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On the Biological Diversity of Ant Alkaloids

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Abstract

Ants have outstanding capacity to mediate inter- and intraspecific interactions by producing structurally diverse metabolites from numerous secretory glands. Since Murray Blum's pioneering studies dating from the 1950s, there has been a growing interest in arthropod toxins as natural products. Over a dozen different alkaloid classes have been reported from approximately 40 ant genera in five subfamilies, with peak diversity within the Myrmicinae tribe Solenopsidini. Most ant alkaloids function as venom, but some derive from other glands with alternative functions. They are used in defense (e.g., alarm, repellants) or offense (e.g., toxins) but also serve as antimicrobials and pheromones. We provide an overview of ant alkaloid diversity and function with an evolutionary perspective. We conclude that more directed integrative research is needed. We suggest that comparative phylogenetics will illuminate compound diversification, while molecular approaches will elucidate genetic origins. Biological context, informed by natural history, remains critical not only for research about focal species, but also to guide applied research.

INTRODUCTION

Equipped with numerous secretory glands, ants (Hymenoptera: Aculeata: Formicidae) are exceptional in producing a wide range of chemicals (13). Ant-derived metabolites likely reflect selective pressures influencing constraints on biosynthesis but also taxa-specific phylogenetic inertia (16). These natural products are often made with hydrocarbon backbones, containing elements like nitrogen and oxygen, and mediate interactions among disparate organisms. Research interest in natural products—driven by the search for alternative therapeutics using advanced chromatography—has led to an increase in novel compound discovery (110).

Alkaloids are a diverse class of 10,000–14,000 bioactive compounds (107). Our body of knowledge about them is as intricate as their functional and structural subdivisions. Historically, alkaloids were defined to include many amines. We follow Pelletier's (107, p. 26) narrower definition as "biologically-active, heterocyclic organic compounds containing nitrogen in a negative oxidation state, of limited distribution among organisms." There has been a growing interest in the biochemistry of ant alkaloids (132), with researchers coming from various fields (chemists, chemical and behavioral ecologists, evolutionary biologists). However, lack of interdisciplinary collaboration has resulted in informational disconnection, limiting broader interpretations (12). Much research has focused on describing alkaloids and their functions and then eventually moving to biomedical applications. While identification is an important first step, we must also consider how metabolites affect differential survival and explain variation across lineages. This review offers an overview of ant alkaloids, including comments on evolution, ecology, and applied research.

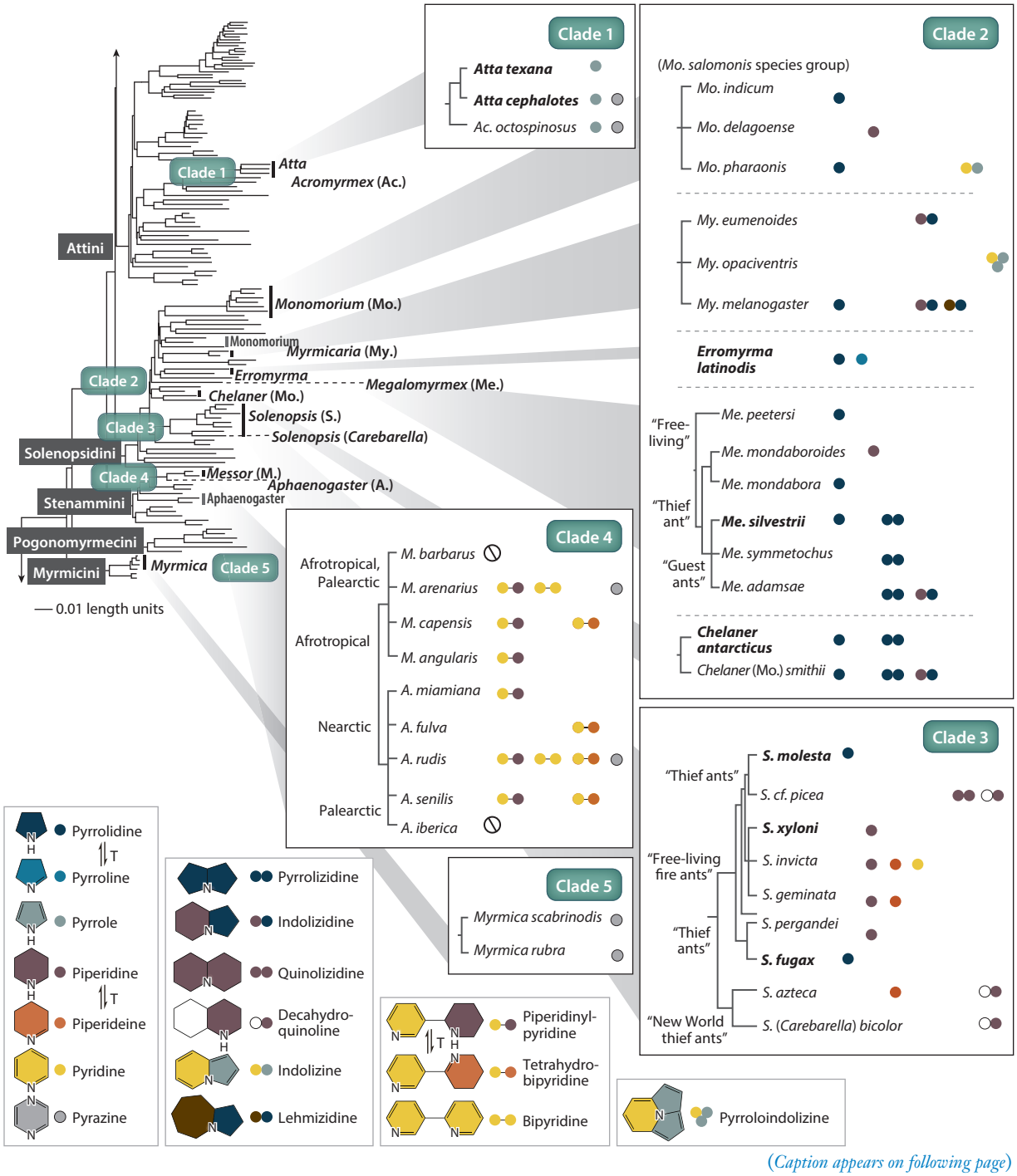
ANT ALKALOID DIVERSITY

Ants have demonstrated an outstanding capacity to produce a diversity of secondary metabolites, secreted by several glands (100). Alkaloids have been recorded in some lineages from mandibular, pygidial, and especially venom gland secretions. Most species produce acid- or protein-rich water-soluble compounds, but stinging lineages such as Pseudomyrmecinae and Myrmicinae employ alkaloidal venom (132).

Despite decades of research, knowledge about alkaloid diversity and evolution remains limited (132). Pioneering investigations date from the 1950s, with scientists looking for causative factors behind fire ant sting injury (e.g., 21, 30, 134). The venom of the red imported fire ant, *Solenopsis invicta* (Myrmicinae: Solenopsidini), is an oily liquid with traces of water and peptides (58, 105), characterized mainly by piperidine alkaloids (93). Solenopsins have remained the most intensively studied animal-derived alkaloids and dominate the literature. Alkaloids have been recorded from five ant subfamilies (Ponerinae, Formicinae, Dolichoderinae, Pseudomyrmecinae, and Myrmicinae), but mainly from the Myrmicinae in the tribe Solenopsidini. We present a phylogeny of Myrmicinae, focusing on lineages rich with venom alkaloid diversity, in **Figure 1**.

Structural Classes of Alkaloids

Of the 300 ant genera worldwide, few species have been investigated for their chemistry (**https://www.pherobase.com/database/family/family-Formicidae.php**), and of the 40 genera studied, 200 species contain alkaloids. Out of 30 structural classes of animal alkaloids, 10–13 (depending on classification) have been found in ants (120). Considering that ants comprise approximately 16,000 species (337 genera), their true alkaloidal diversity is largely unknown. In following a strict definition for alkaloids (107), we refer to some amines (e.g., pyrazines) that are sometimes categorized as alkaloids and sometimes as alkaloid-like compounds. To facilitate crosstalk among fields, we provide structural class names along with trivial names. We summarize alkaloids structurally as



(Caption appears on following page)

Figure 1 (*Figure appears on preceding page*)

Myrmicinae ant phylogeny with an overview of the diversity of venom alkaloids. The main phylogeny is a maximum likelihood tree of the ant subfamily Myrmicinae, excluding Crematogastrini (*top arrow*) and formicoid and poneroid outgroup clades (*bottom arrow*) (for a similar tree topology, with species names, see 142). Clades in boxes represent ant lineages where alkaloids have been identified from the venom apparatus (i.e., venom gland plus associated structures); taxa in bold are included in the Ward phylogeny (142); lifestyles are indicated in quotes. Names (i.e., *Erromyrma*, *Chelaner*, *Solenopsis*) have been updated to reflect current taxonomy (**https://www.antweb.org/**, **https://www.antcat.org/**, **https://www.antwiki.org/**, and references therein). Alkaloid structural classes are indicated in boxes; ring structure and aromaticity are indicated by different colors. Double arrows and Ts indicate tautomerization. Clade 1 represents fungus-growing ant species in the genera *Atta* Fabricius, 1804, and *Acromyrmex* Mayr, 1865 (approximately 240 fungus-growing ant species). Clade 2 includes several genera, containing the paraphyletic genus *Monomorium* Mayr, 1855 (Mo.; 325 species and subspecies). Following *Monomorium*, *Myrmicaria* Saunders, 1842 (My.; 71 species and subspecies), *Erromyrma* (2 species), *Megalomyrmex* Forel, 1885 (Me.; 45 species), and *Chelaner* Emery, 1914 (53 species), are represented in subsections within the box. The *Megalomyrmex* cladogram is approximated based on morphology. Clade 3 is a cladogram of *Solenopsis* Westwood, 1840 (S.; 216 species and subspecies), where thieves are paraphyletic, and fire ants are a monophyletic group of free-living omnivores. Clade 4 represents the tribe Stenammini. Genera such as *Messor* Forel, 1890 (M.; 163 species and subspecies), and *Aphaenogaster* Mayr, 1853 (A.; 227 species and subspecies), are not monophyletic, and while some species produce alkaloids, others do not (*crossed circle*). Clade 5 represents *Myrmica* Latreille, 1804 (189 species and subspecies). Citations to reported compounds are provided in **Supplemental Table 1**.

Supplemental Material >

monocyclic, bicyclic, tricyclic, or polycyclic molecules emphasizing functional groups (**Figure 2**; see the sidebar titled Structural Overview). Structural similarity may, however, not imply metabolic relatedness.

Biogenic Amines that Are Considered Alkaloid-Like

Alkaloid-like biogenic amines are more widespread than alkaloids among animals, including ants (13, 153). For instance, metabolites of tyramines are reported from male ants in Solenopsidini (73). Also noteworthy are pteridines, found in most organisms as metabolic cofactors and prevalent as colorants among Hymenoptera (153). Pteridines are metabolically related to pyrazines, which are widespread ant amines sometimes referred to as alkaloids (e.g., 140) but referred to in this review as alkaloid-like amines, since ant pyrazines usually present the N atom in a positive oxidation state. Dialkylpyrazines are the most common pyrazines in Hymenoptera, though trialkyl- and even tetra-alkyl- analogs exist (31, 53, 146). Insect pyrazines are believed to derive from amino acids (e.g., 103, 124), although some are suggested to form by spontaneous (i.e., nonenzymatic) synthesis (46). The tryptophan-derivative 3-methylindole (i.e., skatole) is an aromatic compound described from animal feces but also found in Attini spp. and several army ants (Dorylinae) (e.g., 81, 27). Skatole is regarded as an alkaloid-like amine because it is widespread in nature (107).

Diversity of Alkaloids Within Clades

Over 50 alkaloid analogs are listed for the *Solenopsis* fire ants (**Figure 1**, *Clade 3*), mostly from *S. invicta* (e.g., 34, 35). All fire ant alkaloids occur in venom, and they have been more intensively researched (134) than the alkaloids of other genera. The best-studied ant alkaloids are a dozen analogs of asymmetric piperidines known as solenopsins. Their structural diversity comes from different saturations and lengths of substituent alkyl chains, as well as optical (*cis*-, *trans*-) and chiral (*2S6R*-, *2S6S*-) isometry of asymmetric analogues (35; see 123, figure 1). Their unsaturated analogs are the dehydrosolenopsins abundant in some species (24). Some frogs can sequester ant piperidines (e.g., solenopsin A, 223K, and 225I), but they do this via myrmecophagy (136).

Interspecific variation in alkaloid profiles has been examined (e.g., in *Monomorium*, *Solenopsis*, *Myrmicaria*, *Megalomyrmex*; Myrmicinae: Solenopsidini) and suggests that closely related Solenopsidini species have discrete, species-specific alkaloids, yet species of different genera may share similar analogs (e.g., 6, 24, 55, 63, 75) (**Figure 1**; **Supplemental Table 1**). For example, the thief ant

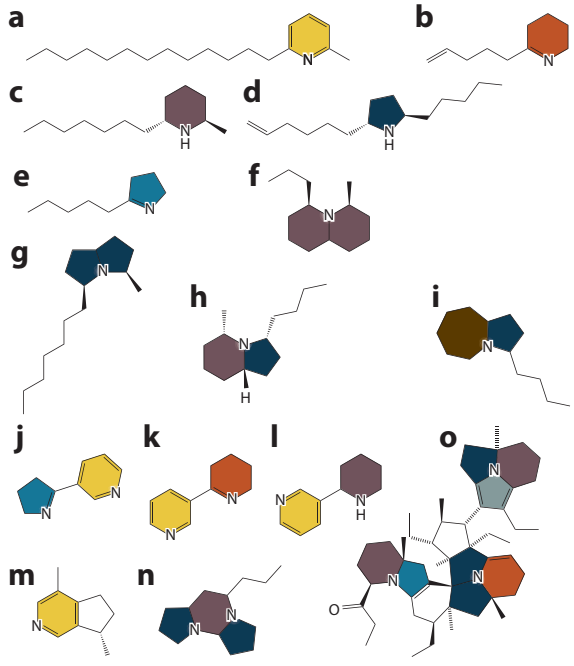


Figure 2

Structural diversity of ant alkaloids. (*a*) 2-Methyl-6-tridecylpyridine from *Solenopsis invicta*. (*b*) 2-(4-Penten-1-yl)-1-piperideine from *Monomorium*. (*c*) *Trans*-2-heptyl-6-methylpiperidine from *Megalomyrmex*. (*d*) *Trans*-2-pentyl-5-(5′-hexenyl)-pyrrolidine from *Monomorium*. (*e*) 2-Pentyl-1-pyrroline from *Monomorium notulum*. (*f*) (4S,6R)-4-methyl-6-propylquinolizidine from the venom of *Solenopsis picea*. (*g*) (3S,5R)-3-heptyl-5-methylpyrrolizidine from *Solenopsis* sp. (*h*) (3R,5S,8aS)-3-butyl-5-methyl-8a-indolizidine from *Monomorium pharaonis*. (*i*) 3-Butyl-pyrrolo[1,2-a]azepane (i.e., 3-butyl-lehmizidine) from *Myrmicaria*. (*j*) 3-(3,4-Dihydro-2H-pyrrol-5-yl)pyridine from *Messor* venom. (*k*) 3,4,5,6-Tetrahydro-2,3′-bipyridine. (*l*) 3-(2-Piperidinyl)pyridine from *Messor* and *Aphaenogaster* venoms. (*m*) (7S)-4,7-dimethyl-6,7-dihydro-5H-cyclopenta[c]pyridine detected from different glands of several ant genera. (*n*) 5-Propyldecahydrodipyrrolo [1,2-a:1′,2′-c]pyrimidine (i.e., tetraponerine 2). (*o*) The dimeric myrmicarin 663 from *Myrmicaria*. Citations for reported compounds are in **Supplemental Table 2**.

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Megalomyrmex mondaboroides (**Figure 1**, *Clade 2*), produces 3-hexyl-5-methylindolizidine isomers that are shared with *Solenopsis* but not with closely related *Megalomyrmex* species (6) (**Figure 1**, *Clade 3*). Some inconsistencies have been observed within tribes (**Figure 1**, *Clades 1 and 4*). In Stenammini, the presence of the pyridine- and piperidine-containing anabasine varies with species and age (**Supplemental Table 1**). Similarly, in Attini, some alkaloids are found in some but not all leaf-cutting species, and other Attini tribe lineages lack alkaloidal compounds completely (5).

Sex variation has been described in *Solenopsis*, *Megalomyrmex*, *Myrmicaria*, and *Monomorium* species (4, 33, 73). Male ants have no venom gland but produce tyramides in their abdomens (33). Tyramides are metabolites of tyramine, which is neuroactive for insects and a mediator of physiological and behavioral functions (33). Males also produce pyrazines (140), while females (i.e., workers and queens) have pyrazines in their mandibular glands and other alkaloids in their venom (134). Different alkaloidal profiles among female sexuals and worker castes have been examined in *Solenopsis* species (55, 121, 131). Minor workers have complex venoms with *trans*-isomeric solenopsin analogs, and major workers tend to have less diverse *cis*-isomeric analog

STRUCTURAL OVERVIEW

Monocyclic fatty acid derivatives in the venoms of the Solenopsidini (**Figure 1**, *Clades 2 and 3*) are the best known and most abundant ant alkaloids. Pyridines (**Figure 2*a***) are detectable from *S. invicta*, along with piperideines (**Figure 2*b***) and piperidines (**Figure 2*c***) (i.e., solenopsins) (34). Another large division includes venom pyrrolidines (**Figure 2*d***), first described from *Monomorium* flower ants (128) and typically volatile and smelly at room temperature. Pyrrolines (**Figure 2*e***) are less common; they are illustrated by the main venom alkaloid in African *Monomorium notulum* (77).

Bicyclic alkaloids are also hypothesized to derive from fatty acid acetate units, where there are a number of izidines (59) in the venoms of small ants; these izidines have been repeatedly recorded to be sequestered by predatory poisonous frogs (e.g., pumiliotoxins). These compounds often occur with their concomitant monocyclic homologs. Among these are quinolizidines (**Figure 2*f***), such as the compound 195A; pyrrolizidines (**Figure 2*g***), such as xenovenine from *Solenopsis* thief ants (42, 74); and indolizines, such as monomorine 1 (**Figure 2*h***). In addition, an analog of a five-seven-ring fused alkaloid from a frog lehmizidine (**Figure 2*i***) was found in the venom of *Myrmicaria melanogaster*.

Bicyclic alkaloids from other pathways include pyridine nicotinoids, originally known from tobacco and recurrent in *Messor* and *Aphaenogaster* ants, in which a pyridine ring combines with either a pyrrole, as in myosmine (**Figure 2*j***), or a piperidine, as in anabaseine (**Figure 2*k***) and its derivative anabasine (**Figure 2*l***). In addition, terpenoid alkaloids (**Figure 2*m***) like actinidine are common in the mandibular and pygidial glands of several taxa.

Tricyclic and otherwise polycyclic ant alkaloids form by concatenation of tricyclic moieties (104). Tricyclic alkaloids include a group of eight pyrido-pyrrolo-pyrimidines (**Figure 2*n***) from the venom of *Tetraponera* (Pseudomyrmecinae) ants, called tetraponerines (96) and also demonstrated to derive from acetate (45). In addition, there are the pyrroloindolizidines, such as the venom myrmicarins of *Myrmicaria* ants, which can nonenzymatically combine into air-sensitive dimers (106) like isomyrmicarin 430A and others (**Figure 2*o***).

mixtures (44, 55). Queens, in contrast, are more uniform, predominantly producing isosolenopsin A across different *Solenopsis* species (36, 131).

ANT ALKALOID FUNCTIONS

Ant alkaloids are often volatile and may function as pheromones and toxins within or between species, depending on context and application. A single compound can work differently depending on whether it is sprayed (144), injected, or applied topically (84). Bioassays using ant-derived and synthetic compounds (as proxies) have formed our current understanding of function but have limitations because pure *trans-* or *cis-* configurations can be difficult to attain (108, 121). Still, synthetics enable researchers to test the function of alkaloids outside the scope of myrmecology (e.g., cytotoxicity of solenopsin A in cancer research), an important avenue of research.

Trail Pheromones

Ants deploy chemical trails of one or more compounds that can originate from different glands to exploit resources (32). Alkaloidal trail pheromones have been mapped to workers' venom sacs (100) and may synergize with other secretions (41). Different alkaloid classes are used (e.g., pyrroles, pyrazines; 32), but different species often employ the same compounds (9 and references therein). For example, methyl 4-methylpyrrole-2-carboxylate, a trail component of leaf-cutter *Atta texana* (135) (**Figure 1**, *Clade 1*; **Figure 2*n***), is found in some but not all attine species (100). A set of indolizidines and pyrrolidines described from *Monomorium pharaonis* (i.e., monomorines)

may work as trail pheromones or attractants, depending on concentrations (70). Pyrazines (e.g., dialkyl-pyrazines) (**Figure 1**) are also often found in venom and employed as trail pheromones by numerous distantly related genera (e.g., *Tetramorium*, *Atta*, *Myrmica*, *Aphaenogaster*, *Messor*, *Eutetramorium*, and *Monomorium*) (**Figure 1**; **Supplemental Table 1**).

Supplemental Material >

Alarm Pheromones

Alarm pheromones are chemicals that diffuse rapidly to initiate recruitment, arousal, and defensive behaviors necessary for colony protection (17). Their glandular origin varies greatly among ant subfamilies; in this section, we provide examples of heterocyclic amines. Among the known substances are anabasine, actinidine, and pyrazines, the latter being the most frequently reported compounds from nearly all ant subfamilies (90, 148) (**Figures 1** and **2*m***). The monoterpenoid actinidine was recorded from the mandibular gland of *Platythyrea punctata* (Ponerinae: Platythyreini) (109) and the pygidial gland of *Megaponera analis* (Ponerinae: Ponerini) (71). The genera *Messor* and *Aphaenogaster* (Myrmicinae: Stenammini) (**Figure 1**, *Clade 4*) have complex venom mixtures containing pyrazines, anabasine, and anabaseine (26 and references therein; 38) (**Figure 2*k***). When disturbed, *Messor ebeninus* emits an emulsion containing anabasine, which suggests that anabasine has an alarm function (38). Some species use the same pyrazine analogs as trail (e.g., 40) and as alarm pheromones (e.g., 69). Pyrazines coming from mandibular glands usually work as alarm substances (13) and have been recorded from *Aphaenogaster* (145), *Solenopsis* (140), *Iridomyrmex* (31), *Wasmannia* (122), *Odontomachus* (Formicidae: Ponerinae) (148), and *Rhytidoponera* (Ectatomminae; Ectatommini).

Sex Pheromones

The only sex pheromone recorded is a mixture of venom pyrrolidines of unmated queens of *Leptothorax kutteri* (Myrmicinae: Crematogastrini) and some parasitic leptothoracine ants (*Formicoxenus nitidulus*, *Harpagoxenus* spp.); this mixture was demonstrated to attract males of these species and induce mating behavior (113). A pheromonal role is suspected for venom piperidines of *S. invicta* fire ant queens due to the relative proportion of these piperidines in association with the queens' reproductive status (49).

Repellents and Insecticides

Insect alkaloids are frequently used as interspecific repellents and insecticides. Most are toxic; therefore, it is not surprising that ant venom alkaloids are employed to overcome competitors (see 57 and references therein), nest assailants (61), and prey (80). The venom delivery mechanism is either injection or dabbing with the stinger tip (132). Interspecific reactions to alkaloidal venom are likely linked to toxic effects (20), but more experimental tests are needed.

Slow-moving predators and scavengers (e.g., *Megalomyrmex peetersi*, *Monomorium rothsteini*, and many *Solenopsis* spp.; 10, 65, 87, 125) have been found to repel competing species by applying alkaloids near food (65, 105). This is analogous to the use of mandibular gland pyrazines (67) applied to food by the distantly related *Wasmannia auropunctata* (82, 122). In addition, *Solenopsis* and *Megalomyrmex* thief ants (**Figure 1**) specialize in pillaging resources from other species by employing venom pyrrolidines and piperidines as effective, long-lasting repellants (6, 10, 20). Venom pyrrolizidines from *Megalomyrmex* thief ants can induce repellency, aggression, and submission in host colonies (6, 8), whereas those from *Megalomyrmex* agropredator scout ants induce immediate escape behavior in their host ants (3). The venom of the guest ant social parasite *Megalomyrmex symmetochus* is lethal against competitor species, like *Gnamptogenys hartmani* raiders (7). When

Gnamptogenys invades the host nest, *Megalomyrmex* defends its host by killing the raiders. In addition to being toxic, its pyrrolizidine venom alkaloids induce raider ants to attack one another (7).

Alkaloid-rich venoms can also serve as nicotinic antagonists, inducing rapid prey paralysis and death, as demonstrated with *Solenopsis* piperidines (14, 19, 50, 57) and *Monomorium* and *Megalomyrmex* pyrrolidines (125). Specifically, ant pyrrolidines show strong inhibition of acetylcholinesterase (116) and neurocontractions (83) in vitro. *Solenopsis* piperideines are less potent against pea aphids than are piperidines; however, piperideines have been less studied than other *Solenopsis* alkaloids (112, 150). Different analogs of the same class may yield different effects. For instance, different piperidine dehydrosolenopsins and solenopsins vary in paralysis times and lethality (57, 70), shaping the biology (e.g., invasiveness, symbioses) of ant species and caste-specific tasks (e.g., nest foundation, defense).

Finally, as alkaloids are usually bitter and poisonous, alkaloid-rich insects may provide group protection against predators (133). The alkaloid-like pyrazine analogs are ecologically described in numerous animals as deterrents against predators (28), but this is not clearly demonstrated with ants. Skatole (3-methyl-1*H*-indole)—a major venom constituent in *Pheidole phallax*—is also secreted by the army ants *Eciton*, *Neivamyrmex*, and *Labidus* (e.g., 81) and has been hypothesized to play a dual role as a vertebrate predator deterrent (143) and as an alarm pheromone. Actinidine (**Figure 2*m***) has been recorded as a repellent from the pygidial glands of *Tapinoma* (Dolichoderinae: Tapinomini) ghost ants (130).

Vertebrate Toxins

Some aggressive stinging ants produce alkaloids that are harmful to vertebrates (132). The best studied are the stings of *Solenopsis* and *Tetraponera* species, common symptoms of which are local pain (30) followed by pustule formation (141). *Solenopsis* fire ants attack reptiles, birds, and mammals (134), and critical alkaloid envenomation in smaller animals causes seizures by cardiac depression and neuronal damage (68). These ants' piperidine solenopsins are highly toxic to cultured human and murine cells (79), even at low concentrations (151). Toxicity mechanisms are better understood for *Solenopsis* piperidines, which cause local tissue damage and necrosis (39, 64, 111) and affect neuronal cells (e.g., 95). Local inflammation is further promoted by direct hemolysis and platelet aggregation (e.g., 72). Bioassays with different animal models indicate that the longer-chain analogs are more toxic (57, 79), particularly the *trans*- isomers. This pattern is also observed with venom tricyclic quinolizidines and indolizidines from *Tetraponera* (tetraponerines; **Figure 2*n***) that are toxic against human cell lines (22, 96), with longer-chained analogs being the most potent (22). A suggested mechanism for the higher toxicity of analogs with longer side chains is a greater exposure of the hydrogen atom in the ring nitrogen and unpaired electrons (24), especially in *trans*- isomers.

Antimicrobial Activity

Antimicrobial activity is common among amines in general; however, few ant compounds have been tested, with the exception of *Solenopsis* and *Monomorium* alkaloids (e.g., 2, 78). The fact that some species are covered in their own venom secretions (14, 55) suggests broad pathogen protection (18). Indeed, venom piperidines from *Solenopsis* fire ants kill various microorganisms [e.g., fungi (89, 126)], Gram-positive and Gram-negative bacteria (78, 127), and trypanosomatid protozoans (123). Additionally, a pyrrolidine from *Megalomyrmex* venom inhibits Gram-positive and Gram-negative bacteria (125). To consider another alkaloid class, minor venom piperideines from fire ants (150) inhibited phytopathogenic fungi (89) but displayed limited activity against pathogenic fungi, bacteria, and protozoa (149). Because of caste-specific adaptations, fire ants can

use antibiotic alkaloids in various ways; workers dispense venom on surfaces inside their nests (105), and young queens deposit venom on eggs (137, 139). Anabaseine has also been found to work as an antibiotic, suggesting that *Messor* might use venom to sanitize their nests (1, 137). Finally, mandibular pyrazines from formicine *Calomyrmex* (Formicinae; Camponotini) and skatole from *P. phallax* venom are antimicrobials (81). The exact mechanism(s) for this antimicrobial action remain unknown, but piperidines affect cell cycle enzymes (136) and have membrane-active properties (43, 91).

EVOLUTION AND PROMISING RESEARCH TRENDS

Ancestral States

Within the order Hymenoptera, insects of the infraorder Aculeata (i.e., wasps, ants, and bees) share a modification of the ovipositor into a sting. In this infraorder, ants are a sister lineage to Apoidea (e.g., bees, digger wasps), with Pompiloidae (e.g., velvet ants) and Vespoidae (e.g., social wasps) being the basal groups (52). Ants are unique in producing alkaloids, as venoms of other Aculeata often contain nonalkaloidal amines (18, 104) or pain-inducing monoterpenes (99). The ability to synthesize pyrazines and actinidine is present in solitary ancestral bees (e.g., Anthophoridae, Eumenidae) and wasps (e.g., Sphecidae, and Tiphiidae) (102) and has been identified from basal Ponerine and Dolichoderine ant lineages (e.g., 109). Instances of alkaloids reported from Formicinae (56, 118, 129) have not been traced to and confirmed to come from any gland, and thus could be coming from associated organisms [e.g., mites (119)]. We conservatively suggest that alkaloids were present early in ant diversification, and that it is still impossible to establish when the biosynthesis of pyrrole- and pyridine-containing compounds arose.

The diversity of ant alkaloids is almost entirely derived from the venoms of stinging ant lineages. Venom genes evolve by neofunctionalization, where traits previously fulfilling a different function get selected for novel adaptations, generally by gene duplication followed by independent evolution (48, 132). The venoms of basal lineages like Ponerinae include water-soluble chemicals (85, 132) that represent a hypothetical ancestral state, which persisted up into Myrmicinae such as *Myrmica* (Myrmicini) and *Pogonomyrmex* (Pogonomyrmecini). Somewhere over the course of the evolution of the Stenammini lineages (e.g., *Messor* and *Aphaenogaster*) (**Figure 1**, *Clade 4*), venom alkaloids arose, and diversity peaked among the Solenopsidini genera (**Figure 1**, *Clades 2 and 3*). In particular, pyrrole-division derivatives became widespread within the tribe Solenopsidini, culminating in the high diversity of alkaloids per species among fire ants [e.g., *S. invicta* and *Solenopsis richteri* (35)].

It is currently impossible to use a single theorized evolutionary path to explain the origin of ant alkaloids, in part because of the broad definition but also because some alkaloidal compounds may not be heritable. Technology with improved resolution has allowed research to progress rapidly and reveal convergent and novel compounds; some alkaloids vary quantitatively and qualitatively among species, and many influence the fitness of organisms. In addition, taxonomists have been revising and renaming lineages so that evolutionary patterns can emerge. The phylogeny in **Figure 1** provides an overview to inspire comparative studies that examine ecological and evolutionary questions relating to alkaloidal compounds in ants.

Widespread Pyrazines

Odoriferous and distasteful pyrazines are widespread in nature (97) and are better known as predator deterrents in aposematic insects (60, 117). However, they do not always confer protection, especially against invertebrate predators (28, 115), and may have other functions. For example,

the major component of *Atta sexdens rubropilosa* trail pheromone 3-ethyl-2,5-dimethylpyrazine is less prevalent in other *Atta* species (40). A similar pattern of variation emerges for *Pogonomyrmex* (Myrmicinae: Pogonomyrmecini) (66) and *Myrmica* (Myrmicinae: Myrmicini) congeners: At least four and eight species, respectively, use 3-ethyl-2,5-dimethylpyrazine as a trail pheromone (51). Trail pheromones are found in the venom sac (100), which is rich in amino acids that likely react enzymatically to form pyrazines (94). Provided that pyrazines are found in the tribe Myrmicini, located at the base of the dated Myrmicinae phylogeny, it appears that some ant lineages may have been producing alkaloids for over 100 million years (142). Interestingly, 3-ethyl-2,5-dimethylpyrazine is also found in the mandibular glands of *S. invicta*, functioning as an alarm pheromone (140). Alkylpyrazines also occur in the mandibular glands of several of the more basal ant lineages, as illustrated by *Ectatomma* (Ectatomminae: Ectatommini), *Odontomachus* (Ponerinae: Ponerini), and *Pachycondyla* (Ponerinae: Ponerini) (101). Whether the production of pyrazines in two distinct ant glands across diverse taxa is a case of convergence remains to be studied.

Nicotinoids in the Stenammini

Nicotinoid pyridine alkaloids have been reported from Stenammini (**Figure 1**, *Clade 4*). In a comparative study, Co et al. (38) noted that anabasine (**Figures 1** and **2*l***) and related nicotinoids are found in some, but not all, *Messor* species (e.g., *Messor barbarus*), and a similar pattern holds for *Aphaenogaster*, where *Aphaenogaster iberica* lacks alkaloid production (88). Some ant-derived natural products have been demonstrated to be biosynthesized by microbial symbionts (124) or acquired through trophic interactions (119). This could be the case for *Aphaenogaster senilis* (88) workers, which, when treated with antibiotics, showed a significant reduction in alkaloid production. Furthermore, oribatid mites, prey to some ant species, can also be a source of alkaloids [e.g., pumiliotoxins in *Brachymyrmex* (147)]. Thus, clarifying symbiotic interactions becomes essential before alkaloid trait evolution can be adequately investigated in these ant lineages.

Solenopsidini: Puzzling Patterns and Polyacetate Chains

In the 1970s, Brand (23) and others (75, 92, 138) suggested that the progression of chemosystematics and biochemical evolution is dependent on the description of venom alkaloids, broad sampling, and informative taxonomic characters. Furthermore, it is necessary to establish the minor variation of venom profiles within species (e.g., 6, 23, 55, 75) and consistent differences between species (e.g., 75, 121). Finally, biochemists with expertise in the development and consequences of metabolic pathways should provide valuable insight, as the pathways themselves may be more important than the mere description of an end product (23). Not surprisingly, research endeavors involving chemosystematics and biochemical evolution have focused on alkaloid-rich Solenopsidini (**Figure 1**, *Clades 2 and 3*).

Solenopsidini taxonomy is particularly challenging and has been an impediment to venom alkaloid evolution research. Still, many authors have proposed hypotheses based on emergent patterns. Our phylogenetic overview leads to more questions than answers. While phylogenetic inertia is at play, we see much variation within and among tribes and genera. Pyrrolidines are found in several genera in Clade 2, but also in *Solenopsis fugax* (**Figure 1**, *Clade 3*). Piperidines appear consistently across *Solenopsis* species but are also found in bicyclic compounds from Stenammini (*Messor*, *Aphaenogaster*; **Figure 1**, *Clade 4*) and other Solenopsidini genera (**Figure 1**, *Clades 2 and 3*; **Supplemental Table 1**). We provide a simplified view, but what is needed next are integrative hypotheses tested with comparative phylogenetics tools (12).

Supplemental Material >

Alkaloid classes found in Solenopsidini are hypothesized to come from the linear concatenation of fatty acid–derived acetate units, akin to the biosynthesis of coniine (86). The mechanism

is suggested to form the skeleton of the related alkaloid classes piperidines, pyrrolizidines, and tetraponerines (63 and references therein) (**Figure 2*n***). These carbon chains will fold into cyclic structures depending on chain length and related enzymes, influencing venom composition. Brand (23) proposed that more basal *Solenopsis* species produce shorter-chained piperidines compared to more derived lineages, which produce longer-chained analogs (see also 92). The mechanism proposed was a thermodynamic chemical equilibrium barrier preventing more ancestral species (e.g., *Solenopsis geminata* and *Solenopsis xyloni*) from producing longer solenopsins (25). As more data were gathered, this hypothesis lost support (121) because the evidence indicated that the observed patterns may involve different mechanisms, perhaps influenced by unique species-specific selective pressures.

Another facet to this evolutionary trend was offered by the observation that different groups of species of *Solenopsis* thief ants and *Monomorium* produce sets of alkaloids following fixed chain-length patterns (76). This scenario considers the possibility that the existence of monocyclic (e.g., piperidines) and bicyclic alkaloids [indolizidines (**Figure 2*b***)] with the same chain length in the same species would be indicative of a common origin through acetate concatenation. This pattern seems compatible with the observations by Brand et al. (25) regarding fire ants belonging to different species groups. This observation warrants an expansion of essential information, mainly by revisiting some of these species for identification and the tracking of key enzymes, as the evidence suggests an evolutionary origin for ant venom alkaloids centered on fatty acids.

Convergent Chemistry: Understanding the Interplay of Ants and Microbes

Microbial symbionts have been suggested to play a role in the biosynthesis of alkaloids. Two related *Aphaenogaster* species—*Ap. senilis* and *Aphaenogaster iberica*—differ in that the former contains alkaloids, while the latter does not (88). Treatment with antibiotics significantly diminished alkaloids in *Ap. senilis* individuals, suggesting that alkaloid production by *Aphaenogaster* may involve endosymbiotic bacteria. It may also provide clues about why venom alkaloids are inconsistently found among Stenammini species (**Figure 1**, *Clade 4*). Venom alkaloids are believed to be biosynthesized at the convoluted portion of the venom gland (29, 54). However, no study has yet suggested the presence of symbionts inside the venom gland or indicated which step of the biosynthesis pathway, if any, gets disrupted by antibiotics (87). However, an *Atta* symbiont, the *Serratia marcescens* 3B2 bacterium, produces pyrazines in vitro—the same compounds that are used as trail pheromones (124). This was the first report of ant symbionts producing alkaloid-like compounds; the biosynthetic pathways observed in *Serratia* suggested that the amino acid L-threonine and acetate are precursors for the synthesis of some pheromone dimethylpyrazines (124). This example of convergent chemistry between ants and associated bacteria warrants further investigation to understand the possible role that bacteria play in ant communication and, consequently, compound evolution.

Alkaloid Origin and Biosynthesis in Ants Remain Largely Unknown

As most alkaloids have been described from plants (15, 107, 133), nonspecialists often assume that ants obtain them from their diet (53, 98, 107). While this is true for many insects, Solenopsidini ants maintain alkaloid-based venom in captivity regardless of diet (62). However, notwithstanding the fact that terpenoids abound in *Solenopsis* and *Monomorium* (152), it remains notable that the monoterpenoid actinidine (**Figure 2*m***) was never reported among these ants, despite the availability of necessary metabolic pathways. Little is known about ant alkaloid biosynthesis, especially regarding precursors and pathways in insects (15, 47). In ants, molecular precursors discovered to date include amino acids (e.g., L-ornithine generating pyrroles), isoprenoids (e.g., the terpenoid actinidine) and polyacetate units [e.g., solenopsins and tetraponerines) (114). Apart from a few

classes, such as solenopsins (86) and the tetraponerines (114) (**Figure 2*n***), biosynthetic routes remain speculative. Tentative pathways through which to study the origin of ant alkaloids based on their chemical precursors include mevalonate (terpenoids), shikimate (aromatic amino acids), and acetate cyclization (**Figure 2**; see the sidebar titled Structural Overview). If key enzymes were to be described, then ant species could be scrutinized using the latest molecular and integrative techniques (e.g., metabolomics) to understand gene expression patterns related to biosynthesis, helping to clarify evolutionary patterns.

Biotech Applications

Alkaloid biotech applications have remained a largely neglected topic in chemical entomology. Applied entomological research attention has highlighted venom peptides; however, alkaloids are more diverse, more stable, and cheaper to isolate (132) and thus have great potential as medicines (98). The biomedical and biotech applications for fire ant piperidines range from novel antibiotics (78) to antiparasitics (123) and chemotherapeutics (11). These alkaloids were even shown to form a stable protic ionic liquid (37) when mixed with organic acids, a likely widespread property allowing various applications ranging from universal solvents to industrial lubricants. The potential for technological development based on novel natural alkaloids is great.

CONCLUSIONS

As the diversity of ant alkaloids continues to be revealed, there is an opportunity to pursue hypothesis-driven integrative studies that will not only shed light on focal taxa, but also inspire biotechnological and biomedical applications. In this review, we provide an overview of the topic to encourage comparative ecological and evolutionary studies. A structured research approach should target lineages with (*a*) well-resolved phylogenies; (*b*) robust species diagnosing characteristics for taxonomy; and (*c*) varied lifestyles (e.g., free-living to social parasite species), diet, symbionts, or other factors that could influence alkaloid presence. Many questions remain. We currently do not know the eco-evolutionary factors driving alkaloid diversification, nor do we understand all biosynthetic pathways. Genomic approaches need to be applied to elucidate underlying genetic mechanisms for biosynthesis, as well as to unveil loci associated with species interactions. Moving forward, we hope to see more holistic research that utilizes interdisciplinary teams including biologists, geneticists, biochemists, and natural products chemists.

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