potential evolutionary consequences (LITTLE & al. 2005).

Ants are threatened and attacked by numerous predators, parasites, and pathogens from various taxa: animals, fungi, eukaryotic microorganisms, bacteria, and viruses (SCHMID-HEMPEL 1998). In this review, we focus mainly on microbial diseases and the respective immune defense mechanisms mounted by ants. Nonetheless, the innate immune system also combats macroparasites and parasitoids when they invade an ant. Following such invasions ants can react using physiological and biochemical mechanisms

for which we will present many examples and references throughout this review.

In addition to immune defenses at the individual level (i.e., molecular, biochemical, physiological and behavioral, Box 1), defenses against parasites and pathogens occur also at the colony level in eusocial species. Collective defenses are defined by the fact that an individual animal cannot perform them on its own, e.g., allogrooming or necrophoric behavior (CREMER & al. 2007 and references therein, CREMER & SIXT 2009 and references therein).

Individual immune defenses comprise genetical, biochemical, physiological, and behavioral responses. Individual ants use exocrine gland secretions to fight microbial pests and pathogens. Very important examples in ants are the metapleural gland secretions that we will discuss in detail below. In comparison with chemical and behavioral defense mechanisms, the innate immune system of ants has yet received little attention. Nevertheless, hymenopteran immunology is an emerging new field with the Western honey bee *Apis mellifera*, the parasitoid jewel wasp *Nasonia vitripennis*, and the invasive fire ant *Solenopsis invicta* as key species being investigated in more and more detail (e.g., EVANS & al. 2006, VALLES & al. 2008). In this review we give a brief overview of microbial diseases pestering ants before we discuss chemical and molecular immune defenses in greater detail. We also summarize ant behavior, sociality, ecology, and evolution with respect to immune defenses in ants.

Microbial diseases of ants

Parasites and pathogens from many taxa infect ants (SCHMID-HEMPEL 1998). Since Paul SCHMID-HEMPEL published his seminal book on parasites in social insects a decade ago (SCHMID-HEMPEL 1998) many more parasites and pathogens of ants have been described. Here, we will not give an exhaustive list of all known parasite species attacking ants, rather we present an overview of parasites from major parasitic taxa associated with ants. Closely related pathogens interfere with the same components or at least very similar components of ants' immune systems and very dissimilar pathogens might be detected and combated by different components of their immune systems. Hence, an outline of major groups of parasites will facilitate an understanding of possible interactions between pathogens and the immune system of ants. To understand the immune system in its whole complexity, it will be useful to study a variety of pathogens (rather than many similar ones) since this will shed light on different components of the immune system.

A few different viruses have been reported to occur in ants. In addition to those reviewed by SCHMID-HEMPEL (1998), there is an increasing number of viruses known to be associated with the fire ant *Solenopsis invicta*. This invasive pest species has been studied in more detail with respect to parasites than other ants, an obvious reason being the search for biological control agents. Two recently discovered viruses have been named Solenopsis invicta virus-1 (SINV-1) (VALLES & al. 2004b, VALLES & STRONG 2005) and Solenopsis invicta virus-2 (SINV-2) (VALLES & al. 2007a). SINV-1 is a single-stranded, dicistronic positive-strand RNA virus found in all stages of *Solenopsis invicta* (see VALLES & al. 2004b). It is a picorna-like virus and has significant similarity to the acute bee paralysis virus (VALLES & al. 2008). SINV-1 has a wide distribution occurring in *Solenopsis invicta* in Argentina and the USA (VALLES & al. 2007b). Furthermore, it has been detected in other *Solenopsis* species (e.g., *S. richteri* and *S. geminata*) (VALLES & al. 2007b). SINV-2 is probably, as for SINV-1, a positive-strand RNA virus but in contrast to SINV-1, SINV-2 is polycistronic and its systematic position less well established (it belongs possibly neither in the Dicistroviridae nor the Picornaviridae) (VALLES & al. 2007a). In a *Solenopsis invicta* cDNA library, which we will discuss further below, WANG & al. (2007) detected 26 putative genes of viruses that might belong to at least 5 different viruses. A honey bee virus (chronic bee paralysis virus = CBPV) was detected in two ant species (*Camponotus vagus* and *Formica rufa*) (CELLE & al. 2008). While the infected ants were found close to infected honey bee colonies, ways of virus transmission and the potentially adverse effects of the CBPV on the ants remain to be investigated. We assume more cases of viral host switches between different ant species (VALLES & al. 2007b) will be discovered in future. Moreover, it will be interesting to see if indeed viruses readily switch between ants and non-ant hosts.

Little is known about bacterial diseases in ants. Bacteria of the genus *Pseudomonas* are an early and rare example of bacteria highly detrimental to ants. These bacteria killed all *Solenopsis invicta* larvae fed vegetative cells within five days (LOFGREN & al. 1975). In *Formica truncorum*, *Wolbachia* (α-proteobacteria) infections cause detrimental effects on colony fitness (WENSELEERS & al. 2002). The occurrence of *Wolbachia* is widespread among many subfamilies of ants (e.g., Dorylinae, Formicinae, Myrmicinae, Ponerinae, Pseudomyrmecinae) (WENSELEERS & al. 1998, VAN BORM & al. 2001). Other bacteria associated with ants have been reported more recently (BAIRD & al. 2007, LEE & al. 2008a) but they are not known to cause disease in ants. There are examples of beneficial or potentially beneficial relationships between ants and bacteria. For example, leaf-cutter ants use actinomycete bacteria in order to maintain stable fungus-ant relationships (MUELLER & al. 2008). In other cases, bacteria (*Blochmannia*) provide nutritional benefits to carpenter ants (FELDHAAR & al. 2007) of the genus *Camponotus*. Some species of the pseudomyrmecine ant genus *Tetraponera* house bacteria in specialized parts of the gut. These bacteria have been proposed to serve *Tetraponera* ants by improving their nitrogen-poor diet (VAN BORM & al. 2002, STOLL & al. 2007). An interesting topic in immunology is how immune systems distinguish between beneficial and harmful microbes. This question remains largely a conundrum though *Acromyrmex* leaf-cutter ants seem to be able to distinguish even between different strains of actinomycete bacterial species (ZHANG & al. 2007).

Eukaryotic microorganisms causing disease in ants span an enormous array of phyla. The full range has presumably not been discovered yet considering what is known from other insects, especially from hymenopterans. Gregarines are unicellular parasites related to malaria parasites (*Plasmodium*), both belonging to the phylum Apicomplexa (LEANDER & al. 2003). A gregarine species conspicuously changing the ant's body color has been found by CROSLAND (1988) in *Myrmecia pilosula*. Microsporidia are also unicellular parasites of ants but they are either fungi (possibly Zygomycota) or at least closely related to fungi (KEELING & FAST 2002, LEE & al. 2008b, WALLER & al. 2009).

Box 1: Glossary.

Acquired immunity: describes an enhanced protection against re-infections with the same kind of pathogen. In jawed vertebrates, it comprises a highly pathogen-specific long-term memory and somatic recombination on the molecular level resulting in high diversity of some immune system proteins (e.g., T-cell receptors).
Behavioral defense: describes an action taken by an animal to avoid contact with pathogens or to remove pathogens from the animal's immediate environment.
Biochemical immune defense: describes combating pathogens inside an organism's body using proteins or other biomolecules.
Chemical immune defense: describes combating pathogens using chemicals externally (including proteins and other biomolecules) produced by exocrine glands of an organism or acquired from the environment the organism inhabits.
Immunity: a characteristic trait of organisms not to become diseased upon exposure to potential parasites or pathogens. Protection against and combating of pathogens is achieved through a variety of mechanisms ranging from molecules to behavior.
Innate immunity: describes a relatively fixed hereditary protection against infection with little capacity for immunological memory. It comprises germ-line encoded recognition molecules that detect pathogen-associated molecular patterns, biochemical signaling pathways, and effector molecules directly affecting pathogens.
Molecular immune defense: describes combating pathogens using nucleic acids or proteins at the cellular level.
Physiological immune defense: describes combating pathogens using mechanical, physical or chemical mechanisms.

They have a number of highly reduced character states (e.g., small genomes and no functional mitochondria). *Thelohania solenopsae* and *Vairimorpha invictae* are two examples of microsporidian parasites (VALLES & al. 2004a) infecting the fire ant *Solenopsis invicta*. Many true fungi (Eumycota) attacking ants have been described as well (SCHMID-HEMPEL 1998). A large number of the entomopathogenic species belong to the phyla Zygomycota (particularly species of the Entomophthorales) and Ascomycota (ROY & al. 2006). *Metarhizium* and *Beauveria* are two ant-killing genera of the Ascomycota (Hypocreales) that have been investigated in a variety of studies. We will discuss immune defenses of ants with regard to these parasitic fungi in several paragraphs of this review.

Defenses of individual ants

The individual animal's immunity armament can be divided into several distinct components (the defense component model, SCHMID-HEMPEL & EBERT 2003): avoidance behavior, prevention of penetration, molecular recognition, and combating infection. Each of these components has to be surmounted by parasites for successful host exploitation (SIVA-JOTHY & al. 2005). Ants share many components of protective defense mechanisms with other insects. Before we discuss these generally occurring components we will highlight an element of ant immunity that is special to the ants.

Exocrine gland secretions

The metapleural gland (also called metasternal or metathoracic gland) is found only in ants and is presumably of very old phylogenetic origin as it is present in fossil specimens from the Cretaceous (HÖLLDOBLER & WILSON 1990). The metapleural gland is a paired structure situated at the posterolateral corners of the alitrunk (= mesosoma) (HÖLLDOBLER & ENGEL-SIEGEL 1984). BROWN (1968) suggested a role of the metapleural gland secretions as being involved in nestmate recognition or as he put it then: "I am an enemy" signaling. Two years later MASCHWITZ & al. (1970) did not find evidence for this hypothesis in a large number of ant species (*Myrmica rubra* [as "*Myrmica laevinodis*", throughout that study], *Manica rubida* [as "*Myrmica rubida*", throughout that study], *Formica polyctena*, *F. rufa*, *Tetramorium caespitum*, *Myrmecina graminicola*, *Harpagoxenus sublaevis*, and *Atta sexdens*) using behavioral experiments. Instead the authors demonstrated antimicrobial efficacy of metapleural gland secretions of three species (*Manica rubida*, *Myrmica rubra*, and *Atta sexdens*) against two bacterial species (*Escherichia coli* and *Staphylococcus aureus*). Metapleural gland secretions of *Manica rubida* and *Atta sexdens* were also effective against the mold *Penicillium glaucum*. The major antimicrobially active component in *Atta sexdens* was phenylacetic acid. This acid was also detected in *Messor barbarus* and *Myrmica rubra* [as "*Myrmica laevinodis*", throughout that study] (SCHILDKNECHT & KOOB 1971). Other substances detected in *Atta sexdens* metapleural secretions are indole-3-acetic acid (SCHILDKNECHT & KOOB 1970), 3-hydroxydecanoic acid (myrmicacin) and two further 3-hydroxy acids (3-hydroxyhexanoic acid and 3-hydroxyoctanoic acid) (SCHILDKNECHT & KOOB 1971). *Atta sexdens* metapleural gland secretions and their major components show bactericidal (strong growth inhibition against *Bacillus sphaericus* and *Pseudomonas putida*) and fungicidal actions (against *Trichoderma viride*) (DO NASCIMENTO & al. 1996). Interestingly, DO NASCIMENTO & al. (1996) discovered great differences among different bacteria and fungi in their susceptibility to metapleural gland secretions even between related parasite taxa. In addition to *Atta sexdens*, myrmicacin was also present in an *Acromyrmex* species and in *Messor barbarus* and *Myrmica rubra* (see SCHILDKNECHT & KOOB 1971). SCHILDKNECHT & KOOB (1970) found indole-3-acetic acid in *Myrmica rubra* metapleural gland secretions and later in a species of the genus *Acromyrmex* and in *Myrmica rubra* (see SCHILDKNECHT & KOOB 1971). Furthermore, antimicrobial properties of metapleural gland secretions against *Escherichia coli* were found in myrmicine (*Crematogaster scutellaris*, *Crematogaster difformis*), ponerine (*Leptogenys processionalis* [as "*Leptogenys ocellifera*"], *Odontomachus* sp. [as "*Odontomachus haematodes*"]), amblyoponine (*Amblyopone australis*), ectatommine (*Rhytidoponera metallica*), myrmeciine (*Myrmecia forficata*), pseudomyrmecine (*Tetraponera* sp.), and in two dolichoderine ants (*Dolichoderus quadripunctatus*, *Liometopum microcephalum*) (MASCHWITZ 1974).

About ten years after the initial demonstration of metapleural gland secretion antimicrobial activities BEATTIE & al. (1985, 1986) studying the Australian myrmeciine ant *Myrmecia nigriscapa* extended the knowledge of metapleural gland function against a large number of fungal species (*Aspergillus niger*, *Beauveria bassiana*, *Cladosporium resinae*, *Colletotrichum gloeosporioides*, *Gliocladium roseum*, *Mucor plumbeus*, *Paecilomyces lilacinus*, *Penicillium aurantiogriseum*, *Metarhizium brunneum*, and *Trichoderma viride*). Interestingly, some of these fungi (*Beauveria bassiana*, *Paecilomyces lilacinus*, and *Metarhizium brunneum*) are entomopathogenic. In another myrmeciine ant, *Myrmecia gulosa* (the quintessential Australian "bulldog" ant), VEAL & al. (1992) showed effects of metapleural gland secretions against two fungi (*Candida albicans*, *C. tropicalis*) and three Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*, and *Bacillus cereus*) and six Gram-negative bacteria (*Escherichia coli*, *Pseudomonas vulgaris*, *P. aeruginosa*, *P. tolaasii*, *P. putida*, and *Salmonella typhimurium*). Secretions from metapleural glands of *Myrmecia gulosa* exhibit broad-spectrum antimicrobial activity. Treatment of a yeast (*Candida albicans*) and of three bacterial species (*Bacillus cereus*, *Escherichia coli*, and *Pseudomonas aeruginosa*) with metapleural gland secretions from *Myrmecia gulosa* led to damage to cell membranes (MACKINTOSH & al. 1995).

A mixture of phenols (3-propylphenol, 3-pentylphenol, 5-propylresorcinol, 5-pentylresorcinol, and 3,4-dihydro-8-hydroxy-3-methylisocoumarin (= mellein)), which in general have bactericidal effects, has been isolated from metapleural gland secretions of *Crematogaster difformis* [as "*Crematogster deformis*"] (ATTYGALLE & al. 1989). Phenols are a major group of substances found in plant resins (BANKOVA & al. 2000) used by bees and ants to combat harmful microbes.

DO NASCIMENTO & al. (1996) studied metapleural gland secretions in three attine species (*Atta sexdens*, *A. cephalotes*, and *Acromyrmex octospinosus*) and confirmed that phenylacetic acid is the major component in the two *Atta* species whereas it is absent from *Acromyrmex octospinosus*. In metapleural gland secretions of *Atta sexdens*, an aqueous emulsion of protein (or peptide) was inferred from infrared spectroscopy and ninhydrin tests (DO NASCIMENTO & al. 1996). Later, ORTIUS-LECHNER & al. (2000) detected more than 20 compounds in metapleural gland secretions of the same leaf-cutter ant species (*Acromyrmex octospinosus*). More recently, the composition of metapleural gland secretions were reported from two fire ant species (*Solenopsis invicta* and *S. geminata*, CABRERA & al. 2004). In *Acromyrmex octospinosus*, the major chemicals were acetic acid, myristic acid, pentadecanoic acid, palmitic acid, indole-3-acetic acid, γ-octalactone, 4-oxooctanoic acid (ORTIUS-LECHNER & al. 2000). Hence, the metapleural gland secretions of *Acromyrmex octospinosus* contain a diverse array of compounds, many carboxylic acids, alcohols, lactones, and keto acids (ORTIUS-LECHNER & al. 2000). The antimicrobial efficacies of various *Acromyrmex octospinosus* metapleural gland compounds were tested against spores and hyphae from the mutualistic fungus (*Leucoagaricus gongylophorus*), and from fungal-parasitic (*Escovopsis* sp., *Trichoderma* sp.), commensal (*Gliocladium virens*, *Aspergillus niger*), and entomopathogenic fungal species (*Metarhizium anisopliae*, *Beauveria bassiana*) as well as against two bacterial species (*Bacillus subtilis*, *Pseudomonas stutzeri*) (BOT & al. 2002). All groups of compounds had inhibitory effects against some microorganisms. In some fungal species (*Escovopsis* sp., *Aspergillus niger*), spores and hyphae differed in their sensitivities. The two entomopathogenic fungi tested were among the most sensitive species. Notably, the mutualistic fungus was sensitive to all classes of metapleural gland compounds (BOT & al. 2002) raising the question of the utility of the glands' secretions in maintaining the fungus garden.

Further evidence for the function of metapleural glands as contributing to the immune system of ants comes from a recent study demonstrating the active usage of the glands' secretions (FERNÁNDEZ-MARÍN & al. 2006). In all 26 ant species studied (from 19 genera and five subfamilies; i.e., Myrmicinae, Pseudomyrmecinae, Dolichoderinae, Formicinae, and Ponerinae), the ants touch the glands' orifices using their forelegs and then groom themselves. Furthermore, five leaf-cutter ant species from the genera *Atta* and *Acromyrmex* spread metapleural gland secretions onto their nestmates, queens, brood, and fungal gardens. When challenged with conidia from any of nine different fungal species, *Atta columbica* ants intensify metapleural gland grooming (FERNÁNDEZ-MARÍN & al. 2006). Earlier, POULSEN & al. (2002) experimentally sealed metapleural glands of *Acromyrmex octospinosus* workers, infected colonies with the entomopathogenic fungus *Metarhizium anisopliae*, and found substantially increased mortality demonstrating the importance of metapleural glands to individual ants and their colonies.

The sizes of metapleural gland reservoirs relative to body size are significantly larger in species of the genera *Acromyrmex* and *Atta* compared to other fungus-growing ants (Attini) (HUGHES & al. 2008). The leaf-cutter ants (*Acromyrmex* and *Atta*) provide their fungus exclusively with fresh vegetation and have very populous colonies (HÖLLDOBLER & WILSON 1990). The larger capacity of their metapleural glands is suggestive of an exceptional relationship between leaf-cutter ants and their parasites (HUGHES & al. 2008). In accordance with this hypothesis, *Acromyrmex subterraneus* minor workers, which work the fungus garden, have relatively larger metapleural gland reservoirs than major workers (DE SOUZA & al. 2006). Further, metapleural gland size differences had a positive impact on survival times of *Acromyrmex echinatior* minor and major workers (POULSEN & al. 2006) being challenged with the entomopathogenic fungus *Aspergillus nomius*.

In many ant species, males lack metapleural glands (e.g., *Nothomyrmecia macrops*). Living together with their numerous sisters might provide sufficient immune defenses for them. Indeed, *Myrmecia gulosa* males kept without workers, which possess metapleural glands, acquire larger numbers of microbes than males kept together with *M. gulosa* workers (MACKINTOSH & al. 1999). Moreover, males are relatively short-lived which might reduce the need for strong immune defenses (HÖLLDOBLER & ENGEL-SIEGEL 1984). However in a few species (e.g., *Temnothorax allardycei*, *Novomessor cockerelli*, *Formica perpilosa*, *Myrmecocystus mimicus*, *Iridomyrmex purpureus*), the existence of metapleural glands has been established in males (HÖLLDOBLER & ENGEL-SIEGEL 1984). Furthermore, in all weaver ants (*Oecophylla*, *Polyrhachis*, *Dendromyrmex*) and in most *Camponotus* species as well as some social parasitic ants studied by HÖLLDOBLER & ENGEL-SIEGEL (1984) the meta-

pleural glands are either considerably atrophied or entirely absent leading the authors to hypothesize "that these arboreal ants are much less exposed to microorganisms than terrestrial ant species, and therefore an antiseptic metapleural gland became unnecessary" (HÖLLDOBLER & ENGEL-SIEGEL 1984). We believe the proposed link between little exposure to microorganisms and the loss of metapleural glands is quite plausible, however, as HÖLLDOBLER & ENGEL-SIEGEL (1984) also point out, there are many arboreal species having metapleural glands. It might be that weaving is the key behavior that results in an antiseptic nest environment (JOHNSON & al. 2003).

Secretions from exocrine glands other than the metapleural glands also contribute to ant immune defenses. Mandibular gland secretions from *Calomyrmex* sp. show bacteriocidal activity and inhibit the growth of fungi (BROUGH 1983). Since the chemical nature of the antimicrobial activity had not been studied then, it would be interesting to see if the antimicrobial peptide defensin is expressed in mandibular glands of *Calomyrmex* ants, as it is in the honey bee *Apis mellifera* (see KLAUDINY & al. 2005). Techniques such as RT-PCR could be used to clarify which proteins occur where. Antimicrobial activity of mandibular gland secretions was also reported in another formicine ant, *Lasius fuliginosus* (PAVAN 1958 cited by BROUGH 1983). Recently, effects of *Atta sexdens* mandibular gland secretions on fungal spore germination have been tested (RODRIGUES & al. 2008). The fungi *Fusarium solani, Trichoderma harzianum*, *Cunninghamella elegans*, and *Syncephalastrum racemosum* have all been negatively affected by mandibular gland secretions, however, spore germination was not at all inhibited in the fungal-parasitic fungus *Escovopsis weberi* (see REYNOLDS & CURRIE 2004) and in the entomopathogenic fungus *Metarhizium anisopliae* (see RODRIGUES & al. 2008).

External surfaces

Prevention of penetration is often achieved through the ant's integument if avoidance behavior is not possible or does not succeed. The integument of insects (including ants) comprises the epidermis and the cuticle that again is divided into epicuticle, exocuticle, and endocuticle (CHAPMAN 1998, GOBIN & al. 2003). The chitinous cuticle has possibly contributed to the general success of arthropods and forms an important mechanical barrier for parasites and pathogens. Fungi (e.g., *Metarhizium anisopliae*) are the only microbial pathogens that have been reported to be able to penetrate the cuticle directly (SIVA-JOTHY & al. 2005). The integument lines the very outside surface of ants and also parts of the gut and the reproductive tract which are both possible entry points of pathogens. Gut tissue has been shown to produce antimicrobial peptides and highly reactive nitrogen and / or oxygen containing molecules (HAO & al. 2003) upon oral infections with *Trypanosoma brucei* in the tsetse fly *Glossina morsitans*. There are no such studies to our knowledge in ants but we believe it would be worthwhile studying immune responses in ant guts because of their obvious exposure to microbial parasites, using similar methods. The reproductive tract is another potential site of microbial invasions. In fact, viruses can be horizontally transmitted during copulation in honey bees (YUE & al. 2006). In the reproductive tract of *Drosophila*, antimicrobial peptides are expressed in a sex-specific manner (TZOU & al. 2000). To our knowledge, no studies have been carried out on immunity gene expression in ants' reproductive tracts. However, sperm storage in *Atta columbica* resulted in an up-regulation of the queen's immune system nine days after mating (BAER & al. 2006). The up-regulation cannot be a direct consequence of the amount of stored sperm because the immune response was negatively correlated with the number of sperm (BAER & al. 2006) indicating trade-offs between copulating (or storing sperm) and other life-history traits. In addition to a lower immune response (when more sperm had been transmitted), the immune response was also lower if the queen had mated with more males (BAER & al. 2006). An untested possible explanation could be that males transmitted antimicrobials in their ejaculates. More genetically diverse ejaculates could thus prove being beneficial. Recently, CASTELLA & al. (2009) found an up-regulation of a different part of the immune system after mating in *Formica paralugubris*. Queens of this species showed increased levels of antibacterial activity of their hemolymph seven days after mating (CASTELLA & al. 2009). But in contrast to *Atta columbica* queens (BAER & al. 2006), there was no significant increase in phenoloxidase activity (CASTELLA & al. 2009). The difference between the two species might be due to different pathogen exposure during nest founding (CASTELLA & al. 2009).

Innate immune system

Innate immunity in insects has been reviewed extensively (GILLESPIE & al. 1997, BREY & HULTMARK 1998, TZOU & al. 2002, SCHMID-HEMPEL 2005, BECKAGE 2008). The fruit fly *Drosophila melanogaster* is probably the insect best studied with regard to the function of specific genes and proteins of its immune system (for a review see LEMAITRE & HOFFMANN 2007). Immune systems of 12 *Drosophila* species (SACKTON & al. 2007), the red flour beetle *Tribolium castaneum* (see ZOU & al. 2007), and the Western honey bee *Apis mellifera* (see EVANS & al. 2006) have been analyzed drawing on their whole genome sequences. The general picture emerging from these comparative genome studies is one of considerable similarity among these holometabolous insects with respect to molecular pattern recognition molecules, biochemical signal transduction pathways, and classes of proteins used to combat infections. Nonetheless, the honey bee *Apis mellifera*, which is probably most similar to ants among the insects analyzed, had an unexpectedly small number of pathogen recognition and effector genes (EVANS & al. 2006). It will be interesting to see whether the immune system of the jewel wasp *Nasonia vitripennis*, a parasitoid whose genome sequence is imminent, is essentially similar to the one of *Apis mellifera*. This would point to hymenopterans being exceptional among the Holometabola and indeed they are sister group to all the others (SAVARD & al. 2006). This is also going to be of interest for future work on ant immunity. While there is no whole genome sequence yet, two expressed sequence tag (cDNA) libraries for the fire ant *Solenopsis invicta* have been made publicly available (WANG & al. 2007, VALLES & al. 2008, WURM & al. 2009). To our knowledge, these databases have not been searched comprehensively for immunity-related genes so that comparisons cannot yet be made between the fire ant's immune system and immune systems of other insects. Insects do not possess major histocompatibility complex molecules, T-cell receptors, and

immunoglobulins (also known as antibodies) that are important in self and also in non-self recognition (LITTLE & al. 2005). These three groups of immunity-related molecules occur only in Gnathostomata (jawed vertebrates). T-cell receptor and immunoglobulin genes acquire very high variability through somatic recombination and mutation. These mechanisms have not been demonstrated to play any role in insect immunity so far. However, in the bumble bee *Bombus terrestris* some characteristics of adaptive immunity have been demonstrated (i.e., increased protection and narrow specificity upon secondary exposure to bacteria, trans-generational immune priming, SADD & al. 2005, SADD & SCHMID- HEMPEL 2006). To our knowledge no such studies have been carried out on ants. The molecular mechanisms facilitating increased protection and immunological memory in insects are not known (SCHULENBURG & al. 2009). However, the Down Syndrome cell adhesion molecule (Dscam) proteins, which can potentially produce thousands of splice variants, are involved in phagocytosis of *Escherichia coli* bacteria in *Drosophila* flies (WATSON & al. 2005). This discovery has opened up new insights into the possibility of the recognition of a large variety of different microbes. Dscam proteins have also been found in other insects (CRAYTON & al. 2006) including the honey bee *Apis mellifera* (see GRAVELEY & al. 2004). Intriguingly, Dscam proteins contain immunoglobulin protein domains typical for gnathostome immune proteins.

Molecular recognition of parasites and pathogens

Molecular recognition of parasites and pathogens is the next component of the immune system once the barrier set by external surfaces has been surmounted. Tissues and cells in the hemocoel produce molecules that recognize pathogen-associated molecular patterns (BRENNAN & ANDERSON 2004). For example, peptidoglycan from bacterial cell walls facilitates recognition of non-self (DZIARSKI & GUPTA 2006). In ants, some recognition genes have recently been cloned and sequenced (VILJAKAINEN 2008): a peptidoglycan recognition protein (*PGRP-SC2*) and a Gram-negative bacteria binding protein (*GNBP1*) from *Myrmica ruginodis* and another peptidoglycan recognition protein (*PGRP-SA*) from *Formica aquilonia*. We have cloned and sequenced *GNBP1* from *Nothomyrmecia macrops* and a range of *Myrmecia* species (SCHLÜNS & al. 2008). The function of these genes in ants has not been established so far although work on *Drosophila melanogaster* suggests they are involved in recognition of bacterial infections (LEMAITRE & HOFFMANN 2007). Overall, there are not yet many reports of genes involved in the immune system of ants. The lack of a full genome sequence of any ant species at all so far clearly impedes the study of innate immunity in one of the most abundant and conspicuous terrestrial animal taxa.

Signal transduction molecules

Subsequent to the recognition of pathogen invasions into the hemocoel, innate immune systems use biochemical signaling pathways that transmit the information of being infected. Two important cellular signaling cascades in insects are the Toll pathway and the imd pathway (SIVA-JOTHY & al. 2005). Recently some signaling pathway genes have been cloned and sequenced from *Formica aquilonia*: *Toll*, *Pelle*, and *Dorsal*, which are parts of the Toll pathway, and *TAK-1* that is a gene of the imd pathway (VILJAKAINEN 2008). We have cloned and partially sequenced *Relish* from *Nothomyrmecia macrops* and several *Myrmecia* species (SCHLÜNS & al. 2008). Relish is a NF-κB-like transcription factor, the last protein of the imd pathway before effector molecules are produced (STÖVEN & al. 2000). We assume Relish enhances transcription of antimicrobial peptide genes in ants since we could demonstrate regulation of two antimicrobials by *Relish* in the honey bee *Apis mellifera* (see SCHLÜNS & CROZIER 2007).

Effector mechanisms

Eventually the infection signal is converted into cellular and / or humoral effector mechanisms combating the invading pathogens. In insects, mechanisms to fight pathogens include (but are not limited to) phagocytosis, nodule formation and encapsulation (GILLESPIE & al. 1997). Cellular and / or melanotic encapsulation of foreign invading organisms is an important ability of the immune system (GILLESPIE & al. 1997). Encapsulation experiments are often used to generate a general idea of immunocompetence (e.g., VAINIO & al. 2004, BAER & al. 2005, BAER & al. 2006, SORVARI & al. 2007, DE SOUZA & al. 2008, DE SOUZA & al. 2009), for example, in the experiment described above of the impact of matings on immune system responses (BAER & al. 2006). Likewise immunocompetence of *Acromyrmex echinatior* workers and males, which had been challenged with the entomopathogenic fungus *Metarhizium anisopliae*, was tested using encapsulation experiments (BAER & al. 2005). Interestingly, workers showed a larger immune response than males, a phenomenon which might be explained by the haploid susceptibility hypothesis that predicts haploid males are more affected by parasites than diploid females due to the complete lack of heterozygous loci in males (O'DONNELL & BESHERS 2004). A similar pattern had been demonstrated earlier (VAINIO & al. 2004) in males and queens of *Formica exsecta*. In the latter experiment as in the other examples, encapsulation of a nylon thread was applied to test for immunocompetence. Moreover, two different sized male morphs (micraners and macraners) were compared and differed in the immune response (VAINIO & al. 2004).

Melanization is a significant process in wound healing but it also can be part of the encapsulation process (GILLESPIE & al. 1997). Melanin is generated through oxidation of monophenols to o-diphenols and oxidation of o-diphenols to o-quinones (CERENIUS & al. 2008). The enzyme catalyzing these reactions is phenoloxidase and the corresponding gene has been detected in all arthropod genomes investigated so far including that of the honey bee (CERENIUS & al. 2008). WILSON-RICH & al. (2008) have recently reported on honey bee phenoloxidase activity in larvae, pupae, and adults.

A very significant group of hemocoelic immunity effector molecules are antimicrobial peptides (BREY & HULTMARK 1998, KUHN-NENTWIG 2003). These peptides can be grouped according to charge (anionic or cationic) and amino acid composition. They often contain hydrophilic and hydrophobic regions (i.e., they are amphipathic). Furthermore, they are often rich in specific amino acids. Antimicrobial peptides attach to and insert into membranes to form pores but other mechanisms of interfering with pathogen biology, like inhibition of enzyme activity, have also been proposed (BROGDEN 2005). A number of such peptides have been isolated from ants, the first being a defen-

sin from *Formica rufa*, described and investigated by TAGUCHI & al. (1998). This defensin is not C-terminally amidated in contrast to defensins from two bee species and is active against a Gram-positive bacterium (*Micrococcus luteus*) (TAGUCHI & al. 1998). The gene encoding defensin of *Formica aquilonia* has been cloned and sequenced including the signal peptide and propeptide sequence (VILJAKAINEN & PAMILO 2005). The two *Formica* defensins do not differ in a single amino acid in their mature parts. Recently, VILJAKAINEN & PAMILO (in press) sequenced defensin genes from 25 formicine ant species (from six different genera) and two *Myrmica* species. Analysis of the molecular evolution of these defensins revealed that positive selection has shaped ant defensins in a similar manner to termicins in termites (BULMER & CROZIER 2004) but in contrast to *Drosophila* defensins (VILJAKAINEN & PAMILO in press). Defensin is also known from the fire ant *Solenopsis invicta* and is similar to defensin-2 from the honey bee *Apis mellifera* (see WANG & al. 2008). Gene expression of three genes believed to be involved in somatic maintenance and in immune defense (GRÄFF & al. 2007) differed between castes (workers and queens) in the ant *Lasius niger*. One of the genes codes for a serine protease inhibitor and could be part of an immune signaling pathway. *Solenopsis invicta* queens, which had recently mated and shed their wings (dealate queens), expressed two antimicrobial peptides (similar to hymenoptaecin and abaecin from the honey bee *Apis mellifera*) more strongly than non-mated alate queens (TIAN & al. 2004). This suggests an immunity burden due to mating in *Solenopsis invicta* reminiscent of the up-regulation of encapsulation processes in *Atta columbica* queens after mating (BAER & al. 2006). In addition to abaecin and hymenoptaecin from *Solenopsis invicta*, these two antimicrobials have recently been cloned and sequenced (partially in the case of hymenoptaecin) from another myrmicine ant (*Myrmica ruginodis*) (VILJAKAINEN 2008). We have partially cloned and sequenced hymenoptaecin from *Nothomyrmecia macrops* and several *Myrmecia* species (SCHLÜNS & al. 2008). Two further antimicrobial peptides have been isolated from hemolymph of *Myrmecia gulosa* (Formaecin-1 and Formaecin-2) (MACKINTOSH & al. 1998). Formaecin-1 is made up of only 16 amino acids, five of which are prolines. Formaecin-1 is glycosylated on threonine 11 by a monosaccharide (N-acetylgalactosamine). Formaecins negatively affected growing cells of *Escherichia coli* (Gram-negative) but not the yeast *Candida albicans* or Gram-positive bacteria such as *Bacillus thuringiensis* (see MACKINTOSH & al. 1998). Recently, HAINE & al. (2008) proposed a special role of antimicrobial peptides in the immune system of insects. In their experiments, most pathogens were eliminated from the hemolymph of *Tenebrio molitor* beetles before antimicrobial peptides were supposedly produced. HAINE & al. (2008) hypothesized that the main function of antimicrobials is to "mop up" potentially resistant microbes that have survived "constitutive" immune defenses such as hemocytes and the phenoloxidase cascade.

Antimicrobial peptides are not only found in the hemolymph but also in venom of ants (KUHN-NENTWIG 2003). Antimicrobial and cytolytic peptides might originally have had a function in combating pathogens and been co-opted to subdue larger prey or to fight off predators later on in hymenopteran evolution. Intriguingly, venom molecules evolved several times independently from immune genes in vertebrates (WHITTINGTON & al. 2008). The efficacy of inducing pores in cell membranes, a characteristic of antimicrobials, has been demonstrated (PLUZHNIKOV & al. 2006) from venom of the ponerine ant *Paraponera clavata*. Venoms of Australian "jumper" ants (*Myrmecia pilosula* species complex) contain several pilosulins (DONOVAN & al. 1993, STREET & al. 1996, INAGAKI & al. 2004, INAGAKI & al. 2008). Five of these antimicrobial peptides share high sequence similarity in their first 47 amino acids including the signal peptide sequence (INAGAKI & al. 2008). By contrast, the mature peptide coding regions vary substantially among pilosulins. Pilosulin 3 and 4 were isolated (INAGAKI & al. 2004) from *Myrmecia banksi*. Interestingly, they show some similarities to melittin from the venom of the honey bee *Apis mellifera* which is also antimicrobial and hemolytic (INAGAKI & al. 2004). Pilosulin 3 was to some degree effective against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive), but was not effective against *Lactococcus garvieae* (Gram-positive), *Pseudomonas aeruginosa* (Gram-negative), and the yeasts *Candida albicans* and *Saccharomyces cerevisiae*. Pilosulin 4 was effective against *Escherichia coli* and *Staphylococcus aureus*, and had, as Pilosulin 3, no effect against *Lactococcus garvieae*, *Candida albicans*, and *Saccharomyces cerevisiae* (see INAGAKI & al. 2004). In contrast to other pilosulins, pilosulin 5 shows only weak antimicrobial activity (INAGAKI & al. 2008). Furthermore, the pilosulin 5 gene shares little similarity with the genes of pilosulins 1 - 4 in the 5'- and 3'-untranslated regions in contrast to the other pilosulins that are quite similar in these regions (INAGAKI & al. 2008).

Venom of the ponerine ant *Pachycondyla goeldii* contains at least 15 different ponericins (ORIVEL & al. 2001). These venom peptides show antimicrobial and cytolytic activities against a wide range of Gram-positive (e.g., *Staphyloccocus aureus*) and Gram-negative (e.g., *Escherichia coli*) bacteria and a yeast (*Saccharomyces cerevisiae*) (ORIVEL & al. 2001). Ponericins fall into three groups (G, W, L) based on their primary structure and are 24 (L) to 31 (G) amino acids long. Ponericins W share sequence similarity with mellitin from venom of the honey bee *Apis mellifera*. Ponericins G share sequence similarity with cecropin-like peptides from Lepidoptera and Diptera (ORIVEL & al. 2001). These similarities suggest the amphipathic ponericins might fold into α-helices (ORIVEL & al. 2001).

Antimicrobial peptides are not the only gene products (i.e., RNAs and proteins) ants can employ to combat invading pathogens. Transferrins are iron metabolism proteins. The transferrin genes are up-regulated upon infection in various animals including the fire ant *Solenopsis invicta* challenged with conidia (VALLES & PEREIRA 2005) from the entomopathogenic fungus *Beauveria bassiana*. Hence, transferrins play a significant role in immune defenses presumably by depriving microorganisms of iron. Ants of the formicine genus *Polyrhachis* lack metapleural glands and thus might be particularly vulnerable to microbial attacks. In a study on the molecular evolution of transferrins (including 14 *Polyrhachis* species), three lineages were found to be under positive selection (SHUETRIM 2007). It would be interesting to investigate if these lineages were more exposed to pathogenic microorganisms than the others that possess more slowly evolving transferrins by using the approach of ROSENGAUS & al. (2003) for total microbial bur-

den or TRFLP methods to assess microbial species richness (FIERER & JACKSON 2006).

Behavior of individual ants

Behavioral defenses of individuals are an important line of defense that can start before molecular defense mechanisms come into play. Workers and soldiers of *Pheidole titanis* seek cover to avoid attacks by parasitic phorid flies (*Apocephalus* sp., FEENER 1988). By contrast, in another *Pheidole* species (*Pheidole dentata*) only soldiers are attacked and only they make efforts to avoid being parasitized by phorid flies (FEENER 1981). A social variant of such defensive behavior can be observed in leaf-cutter ants. Major workers of leaf-cutter ants often carry minor workers sitting on leaf fragments that are brought back to the nest. In *Atta sexdens* and *Atta laevigata*, the proportion of fragments with minor workers being carried increased if the fungus *Rhizopus* sp. was present (VIEIRA-NETO & al. 2006). Further, in *Atta sexdens* more minor workers are carried if parasitic phorid flies (*Neodohrniphora erthali*) are nearby, suggesting a defensive role of the minor workers (VIEIRA-NETO & al. 2006). In addition to generating behavioral differences during evolution, parasitoid fly attacks might have even shaped morphological caste differences in *Atta* leafcutter ants (ORR 1992). *Acromyrmex striatus* ants are able to distinguish between clean food (rice grains and wheat flour) and the same food contaminated with entomopathogenic fungi (*Metarhizium anisopliae* and *Beauveria bassiana*) or their spores (DIEHL-FLEIG & LUCCHESE 1991). The workers try to remove the contaminated food from the nest (DIEHL-FLEIG & LUCCHESE 1991). *Formica rufa* workers avoid contact with nestmates heavily infected with an ant-killing fungus (MARIKOVSKY 1962). *Linepithema humile* workers were also able to avoid scavenging on insect cadavers killed by certain nematodes (Heterorhabditidae) while they were not deterred if the insects were killed by other nematodes (Steinernematidae) (BAUR & al. 1998). This avoidance behavior was beneficial to the heterorhabditid nematodes, but it remains to be shown that it indeed constitutes parasite avoidance behavior by the ants.

Sociality and defenses

Colony level defenses in social insects receive increasing attention and several reviews on this topic have been published, some of them very recently (SCHMID-HEMPEL 1998, CREMER & al. 2007, CREMER & SIXT 2009, WILSON-RICH & al. 2009). Therefore, we refer the interested reader to these reviews. Here, we will focus on highlighting some mechanisms that are especially intriguing because of the ant's collective usage of chemicals. As we have discussed in detail above, ants can prevent the establishment of harmful microorganisms in nests using secretions from exocrine glands which can be viewed as both collective and individual immune defenses. A different way of using chemicals to fend off microbes has been investigated by CHAPUISAT, CASTELLA, CHRISTE and co-workers in a number of studies (CHAPUISAT & al. 2007, CHRISTE & al. 2003, CASTELLA & al. 2008a, b). Wood ants (*Formica paralugubris*) collect plant resins and incorporate these in their nests (CHRISTE & al. 2003). This behavior is also common in other social Hymenoptera such as the honey bee *Apis mellifera*. The plant resins found in bee hives (there called propolis) are chemically unchanged from the original plant resins and known to exhibit antimicrobial properties (BANKOVA & al. 2000). Likewise, the conifer resin used by *Formica paralugubris* inhibited the growth of microbes (CHRISTE & al. 2003). Moreover, when resin was present in nests fewer bacteria and fungi were found compared to nests containing only very little resin (CASTELLA & al. 2008b). The wood ants benefited directly from the antimicrobial property of resin as they survived for longer if attacked by *Pseudomonas fluorescens* bacteria or the entomopathogenic fungus *Metarhizium anisopliae* (CHAPUISAT & al. 2007). Infections with *Metarhizium anisopliae* did not increase the ants' collection of resin, however, suggesting prophylactic rather than therapeutic resin usage (CASTELLA & al. 2008a). Ants reduced the activity of their immune system if they lived in resin-rich nests possibly because of the reduced number of potentially harmful microbes in these nests (CASTELLA & al. 2008b).

Within-colony genetic diversity and immunity

Genotypic diversity among individuals within ant colonies can result in disease resistance similar to herd immunity achieved through vaccinations of people in human populations (SCHMID-HEMPEL 1994). An improved overall colony immunity is one facet of genetic variance hypotheses for the evolution of polyandry and / or polygyny (CROZIER & FJERDINGSTAD 2001). There is also empirical evidence that the level of relatedness within colonies is indeed significantly associated with parasite loads (SCHMID-HEMPEL & CROZIER 1999). Further, intracolonial diversity improved survival of the ant *Formica selysi* infected with the entomopathogenic fungus *Metarhizium anisopliae* while diversity was detrimental in the absence of this parasite, perhaps because of increased intragroup discord (REBER & al. 2008). In another study supporting the genetic variance hypothesis for improved immunity, HUGHES & BOOMSMA (2006) carried out serial passages of *Metarhizium anisopliae* on *Acromyrmex* leafcutter ant hosts for nine generations, varying the degree of genetic difference between hosts in different experimental lines. Higher genetic diversity between host generations led to increased extinction of the fungus, whereas lower genetic diversity between host generations led to higher spore production by the ninth passage.

Two different social forms, monogynous and polygynous colonies, exist in the fire ant *Solenopsis invicta* depending on the genotypes of queens and workers at a single locus (*Gp-9*). WANG & al. (2008) found higher expression of the defensin gene and lower rates of virus infection in monogynous than in polygynous colonies. At first sight, this contradicts the hypothesis that group diversity promotes immunity, but WANG & al. (2008) note that haplometrosis promotes stringent selection against susceptible monogynous queens whereas such queens can survive in the dependent colony foundation of the polygynous form. A focussed test of the genetic diversity hypothesis in fire ants could be carried out by varying groups of polygynous ants.

Nest architecture and disease

Nest architecture might have an important impact on pathogen transmission in ant colonies. Results from theoretical modeling using an individual-based approach suggest that certain kinds of nest architecture (e.g., a simple spatial separation of chambers) could considerably delay the spread of infections (PIE & al. 2004). Further, worker density and

activity level had also a strong impact on dynamics of epidemics (PIE & al. 2004). In another theoretical study, FEFFERMAN & al. (2007) found a slight beneficial effect of non-random spatial distribution of younger and older individuals in social insect nests, which could in principle be tested using artificial laboratory nests of different architecture.

Habitats and microbial loads in nests

Evidence for effects of habitats on microbial loads in social insect nests comes from a study on termites living in either damp or in dry wood (ROSENGAUS & al. 2003). To our knowledge there are no such studies demonstrating a direct link between habitat and microbial loads in ants. Nonetheless, microbial biomass was higher in nest soil of three ant species (*Myrmica scabrinodis*, *Lasius flavus*, *L. niger*) than in control areas (DAUBER & al. 2001). However, there was no difference among the three species (DAUBER & al. 2001). Likewise, greater fungal abundance was detected in mounds of the fire ant *Solenopsis invicta* and in *Aphaenogaster texana* nests (ZETTLER & al. 2002). By contrast, fungal species richness and diversity was lower in nests of both ant species than in non-mound soil (ZETTLER & al. 2002).

Conclusions and outlook

The first studies on ant immunity date back several decades. Since then metapleural gland secretions and their impact on bacteria and fungi have been investigated in some detail. There is, however, a gap in our knowledge with regard to metapleural gland secretions and their impact on naturally occurring microbial enemies of ants. Furthermore, the comparatively small number of microbial pathogens and parasites known from ant species weighed against the number known from honey bees suggests that many microbial diseases are still to be discovered in ants. It would be desirable in future research to elucidate ecological and epidemiological factors of ant diseases as well as abundance and diversity of ant pathogens.

The advances in molecular biology technology are increasingly rapid, facilitating comparative genomic analyses of innate immune systems among insects and other organisms. However, there is yet no whole genome sequence of any ant species, and no comprehensive analysis of ant immunity genes has been carried out so far. There is a strong need to search for more genes drawing on the available fire ant cDNA libraries using bioinformatic methods. Functions of the newly identified genes should be experimentally tested applying techniques such as RNA interference. Moreover, a whole genome sequence of any ant species is highly desirable or at least more cDNA libraries from a range of ant subfamilies could facilitate phylogenetic comparisons of immune genes.

We propose that future research links molecular and organismic levels of ant-parasite interactions to achieve a comprehensive understanding of immune defense mechanisms in ants.

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Zusammenfassung

Ebenso wie alle anderen Organismen können Ameisen von einer großen Zahl mikrobieller Parasiten und Pathogenen befallen werden. Infolgedessen haben Ameisen eine Reihe von Immunabwehrmechanismen evolviert. Hier geben wir einen Überblick über den derzeitigen Kenntnisstand der Immunabwehrmechanismen von Ameisen mit einem Schwerpunkt auf Genen, Proteinen und chemischen Substanzen, die an der angeborenen Immunität der Ameisen beteiligt sind. Einige Erkennungs-, Signalketten- und Effektormoleküle (wie zum Beispiel antimikrobielle Peptide) sind von einer Reihe zu verschiedenen Unterfamilien gehörenden Ameisenarten beschrieben worden. Jedoch sind eine sehr große Zahl an Komponenten der angeborenen Immunität bisher nicht identifiziert worden. Sekrete der Metapleuraldrüsen haben eine besondere Bedeutung als ameisenspezifische Immunkomponenten. Es ist bekannt, dass die chemisch diversen Substanzen dieser Drüsen antimikrobielle Eigenschaften besitzen und daher eine wichtige Rolle im Leben der Ameisen spielen. Zusätzlich zu den molekularen und chemischen Abwehrmechanismen der individuellen Physiologie tragen auch individuelle und gruppenspezifische Verhaltensmuster erheblich zur Krankheitsbekämpfung bei. Beispiele zur Krankheitsresistenz beitragender Verhaltensmechanismen beinhalten Parasitenvermeidung, aktive Einbeziehung antimikrobieller Pflanzenharze in das Nestmaterial und erhöhte genetische Kolonievariabilität durch Polyandrie oder Polygynie (oder beides). Wir schlagen vor, dass zukünftige Forschung die Immunität der Ameisen auf molekularer Ebene mit ihrer Ökologie und der Ökologie ihrer Pathogene verbindet und evolutive Mechanismen untersucht, um ein umfassendes Verständnis der Immunabwehrmechanismen der Ameisen zu erlangen.

References

ATTYGALLE, A.B., SIEGEL, B., VOSTROWSKY, O., BESTMANN, H. J. & MASCHWITZ, U. 1989: Chemical composition and function of metapleural gland secretion of the ant, *Crematogaster deformis* SMITH (Hymenoptera, Myrmicinae). – Journal of Chemical Ecology 15: 317-328.

BAER, B., ARMITAGE, S. & BOOMSMA, J. 2006: Sperm storage induces an immunity cost in ants. – Nature 441: 872-875.

BAER, B., KRUG, A., BOOMSMA, J. & HUGHES, W. 2005: Examination of the immune responses of males and workers of the leaf-cutting ant *Acromyrmex echinatior* and the effect of infection. – Insectes Sociaux 52: 298-303.

BAIRD, R., WOOLFOLK, S. & WATSON, C.E. 2007: Survey of bacterial and fungal associates of black/hybrid imported fire ants from mounds in Mississippi. – Southeastern Naturalist 6: 615-632.

BANKOVA, V.S., DE CASTRO, S.L. & MARCUCCI, M.C. 2000: Propolis: recent advances in chemistry and plant origin. – Apidologie 31: 3-15.

BAUR, M.E., KAYA, H.K. & STRONG, D.R. 1998: Foraging ants as scavengers on entomopathogenic nematode-killed insects. – Biological Control 12: 231-236.

BEATTIE, A.J., TURNBULL, C., HOUGH, T., JOBSON, S. & KNOX, R.B. 1985: The vulnerability of pollen and fungal spores to ant secretions – evidence and some evolutionary implications. – American Journal of Botany 72: 606-614.

BEATTIE, A.J., TURNBULL, C.L., HOUGH, T. & KNOX, R.B. 1986: Antibiotic production – a possible function for the metapleural

glands of ants (Hymenoptera, Formicidae). – Annals of the Entomological Society of America 79: 448-450.

BECKAGE, N.E. (Ed.) 2008: Insect immunology. – Elsevier, Amsterdam, The Netherlands, 348 pp.

BOT, A.N.M., ORTIUS-LECHNER, D., FINSTER, K., MAILE, R. & BOOMSMA, J.J. 2002: Variable sensitivity of fungi and bacteria to compounds produced by the metapleural glands of leaf-cutting ants. – Insectes Sociaux 49: 363-370.

BRENNAN, C. & ANDERSON, K. 2004: *Drosophila*: the genetics of innate immune recognition and response. – Annual Review of Immunology 22: 457-483.

BREY, P.T. & HULTMARK, D. (Eds.) 1998: Molecular mechanisms of immune responses in insects. – Chapman & Hall, London, UK, 325 pp.

BROGDEN, K.A. 2005: Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? – Nature Reviews Microbiology 3: 238-250.

BROUGH, E.J. 1983: The anti-microbial activity of the mandibular gland secretion of a formicine ant, *Calomyrmex* sp. (Hymenoptera, Formicidae). – Journal of Invertebrate Pathology 42: 306-311.

BROWN Jr, W. 1968: An hypothesis concerning the function of the metapleural glands in ants. – American Naturalist 102: 188-191.

BULMER, M.S. & CROZIER, R.H. 2004: Duplication and diversifying selection among termite antifungal peptides. – Molecular Biology and Evolution 21: 2256-2264.

CABRERA, A., WILLIAMS, D., HERNANDEZ, J.V., CAETANO, F.H. & JAFFE, K. 2004: Metapleural- and postpharyngeal-gland secretions from workers of the ants *Solenopsis invicta* and *S. geminata*. – Chemistry & Biodiversity 1: 303-311.

CASTELLA, G., CHAPUISAT, M. & CHRISTE, P. 2008a: Prophylaxis with resin in wood ants. – Animal Behaviour 75: 1591-1596.

CASTELLA, G., CHAPUISAT, M., MORET, Y. & CHRISTE, P. 2008b: The presence of conifer resin decreases the use of the immune system in wood ants. – Ecological Entomology 33: 408-412.

CASTELLA, G., CHRISTE, P. & CHAPUISAT, M. 2009: Mating triggers dynamic immune regulations in wood queens. – Journal of Evolutionary Biology 22: 564-570.

CELLE, O., BLANCHARD, P., OLIVIER, V., SCHURR, F., COUGOULE, N., FAUCON, J. & RIBIÈRE, M. 2008: Detection of Chronic bee paralysis virus (CBPV) genome and its replicative RNA form in various hosts and possible ways of spread. – Virus Research 133: 280-284.

CERENIUS, L., LEE, B. K. & SÖDERHÄLL, K. 2008: The proPO-system: pros and cons for its role in invertebrate immunity. – Trends in Immunology 29: 263-271.

CHAPMAN, R.F. 1998: The insects: structure and function. 4th edn. – Cambridge University Press, Cambridge, UK, 768 pp.

CHAPUISAT, M., OPPLIGER, A., MAGLIANO, P. & CHRISTE, P. 2007: Wood ants use resin to protect themselves against pathogens. – Proceedings of the Royal Society B: Biological Sciences 274: 2013-2017.

CHRISTE, P., OPPLIGER, A., BANCALA, F., CASTELLA, G. & CHAPUISAT, M. 2003: Evidence for collective medication in ants. – Ecology Letters 6: 19-22.

CRAYTON, M.E., POWELL, B.C., VISION, T.J. & GIDDINGS, M.C. 2006: Tracking the evolution of alternatively spliced exons within the Dscam family. – BMC Evolutionary Biology 6: 16.

CREMER, S., ARMITAGE, S. & SCHMID-HEMPEL, P. 2007: Social immunity. – Current Biology 17: R693-R702.

CREMER, S. & SIXT, M. 2009: Analogies in the evolution of individual and social immunity. – Philosophical Transactions of the Royal Society B: Biological Sciences 364: 129-142.

CROSLAND, M.W.J. 1988: Effect of a gregarine parasite on the color of *Myrmecia pilosula* (Hymenoptera: Formicidae). – Annals of the Entomological Society of America 81: 481-484.

CROZIER, R.H. & FJERDINGSTAD, E.J. 2001: Polyandry in social Hymenoptera – disunity in diversity? – Annales Zoologici Fennici 38: 267-285.

DAUBER, J., SCHROETER, D. & WOLTERS, V. 2001: Species specific effects of ants on microbial activity and N-availability in the soil of an old-field. – European Journal of Soil Biology 37: 259-261.

DE SOUZA, A.L.B., SAORES, I.M.F., CYRINO, L.T. & SERRÃO, J.E. 2006: The metapleural gland in two subspecies of *Acromyrmex subterraneus* (Hymenoptera: Formicidae). – Sociobiology 47: 19-25.

DE SOUZA, D.J., BÉZIER, A., DEPOIX, D., DREZEN J.-M. & LENOIR, A. 2009: *Blochmannia* endosymbionts improve colony growth and immune defence in the ant *Camponotus fellah*. – BMC Microbiology 9: 29.

DE SOUZA, D.J., VAN VLAENDEREN, J., MORET, Y. & LENOIR, A. 2008: Immune response affects ant trophallactic behaviour. – Journal of Insect Physiology 54: 828-832.

DIEHL-FLEIG, E. & LUCCHESE, M.E.P. 1991: Reações comportamentais de operárias de *Acromyrmex striatus* (Hymenoptera, Formicidae) na presença de fungos entomopatogênicos. – Revista Brasileira de Entomologia 35: 101-107.

DO NASCIMENTO, R., SCHOETERS, E., MORGAN, E.D., BILLEN, J. & STRADLING, D.J. 1996: Chemistry of metapleural gland secretions of three attine ants, *Atta sexdens rubropilosa*, *Atta cephalotes*, and *Acromyrmex octospinosus* (Hymenoptera: Formicidae). – Journal of Chemical Ecology 22: 987-1000.

DONOVAN, G.R., BALDO, B.A. & SUTHERLAND, S. 1993: Molecular cloning and characterization of a major allergen (Myr pI) from the venom of the Australian jumper ant, *Myrmecia pilosula*. – Biochimica et Biophysica Acta 1171: 272-280.

DZIARSKI, R. & GUPTA, D. 2006: The peptidoglycan recognition proteins (PGRPs). – Genome Biology 7: 232.

EVANS, J., ARONSTEIN, K., CHEN, Y.P., HETRU, C., IMLER, J., JIANG, H., KANOST, M., THOMPSON, G.J., ZOU, Z. & HULTMARK, D. 2006: Immune pathways and defence mechanisms in honey bees *Apis mellifera*. – Insect Molecular Biology 15: 645-656.

FEENER, D.H. 1981: Competition between ant species: outcome controlled by parasitic flies. – Science 214: 815-817.

FEENER, D.H. 1988: Effects of parasites on foraging, and defense behavior of a termitophagous ant, *Pheidole titanis* WHEELER (Hymenoptera: Formicidae). – Behavioral Ecology and Sociobiology 22: 421-427.

FEFFERMAN, N., TRANIELLO, J., ROSENGAUS, R. & CALLERI, D. 2007: Disease prevention and resistance in social insects: modeling the survival consequences of immunity, hygienic behavior, and colony organization. – Behavioral Ecology and Sociobiology 61: 565-577.

FELDHAAR, H., STRAKA, J., KRISCHKE, M., BERTHOLD, K., STOLL, S., MUELLER, M. & GROSS, R. 2007: Nutritional upgrading for omnivorous carpenter ants by the endosymbiont *Blochmannia*. – BMC Biology 5: 48.

FERNÁNDEZ-MARÍN, H., ZIMMERMAN, J., REHNER, S. & WCISLO, W. 2006: Active use of the metapleural glands by ants in controlling fungal infection. – Proceedings of the Royal Society B: Biological Sciences 273: 1689-1695.

FIERER, N. & JACKSON, R.B. 2006: The diversity and biogeography of soil bacterial communities. – Proceedings of the National Academy of Sciences of the United States of America 103: 626-631.

GILLESPIE, J.P., KANOST, M.R. & TRENCZEK, T. 1997: Biological mediators of insect immunity. – Annual Review of Entomology 42: 611-643.

GOBIN, B., ITO, F. & BILLEN, J. 2003: The subepithelial gland in ants: a novel exocrine gland closely associated with the cuticle surface. – Acta Zoologica (Stockholm) 84: 285-291.

GRÄFF, J., JEMIELITY, S., PARKER, J., PARKER, K. & KELLER, L. 2007: Differential gene expression between adult queens and workers in the ant *Lasius niger*. – Molecular Ecology 16: 675-683.

GRAVELEY, B.R., KAUR, A., GUNNING, D., ZIPURSKY, S.L., ROWEN, L. & CLEMENS, J.C. 2004: The organization and evolution of the dipteran and hymenopteran Down syndrome cell adhesion molecule (Dscam) genes. – RNA 10: 1499-1506.

HAINE, E.R., MORET, Y., SIVA-JOTHY, M. & ROLFF, J. 2008: Antimicrobial defense and persistent infection in insects. – Science 322: 1257-1259.

HAO, Z., KASUMBA, I. & AKSOY, S. 2003: Proventriculus (cardia) plays a crucial role in immunity in tsetse fly (Diptera: Glossinidiae). – Insect Biochemistry and Molecular Biology 33: 1155-1164.

HAUTON, C. & SMITH, V. 2007: Adaptive immunity in invertebrates: a straw house without a mechanistic foundation. – Bioessays 29: 1138-1146.

HÖLLDOBLER, B. & ENGEL-SIEGEL, H. 1984: On the metapleural gland of ants. – Psyche 91: 201-224.

HÖLLDOBLER, B. & WILSON, E.O. 1990: The ants. – Belknap Press of Harvard University Press, Cambridge, MA, 732 pp.

HUGHES, W.O. & BOOMSMA, J.J. 2006: Does genetic diversity hinder parasite evolution in social insect colonies? – Journal of Evolutionary Biology 19: 132-143.

HUGHES, W.O., PAGLIARINI, R., MADSEN, H., DIJKSTRA, M. & BOOMSMA, J. 2008: Antimicrobial defense shows an abrupt evolutionary transition in the fungus-growing ants. – Evolution 62: 1252-1257.

INAGAKI, H., AKAGI, M., IMAI, H.T., TAYLOR, R.W. & KUBO, T. 2004: Molecular cloning and biological characterization of novel antimicrobial peptides, pilosulin 3 and pilosulin 4, from a species of the Australian ant genus *Myrmecia*. – Archives of Biochemistry and Biophysics 428: 170-178.

INAGAKI, H., AKAGI, M., IMAI, H.T., TAYLOR, R.W., WIESE, M.D., DAVIES, N.W. & KUBO, T. 2008: Pilosulin 5, a novel histamine-releasing peptide of the Australian ant, *Myrmecia pilosula* (Jack Jumper Ant). – Archives of Biochemistry and Biophysics 477: 411-416.

JOHNSON, R.N., AGAPOW, P.M. & CROZIER, R.H. 2003: A tree island approach to inferring phylogeny in the ant subfamily Formicinae, with especial reference to the evolution of weaving. – Molecular Phylogenetics and Evolution 29: 317-330.

KAUFMANN, S.H. 2008: Elie Metchnikoff's and Paul Ehrlich's impact on infection biology. – Microbes and Infection 10: 1417-1419.

KEELING, P.J. & FAST, N.M. 2002: Microsporidia: biology and evolution of highly reduced intracellular parasites. – Annual Review of Microbiology 56: 93-116.

KLAUDINY, J., ALBERT, S., BACHANOVÁ, K., KOPERNICKÝ, J. & SIMÚTH, J. 2005: Two structurally different defensin genes, one of them encoding a novel defensin isoform, are expressed in honeybee *Apis mellifera*. – Insect Biochemistry and Molecular Biology 35: 11-22.

KLEIN, J. & HOREJSI, V. 1997: Immunology. 2nd edn. – Blackwell Publishing, Oxford, UK, 722 pp.

KUHN-NENTWIG, L. 2003: Antimicrobial and cytolytic peptides of venomous arthropods. – Cellular and Molecular Life Sciences 60: 2651-2668.

LEANDER, B.S., CLOPTON, R.E. & KEELING, P.J. 2003: Phylogeny of gregarines (Apicomplexa) as inferred from small-subunit rDNA and beta-tubulin. – International Journal of Systematic and Evolutionary Microbiology 53: 345-354.

LEE, A.H., HUSSENEDER, C. & HOOPER-BÙI, L. 2008a: Culture-independent identification of gut bacteria in fourth-instar red imported fire ant, *Solenopsis invicta* BUREN, larvae. – Journal of Invertebrate Pathology 98: 20-33.

LEE, S.C., CORRADI, N., BYRNES, E.J., TORRES-MARTINEZ, S., DIETRICH, F.S., KEELING, P.J. & HEITMAN, J. 2008b: Microsporidia evolved from ancestral sexual fungi. – Current Biology 18: 1675-1679.

LEMAITRE, B. & HOFFMANN, J. 2007: The host defense of *Drosophila melanogaster*. – Annual Review of Immunology 25: 697-743.

LEMAITRE, B., NICOLAS, E., MICHAUT, L., REICHART, J.M. & HOFFMANN, J.A. 1996: The dorsoventral regulatory gene cassette spätzle/Toll/cactus controls the potent antifungal response in *Drosophila* adults. – Cell 86: 973-983.

LITTLE, T., COLEGRAVE, N., SADD, B. & SCHMID-HEMPEL, P. 2008: Studying immunity at the whole organism level. – BioEssays 30: 404-405.

LITTLE, T., HULTMARK, D. & READ, A. 2005: Invertebrate immunity and the limits of mechanistic immunology. – Nature Immunology 6: 651-654.

LOFGREN, C.S., BANKS, W.A. & GLANCEY, B.M. 1975: Biology and control of imported fire ants. – Annual Review of Entomology 20: 1-30.

MACKINTOSH, J.A., FLOOD, J.A., VEAL, D.A. & BEATTIE, A.J. 1999: Increase in levels of microbiota recoverable from male and larval *Myrmecia gulosa* (FABRICIUS) (Hymenoptera: Formicidae) following segregation from worker ants. – Australian Journal of Entomology 38: 124-126.

MACKINTOSH, J.A., TRIMBLE, J.E., JONES, M.K., KARUSO, P.H., BEATTIE, A.J. & VEAL, D.A. 1995: Antimicrobial mode of action of secretions from the metapleural gland of *Myrmecia gulosa* (Australian bull ant). – Canadian Journal of Microbiology 41: 136-144.

MACKINTOSH, J.A., VEAL, D.A., BEATTIE, A.J. & GOOGLEY, A.A. 1998: Isolation from an ant *Myrmecia gulosa* of two inducible O-glycosylated proline-rich antibacterial peptides. – Journal of Biological Chemistry 273: 6139-6143.

MARIKOVSKY, P. 1962: On some features of behavior of the ants *Formica rufa* L. infected with fungous disease. – Insectes Sociaux 9: 173-179.

MASCHWITZ, U. 1974: Vergleichende Untersuchungen zur Funktion der Ameisenmetathorakaldrüse. – Oecologia 16: 303-310.

MASCHWITZ, U., KOOB, K. & SCHILDKNECHT, H. 1970: Ein Beitrag zur Funktion der Metathoracaldrüse der Ameisen. – Journal of Insect Physiology 16: 387-404.

MUELLER, U.G., DASH, D., RABELING, C. & RODRIGUES, A. 2008: Coevolution between attine ants and actinomycete bacteria: a reevaluation. – Evolution 62: 2894-2912.

O'DONNELL, S. & BESHERS, S.N. 2004: The role of male disease susceptibility in the evolution of haplodiploid insect societies. – Proceedings of the Royal Society B: Biological Sciences 271: 979-983.

ORIVEL, J., REDEKER, V., LE CAER, J.P., KRIER, F., REVOL-JUNELLES, A.M., LONGEON, A., CHAFFOTTE, A., DEJEAN, A. & ROS-

SIER, J. 2001: Ponericins, new antibacterial and insecticidal peptides from the venom of the ant *Pachycondyla goeldii*. – Journal of Biological Chemistry 276: 17823-17829.

ORR, M.R. 1992: Parasitic flies (Diptera, Phoridae) influence foraging rhythms and caste division of labor in the leaf-cutter ant, *Atta cephalotes* (Hymenoptera, Formicidae). – Behavioral Ecology and Sociobiology 30: 395-402.

ORTIUS-LECHNER, D., MAILE, R., MORGAN, E.D. & BOOMSMA, J.J. 2000: Metapleural gland secretion of the leaf-cutter ant *Acromyrmex octospinosus*: new compounds and their functional significance. – Journal of Chemical Ecology 26: 1667-1683.

PIE, M.R., ROSENGAUS, R. & TRANIELLO, J.F. 2004: Nest architecture, activity pattern, worker density and the dynamics of disease transmission in social insects. – Journal of Theoretical Biology 226: 45-51.

PLUZHNIKOV, K.A., BOCHAROV, D.N., KONONOVA, N.V., SUKHANOV, S.V., BALASHOVA, T.A., ARSENIEV, A.S. & GRISHIN, E.V. 2006: Identification and structural analysis of a glycophospholipid component from the venom of ant *Paraponera clavata*. – Russian Journal of Bioorganic Chemistry 32: 478-484.

POULSEN, M., BOT, A.N.M., NIELSEN, M.G. & BOOMSMA, J.J. 2002: Experimental evidence for the costs and hygienic significance of the antibiotic metapleural gland secretion in leaf-cutting ants. – Behavioral Ecology and Sociobiology 52: 151-157.

POULSEN, M., HUGHES, W. & BOOMSMA, J. 2006: Differential resistance and the importance of antibiotic production in *Acromyrmex echinatior* leaf-cutting ant castes towards the entomopathogenic fungus *Aspergillus nomius*. – Insectes Sociaux 53: 349-355.

REBER, A., CASTELLA, G., CHRISTE, P. & CHAPUISAT, M. 2008: Experimentally increased group diversity improves disease resistance in an ant species. – Ecology Letters 11: 682-689.

REYNOLDS, H.T. & CURRIE, C.R. 2004: Pathogenicity of *Escovopsis weberi*: the parasite of the attine ant-microbe symbiosis directly consumes the ant-cultivated fungus. – Mycologia 96: 955-959.

RODRIGUES, A., CARLETTI, C.D., BUENO, O.C. & PAGNOCCA, F.C. 2008: Leaf-cutting ant faecal fluid and mandibular gland secretion: effects on microfungi spore germination. – Brazilian Journal of Microbiology 39: 64-67.

ROSENGAUS, R.B., MOUSTAKAS, J.E., CALLERI, D.V. & TRANIELLO, J.F.A. 2003: Nesting ecology and cuticular microbial loads in dampwood (*Zootermopsis angusticollis*) and drywood termites (*Incisitermes minor*, *I. schwarzi*, *Cryptotermes cavifrons*). – Journal of Insect Science 3: 31.

ROY, H.E., STEINKRAUS, D.C., EILENBERG, J., HAJEK, A.E. & PELL, J.K. 2006: Bizarre interactions and endgames: entomopathogenic fungi and their arthropod hosts. – Annual Review of Entomology 51: 331-357.

SACKTON, T., LAZZARO, B., SCHLENKE, T., EVANS, J., HULTMARK, D. & CLARK, A. 2007: Dynamic evolution of the innate immune system in *Drosophila*. – Nature Genetics 39: 1461-1468.

SADD, B.M., KLEINLOGEL, Y., SCHMID-HEMPEL, R. & SCHMID-HEMPEL, P. 2005: Trans-generational immune priming in a social insect. – Biology Letters 1: 386-388.

SADD, B.M. & SCHMID- HEMPEL, P. 2006: Insect immunity shows specificity in protection upon secondary pathogen exposure. – Current Biology 16: 1206-1210.

SAVARD, J., TAUTZ, D., RICHARDS, S., WEINSTOCK, G.M., GIBBS, R.A., WERREN, J.H., TETTELIN, H. & LERCHER, M.J. 2006: Phylogenomic analysis reveals bees and wasps (Hymenoptera) at the base of the radiation of holometabolous insects. – Genome Research 16: 1334-1338.

SCHILDKNECHT, H. & KOOB, K. 1970: Plant bioregulators in the metathoracic glands of myrmicine ants. – Angewandte Chemie International Edition 9: 173.

SCHILDKNECHT, H. & KOOB, K. 1971: Myrmicacin, the first insect herbicide. – Angewandte Chemie International Edition 10: 124-125.

SCHLÜNS, H. & CROZIER, R.H. 2007: Relish regulates expression of antimicrobial peptide genes in the honeybee, *Apis mellifera*, shown by RNA interference. – Insect Molecular Biology 16: 753-759.

SCHLÜNS, H. CROZIER, Y.C. & CROZIER, R.H. 2008: Sequence evolution of immune genes in bulldog ants (Myrmeciinae). – Proceedings of the 4th European meeting of the International Union for the Study of Social Insects, La-Roche-en-Ardenne, Belgium, p. 65.

SCHMID-HEMPEL, P. 1994: Infection and colony variability in social insects. – Philosophical Transactions of the Royal Society B: Biological Sciences 346: 313-321.

SCHMID-HEMPEL, P. 1998: Parasites in social insects. – Princeton University Press, Princeton, NJ, 409 pp.

SCHMID-HEMPEL, P. 2005: Evolutionary ecology of insect immune defenses. – Annual Review of Entomology 50: 529-551.

SCHMID-HEMPEL, P. & CROZIER, R.H. 1999: Polyandry versus polygyny versus parasites. – Philosophical Transactions of the Royal Society B: Biological Sciences 354: 507-515.

SCHMID-HEMPEL, P. & EBERT, D. 2003: On the evolutionary ecology of specific immune defence. – Trends in Ecology & Evolution 18: 27-32.

SCHULENBURG, H., KURTZ, J., MORET, Y. & SIVA-JOTHY, M. 2009: Introduction. Ecological immunology. – Philosophical Transactions of the Royal Society B: Biological Sciences 364: 3-14.

SHUETRIM, A.J. 2007: Molecular evolution of the immune related gene transferrin in *Polyrhachis* ants and distantly related insect taxa. – PhD thesis, James Cook University, Townsville, Australia, 118 pp.

SIVA-JOTHY, M., MORET, Y. & ROLFF, J. 2005: Insect immunity: an evolutionary ecology perspective. – Advances in Insect Physiology 32: 1-48.

SORVARI, J., RANTALA, L.M., RANTALA, M.J., HAKKARAINEN, H. & EEVA, T. 2007: Heavy metal pollution disturbs immune response in wild ant populations. – Environmental Pollution 145: 324-328.

STOLL, S., GADAU, J., GROSS, R. & FELDHAAR, H. 2007: Bacterial microbiota associated with ants of the genus *Tetraponera*. – Biological Journal of the Linnean Society 90: 399-412.

STÖVEN, S., ANDO, I., KADALAYIL, L., ENGSTRÖM, Y. & HULTMARK, D. 2000: Activation of the *Drosophila* NF-κB factor Relish by rapid endoproteolytic cleavage. – EMBO Reports 1: 347-352.

STREET, M.D., DONOVAN, G.R. & BALDO, B.A. 1996: Molecular cloning and characterization of the major allergen Myr p II from the venom of the jumper ant *Myrmecia pilosula*: Myr p I and Myr p II share a common protein leader sequence. – Biochimica et Biophysica Acta 1305: 87-97.

TAGUCHI, S., BULET, P. & HOFFMANN, J.A. 1998: A novel insect defensin from the ant *Formica rufa*. – Biochimie 80: 343-346.

TIAN, H.S., VINSON, S.B. & COATES, C.J. 2004: Differential gene expression between alate and dealate queens in the red imported fire ant, *Solenopsis invicta* BUREN (Hymenoptera: Formicidae). – Insect Biochemistry and Molecular Biology 34: 937-949.

TZOU, P., DE GREGORIO, E. & LEMAITRE, B. 2002: How *Drosophila* combats microbial infection: a model to study innate immunity and host-pathogen interactions. – Current Opinion in Microbiology 5: 102-110.

TZOU, P., OHRESSER, S., FERRANDON, D., CAPOVILLA, M., REICHHART, J.M., LEMAITRE, B., HOFFMANN, J.A. & IMLER, J.L. 2000: Tissue-specific inducible expression of antimicrobial peptide genes in *Drosophila* surface epithelia. – Immunity 13: 737-748.

VAINIO, L., HAKKARAINEN, H., RANTALA, M.J. & SORVARI, J. 2004: Individual variation in immune function in the ant *Formica exsecta*; effects of the nest, body size and sex. – Evolutionary Ecology 18: 75-84.

VALLES, S.M., OI, D.H., BRIANO, J.A. & WILLIAMS, D.F. 2004a: Simultaneous detection of *Vairimorpha invictae* (Microsporidia: Burenellidae) and *Thelohania solenopsae* (Microsporidia: Thelohaniidae) in fire ants by PCR. – Florida Entomologist 87: 85-87.

VALLES, S.M. & PEREIRA, R.M. 2005: *Solenopsis invicta* transferrin: cDNA cloning, gene architecture, and up-regulation in response to *Beauveria bassiana* infection. – Gene 358: 60-66.

VALLES, S.M. & STRONG, C.A. 2005: *Solenopsis invicta* virus-1A (SINV-1A): distinct species or genotype of SINV-1? – Journal of Invertebrate Pathology 88: 232-237.

VALLES, S.M., STRONG, C.A., DANG, P.M., HUNTER, W.B., PEREIRA, R.M., OI, D.H., SHAPIRO, A.M. & WILLIAMS, D.F. 2004b: A picorna-like virus from the red imported fire ant, *Solenopsis invicta*: initial discovery, genome sequence, and characterization. – Virology 328: 151-157.

VALLES, S.M., STRONG, C.A. & HASHIMOTO, Y. 2007a: A new positive-strand RNA virus with unique genome characteristics from the red imported fire ant, *Solenopsis invicta*. – Virology 365: 457-463.

VALLES, S.M., STRONG, C.A., HUNTER, W.B., DANG, P.M., PEREIRA, R.M., OI, D.H. & WILLIAMS, D.F. 2008: Expressed sequence tags from the red imported fire ant, *Solenopsis invicta*: annotation and utilization for discovery of viruses. – Journal of Invertebrate Pathology 99: 74-81.

VALLES, S.M., STRONG, C.A., OI, D.H., PORTER, S.D., PEREIRA, R.M., MEER, R.K.V., HASHIMOTO, Y., HOOPER- BÙI, L.M., SANCHEZ-ARROYO, H., DAVIS, T., KARPAKAKUNJARAM, V., VAIL, K.M., GRAHAM, L.C., BRIANO, J.A., CALCATERRA, L.A., GILBERT, L.E., WARD, R., WARD, K., OLIVER, J.B., TANIGUCHI, G. & THOMPSON, D.C. 2007b: Phenology, distribution, and host specificity of *Solenopsis invicta* virus-1. – Journal of Invertebrate Pathology 96: 18-27.

VAN BORM, S., BUSCHINGER, A., BOOMSMA, J. & BILLEN, J. 2002: *Tetraponera* ants have gut symbionts related to nitrogen-fixing root-nodule bacteria. – Proceedings of the Royal Society B: Biological Sciences 269: 2023-2027.

VAN BORM, S., WENSELEERS, T., BILLEN, J. & BOOMSMA, J.J. 2001: *Wolbachia* in leafcutter ants: a widespread symbiont that may induce male killing or incompatible matings. – Journal of Evolutionary Biology 14: 805-814.

VEAL, D.A., TRIMBLE, J.E. & BEATTIE, A.J. 1992: Antimicrobial properties of secretions from the metapleural glands of *Myrmecia gulosa* (the Australian bull ant). – Journal of Applied Bacteriology 72: 188-194.

VIEIRA-NETO, E.H.M., MUNDIM, F.M & VASCONCELOS, H.L. 2006: Hitchhiking behaviour in leaf-cutter ants: an experimental evaluation of three hypotheses. – Insectes Sociaux 53: 326-332.

VILJAKAINEN, L. 2008: Evolutionary genetics of immunity and infection in social insects. – PhD thesis, University of Oulu, Finland, 50 pp.

VILJAKAINEN, L. & PAMILO, P. 2005: Identification and molecular characterization of defensin gene from the ant *Formica aquilonia*. – Insect Molecular Biology 14: 335-338.

VILJAKAINEN, L. & PAMILO, P. in press: Selection on an antimicrobial peptide defensin in ants. – Journal of Molecular Evolution, DOI 10.1007/s00239-008-9173-6

WALLER, R.F., JABBOUR, C., CHAN, N.C., CELIK, N., LIKIC, V.A., MULHERN, T.D. & LITHGOW, T. 2009: Evidence of a reduced and modified mitochondrial protein import apparatus in microsporidian mitosomes. – Eukaryotic Cell 8: 19-26.

WANG, J., JEMIELITY, S., UVA, P., WURM, Y., GRAEFF, J. & KELLER, L. 2007: An annotated cDNA library and microarray for large-scale gene-expression studies in the ant *Solenopsis invicta*. – Genome Biology 8: R9.

WANG, J., ROSS, K. & KELLER, L. 2008: Genome-wide expression patterns and the genetic architecture of a fundamental social trait. – PLoS Genetics 4: e1000127.

WATSON, F.L., PUTTMANN-HOLGADO, R., THOMAS, F., LAMAR, D.L., HUGHES, M., KONDO, M., REBEL, V.I. & SCHMUCKER, D. 2005: Extensive diversity of Ig-superfamily proteins in the immune system of insects. – Science 309: 1874-1878.

WENSELEERS, T., ITO, F., VAN BORM, S., HUYBRECHTS, R., VOLCKAERT, F. & BILLEN, J. 1998: Widespread occurrence of the micro-organism *Wolbachia* in ants. – Proceedings of the Royal Society B: Biological Sciences 265: 1447-1452.

WENSELEERS, T., SUNDSTRÖM, L. & BILLEN, J. 2002: Deleterious *Wolbachia* in the ant *Formica truncorum*. – Proceedings of the Royal Society B: Biological Sciences 269: 623-629.

WHITTINGTON, C., PAPENFUSS, A., BANSAL, P., TORRES, A.M., WONG, E., DEAKIN, J., GRAVES, T., ALSOP, A., SCHATZKAMER, K., KREMITZKI, C., PONTING, C., TEMPLE-SMITH, P., WARREN, W., KUCHEL, P.W. & BELOV, K. 2008: Defensins and the convergent evolution of platypus and reptile venom genes. – Genome Research 18: 986-994.

WILSON-RICH, N., DRES, S.T. & STARKS, P. 2008: The ontogeny of immunity: development of innate immune strength in the honey bee (*Apis mellifera*). – Journal of Insect Physiology 54: 1392-1399.

WILSON-RICH, N., SPIVAK, M., FEFFERMAN, N. & STARKS, P. 2009: Genetic, individual, and group facilitation of disease resistance in insect societies. – Annual Review of Entomology 54: 405-423.

WURM, Y., UVA, P., RICCI, F., WANG, J., JEMIELITY, S., ISELI, C., FALQUET, L. & KELLER, L. 2009: Fourmidable: a database for ant genomics. – BMC Genomics 10: 5.

YUE, C., SCHRÖDER, M., BIENEFELD, K. & GENERSCH, E. 2006: Detection of viral sequences in semen of honeybees (*Apis mellifera*): evidence for vertical transmission of viruses through drones. – Journal of Invertebrate Pathology 92: 105-108.

ZETTLER, J.A., MCINNIS, T.M., ALLEN, C.R. & SPIRA, T.P. 2002: Biodiversity of fungi in red imported fire ant (Hymenoptera: Formicidae) mounds. – Annals of the Entomological Society of America 95: 487-491.

ZHANG, M., POULSEN, M. & CURRIE, C. 2007: Symbiont recognition of mutualistic bacteria by *Acromyrmex* leaf-cutting ants. – The ISME Journal 1: 313-320.

ZOU, Z., EVANS, J., LU, Z., ZHAO, P., WILLIAMS, M., SUMATHIPALA, N., HETRU, C., HULTMARK, D. & JIANG, H. 2007: Comparative genomic analysis of the *Tribolium* immune system. – Genome Biology 8: R177.