REVIEW ARTICLE

Ant Interactions with Soil Organisms and Associated Semiochemicals

Robert Vander Meer

Abstract This review focuses on the semiochemical interactions between ants and their soil environment. Ants occupy virtually every ecological niche and have evolved mechanisms to not just cope with, but also manipulate soil organisms. The metapleural gland, specific to ants was thought to be the major source of semiochemical antimicrobial compounds targeting general or specific deleterious microbes. The extremely diverse variety of semiochemicals and their sources with antimicrobial activity or potential activity is highlighted. The leaf-cutting ants and fire ant provide the most researched species, in part because they cause significant economic damage. The leaf-cutting ant is particularly interesting because researchers have uncovered unexpected interactions between leaf-cutting ant fungal farm, parasitic fungi, bacteria, yeasts, and ant defensive semiochemicals. These complex relationships highlight the multidimensional aspects of ants and the soil environment in which they live.

Keywords Formicidae · Microbes · Fungi · Bacteria · Yeast · Parasite · Semiochemical · Defense

Introduction

Social insects are characterized by three factors: 1. Individuals of a species cooperate in taking care of immature forms; 2. Reproductive division of labor occurs where non-reproductive individuals work for fecund individuals;

R. Vander Meer (✉)
ARS, USDA,
1600 SW 23rd Drive,
Gainesville, FL 32608, USA
e-mail: bob.vandermeer@ars.usda.gov

and 3. There is an overlap of generations such that offspring assist parents. Insect species exhibiting all of these traits often are referred to as eusocial. Only some highly evolved wasps and bees, but all ants and termites are eusocial. Termites are restricted to habitats with cellulose resources, but ants, the subject of this review, have evolved to take advantage of a wide variety of resources and inhabit virtually every terrestrial/arboreal ecological niche–worldwide. There are over 14,000 extant described ant species (Hymenoptera: Formicidae) in 21 extant and 5 extinct sub-families and 298 genera (see Fig. 1). There are an estimated 8,000 species yet to be described (Bolton 2012). The number of scientific citations from the FORMIS (51,000 references) database (Wojcik and Porter 2012) associated with extant and extinct subfamilies is highly correlated with the number of species in each subfamily (Fig. 1). However, certain genera (and species) within the three major subfamilies dominate, e.g., about 75 % of the Myrmicinae citations are due to 4 of 141 genera: *Solenopsis*, *Atta*, *Acromyrmex*, and *Monomorium*; about 75 % of subfamily Formicinae citations are due to 2 of 49 genera: *Formica* and *Camponotus*; and in subfamily Dolichoderinae, about 64 % of the citations are due to 2 of 6 genera: *Linepithema* and *Tapinoma*.

Ants do not fly except when winged female and male sexuals have mating flights. Thus, they are primarily terrestrial although some species occupy arboreal habitats. Terrestrial ant colonies come in all sizes from tens of workers to millions; from a single queen to multiple queens; form a variety of nests types from leaf litter to deep intricately excavated earthen nests that are works of art to the human eye (Tschinkel 2004, 2010). In solitary (non-social) insects, males and females have evolved a wide variety of mechanisms (including pheromones) to find each other, mate, and produce a new generation of reproductive males and females. For ants, the colony is the reproductive entity,
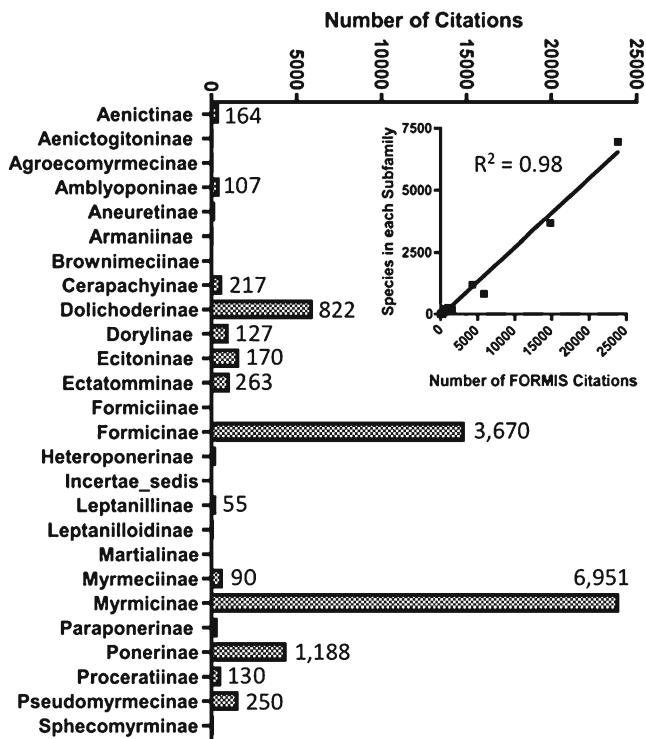
Fig. 1 Number of citations in Formis (Wojcik and Porter 2012) associated with each of the 21 extant and 5 extinct Formicidae subfamilies. The number of species and subspecies in each subfamily is shown above or near the subfamily bar (Bolton 2012). Linear regression of species in each subfamily vs. the number of citations was highly significant (inserted graph)

and is comprised of the mated queen(s) that may live for a decade or more and a non-reproductive worker force that brings in resources for the queen and developing brood, maintains nest structure, and defends the colony (queen) from outside intrusions. A colony can lose 90 % of the worker force and the colony will recover. Once mature, an ant colony usually undergoes annual ergonomic (worker production) and reproductive (sexual production) stages until the queen dies.

Soil is home to a wide variety and huge numbers of organisms that for a particular ecological niche attempts to maintain homeostasis. In this milieu of soil organisms, ants represent an important group of macro-organisms that dramatically affect the soil in which they live. For example, they are second in animal turbation only to earthworms: $5,000 \text{ gm}^{-2} \text{ y}^{-1}$ vs. $15,000 \text{ gm}^{-2} \text{ y}^{-1}$ for earthworms, but ants likely are more important because their geographic distribution is much wider than that of worms (Paton et al. 1995), e.g., in the deserts of Australia, soil turnover rates are reported to be $420 \text{ kg ha}^{-1} \text{ y}^{-1}$ (Briese 1982). On the other end of the spectrum, in Argentina, *Camponotus punctulatus* worker ants moved $2,100 \text{ kg ha}^{-1} \text{ y}^{-1}$ of soil in improved pastures (Folgarait 1998). In addition to soil turbation, the soil in the vicinity of ant mounds usually is enriched with

nutrients due to the concentration of resources needed to maintain their large populations (Dostál et al. 2005; Wagner and Jones 2006; Wagner and Fleur Nicklen 2010).

The above highlights the dynamic and intimate nature of the interaction between ants and soil, thus it is not surprising that ants have a high probability of contacting a myriad of soil microorganisms, some of which are mutualistic, symbiotic, or entomopathogenic. The latter, often highly coevolved interactions, represent a classic "arms race" where ants have evolved defenses similar to those for non-social insects, such as avoidance behavior and use of the integument as a protective shield from pathogens. Once parasite penetration occurs, an immune response may be elicited that can take many forms, e.g., biochemical, innate, and molecular (Brennan and Anderson 2004; Schmidt et al. 2008). Specifically for ants, *Formica rufa* and *Myrmecia gulosa* respond to bacterial challenge by producing antimicrobial "defensin" peptides, 40 amino acids long (Taguchi et al. 1998) and 2×16 amino acids long (Mackintosh et al. 1998), respectively. In *Lasius neglectus*, transfer of *Metarhizium anisopliae* spores from infected to naïve workers through grooming leads to up-regulation of "defensin" and prophenoloxidase (PPO) in the previously naïve workers, resulting in sub-lethal infections. *Solenopsis invicta* responds to premeditated fungal infection by up-regulating the transferrin gene, an immune response (Valles and Pereira 2005; Schlüns and Crozier 2009).

Social insects rely on a wide variety of semiochemicals for maintenance of social structure, territory, defense, and food procurement. Illustrative of their potential complexity are the 75 recently described exocrine glands (Billen 2009a), including 20 from ant legs (Billen 2009b). See Fig. 2 for a few of these exocrine glands. Some of these exocrine glands are involved in chemical defense and may directly influence organisms in their underground habitat. While many of these exocrine glands are probably involved in the lubrication of moving parts, there is ample documentation for the synthesis of a wide variety of structural types (Morgan 2008), as well as functions (Vander Meer and Alonso 1998). This review will focus on the semiochemicals
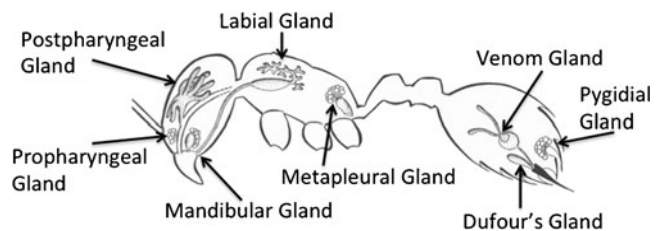

Fig. 2 Common exocrine glands of ants are shown in this schematic profile drawing of an ant. Mandibular, Dufour's, pygidial, and venom glands are known to produce pheromones. The metapleural gland produces antibiotics. Drawing by Johan Billen, University of Leuven, Belgium

and other compounds that may influence ant and soil microorganism interactions.

The Metapleural Gland

The metapleural gland (MG) is unique to ants (Hölldobler and Engel-Siegel 1985) and is located in the metathorax (Fig. 2). Secretory cells open to a holding chamber that leads to the surface through an always-open orifice, thus gland products can flow freely to the outside cuticle (Schoeters and Billen 1993). The location of the gland is conducive for worker ants to use their legs to apply the exocrine products to themselves, nestmates, and/or substrates within the nest. This gland produces compounds that have antibiotic activity (Maschwitz et al. 1970; Maschwitz 1974) that could function to protect terrestrial ant colonies from soil pathogens.

A comparative study (Hölldobler and Engel-Siegel 1985) has shown that not all ant species have functional MGs, e.g., arboreal ants, slave-making ants, and males of many species. All of these apparent anomalies have been rationalized by hypothesized lower pathogen exposure levels (arboreal species), functional usurped colony workers (slave raiders), or alternative uses where males have functional glands (Hölldobler and Engel-Siegel 1985). Other functions for the MG have been proposed, e.g., nestmate recognition (Brown 1968) and territorial marking (Jaffe and Puche 1984). Certainly with over 14,000 described ant species, multiple functions are not unexpected, especially since functional parsimony is well documented in social insects (Blum 1996; Trhlin and Rajchard 2011; Matsuura 2012, this issue). These areas are interesting, however, I restrict this review to ant relationships with soil microorganisms and ant derived antimicrobial activity starting with MGs and adding other chemistry that relates to ant/microorganism interactions. See Yek and Mueller (2011) for a comprehensive review of the metapleural gland in ants.

Metapleural gland secretions from all ant species investigated (12) have anti-bacterial activity (Yek and Mueller 2011), but in limited screening, *Pseudomonas* and *Bacillus* bacteria species were resistant to the secretions (Iizuka et al. 1979; Mackintosh et al. 1995). Metapleural glands from three Attine leaf-cutting ants (2 × *Atta* and 1 × *Acromyrmex*) and one bulldog ant (*Myrmecia*) species have been evaluated against 18 fungi species. Five fungi were resistant to MG secretions, primarily from *Atta sextens* (4 of 5). The five resistant fungi were entomopathogenic (2) or phytopathogenic (3) (Yek and Mueller 2011). It is clear that MG secretions have antibiotic activity, but the activity is not uniform against potential pathogens (Yek and Mueller 2011). The chemistry associated with MG products generally has acidic characteristics, expressed in the form of carboxylic acid or phenol moieties. Examples of these along

with the ant species they are associated with are shown (Table 1, Fig. 3).

The remainder of this review is ordered based on ant subfamilies.

Subfamily Myrmicinae

The Attines

All Attines are fungus (Basidiomycota: Agaricales) growing species. The lower and higher Attines differ in that the lower Attine fungus gardens (families Pterulaceae/Lepiotaceae) can survive independently from the ants, whereas the higher Attines have evolved a sophisticated obligate mutualism with the family Lepiotaceae fungi that supply the ants with a required food resource. The ants culture the fungus by providing necessary physical conditions and plant substrates needed for its growth (Villesen et al. 2004; Schultz and Brady 2008). In this review, higher Attine species are designated Attine, and the lower Attines are indicated as necessary. The Attine species are of special interest for this review. The group is composed of four major genera (Bolton 2012): *Trachymyrmex* (47 spp. and 1 subspecies), *Sericomyrmex* (19 spp. and 3 subspecies), *Atta* (15 spp.), and *Acromyrmex* (30 spp.). *Atta* and *Acromyrmex* species are major leaf harvesters in the neotropics and cause significant economic problems (Cherrett 1986; Fowler et al. 1986), which have resulted in much basic and applied research. The obligate mutualism has been a driving force in MG investigations, because not only do the Attines need a way to combat microorganisms that negatively affect their workers and other life stages, but their obligate fungi also are susceptible to parasitism, usurpation, and/or mortality from competing organisms. Therefore, it is possible that antibiotic compounds produced in the MG could serve to protect both colony members and their co-evolved highly specific fungal garden. The two functions have different requirements: general antibiotic activity works for protection of colony members; however, protection of the specific fungal garden requires a targeted rather than a generalist antibiotic.

Forty-three compounds have been identified from Attine MGs, primarily from *Acromyrmex octospinosus* (Ortius-Lechner et al. 2000, 2003) and to a lesser extent from *Ac. subterraneus*, *Atta sextens*, and *A. cephalotes* (Schildknecht 1976; Beattie et al. 1984; do Nascimento et al. 1996). Compounds A, B, and C (Table 1, Fig. 3) are often most abundant. Component A, 3-hydroxyl decanoic acid (myrmicacin) has antimicrobial activity (Iizuka et al. 1979), but the compound is not specific to Attines (see Table 1), and no activity against antagonists of the obligate fungal garden has been demonstrated. Indoleacetic acid (compound B, Table 1, Fig. 3) is interesting in that it is a well-known plant growth

Table 1 Identified components from the metapleural glands of ant species are indicated by an X. Column 2, Structural Type, corresponds to the letter under each structure in Fig. 3. For *C. difformis*: x = Attygalle et al. 1989; X = Jones et al. 2005. Compounds <1 % not included

Metapleural gland components	Structure type	*Atta sextens*	*Acromyrmex octospinosus*	*Ac. subterraneus*	*Myrmica rubra*	*Messor barbarus*	*Crematogaster defformis*	*Crematogaster inflata*	*Solenopsis invicta*
3-hydroxyldecanoic acid (myrmicacin)	A	X	X	X	X	X			
3-hydroxyloctanoic acid	A-1	X							
3-hydroxylhexanoic acid	A-2	X							
Indoleacetic acid	B	X	X	X	X				
Phenylacetic acid	C	X			X	X			
4-oxo-octanoic acid	D		X						
4-oxo-decanoic acid	D-1		X						
γ-octalactone	E		X						
3,4-diH-8-hydroxyisocoumarin	F								
Mellien	G						X		
6-propylsalicylic acid	H						xX		
6-pentylsalicylic acid	H-1						X		
6-heptylsalicylic acid	H-2						X		
6-pentyl resorcylic acid	I						X	X	
6-heptyl resorcylic acid	I-1						X	X	
6-nonyl resorcylic acid	I-2						X		
5-propyl resorcinol	J						xX		
5-pentyl resocinol	J-1						xX	X	
5-heptyl resorcinol	J-2							X	
5-nonyl resorcinol	J-3								
3-propyl phenol	K						xX		
3-pentyl phenol	K-1						xX		
3-heptyl phenol	K-2						x		
Carboxylic acid	L		X						X

Fig. 3 Representative structures for compounds isolated from the metapleural gland of ants. Compound letters match the Structure Type column in Table 1. See Table 1 for the structural name and the species from which the compounds were isolated
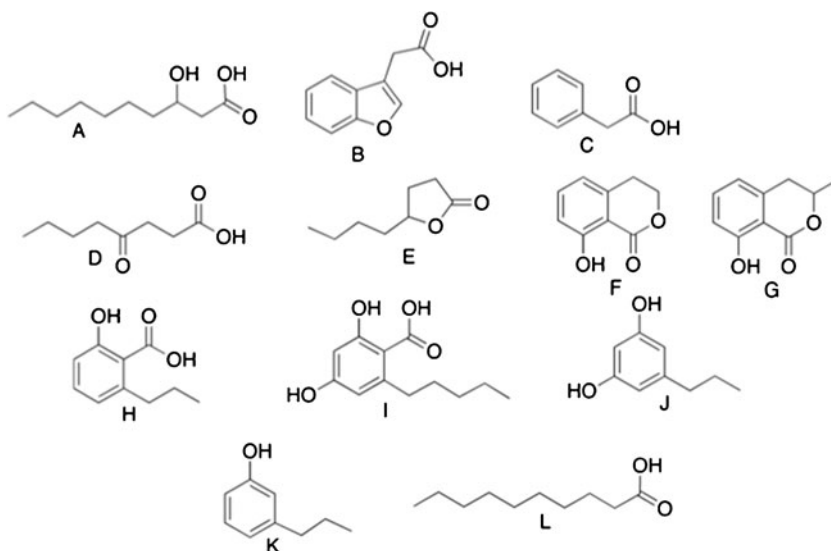
hormone (Wrightman 1962), and is a major MG component in *Ac. octospinosus*, but a minor component in *A. sextans* and *A. cephalotes*, except for soldiers (18 %) of the latter species (do Nascimento et al. 1996). Phenyl acetic acid (Component C) also is a plant auxin (Wightman and Lighty 1982) and a major component in the MG of *A. sextans* and *A. cephalotes*, but it is absent from *Acromyrmex* species (do Nascimento et al. 1996). These three compounds individually and in combination have been evaluated for their effects on the growth of the fungal garden of *A. sextans*. Both growth enhancement and inhibition were observed depending on concentrations, suggesting that workers may be able to stimulate garden growth or increase usage of MG chemistry to cope with a negative invader, even at the expense of fungal garden growth (Schildknecht et al. 1973). After contacting the MG, *Atta* and *Acromyrmex* spp. groom themselves, each other, and their fungal gardens, distributing the MG products. In addition, when *Atta* workers are challenged with foreign fungal conidia, they respond by increasing their grooming rate, and thus MG product distribution (Fernández-Marín et al. 2006). Interestingly, ants in general have evolved an excellent particulate filtration mechanism for ingested liquid food or from the grooming process (Eisner and Happ 1962; Glancey et al. 1981). Particles are compressed by the mouthparts into the infrabuccal pocket where a pellet is eventually formed and ejected. *Atta* workers with their MGs sealed produce fewer infrabuccal pellets than normal workers. Conidia in pellets from workers with sealed MGs germinated with greater frequency than those from normal workers. This demonstrated the effect of MG products, and that workers actively respond to microorganism challenge with increased use of MG secretions (Fernández-Marín et al. 2006). Another fungal challenge experiment has shown that small *Ac. echinatior* workers have more developed MGs than large workers

and are better capable of resisting size equivalent spore doses (Poulsen et al. 2006).

Attine MG secretions likely play a role in protecting workers from microbial attack, in maintaining fewer microorganisms in the nest soil, and even in sustaining the integrity of the fungal garden; however, they must act in concert with other mechanisms and probably other exocrine gland products. For example, Attine mandibular glands (Fig. 2) contain citral, 4-methyl-3-heptanol,, 2-heptanone, 3-octanone, 4-methyl-2-heptanone, β-citronellol, and geraniol (Blum et al. 1968). All of these compounds except 4-methyl-2-heptanone and β-citronellol exhibit anti-fungal activity as great as the MG components: phenylacetic acid, indoleacetic acid, hexanoic and octanoic acids (Mendonça et al. 2009). Recently, 3-octanol, 3-octanone, 3-heptanone, 4-methyl-3-heptanone, perillene, and α,α-acariolide were isolated from one or more of ten Attine *Trachymyrmex* and one *Sericomyrmex* head extracts (Adams et al. 2012), likely mandibular gland products. Abdominal extracts yielded *E*, *E*-α- and *Z*-β-farnesene along with alkanes (Adams et al. 2012). The trail pheromone of some *Atta* and *Acromyrmex* species has been determined to be from the poison gland, and a common active component was methyl 4-methylpyrrole-2-carboxylate (Tumlinson et al. 1972; Evershed and Morgan 1983). In addition, some *Atta* species also produced 3-ethyl-2,5-dimethylpyrazine (see Fig. 4) in their poison glands (Evershed and Morgan 1983), thus illustrating the diversity of exocrine gland compounds available to influence Attine surroundings. In an Atta species, 29 unique worker tasks have been identified including: caring for hyphae, licking substrate, implanting hyphae, transporting hyphae, and degrading vegetation, all activities that could involve both mandibular gland, MG, and or other glandular products (Wilson 1980). It is clear that we are far from understanding the synergies and interactions of multiple glandular products
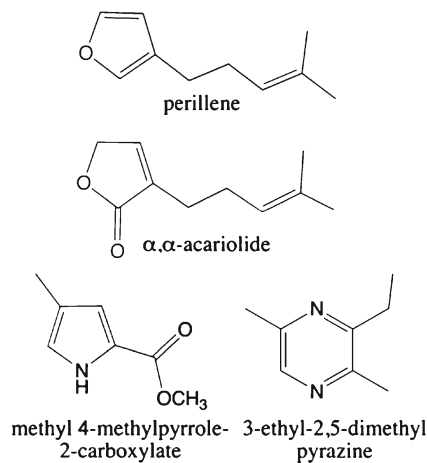
Fig. 4 Perillene and α,α-acariolide were isolated from Attine head (Mandibular glands?) extracts (Adams et al. 2012). The pyrrole and pyrazine are trail pheromone components isolated from Attine poison glands (Evershed and Morgan 1983)

in the complicated world of the Attines, and other ant species.

The last publication that dealt with new chemistry and components of Attine MGs was over 10 years ago (Ortius-Lechner et al. 2000). Lack of a clear association between MG components and Attine fungal gardens, along with the fascinating discovery that the Attines use antibiotic producing bacteria to control parasites of their fungal garden (Currie et al. 1999), shifted and multiplied research efforts to the latter subject.

The fungal gardens of Attine species are attacked by highly co-evolved micro-fungi parasites (Escovopsis spp.) that if unchecked can quickly destroy the fungal garden. The parasitic fungi is specifically attracted to chemicals (unknown structures) released by its host fungal garden, as evidenced by preferential growth of the Escovopsis parasite toward their fungal host rather than to non-host fungi (Gerardo et al. 2006). Fungi are known to produce volatiles that attract insects (Pierce et al. 1991; Schiestl et al. 2006) and/or have antimicrobial activity, e.g., penicillin. In addition, the Escovopsis strain isolated from host A will move quicker toward host A cultivar than toward other host cultivars, perpetuating Escovopsis strain and host fungal garden fidelity and the mutualism between the two fungi (Gerardo et al. 2006).

A bacterium has been isolated from fungus garden material that inhibits germination of the conidia of a Escovopsis sp. parasite and several entomopathogenic fungi, but not the mutualistic fungal garden of the ant (Santos et al. 2004). This is the magic bullet that gets around the apparent non-specific antibiotic activity of MG products. Filamentous Actinomycete bacteria from Pseudonocardia (Currie et al. 1999), Streptomyces (Haeder et al. 2009), and Burkholderia (Santos et al. 2004) genera have been isolated from fungal gardens. Remarkably, Attine workers have evolved

specialized cuticle structures (crypts) and associated glands that hold these special bacteria–essential in the Attine defense against Escovopsis spp. parasites (Currie et al. 2006; Haeder et al. 2009). An antifungal macrocyclic compound has been visualized on the cuticle of workers by using a MALDI imaging technique, thus demonstrating that these symbiotic bacteria can protect workers from microbes, while the workers actively or passively distribute the bacteria within their nest and fungal garden (Schoenian et al. 2011). The special cuticular structures support a co-evolution developmental process; however, the actinomycete symbionts may be the result of frequent environmental acquisition rather than co-evolution (Mueller et al. 2008; Barke et al. 2011).

Importantly, several antifungal compounds have been isolated and identified from Attine symbiotic bacteria, e.g., Candicidin-D (Streptomyces spp.), Antimycin (Streptomyces spp.). Structures of these compounds are shown in Fig. 5. Valinomycins, antimycins, and actinomycins have been identified from Streptomyces isolates from three Acromyrmex spp. via LC-MS (Schoenian et al. 2011). Each of these compound groups shows varying activities against fungi, including the Escovopsis parasite of the Attine obligate mutualist fungus, as well as bacterial Streptomyces and Pseudonocardia symbionts. Antifungal compound combinations exhibit synergistic effects against the fungal garden parasite, Escovopsis (Schoenian et al. 2011). Similarly, candicidin and antimycin fungicides are made by a Streptomyces bacterial symbiont of Ac. octospinosis. Mutants of this Streptomyces that were unable to produce candicidins or antimycins still maintained activity against the Escovopsis parasite, indicating that additional antimicrobial compounds are being produced (Seipke et al. 2011).

To add further complexity to Attine symbiotic relationships (at this point–ants: bacteria: fungal garden: parasitic fungus) was the discovery of a black yeast (Ascomycota; Phialophora) growing on the worker cuticle of a lower Attine species (Apterostigma), which was localized on the cuticle with the symbiotic bacteria (Little and Currie 2007). The black yeast could not be isolated (cultured) from other Attine genera; however, the yeast was detected from Attines by using molecular techniques. A related molecular phylogeny study showed that the black yeasts from the Attines formed a derived monophyletic group, thus supporting the hypothesis that the yeasts are symbionts of the Attines (Little and Currie 2007). This represents a fifth symbiotic relationship. These same authors further demonstrated that the black yeast received nutrients from the mutualistic bacterial biomass on the ants (Little and Currie 2008). Yeast manipulation experiments have demonstrated that yeast infected colonies have reduced capacity to produce antifungal agents (via the bacteria),

Fig. 5 Five anti-fungal agents isolated from leaf-cutting ant Actinomycete symbiotic bacteria. spp., Dentigerumycin (*Pseudonocardia* spp.), Valinomycin (*Streptomyces* spp.) and a Nystatin-like compound (Haeder et al. 2009; Oh et al. 2009; Barke et al. 2010; Schoenian et al. 2011)
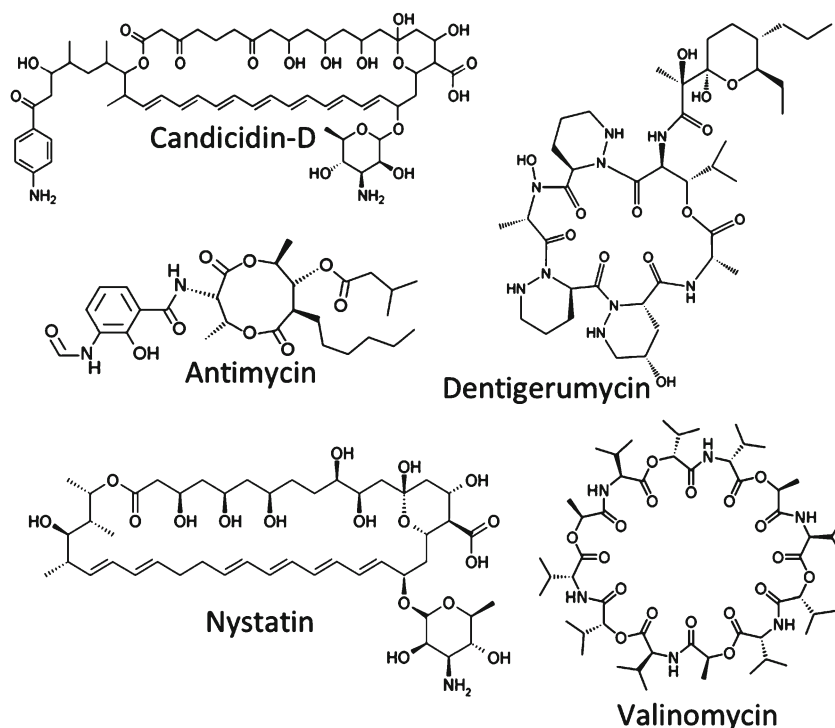
with a consequent reduction in their ability to defend against the parasitic fungi (*Escovopus*), which in turn lowers fungal garden/colony health (Little and Currie 2008).

There are many more chapters to go in this intriguing and complicated story of Attine survival in the soil environment. The real challenge is understanding the dynamics of what has become a powerfully linked community of symbionts, mutualists, and parasites to create a holistic picture that includes the interactions of Attine worker behaviors, exocrine gland products, suites of symbiotic bacteria, obligate host fungal gardens, specific fungal garden parasites, and black yeasts. While experimentally it is expeditious to take a reductionist approach and isolate parts of complex systems, e.g., ants and fungal cultivar, we all too often stop there and neglect to attempt to put the parts together in a holistic approach. Attine research forces this issue, as exemplified by the informative web diagram constructed by Little and Currie (2008) that helps visualize the complex sets of interactions associated with Attine ants, bacteria, yeast, fungal cultivar, and cultivar parasite.

Solenopsis–Fire Ants

Solenopsis currently has 183 described species (Bolton 2012). Most of these are "thief ants" that have small monomorphic workers living in small colonies, generally near nests of other ant species where they manage to steal that colony's food and brood (Thompson 1989). The *Solenopsis saevissima* species group is comprised of 20 new world species that have very different characteristics from the thief ants (Pitts et al. 2005). Members of this group generally are called fire ants, due to the burning sensation associated with their stings. *Solenopsis richteri* and *S. invicta* are the most notorious of the fire ants, because they were accidentally introduced into the United States in the 1910s and 1930s, respectively, into Mobile, Alabama, where *S. invicta* quickly outcompeted *S. richteri* and adapted to the lack of natural enemies and non-competitive native ant species (Wilson 1958; Lofgren 1986). The ant spread through natural mating flight activities, but most aggressively through human movement of infested nursery stock throughout the southern United States (Lofgren 1986). Imported fire ants now infest more than 130 million ha in 13 southern tier states and Puerto Rico and are spreading northward (APHIS 2011). In the last decade, *S. invicta* has changed from an invasive pest ant in the United States to a global problem, with infestations occurring in Australia (Henshaw et al. 2005), Taiwan (Chen et al. 2006), mainland China (Zeng et al. 2005; Zhang et al. 2007), Mexico (Sánchez-Peña et al. 2005), and many Caribbean Island countries (Davis et al. 2001).

Fire ants are a central foraging species that has a nest tumulus made from excavated below ground soil. Nests may extend 2–3 m in depth. Mature colonies can have up to 250,000 workers and a density of 120 mounds/ha (monogyne social form). Underground foraging tunnels radiate from the central nest with exit holes to the surface (Markin et al. 1975). However, resource gathering occurs both on the surface and underground (Tennant and Porter 1991).

Fire ants use a variety of exocrine gland (Fig. 2) products to maintain social structure and defend territory, e.g., Dufour's gland regulates foraging (Vander Meer 1986b), queen poison gland induces retinue activity (Vander Meer et al. 1980), mandibular gland products alarm workers (Vander Meer et al. 2010), and worker poison gland products are used in defense and food procurement (Obin and Vander Meer 1985). The subterranean habitat of fire ant colonies puts them at risk from soil microbes and nematodes.

Metapleural Gland Based on previous examples, fire ant metapleural glands would be expected to have antimicrobial activity. The metapleural gland chemistry of *Solenopsis invicta* and *S. geminata* has been reported as a series of four fatty acids: palmitic, linoleic, oleic, and stearic acids, along with minor amounts of hydrocarbons, which are attributed to substances (cuticular hydrocarbons) accumulating in the MG reservoir from the cuticle (Cabrera et al. 2004). Although neither MG extracts nor their individual components have been evaluated for antibiotic activity at physiological concentrations, some fatty acids do negatively impact bacteria (Kabara et al. 1972). When minimum inhibitory concentrations from Kabara et al. (1972) are compared with the amount of each of the four carboxylic acids found by Cabrera et al. (2004), it is clear that one (*S. geminata*) or two of the acids (*S. invicta*) are at a concentration high enough to inhibit bacterial growth (Table 2), especially since the MG reservoir volume is likely much less than 1 µl. The hypertrophied MG reservoir volume for *Crematogaster inflata* is about 0.1 µl (data in Billen et al. 2011).

Poison Gland Chemistry Some 54 years ago, the chemical and antibiotic properties of fire ant venom were reported (Blum et al. 1958), and a venom alkaloid was later unambiguously identified as *trans*-2-methyl-6-n-undecylpiperidine (MacConnell et al. 1970). Since these first reports the chemistry of the major alkaloid components (Fig. 6) have been well defined as series of *cis*- and/or *trans*-2-methyl-6-alkyl or alkenyl piperidines (MacConnell et al. 1971; Brand

Table 2 Carboxylic acid Minimum Inhibitory Concentrations (MIC) for 8 bacteria species and 2 strains (Kabara et al. 1972) compared with the amount of four carboxylic acids found in *S. geminata* and *S. invicta* MGs (Cabrera et al. 2004). NI = Not Inhibitory

Carboxylic acid	MIC (ng/µl)	From *S. geminata* (ng/gland)	From *S. invicta* (ng/gland)
Palmitic	122 (6/10)[a]	47	206
Stearic	NI (0/10)	11	11
Oleic	500 (1/10)	29	207
Linoleic	12.3 (7/10)	69	184

[a] Number of inhibited bacteria/out of a total of 8 bacteria and 2 strains (Kabara et al. 1972)
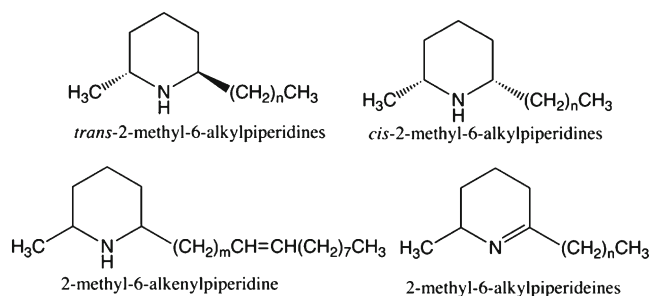

Fig. 6 Structures of common piperidine alkaloids produced by fire ants (N=10, 12, 14, 16; m=3,5,7). The *trans*- and *cis*- isomers have the configurations shown (Leclercq et al. 1994). The piperideines are generally minor compounds and are considered to be biosynthetic precursors to the piperidines (Leclercq et al. 1996)

et al. 1972) that vary with each fire ant species, such that they can be used as chemotaxonomic characters (Brand 1978; Vander Meer et al. 1985; Vander Meer 1986a). The USA native fire ant, *S. xyloni*, in addition to the *cis* and *trans* piperidines, also has a detectable amount of 2-methyl-6-n-undecyl-$\Delta^{1,2}$-piperideine (Fig. 6), which was suggested previously as a likely precursor to the piperidines or as an intermediate in the inter-conversion of the *cis* and *trans* ring isomers (Brand et al. 1972). The absolute configuration of the alkaloids was determined as shown in Fig. 6 (Leclercq et al. 1994). Biosynthetic studies have demonstrated that the piperidine alkaloids are biosynthesized via a polyacetate route and that, indeed, 2-methyl-6-n-undecyl-$\Delta^{1,6}$-piperideine and/or 2-methyl-6-n-undecyl-$\Delta^{1,2}$-piperideine are intermediates to the piperidines (Leclercq et al. 1996). With current separation and detection systems, the piperideines can be observed in extracts of *Solenopsis* poison sacs, but are present in ≤1 % of the total alkaloid (personal observation). Nevertheless, publications on piperideines that have appeared lately have provided support for the Leclercq et al. (1996) biosynthetic scheme (Chen et al. 2009; Chen and Fadamiro 2009a, b).

Fire ant piperidine alkaloids have wide ranging effects on humans, from platelet and neutrophil activation (Javors et al. 1993) to lytic release of histamine from mast cells causing the characteristic sterile pustule formation at the site of the sting (Lind 1982). The venom of stinging Hymenoptera had been shown previously to contain proteins, thus fire ant venom appeared to be an outlier, even though many cases of allergic reactions and anaphylactic shock were being reported from fire ant stings (Lockey 1974; Rhoades et al. 1977). This conundrum was solved when small amounts of proteins were isolated (≈0.1 % of venom weight) and characterized (Baer et al. 1979) as similar to those found in other hymenopteran venoms, namely with phospholipase and hyaluronidase activity. Research on the fire ant allergens continues (Padavattan et al. 2008), driven by the fact that 1 % of the human population is susceptible to developing hypersensitivity to fire ant stings and about 33 % of the

population in the infested areas are stung each year, resulting in significant medical demands (Partridge et al. 2008).

Interestingly, the fire ant has evolved a variety of mechanisms for release of venom alkaloids to the environment, generally called "gaster flagging" (Obin and Vander Meer 1985). In the context of defense, the ants raise their gaster 90° to the substrate and vibrate it while slowly emitting venom from the sting. This is a well-known mechanism for generation of an aerosol (Mason et al. 1963). Small amounts of venom reach the intruder who may be repelled and a physical confrontation avoided. This sequence has been observed when another ant species has surrounded a food source and a single foraging fire ant uses the aerosol mechanism to repel the other ants from the food (Banks and Williams 1989). A similar mechanism has been reported for *Monomorium minimum* (Adams and Traniello 1981). If the aerosol does not work, fire ant workers can accurately throw 0.03 to 0.13 mm diameter droplets at an approaching enemy and, of course, as a last resort they will attempt to bite and sting an intruder (Obin and Vander Meer 1985). Most relevant for this review is the use of gaster flagging within the context of the nest and readily observed in laboratory colonies near brood, where about 1 ng of alkaloid per piece of brood has been detected (Obin and Vander Meer 1985). It is assumed that the ants are also dispersing venom alkaloids on nest soil as a prophylactic or in response to pathogen challenge, although this has not been directly demonstrated.

The antimicrobial activity of fire ant piperidine alkaloids, first described by Blum et al. (1958) was later shown to have greater inhibitory activity against gram-positive than against gram-negative bacteria (Jouvenaz et al. 1972). Fungicide, termiticide, and seed germination inhibition have been added to the growing repertoire of piperidine alkaloid activities (Escoubas and Blum 1990). Germination of the entomopathogenic fungi, *Beauveria bassiana*, *Metarhizium anisopliae*, and *Paecilomyces fumosoroseus* are inhibited at physiologically relevant alkaloid concentrations (Storey et al. 1991). In the field, biodiversity of fungi in fire ant nest soil is less (50 %) than the surrounding soil, although fungal abundance is greater than in non-nest soil (Zettler et al. 2002). Therefore, the fire ant influences the fungal make-up of the nest soil directly, via venom alkaloids, MG carboxylic acids, and/or other undiscovered mechanisms. Another option related to what was already seen with the Attines is that the remaining fungi or bacteria in the fire ant nest soil may inhibit growth of other fungal species (Domsch et al. 1980). There is support for this: when nest soil and sterile soil with fire ants were inoculated with conidial suspensions of entomopathogenic fungus, *Beauveria bassiana*, the LC_{50} conidial concentration for nest soil was 2×10^7 greater than that for sterile soil, thus supporting the concept that fire ant nest soil organisms are producing fungicidal metabolites (Shields et al. 1981; Pereira et al. 1993). Direct evidence is needed.

Recall that MG chemistry and antimicrobial activity are linked to acidic moieties. In contrast, the fire ant piperidine alkaloids are basic and have wide ranging physiological activities that include antimicrobial activity. The fire ant MG produces acidic antibiotics, while the poison gland produces basic antibiotics. The effects of a combination of acidic and basic antibiotics with soil microbes have yet to be determined.

Solenopsis–Thief Ants

Thief ant species are a large world-wide, taxonomically difficult group previously classified in the subgenus, *Diplorhopturum* (Pacheco 2001). Workers are diminutive and live almost exclusively underground where their resources come from larvae stolen from other ant species. These ants, not unlike their larger fire ant relatives, have evolved an excellent recruitment system, as well as defensive chemistry that incapacitates defending workers of the species raided, allowing them to escape with brood (Blum et al. 1980). Across the thief ant species thus far investigated, the venom chemistry shows a diverse array of alkaloid structural types and a variety of isomers within each type. Figure 7 shows an example of each structural type: pyrrolidine (Jones et al. 1979), pyrrolizidine (Jones et al. 1980), indolizidine (Gorman et al. 1998), quinolizidine (Jones et al. 1999), decahydroquinoline (Jones et al. 1999), and pyrroline (Jones et al. 1982). These compounds have been of interest because of their interesting role in food procurement, potent defense against other ant species, possible chemotaxonomic
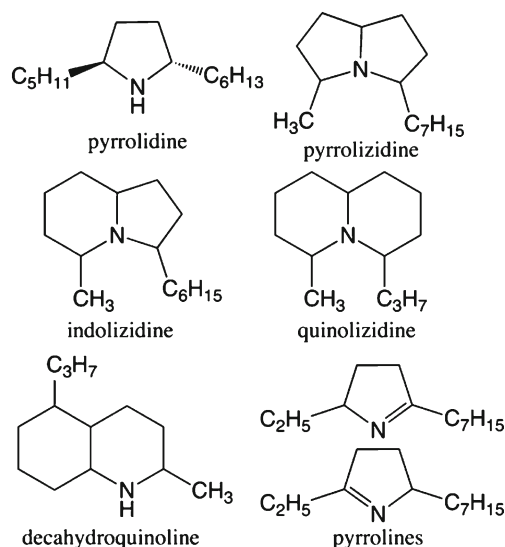

Fig. 7 *Solenopsis* spp. thief ant poison glands produce a wide variety of alkaloids as illustrated by these representative compounds: 2-hexyl-5-pentylpyrrolidine, 3-heptyl-5-methylpyrrolizidine, 3-hexyl-5-methylindolizidine, 4-methyl-6-propylquinolizidine, 2-methyl-5-propyldecahydroquinoline, and 2-heptyl-5-ethylpyrroline (see text for references)

utilization, and the high probability that new alkaloid natural products would be discovered. Insecticidal activity has been-reported for pyrrolidines, pyrrolines, pyrrolizidines, and indolizidines (Bacos et al. 1988; Escoubas and Blum 1990). The former two also inhibit seed germination (Escoubas and Blum 1990). Thief ants also have to cope with microorganisms in their soil habitat; however, their metapleural gland chemistry is unknown. The impressive alkaloid diversity, their demonstrated physiological activities, and evidence that plant-derived derivatives of the alkaloid classes shown in Fig. 7 also have antimicrobial activity, suggests a nest hygiene role for thief ant venom components.

Monomorium

The *Monomorium* genus has over 400 species and subspecies. The Pharaoh's ant, *M. pharaonis*, is the most well-known species in this genus because it is an invasive pest in many parts of the world and has been implicated in transmission of pathogenic bacteria (Hughes et al. 1989). Species in this genus have characteristics similar to thief ants, e.g., they use venom to repel other ant species away from resources (Hölldobler 1973). Investigation of *Monomorium* venom components have shown them to be similar to those alkaloid classes shown in Fig. 7 for *Solenopsis* thief ants, e.g., pyrrolidines, indolizidines, pyrrolines (Jones et al. 1982), and unsaturated pyrrolizidines from the related species *Chelaner antarcticus* (now *Monomorium*) (Jones et al. 1986). Novel dialkylpiperidenes (Jones et al. 1990) and monosubstituted pyrrolines (Jones et al. 2003) can be seen in Fig. 8. As with *Solenopsis* thief ants, *Monomorium* spp. can use these alkaloids for prey procurement and defense (Hölldobler
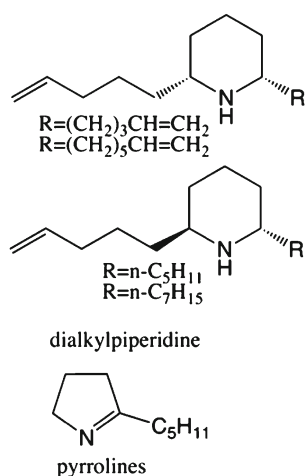

Fig. 8 *Monomorium* species produce a similar suite of compounds to those shown for *Solenopsis* thief ants in Fig. 7. In addition, they produce the specific examples of dialkyl piperidines and mono substituted pyrroline shown in this Figure (Jones et al. 1990, 2003)

1973). The defensive utility can be against other ant species or soil microbes.

Crematogaster

The genus *Crematogaster* has 771 species and subspecies. *Crematogaster difformis* and *C. inflata* are arboreal ants living in myrmecophytic epiphytes in the crowns of canopy trees (Tanaka et al. 2009) and tree cavities (Billen et al. 2011), respectively. Since they do not have the purported pressure that soil inhabiting ant species have to produce antimicrobial defenses, their MGs were expected to be non-functional and atrophied (Hölldobler and Engel-Siegel 1985). Instead, their MGs are highly developed; in fact, *C. inflata* has the largest known MGs (measured by secretory cell counts, Billen et al. 2011). Metapleural gland chemistry has been reported for *C. difformis* in two reports, one using samples collected from Malaysia (Attygalle et al. 1989) and the other from Brunei (Jones et al. 2005). The MG chemistry of each is so unique (combinations of 6-alkylsalicylic and 6-alkylresorcylic acids, 5-alkylresorcinols and 3-alkyl-phenols; see Table 1, Fig. 3) that Jones et al. (2005) suggested that they are likely different species. Comparison with a third species, *C. inflata* (Jones et al. 2005), shows a third blend of components (Table 1, Fig. 3). Metapleural gland chemistry may provide a useful chemotaxonomic character for classifying members of this large and difficult genus. Interestingly, the *C. inflata* MG secretion has been described as viscous (Buschinger and Maschwitz 1984; Ito et al. 2004) and did not inhibit growth of *E. coli*, whereas the secretion of *C. difformis* is fluid and inhibited growth of *E. coli* (Maschwitz 1974). Different functions have been ascribed to each MG secretion, predator and pathogen defense, for *C. inflata* and *C. difformis*, respectively (Buschinger and Maschwitz 1984; Ito et al. 2004). The gland chemistry of both species, though different from Attines, has the typical carboxylic and phenol acidic moieties (Table 1, Fig. 3) associated with the antibiotic role of MGs (see Yek and Mueller 2011). The classes of compounds produced, 6-alkylsalicylic acids (Gellerman et al. 1969); 5-alkylresorcinols (Ayer et al. 1983; Jin and Zjawiony 2006); and related alkylphenols (Fontenelle et al. 2011) have been shown to have antibiotic activity. These data demonstrate that MG products that have antibiotic activity also can have negative effects on predator arthropods and *vice versa*. It is well-known that epiphytes and tree cavities harbor a wide assortment of fungi and bacteria (Gönczöl and Révay 2003; Hashidoko 2005; Yuan et al. 2009), so it follows that *Crematogaster* species or other ants that nest in tree cavities or myrmecophytic epiphytes or any related habitat have a need for protection from pathogens and predators. Likewise, other MG antibiotic compounds may also function as deterrents to predators–functional

parsimony is a common feature in social insects (see Matsuura 2012, this issue). Pheromone function is usually named after the first discovered function, e.g., alarm pheromone, but this sometimes acts like blinders to a horse, retarding the discovery of additional functions.

Pogonomyrmex

The *Pogonomyrmex* genus is composed of 77 species. Ant species that come in contact with the human population– such as the fire ant and those species that change human behavior after being stung–like the "pogos" usually initiate characterization in the context of their physiological effects on people. Harvester ants (*Pogonomyrmex* spp.) have one of the most painful and long lasting stings in the ant world (3 of 4 with 4 = "traumatically painful") (Starr 1985). The components are phospholipases, lipase, hyaluronidase, phosphatase, and several esterases, histamine, and kinin-like peptides (Schmidt and Blum 1978a). A pharmacological and toxicological study of the venom of *P. badius* found it to be the most toxic known insect venom to mice (Schmidt and Blum 1978b). Unfortunately, no antimicrobial evaluations have been conducted. *Pogonomyrmex* live in large colonies underground where defense against microbes is expected to be important.

Aphaenogaster and Messor

The *Aphaenogaster* and *Messor* genera are composed of 215 and 161 species, respectively. The poison glands of several *Aphaenogaster* species contain anabaseine and/or anabasine (Fig. 9), alkaloids also found in tobacco and related to nicotine, e.g., *A. fulva* and *A. tennesseensis* (Wheeler et al. 1981); *A. rudis* (Attygalle et al. 1998); *A. subterranea* and *A. miamiana* (Leclercq et al. 2001). Recently, the poison sac
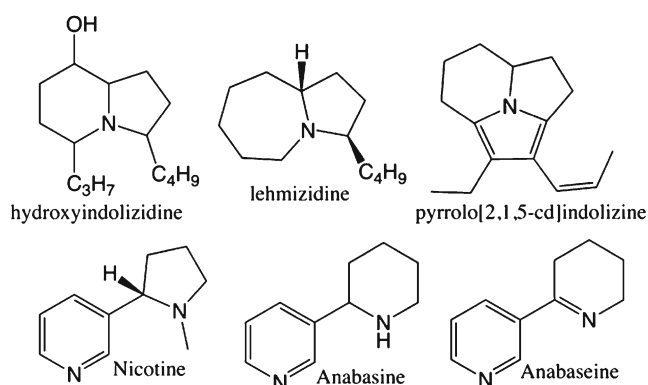
of *A. senilis*, but not *A. iberica* was found to contain anabasine and anabaseine, as well as other unidentified alkaloids (Lenoir et al. 2011). Anabaseine hydrochloride was shown to have strong antibacterial and fungicidal activity (Abdulina et al. 2002), suggesting that *Aphaenogaster* poison gland products could function in nest defense against predators, parasites, competitor ant species, and control microbes in their nest environment. Members of the *Messor* genus also produce antibiotic anabasine (Fig. 9) and several pyrazines as minor components of their poison gland (Cruz López et al. 2006).

Myrmica

The *Myrmica* genus contains 171 species and 2 subspecies. Plant auxins, indoleacetic acid (Schildknecht and Koob 1970) and phenyl acetic acid were detected from MG extracts of *Myrmica rubra* (initially named *M. laevinodis*, but since changed to *M. rubra*), as well as 3-hydroxydecanoic acid (myrmicacin)(Schildknecht 1976). In the same report, *Messor barbarus* was shown to have phenylacetic acid and myrmicacin, but not indoleacetic acid (see Table 1, Fig. 3). All three compounds inhibit fungal (*Botrytis cinerea* and *Altenarea tennis*) sporulation, with myrmicacin especially effective (Schildknecht 1976).

Myrmicaria

The *Myrmicaria* genus is comprised of 67 species. *Myrmicaria melanogaster* is phylogenetically close to *Monomorium* and *Solenopsis* thief ants, and their venom alkaloids show similarities and differences (Jones et al. 2007). They produce pyrrolidines, indolizidines (Fig. 7), and a novel hydroxylated indolizidine, a mono-substituted lehmizidine (Jones et al. 2007), and a tricyclic alkaloid, pyrrolo[2,1,5-cd]indolizines (Fig. 9), as well as dimeric and trimeric versions of the tricyclic alkaloid (Schröder et al. 1996). Antimicrobial activity has not been directly investigated.

Myrmecia

The *Myrmecia* genus is composed of 89 species. These Australian bulldog ants are noted for their aggressive behavior and potent sting. *Myrmecia pilosula* ranks high on the human pain scale (2–3) (Starr 1985). Research has identified allergens (Wiese et al. 2007). A separate study targeting antimicrobial activity, identified a cytotoxic peptide (Zelezetsky et al. 2005), pilosulin 1, from the venom of *M. pilosula*, that had strong antifungal and antibacterial activity. Although obvious, the general point is that chemistry function is driven by bioassay. We have already seen that ants are capable of regulating the release of their venom into the environment, so it would not be surprising if low-

Fig. 9 The genus, *Myrmicaria*, produces alkaloids similar to *Solenopsis* thief ants and *Monomorium* species (Fig. 7), as well as the top three compounds shown here (Jones et al. 2007; Schröder et al. 1996). The poison glands of *Aphaenogaster* and *Messor* genera produce anabaseine and/or anabasine, analogs of nicotine

level dispersal of protein dominated venoms also play a role in nest hygiene. Research is needed.

Subfamily Formicinae

Camponotus and Calomyrmex

The *Camponotus* and *Calomyrmex* genera are composed of over 1,500 and 14 diverse species/subspecies, respectively. The *Camponotus* genus generally does not have a functional metapleural gland, rationalized by the fact that carpenter ants do not live in the soil, but prefer moist decaying wood; therefore, microbial pressure is not as great as on the soil inhabiting ants. They may not have the same problems, but rotting wood is an excellent habitat for a variety of microbes, fungi, bacteria, and yeasts (Blanchette and Shaw 1978; Zhang et al. 2008) including Actinobacteria that produce Attine fungicides (Barke et al. 2010) and basidiomycetes fungi that are related to the Attine fungal garden. Chemical analysis of the contents of mandibular glands from *Camponotus quadrisectus* and *C. irritibilis* (collected from Brunei) led to the identification of three new structures, *E*-2-ethyl-2-hexen-1-ol, 4-hydroxymellein, and *E*-2-ethyl-2-hexenal, as well as mellein (Fig. 10) as major components for one or the other species (Voegtle et al. 2008). There also are minor amounts of m-cresol and 6-methyl salicylic acid. Mandibular glands are usually associated with alarm and defense, but Voegtle et al. (2008) noted that the major mandibular gland products have antibiotic activity (Kanoh and El-Gindi 2004; Lyr and Banasiak 1983) expanding possible roles for the mandibular gland products of *Camponotus* species and subfamily Formicinae in general. In support of the above, an undescribed *Calomyrmex* species produces antibiotic substances from their mandibular glands. Pyrazines were likely responsible for the antibiotic activity (Brough 1983).
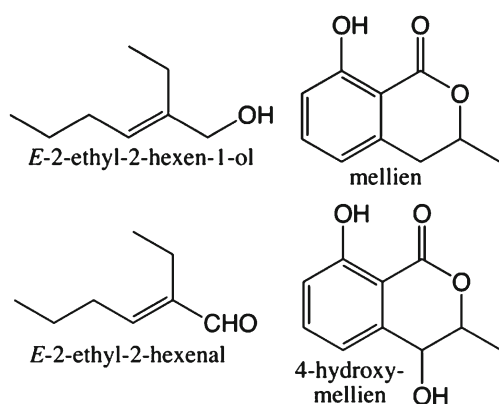
Another possibility: Formic acid is the major component in the venoms of all species of ants in the Formicinae subfamily. Formic acid is cytotoxic, present in concentrations up to 60 %, and the venom also contains small peptides and amino acids (Blum 1984). These ants do not sting, but they have powerful mandibles that can pierce the skin, during which they curl their abdomen toward the bite and spray formic acid. Carpenter ants can control the release rate and direction of release of their venom. Formic acid has antibiotic activity and could contribute to control of soil microbes.

Subfamily Dolichoderinae

Linepithema

The *Linepithema* genus contains 20 species. *Linepithema humile* (previously known as *Iridomyrmex humilis*), the Argentine ant, is a pest species in many parts of the world. The pygidial gland (Fig. 2) is used for defense in this subfamily. The pygidial glands of *L. humile* contain iridomyrmecin and dolichodial (Cavill et al. 1976); the former was demonstrated to be a strong insecticide and have antibiotic activity (Pavan 1949). The antibiotic activity of iridoids from plants have been shown to have a broad activity spectrum (Bakuridze et al. 1987). In addition, the Argentine ant produces a number of pyrazines in the Dufour's glands (see Fig. 11 for structures). Pyrazines have been shown to have antibiotic activity (Premkumar and Govindarajan 2005).

Subfamily Ponerinae

Pachycondyla

The genus *Pachycondyla* contains 252 species and 62 subspecies. One of these species, *Pachycondyla goeldii*, is typical of the subfamily in that it is an aggressive predatory ant that relies on its venom for subduing its prey. The venom was found to contain 15 peptides named ponericins, all of which show antibacterial activity (Orivel et al. 2001). Three structural types have been identified–ponericins G, W, and L. Broad physiological activities were found, including hemolytic, insecticidal, antibacterial (gram positive and negative), and yeast inhibition. The authors suggest that besides prey procurement, these ants are cleaning the prey of microbes prior to being brought into the colony, which presents a second possible function for these broadly active peptide venom components.

Fig. 10 *Camponotus* is a large genus in the subfamily Formicinae. They do not have a functional MG; however, the mandibular gland products shown here have antibiotic activity (Kanoh and El-Gindi 2004; Voegtle et al. 2008)

Summary and Needed Research

While the four ant subfamilies cited here (out of 21) represent the greatest number of species (about 90 % of the 14,300
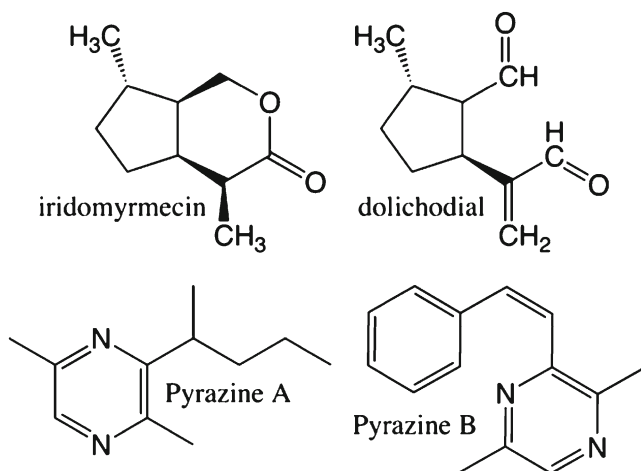
Fig. 11 The Argentine ant is the most studied species in the *Linepithema* genus. The pygidial gland produces compounds shown here. Iridomyrmecin has been shown to have antibiotic activity (Pavan 1949) and the other compounds by analogy are likely to also have antibiotic activity (Bakuridze et al. 1987; Premkumar and Govindarajan 2005)

described ant species) and citations (see Fig. 1), less than 1 % of those species are mentioned in this review. There is a lot of relevant literature that could not be included, and there obviously is a lot more work to be done. Consider this review an appetizer, not a meal. My objective was to explore the complexities of soil organism–ant interactions, which certainly are highlighted by the Attine system, and to indicate where additional research is needed. The latter can be overwhelming.

As I have pointed out in this review, research related to chemical ecology is needed in several areas. For example: a) What are the products and functions of the Attine glands associated with the cuticular crypts that harbor the symbiotic bacteria? b) Are Attine worker products from the mandibular gland and/or other exocrine glands involved in the preparation of cut leaves for the fungal gardens? If so, what is their function? c) What is the chemistry involved in attraction of the *Escovopsis* parasite fungus to its host Attine symbiotic fungus? d) What are the antibiotic effects of combinations of exocrine gland products? Fire ants produce both acidic (MG) and basic (poison gland) antibiotic compounds. How do they interact in the soil/microbial environment? This same question likely applies to most ants in the Myrmicinae subfamily. e) We know little about MG chemistry, despite the fact that it has been highlighted in this review. f) Exocrine gland products can have multiple functions—the same products may elicit defense against microbes and predators/intruders, but this needs to be demonstrated. g) Ants are capable of regulating the release of their venom into the environment, thus low-level dispersal of alkaloid or protein-dominated venoms could play a role in nest hygiene. This has not been demonstrated. h) We have seen that arboreal and decaying wood-inhabiting ant species do have a need for antimicrobials that could be met

through their mandibular or venom gland products, but this needs confirmation. i) In many examples, the chemistry reveals interesting natural product structures, but the function is assumed, not demonstrated, and other functions, such as antibiotic activity, rarely have been investigated.

The beauty of working with ants is that with over 14,000 described species evolving in virtually every ecological niche in the world, it is impossible to generalize and surprises are not the exception. Other areas of research not addressed in the review that also need attention are itemized below:

A) *Cross talk between microorganisms* via *semiochemicals.* This is a developing area of research with a variety of potential outcomes (Shank and Kolter 2009) that is even more exciting when linked to the complexities of the Attine system.

B) *Entomopathogenic nematodes.* These were not addressed here, but some, of course, are specialists on ants (Poinar et al. 2007; Rasman et al. 2012, this issue) and have been considered for biological control of some pest ant species. Besides the ant–nematode interactions, nematophagous fungi are also part of the picture (Soto-Barrientos et al. 2011).

C) *Parasites that manipulate the behavior of their ant hosts, thus optimizing their development.* Zombie ants—A *Camponotus* sp. infected with *Ophiocordyceps* fungi exhibits predictable altered zombie-like behavior that benefits the parasitic fungus (Hughes et al. 2011). *Ophiocordyceps* infections of *Atta* and *Acromyrmex* species have been reported (Hughes et al. 2009).

D) *Ant Viruses.* The first ant viruses were identified using a metagenomic approach, specifically for the fire ant, *Solenopsis invicta*. Three RNA viruses have been isolated and characterized (Valles 2012). The first two, SINV- 1 and SINV-2 are found in all developmental stages, but only chronic asymptomatic infections have been observed, except that SINV-1 sometimes becomes pathogenic if colonies are stressed, e.g., dug up and brought to the lab. A third virus, SINV-3 has proven to be virulent in colonies brought back to the lab and is readily transmitted to healthy colonies through air and mechanical transfer. The virulence has not been observed in the field, but laboratory colonies are short-lived. The main difference, of course, is the soil and the possible negative interactions between soil microorganisms and SINV-3 that may keep the virus in check under field conditions. Another virus has been discovered recently from the crazy ant, *Nylanderia pubens* (Valles et al. 2012) indicating that ant viruses may be common. Virus–soil interactions may be important factors in ant colony health (Yeager and O'Brien 1979).

It should be clear that ants are chemical factories that produce a huge diversity of structural types that have

evolved with all the pressures of the unique, dynamic soil, wood, or arboreal environment encountered by each species. These pressures have led to multiple exocrine gland semiochemical sources that can have multiple contextual functions. It is no wonder that ants are poor pollinators–they are walking pharmacies that just casual contact with can reduce the viability of flower pollen (Beattie et al. 1984). There is no predicting what new chemistry you will find when working with a new ant species, which is why ants have been such a rich source for new natural products. Imagine a fire ant worker covered with hard cuticular armor, oozing with antibiotic carboxylic acids from its metapleural gland, sending off an antibiotic/anti-predator aerosol of poison gland piperidine alkaloids through its sting into the nest environment, releasing pyrazine alkaloid alarm pheromones from its mandibular glands at the slightest disturbance, depositing minute amounts of sesquiterpene and homosesquiterpenes from the Dufour's gland to mass recruit workers to food or initiate colony emigration. These worker ant activities are based on independent pieces of research. We tend, by necessity, to be reductionist in our research, but the leaf-cutting ant story rings out loud and clear that the real world is a dynamic multidimensional system in a delicate equilibrium. Understanding the dynamics is one of our greatest challenges.

> Korean kimchi was recently defined as a "mix of cabbage with spices and salt brine: several strains of naturally occurring *Lactobacillus* will start pumping out acids that lower the pH, killing off pathogens and imparting a mouth tingling tang." (Wired, 060, May 2012).

> Leaf-cutting ants had the concept first!

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