



Functional properties of ant queen pheromones as revealed by behavioral experiments

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Abstract

An ant colony is the epitome of social organization where up to millions of individuals cooperate to survive, compete, and reproduce as a single superorganism. Female members of ant colonies typically are categorized into a reproductive queen caste and a non-reproductive worker caste. The queen(s) conveys her fertility condition and in cases, genotype status, via a suite of queen pheromones whose various functions are crucial to the superorganismal nature of ant colonies. Knowledge of these functional properties is fundamental for identifying constituent chemicals and understanding corresponding modes of actions. In this review, I summarize functional properties of ant queen pheromones learned from seven decades of behavioral experiments, and contextualize this knowledge within the broader understanding of queen pheromones in other major groups of social insects. The effects include promotion of colony integrity and coherence, maintenance of reproductive dominance of the queen, and regulation of colony social structure. Additionally, general characteristics of queen pheromones are discussed and potential avenues for future research are highlighted.

Keywords Chemical communication · Queen pheromones · Eusociality · Behavioral assays

Introduction

The evolutionary success of social insects is attributable to their highly integrated colonial lifestyles, where numerous individual insects cooperate to survive, compete with conspecifics, and produce sexual forms (reproductive individuals) (Wilson 1971; Lin and Michener 1972). Ants (Hymenoptera: Formicidae), in particular, manifest immense diversity, abundance, and ecological impact (Wheeler 1910; Schultheiss et al. 2022). Studies of ants have illuminated the inner workings of insect societies despite the independent emergence of sociality in multiple diverse lineages and the consequent uniqueness of each major eusocial taxon (Wilson 1971).

Male ants contribute little or nothing to the maintenance and growth of the colony, confining their activities to competing for matings during their reproductive seasons.

Females are generally divided into two interdependent castes that showcase a reproductive division of labor. Members of the queen caste can mate and usually monopolize egg-laying in the colony. Meanwhile the workers, normally daughters of the queen and mostly sterile, perform routine yet essential tasks such as foraging, nest building, and brood care, acting as the extended phenotype of the queen (Wheeler 1910; Hölldobler and Wilson 1990; Smith et al. 2008b; Beekman and Oldroyd 2019).

Despite a clear functional separation, the extent of morphological differentiation between the two castes nonetheless varies widely. The queen caste in many ant species is morphologically and physiologically distinct from the worker caste. Such workers are not only smaller in size, but they also lack sperm storage organs (spermathecae) and often functional ovaries, rendering them completely sterile and incapable of laying eggs (Keller et al. 2014; Tribble and Kronauer 2017). In some species, workers retain reproductive potential but exhibit subtle and consistent differences from the queen caste. If the queen is missing, such workers may ascend to be the dominant reproductive females (Powell and Tschinkel 1999; Penick et al. 2021). In a minority of ant species, the two castes are not morphologically or physiologically well differentiated (Peeters 1991). Rarely, species

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have secondarily lost one of these castes (Peeters and Ito 2001; Monnin and Peeters 2008; Goudie and Oldroyd 2018).

Regardless of the degree of caste dimorphism, the colony must be informed of the presence of the queen or the dominant reproductive female to function optimally (Keller and Nonacs 1993). Communication in ant colonies is primarily chemical in nature, which is related to their ancestral subterranean habits (Tschinkel 2015, 2021), although visual, vibrational, and tactile signals or cues are also deployed in some instances (Hölldobler and Wilson 1990; Hölldobler 1995; Barbero et al. 2009; Golden and Hill 2016; Knaden 2019; Yilmaz and Spaethe 2022).

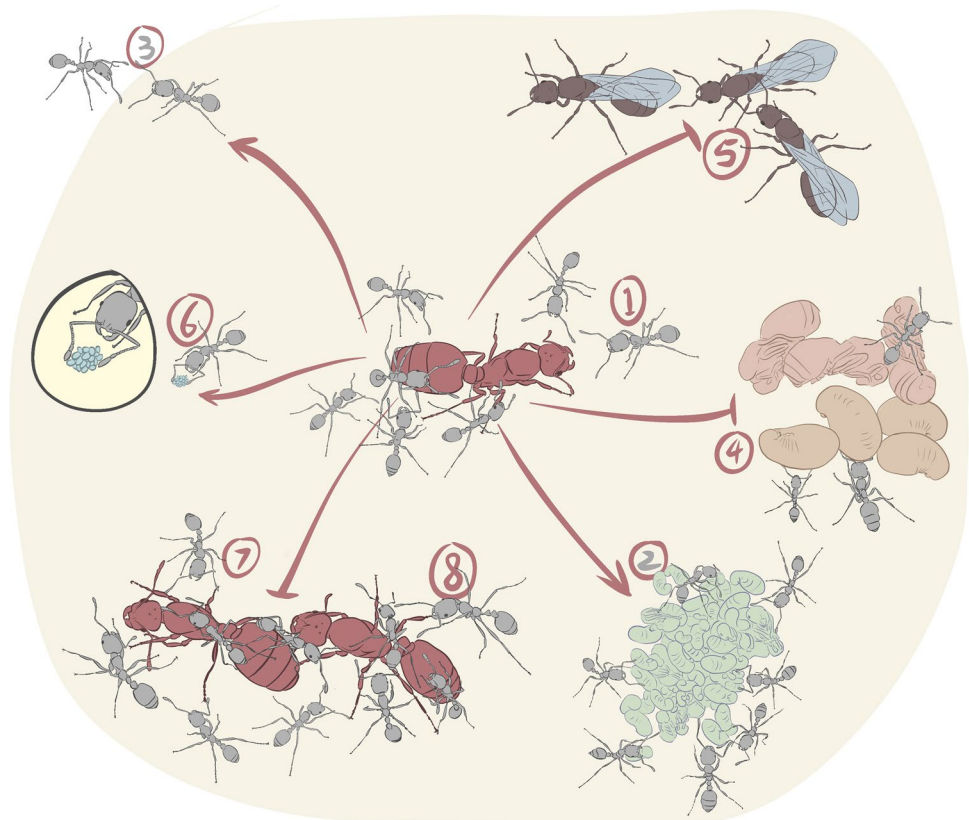
Chemical compounds used for intraspecific communication are termed pheromones (Karlson and Lüscher 1959). The potential durability and transmissibility of pheromones may have helped ants to evolve massive colony sizes and become the dominant invertebrates in most terrestrial ecosystems (Schultheiss et al. 2022). A variety of pheromones are deployed by ants, exemplified by alarm pheromones for threat alerts and trail pheromones for navigational guidance between various locations (Jackson and Ratnieks 2006; Grüter and Keller 2016; Vargo 2019). The studies of ant pheromones have not only deepened our knowledge of their social biology but also inspired such seemingly distinct efforts as designing network computation algorithms (Bonabeau et al. 2000).

In many eusocial insects, although the queen(s) does not actively participate in the colony maintenance and brood-rearing tasks, her presence has dramatic effects on worker brood care activities, nestmate discrimination, developmental trajectories of larvae, and colony coherence, reproductive output, and social structure (Wilson 1971; Hölldobler and Wilson 1990; Kocher and Grozinger 2011; Matsuura 2012; Ayasse and Jarau 2014; Vollet-Neto et al. 2018).

Most, if not all, of these effects are mediated through queen pheromones, a set of pheromones either exclusively produced by the queen or produced in higher quantities compared to workers (Sramkova et al. 2008; Smith et al. 2012b; Traynor et al. 2014). Thus, much like invisible wires that connect the queen to the rest of her colony and extend her influence, queen pheromones are key regulators of colony ontogeny and the emergent superorganismal features of insect societies (Wheeler 1910) (Fig. 1).

Debates remain regarding the function and mode of action of queen pheromones, which were at times considered to comprise means of queen control over reproductive activities of nestmates (Fletcher and Ross 1985) but are now widely acknowledged to be honest signals of identity and quality of the queen (Keller and Nonacs 1993; Villalta et al. 2018). Queen pheromone-induced behavioral shifts in workers thus may be explained ultimately by the alignment of their evolutionary interests with the queen's from a kin

Fig. 1 Summary of generalized functional properties of queen pheromones in ants, as exemplified in a polygyne *Solenopsis invicta* colony. These functions include: 1) Attracting workers to the queen; 2) Promoting colony maintenance tasks, such as brood care; 3) Inducing nestmate discrimination; 4) Inhibiting larval sexual development, through worker behaviors; 5) Inhibiting adult reproductive physiology, thereby preventing dealation, ovarian development, and egg laying in other queens, gynes, and workers; 6) Inducing worker policing (in many cases, eggs serve as agents for the dissemination of queen pheromones); 7) Inducing the execution of supernumerary queens in monogyne colonies; 8) Mediating the regulation of queen acceptance in polygyne colonies. Gray numbers indicate functional categories where a chemical basis has not yet been demonstrated



selection perspective (Hamilton 1964; Foster et al. 2006). Workers are expected to achieve a higher inclusive fitness return associated with the ecological benefits of colony life if they help rear sisters rather than produce their own offspring in social hymenopterans, especially when the colony is headed by a single monogamous queen, the presumed ancestral condition for most eusocial insect lineages (Hughes et al. 2008; Boomsma and Gawne 2018; Kay et al. 2019). Thus, when the kinship structure changes following loss of the queen, workers prioritize their direct fitness and compete for reproduction pending establishment of a new reproductive hierarchy, rather than performing colony maintenance or other duties (Bourke 1988; Wenseleers et al. 2020b). Exceptions occur in species with obligately sterile workers, where workers are selected to maximize the rate of sexualization of the remaining brood because, by definition, they lack any route for direct fitness returns.

Pheromones can be categorized generally as having either releaser or primer effects. Releaser effects include immediate, typically behavioral responses, while primer effects are slower and progressive, often physiological responses (Conte and Hefetz 2008; Vargo 2019). A difficulty in deciphering queen pheromones stems from their multifunctional and multicomponent characteristics: at times, multiple molecules synergistically trigger a response, while in other instances, a single compound can elicit both releaser and primer effects (Grozinger et al. 2007).

Thus far, only the queen pheromones in honey bees are sufficiently well studied that a systematic understanding linking the identities of constituent chemicals, modes of action, and known functions has emerged (Keeling et al. 2003; Bortolotti and Costa 2014; Princen et al. 2019). Despite over seven decades of effort, we have only identified pheromonal compounds in a few ant species that were associated with some, but not all, known and expected functions of queen pheromones. Meanwhile, the past decade has seen considerable progress of queen pheromone identification in otherwise understudied groups of social insects such as sweat bees, wasps and termites.

This review provides a comprehensive discussion of functional properties of queen pheromones in ants, structured with a strong focus on bioassay results and the underlying natural history and sociobiology of ant colonies, which is lacking in recent reviews of similar topics (Table 1). Despite the evident limited focus on ants, the pheromonal effects discussed are largely congruent with other social insects (Table 2). I first cover the effects of queen pheromones on colony integrity and coherence. I then discuss how queen pheromones help to maintain the reproductive dominance of the queens as well as the overall reproductive division of labor in a colony. Next, I review recent studies on how queen pheromones mediate the regulation of colony social structures, specifically regarding the number of queens in

the colony. Lastly, I discuss general characteristics of queen pheromones and highlight unanswered questions as a guide to future research regarding eusocial insect queen pheromones (Box 1).

Effects on colony integrity and coherence

Induction of retinue and rescue behavior

Upon examining the queen in an ant colony, one notices that she is surrounded by “retinue” workers that often lick her cuticle or otherwise groom her, a scenario commonly observed in other social insects such as honeybees and termites (Wilson 1971; Bortolotti and Costa 2014). A prominent releaser effect of the queen pheromone is attraction, enabling workers to locate the queen, so as to care for or rescue her. In a way, the queen acts as the “gravity center” that holds the colony together. This notion is evident in the rock ant *Temnothorax rugatulus*, a frequently emigrating species; when a colony was experimentally split into two nests under equal conditions, all members almost always reunited with the queen in one of the nests (Doering and Pratt 2016).

Early investigations pointed to the existence of chemical pheromones by showing that queens were attractive to their workers, and that this attraction capacity could be transferred in chemical extracts. *Pheidole* workers would adopt corpses of *Lasius* queens treated with *Pheidole* queen extracts (Stumper 1956). Queenless workers of *Myrmica* will generally accept queens of a closely related species (Brian 1986a, 1988a, b), which is likely due to the similarity of the chemical makeup of queen recognition signals among recently diverged taxa, a feature that ultimately may pave the way for the rise of social parasites (Lenoir et al. 2001). The chemical basis of retinue behavior pheromones was verified from experiments showing the same effect by queen corpses or queen cuticular extracts in a wide range of taxa (Watkins and Cole 1966; Jouvenaz et al. 1974; Fowler and Roberts 1982; Hölldobler and Wilson 1983; Wilson and Hölldobler 1985).

Fire ant workers (*Solenopsis invicta*) exhibit emphatic and swift responses to a queen exposed outside of the colony: workers will (1) quickly be attracted to her, (2) cluster around her, (3) move brood items to or around her, (4) form a pheromonal trail that the queen can follow back to the nest, and/or (5) pull the queen towards the nest, should she not move voluntarily (Glancey et al. 1983). Workers exhibit the same series of stereotyped behaviors toward a paper dummy dosed with reproductive queen hexane extract as they do toward live queens or fresh queen corpses, i.e., collectively retrieving the treated dummy into the nest and keeping it there for hours (Trible and Ross 2016; Zeng et al. 2022).

Table 1 List of ant genera experimentally studied and discussed in this review. The column Common Name provides common names for selected invasive species that are representative and extensively researched. In the Caste Dimorphism column, the entry “Pronounced” denotes that workers cannot transition into gamergates to replace the queens, whereas “Modest” indicates the opposite. Cells with reference entries indicate the demonstration of functional properties of queen pheromones through bioassays, including chemical extract assays and queen removal experiments, while compound names or chemical groups in parentheses indicate the identified queen pheromone components. Some studies in the “Attracting workers” functional category are not from controlled experiments but descriptions of natural history. The star sign (*) following a specific reference suggests that this study provided a counter-example to the corresponding queen pheromone function

Subfamily	Genus	Common Names	Caste Dimorphism	Workers Retain Functional Ovaries	Queen Pheromone Effect Category								
					Attracting workers	Promoting colony maintenance and coherence	Inducing nestmate discrimination	Inhibiting larval sexual development	Inhibiting reproductive physiology of adult females	Inducing worker policing	Inducing execution of superfluous reproductive adults	Mediating queen acceptance in polygyne colonies	
Myrmicinae	<i>Myrmica</i>		Pronounced	Yes	Brian 1988a, b	Vienne et al. 1998	Brian and Hibble 1963; Brian and Carr 1960; Brian 1973	Brian and Rigby 1978					
Myrmicinae	<i>Myrmica</i>	Bull ants	Modest	Yes	Dietemann et al. 2003								
Myrmicinae	<i>Leptothorax & Temnothorax</i>	Rock ants; acorn ants	Pronounced	Yes	Doering and Pratt 2016	Keiser et al. 2018; Giehr et al. 2019		Dejean and Passera 1974; Bourke 1993*; Brunner et al. 2011		Stroeymeyt et al. 2007			
Myrmicinae	<i>Novomessor & Aphaenogaster</i>		Pronounced	Yes	Lubertazzi 2012	Ichinose et al. 2009*	Hölldobler and Carlin 1989; Boulay et al. 2007, 2009			Smith et al. 2009, 2011 (unbranched alkanes)	Chéron et al. 2009		
Myrmicinae	<i>Solenopsis</i>	Fire ants	Pronounced	No	Jouvenaz 1974; Tribble and Ross 2016	Obin and Vander Meer 1989; Vander Meer and Alonso 2002	Vargo and Fletcher 1986a, b; Vargo 1988; Klobuchar and Deslippe 2002	Fletcher and Blum 1981, 1983b; Vargo and Laurel 1994		Not expected	Fletcher and Blum 1983a; Fletcher 1986; Vander Meer and Alonso 2002	Zeng et al. 2022 (unsaturated CHC's)	

Table 1 (continued)

		Queen Pheromone Effect Category										
Subfamily	Genus	Common Names	Caste Dimorphism	Workers Retain Functional Ovaries	Attracting workers	Promoting colony maintenance and coherence	Inducing nestmate discrimination	Inhibiting larval sexual development	Inhibiting reproductive physiology of adult females	Inducing worker policing	Inducing execution of superfluous reproductive adults	Mediating queen acceptance in polygynous colonies
Myrmicinae	<i>Monomorium</i>	Pharaoh ants	Pronounced	No	Oliveira et al. 2020 (monocyclic diterpene neocembrene)			Petersen-Braun 1975, 1977; Edwards and Chambers 1984; Edwards 1987; Oliveira et al		Not expected		
Myrmicinae	<i>Atta</i>	Leaf cutting ants	Pronounced	Yes		Della Lucia et al. 2003; Sousa-Souto and Souza 2006						
Myrmicinae	<i>Pheidole</i>	Big-headed ants	Pronounced	No	Stumper 1956; Wilson and Hölldobler 1985					Not expected		
Formicinae	<i>Camponotus</i>	Carpenter ants	Pronounced	Yes	Fowler and Roberts 1982				Endler et al. 2006			
Formicinae	<i>Cataglyphis</i>	Desert ants	Pronounced	Yes		Berton et al. 1992			Van Oystaeyen et al. 2014 (n-C27, n-C29, 3MeC29)			
									Carlín and Hölldobler 1983, 1986, 1987; van Zweden et al. 2009*			
									Carlin and Hölldobler 1983, 1986, 1987; van Zweden et al. 2009*			
									Lahav et al. 1998			

Table 1 (continued)

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Formicinae	<i>Formica</i>	Wood ants; field ants	Pronounced	Yes	Orius and Heinze 1999	Helanterä and Sundström 2005	Helanterä and Sundström 2005	Helanterä and Sundström 2005	Helanterä and Sundström 2005, 2007			
Formicinae	<i>Lasius</i>	Garden ants	Pronounced	Yes	Holman et al. 2010 (3-MeC31)			Holman et al. 2010, 2013; Holman 2012 (3-MeC31)				
Formicinae	<i>Oecophylla</i>	Weaver ants	Pronounced	Yes	Hölldobler and Wilson 1983			Hölldobler and Wilson 1983				
Formicinae	<i>Plagiolepis</i>		Pronounced	Yes				Passera 1980				
Dolichoderinae	<i>Linepithema</i>	Argentine ants	Pronounced	Yes	Vargo and Passera 1991; Passera et al. 1995						Vásquez and Silverman 2008	
Dorylinae	<i>Neivamyrmex</i>	Army ants	Pronounced	No	Watkins and Cole 1966					Not expected		
Ectatomminae	<i>Gnampitogenys</i>		Modest	Yes					Gobin et al. 1999			
Ectatomminae	<i>Rhytidoponera</i>		Modest	Yes			Crosland 1990*					
Ponerinae	<i>Diacamma</i>		Modest	Yes					Tsuji et al. 1999			
Ponerinae	<i>Dinoponera</i>	Giant Amazonian ants	Modest	Yes						Monnin et al. 2002		
Ponerinae	<i>Harpegnathos</i>	Jumping ants	Modest	Yes				Penick and Liebig, 2017	Powell and Tschinkel 1999; Peeters et al. 2000; Penick et al. 2014			

Table 1 (continued)

Subfamily	Genus	Common Names	Caste Dimorphism	Workers Retain Functional Ovaries	Queen Pheromone Effect Category							
					Attracting workers	Promoting colony maintenance and coherence	Inducing nestmate discrimination	Inhibiting larval sexual development	Inhibiting reproductive physiology of adult females	Inducing worker policing	Inducing execution of superfluous reproductive adults	Mediating queen acceptance in polygynous colonies
Ponerinae	<i>Odontomachus</i>	Trap jaw ants	Pronounced	Yes	Smith et al. 2012b [(Z)-9-nona-cosene]							Smith et al. 2012b, 2013 [(Z)-9-non-acosene]
Ponerinae	<i>Pachycondyla</i>		Modest	Yes								D'Ettorre et al. 2004a

Additionally, queenless workers infrequently exhibit vibrant body shaking upon retrieving a queen (Zeng, personal observation), which resembles the jerking response to a queen or king in termites (Funaro et al. 2018, 2019).

A general correlation between the intensity of attraction and the fecundity or weight of a queen was noted by many studies (Sommer and Hölldobler 1995; Hannonen et al. 2002). Such a trend is pertinent to other queen pheromone effects in the discussion that follows, and is most parsimoniously explained by a higher level of pheromone production occurring in more fertile queens (Fletcher and Blum 1983a). This collective, accurate assessment of the fertility condition of an individual is fundamental to the signaling function of queen pheromones (Keller and Nonacs 1993).

Promotion of colony maintenance

In many ant species, a newly mated queen performs all necessary tasks to initiate the growth of the colony by herself. She prepares a nest site (e.g., digs a burrow in the soil) and raises the first cohort of workers from the eggs she then lays. But when worker adults emerge, the queen stops engaging in colony maintenance tasks and transitions to acting strictly as an egg layer (Cassill et al. 2002; Majidifar et al. 2022). The continued presence of the queen not only ensures the steady production of fertilized eggs and, subsequently, additional brood and adults, but a few studies suggest that the queen also boosts the level of worker activity in brood care and acts to maintain the cohesion of the colony. In other words, the queen (or queen pheromones) may function as a “catalyst” to promote colonial development and maintenance, on top of being a “gravity center” to maintain colony cohesion, as shown in the studies below.

In broodless colonies of *Cataglyphis cursor*, overall worker activities were reduced after queen removal (Berton et al. 1992). Queenright workers of *Myrmica* sp. and *Manica* sp. antennated the brood more often and stayed longer with the brood than queenless workers (Vienne et al. 1998). In contrast, queenless workers tend to leave the nest, interacting more often with adult workers instead of the brood. Similarly, in *Atta sexdens*, workers departed more frequently from the nest, exhibited higher mortality and lowered refuse accumulation, but showed no change in foraging efficiency, when the queen was removed (Della Lucia et al. 2003; Sousa-Souto and Souza 2006). In *Temnothorax curvispinosus*, queenright sub-colonies outperformed queenless counterparts in various task efficiencies and were more resistant to fungal pathogens (Keiser et al. 2018). In *Temnothorax crassispinus*, queen presence promoted defecation within the nest which may help to suppress mold (Giehr et al. 2019).

These studies showed that the presence of the queen induced a higher level of brood care, stronger cohesion, and better overall performance of the colony. At the proximate

Table 2 Current knowledge of queen pheromones research in major groups of social insects. Among the color-filled cells, darker green indicates that identified compounds or groups of compounds convey the corresponding pheromonal effect in a social insect group. Lighter green signifies a pheromonal basis demonstrated through the effect of

contact or bodily or tissue extract of the queen. Yellow represents the effect shown through queen removal or isolation experiments. Gray indicates the effect observed when the queen is present, with a speculated pheromonal basis. White denotes that the effect was either not shown or not expected

	Maintaining Colony Cohesion			Maintaining Reproductive Dominance			Mediating Colony Social Structure	
	Attracting workers and eliciting unique response	Promoting colony maintenance and coherence	Inducing nestmate discrimination	Regulating larval sexual development	Inhibiting reproductive physiology of adult females	Inducing worker policing	Inducing execution of superfluous reproductive adults	Mediating queen acceptance in polygynous colonies
Honey Bees (family Apidae, <i>Apis</i> spp.)	Kaminski et al. 1990; Keeling et al. 2003	Winston et al. 1989, 1991 (maintaining swarm)	Breed et al. 1992	Pettis et al. 1995; Melathopolous et al. 1996	Hoover et al. 2003; Katzav-Gozansky et al. 2006	Ratnieks 1995 (Dufour's gland secretion)	Gilley 2001; Tarpay and Fletcher 2003 (honeybee workers typically don't kill queens but will help in queen duels)	
Bumble Bees (family Apidae, <i>Bombus</i> sp.)	van Doorn and Chrambach 1989			Alaux et al. 2004; Lopez-Vaamonde et al. 2007	Van Oystaeyen et al. 2014; Amsalem et al. 2015; Holman et al. 2017; Orlova and Amsalem 2012	Zanette et al. 2012	Van Doorn 1986	
Stingless Bees (family Apidae, <i>Friesella</i> sp.)	Imperatriz-Fonseca and Zucchi 1995; Jarau et al. 2010				Nunes et al. 2014			
Sweat Bees (family Halictidae)	Steitz and Ayasse 2020 (inducing workers to quickly back away)				Steitz et al. 2019; Steitz and Ayasse 2020			
Ants (family Formicidae)	See Table 1	See Table 1	See Table 1	See Table 1	See Table 1	See Table 1	See Table 1	See Table 1
Wasps (family Vespidae)	Ishay et al. 1965	Ishay et al. 1965	Gamboa et al. 1986	Ikan 1969 (initiate queen cell construction); Mori and Otsuka 1985	Oi et al. 2015, 2016	Oi et al. 2015		
Termites (<i>Reticulitermes</i> spp.)	Funaro et al. 2018 (eliciting antennation and shaking of the body by workers and soldiers)	Matsuura et al. 2010 (stimulating egg-carrying and piling behavior)	Konishi and Matsuura 2021	Matsuura et al. 2010 (inhibition of the differentiation of workers into supplementary queens)	Yamamoto and Matsuura, 2011 (suppression of egg laying in secondary queens)	Sun et al. 2020		

level, these shifts in worker behavior might be manifested as secondary effects of the attractiveness of the queen causing, for instance, a more structured spatial distribution of the brood and, as a result, more efficient brood care. These results are consistent with findings in honeybees where queen

mandibular pheromones are shown to stimulate a wide range of worker task performance, including foraging, defense, nest building, and brood rearing (Bortolotti and Costa 2014). However, unlike honey bees, these effects in ants remained to be associated with a chemical basis (Box 1, Q1).

Box 1 Questions and prospective avenues for further study of ant queen pheromones

Q1:

The presence of the queen has been shown to increase task performance efficiency, the health condition, and the cohesion of workers. However, the pheromonal basis of these effects remains to be confirmed through experiments using queen corpses or chemical extracts.

Q2:

Does the effect on nestmate discrimination have a pheromonal basis? If so, what specific mechanisms are involved in how queen pheromones affect worker nestmate discrimination?

Q3:

Experiments have demonstrated that workers respond to queen pheromones by actively suppressing larval sexual development. However, do larvae also sense queen pheromones? And do queen pheromones directly affect larval physiology?

Q4:

Under the influence of the queen, ant workers exhibit targeted destruction of broods based on specific sex, caste, and life-stage. How does a queen pheromone function to trigger only a narrowed subset of the discriminatory behavioral repertoire of nurse workers?

Q5:

There is a strong but variable connection between the queen pheromones responsible for nestmate recognition, fertility signaling, and regulation of reproduction. A major task is to determine whether, in specific cases, these comprise one or more unique semiochemical systems and modes of action. Furthermore, it is important to investigate if the mode of action (physiology) of certain effects is consistent across different ant lineages.

Q6:

As more genetic components have been identified that underlie social polymorphism in ants, What are the roles of queen pheromones in regulating colony social structure (polygyny) in species other than *S. invicta*?

Q7:

Unsaturated CHCs (cuticular hydrocarbons) serve as signals for queen supergene status in *S. invicta*. The identification of the complete blend will reveal the first set of pheromones involved in the regulation of colony social structure in a highly eusocial insect.

Q8:

CHCs have been identified as fertility signals in numerous, but not all, species. How do these fertility signals change across different ant lineages? Is there a relationship between phylogenetic distance and the similarity of fertility signal compounds?

Q9:

In the evolution of ant sociality, at what point did queen pheromones become essential? Is there a relationship between the degree of queen-worker dimorphism and the strength of the effect of queen pheromones (Smith and Liebig, 2017)?

Induction of nestmate discrimination

Similar to other eusocial insects, the nest of an ant colony comprises the physical structure, food storage, and brood items. It requires substantial investment to build and is valuable to looting and raiding, often from sympatric conspecific colonies (Hölldobler and Michener 1980; Tschinkel 1992; Sturgis and Gordon 2012). To safeguard the nest from intruders and ensure sustainable growth, a colony must deploy effective nestmate discrimination. Each ant colony carries a set of cuticular chemical odor labels, which workers use to distinguish nestmate from non-nestmate conspecifics (Ozaki et al. 2005; Sturgis and Gordon 2012).

The presence of the queen has a significant impact on the odor label of a colony, such that queenless colonies exhibit reduced territoriality, acting as if they have lost some component of their distinct colony identity. Compared to queenless workers, workers from queenright colonies are more subject to aggression by non-nestmate conspecific workers, as well as being more aggressive themselves towards such workers.

Queenless *S. invicta* workers of the monogyne social form (single queen per colony) received little aggression from their original nestmates when returned to the natal queenright colony. However, if they tended a foreign queen for only 15 minutes, they were attacked by their original nestmates (Obin and Vander Meer 1989). Queenless workers also became less aggressive themselves after queen removal, becoming completely docile after about two weeks (Vander Meer and Alonso 2002). Thus, in monogyne fire ants, a colony's unique chemical identity is attributable, at least in part, to its sole reproductive queen.

Similar phenomena were demonstrated in other species. Queenless workers of *Cataglyphis niger* did not show aggression towards and did not receive aggression from original nestmate workers from the queenright parent colony (Lahav et al. 1998). In *Camponotus* species, worker aggression towards non-nestmates largely disappeared in queenless colonies and reappeared after an unrelated queen was adopted into the colony (Carlin and Hölldobler 1983, 1986, 1987). Additionally, queens with less developed ovaries or that were incompletely inseminated had a weaker such effect than normal queens (Carlin and Hölldobler 1987).

However, as a counterexample, queen presence had little impact on nestmate discrimination in *Camponotus aethiops*, a discrepancy that was attributed to the presence of heritable chemical cues on workers (van Zweden et al. 2009). Another example came from *Rhytidoponera confusa*, where nestmate discrimination also was not affected by the presence of the queen (Crosland 1990). Queen-worker dimorphism in *R. confusa* is not pronounced and the colonies can reproduce without the queen (Ward 1981, 1983). Because the queen is not an indispensable component of the colony identity, it is

plausible that her absence does not impact worker nestmate recognition abilities (Carlin and Hölldobler 1991).

These results point to the intricate nature of nestmate discrimination in ant colonies, which is governed by both environmentally derived cues and genetic factors (Carlin and Hölldobler 1983; Helanterä et al. 2011; Sturgis and Gordon 2012; Caliani Oliveira et al. 2022). Unraveling the specific mechanisms by which queen pheromones affect nestmate discrimination remains a challenge (Box 1, Q2). One possibility is that workers obtain queen cuticular odor cues in small colonies through common contact with the queen (Lahav et al. 1998). Another possibility is that queenless workers retain the ability to detect non-nestmates but lack the incentives to act aggressively toward them.

Maintenance of reproductive dominance

Inhibition of larval sexual development

As the hallmark of eusociality, reproductive division of labor necessitates that members of the queen caste maintain their reproductive dominance. In small colonies, the queen may manipulate larval caste fate and suppress adult reproduction through physical actions (Heinze and Smith 1990), which is frequently observed in wasp societies where caste dimorphisms are not distinct (Ross and Matthews 1991). However, in populous colonies, the effect is often achieved via an essential and well-studied class of queen primer pheromones that exert their effects onto almost all life stages of female colony members (Smith and Liebig 2017; Holman 2018).

To begin with, queen pheromones have been shown to inhibit sexualization (development as queens) of female larvae, thus biasing female development toward worker production over gyne (virgin winged queen) production.

Early studies on the subject came from *Myrmica* species, where the queen induces improved larval survival, earlier pupation, and lower larval and pupal weights, in apparent accord with the notion of the queen stimulating general colony function and cohesion (Brian 1957, 1986b; Brian and Carr 1960). When the queen was present, large gyne-destined larvae received less care from workers than small worker-destined larvae (Brian and Hibble 1963); moreover, the workers lethally bite gyne-destined larvae (Brian and Carr 1960; Brian 1973). The presumed pheromone was not volatile while, notably, structural and topological features of the queen played a role in the pheromonal effect (Brian 1970, 1973).

In the Pharaoh's ant, *Monomorium pharaonis*, the presence of a fertile queen inhibited development of sexual brood (Petersen-Braun 1975, 1977). Unlike the case in *Myrmica*, this effect was disseminated specifically by the queen-laid eggs, but not queen corpses or solvent extracts (Berndt and

Nitschmann 1979; Edwards 1987; Boonen and Billen 2017). Queenright workers always accept worker broods from a foreign colony but would cannibalize any introduced sexual broods (Edwards 1991). Monocyclic diterpene neocembrene, a compound produced only by egg-laying queens, was found to be a pheromonal component in *M. pharaonis* as it elicited weak “queen retinue” attraction as well as inhibited production of sexuals (Edwards and Chambers 1984; Oliveira et al. 2020).

Evidence from the two above examples and many other species strongly suggested that queen pheromones affect caste development through the behavior of workers, including biting the larvae to suppress growth evident by bite marks on larvae, or simply killing sexualized larvae (see Supplementary Information for more details; Table 1; Box 1, Q3). This is also the case in honey bees and stingless bees where workers control the caste fate of larvae, besides genetic and maternal factors (Bueno et al. 2023).

Adult workers probably sense queen pheromones mainly through antennal chemoreceptors, which was supported by strong electrophysiological responses of worker antennae towards queen extracts and candidate queen pheromones (D’Ettorre et al. 2004b; Holman et al. 2010; de Narbonne et al. 2016). Following perception, workers respond to queen pheromones by actively suppressing the sexual development of some larvae via the behaviors above (Box 1, Q4).

Inhibition of reproductive physiology of adult females

As the best studied effect in many groups of social insects, queen pheromones suppress physiological changes tied to the onset of reproduction in adult females in the colony, which can be measured in diverse behavioral and physiological changes in the trajectory of reproductive development, including dealation, ovarian activation, weight gain, and finally egg laying.





Dealation, or wing shedding, is the first observable indication of the onset of reproduction development in adult

gynes in many ants. Take the example of *S. invicta*, virgin gynes in queenright colonies typically remain winged until a mating flight event, after which the newly mated gynes kick off their wings to initiate colony founding underground (Tschinkel 2013). However, these gynes can dealate as soon as 12 h after separation from fertile queens, with their alary muscles beginning to histolyze simultaneously followed by ovarian development, and oviposition starts in another 2 to 3 days (Fletcher and Blum 1981, 1983b; Vargo and Laurel 1994). By this time, the gyne begins to exhibit attractiveness in the formation of a queen retinue and to produce the inhibitory pheromones (Vargo 1999).

The fecundity of a queen is correlated to her weight and the ability to suppress reproductive development in nestmate queens. The correlation is likely due to a link between weight and the level of pheromone production (Fig. 2). Evidence comes from the fact that queenless monogyne workers consistently recognized and adopted the heavier queen of two presented as their new queen (Fletcher and Blum 1983a). Corpses of heavier queens suppressed dealation for longer than light-weight queen corpses (Fletcher and Blum 1983b; Willer and Fletcher 1986). Such an inhibitory effect acts on other egg-laying reproductive queens as well: the addition of live queens or queen corpses reduced fecundity of all nestmate queens in polygyne colonies (Vargo and Laurel 1994).

Perception of queen pheromones via the antennal sensilla leads to a downregulation of dopamine production, which in turn suppresses the production of juvenile hormones (JH) and inhibits reproductive development (Robinson and Vargo 1997; Boulay et al. 2001). JHs are critical regulators not only of reproduction, but of development and behavior, throughout the lifecycle of insects (Jindra et al. 2013). Topical treatment of alate gynes with JH or JH analogue induced dealation in *S. invicta* even in the presence of the queen, overriding the inhibitory effect of the queen pheromone (Vargo and Laurel 1994). Notably, JH treatment can yield opposing effects on reproductive development depending on the species and the size of the treatment doses used,

Fig. 2 Characteristics of ant queen pheromones exemplified by *Solenopsis invicta*

Types of Female	 Worker	 Alate Gyne	 Polygyne Reproductive Queen (or Virgin Dealate)	 Monogyne Reproductive Queen
Mating Status	Unmated	Unmated	Unmated or mated	Mated
Fertility Condition	Sterile (unable to lay eggs)	None (not laying eggs)	Low	High
Pheromone Quantity	None	Negligible	Low	High
Effect Strength of Queen Pheromone	None	Negligible	Moderate	Strong

suggesting a condition-dependent cost of JH and calling for more studies on the endocrinological regulation of reproduction (Robinson and Vargo 1997; Cuvillier-Hot et al. 2004; Penick et al. 2011; Holman 2012).

The inhibitory effects on worker reproduction received detailed studies in *Camponotus*, where eggs are again the dissemination agents of the pheromones. The addition of queen-laid eggs prohibited workers from laying eggs in queenless colonies; in addition, worker-laid eggs were less prone to destruction when applied with queen cuticular hydrocarbons (CHCs) (Endler et al. 2004). As a further support, surface chemical profiles of eggs corresponded to the cuticular chemical profiles of the respective egg-laying queen or worker (Endler et al. 2006).

In *Lasius* species, the compound 3-methylhentriacontane (3-MeC31) suppressed egg-laying of workers, making it the first identified queen pheromone with inhibitory effects on worker reproduction (Holman et al. 2010, 2013; Holman 2012). Further analysis indicated a slower evolution of this compound compared to other CHCs in *Lasius*, hinting at potential evolutionary constraints on queen signals, but did not agree with the findings in *Temnothorax* species (Brunner et al. 2011; Holman et al. 2013). Other studies have shown inhibitory effects on worker reproductive physiology by live queens, queen corpses, queen-laid eggs, or queen CHCs across various ant taxa (Table 1; Supplementary Information; Box 1, Q5).

Another general effect of such inhibitory pheromones is a shortening of longevity. Reproduction and longevity are typically a trade-off in animals (De Loof 2011; Blacher et al. 2017). However, in social insects, reproduction and longevity are instead positively linked (Blacher et al. 2017), perhaps due to a reproductive division of labor where queens are liberated from costly daily tasks. When worker ants become reproductively active after queen removal, they also showed extended lifespans, as documented in some ant species as well as in other social insects (Tsuji et al. 1996; Kohlmeier et al. 2017; Vollet-Neto et al. 2018; Majoe et al. 2021; Negroni et al. 2021).

Harpegnathos saltator workers are fully capable of reproduction (Peeters et al. 2000). Removal of the queen (and her pheromones) prompted workers to engage in antennal duels, a ritualistic competition to re-establish hierarchy that occurs in many ponerine species (Powell and Tschinkel 1999; Peeters et al. 2000; Penick et al. 2014). Winners of these duels transition into gamergates (Sasaki et al. 2016), which had about five times the lifespan of normal workers (Yan et al. 2022). These gamergates displayed queen-like physiology, with decreased brain and optic lobe volumes, and decreased venom production. They also behaved more like queens, remaining inside the nest and hiding from intruders (Penick et al. 2021). Nevertheless, these queen-like traits can

revert back to a worker-like state if a gamergate is exposed to a strong source of queen pheromones (Penick et al. 2021), such is the case in other social insects (Van Oystaeyen et al. 2014).

Induction of worker policing

Although the queen is the dominant reproductive member, workers in many ant species can potentially produce males by laying unfertilized eggs. These egg-laying workers pose a source of conflict over male parentage within the colony, as well as a cost to the colony productivity (Helanterä and Sundström 2007; Bourke and Franks 2019). The queen(s) represses reproduction of workers through pheromonal inhibition as discussed above, or through behaviors such as destruction of worker-laid eggs (Bourke 1991). Workers themselves also police reproduction of nestmate workers, a behavior documented in many social hymenopterans that might have evolved concurrently with eusociality (Ratnieks 1988; Frank 1995, 2003; Wenseleers et al. 2020a).

Typical acts of policing in social insect colonies include direct aggression toward adults or destruction of their eggs (Ratnieks and Visscher 1989; Beekman and Oldroyd 2005). Mechanistically, workers must (i) recognize queen presence through queen pheromones and (ii) correctly assess the fertility status of colony members, as well as recognize the origin of offspring through pheromones present on the egg surface or post-embryonic cuticle (Ratnieks 1995; Oi et al. 2015b). In some species, queens actively mark suspect individuals with pheromones to “command” worker policing.

Pachycondyla workers would lay viable embryonated eggs when the workers were physically separated from the queen (Dietemann and Peeters 2000). Worker-laid eggs were eaten more frequently by nestmate workers than queen-laid eggs, and such policing was more prominent when the queen was present (D’Ettorre et al. 2004a). Notably, the potential pheromonal cues by which workers distinguish egg origin were persistent and non-transferable through mutual contact between the eggs (D’Ettorre et al. 2006).

Workers in the genus *Formica* could distinguish nestmate eggs from non-nestmate eggs (Helanterä and Ratnieks 2009; Helanterä et al. 2014), and worker-laid eggs from queen-laid eggs, but the latter ability was displayed only when an adult queen was present (Helanterä and Sundström 2005, 2007). Corresponding to the above finding, hydrocarbon profiles of eggs displayed robust and consistent differences among species, colonies, and even among matriline within a colony, demonstrating a link between genetic variation and potential pheromonal variation (Helanterä et al. 2014; Helanterä and d’Ettorre 2015).

In some cases, the egg-laying workers themselves, but not their eggs, were subject to policing. In *Temnothorax*

unifasciatus, that reproductive workers were attacked, not by random nestmates, but only by a select few workers who would become dominant reproductives upon queen removal (Stroeymeyt et al. 2007). Likewise, in *Novomesor cockerelli* (previously *Aphaenogaster cockerelli*), worker-laid eggs did not differ from queen-laid eggs in their surface chemical profiles and were not policed (Smith et al. 2008a). Instead, egg-laying workers were attacked by nestmate workers (Smith et al. 2011). Reproductive status is signaled by unbranched alkanes, as the application of these compounds on non-reproductive workers induced nestmate aggression, but only in the presence of a queen (Smith et al. 2009). The queen also attacked and marked reproductive workers for aggression by discharging compounds from her Dufour's gland onto the target worker (Smith et al. 2012a). This is similar to the finding in *Dinoponera quadriceps*, where high-ranking gamergates mark challengers with Dufour's gland secretion to direct aggression by low-ranking workers (Monnin et al. 2002).

In *Odontomachus brunneus*, a hydrocarbon, (Z)-9-nonacosene, was identified as a fertility signal, based on three lines of evidences: (i) its higher abundance in reproductive individuals, (ii) the typical submissive gesture of nestmate workers towards workers treated with the compound (Fig. 3), and (iii) the nestmate policing (biting and pulling) of treated workers in queenright colonies (Medeiros et al. 1992; Smith et al. 2012b, 2013). The role of this compound was conserved across geographic populations, but it must function synergistically with other pheromonal chemicals (Smith et al. 2013, 2015).

Induction of execution of superfluous reproductive adults

A queenright monogyne colony is generally not expected to accept additional reproductive queens as this would decrease indirect fitness benefits to workers despite the apparent benefit of larger social groups (Hamilton 1964; Gardner et al. 2011). In species with strong caste dimorphism, superfluous queens are eliminated by workers, which may be considered as an extreme form of policing, as these queens cannot transition back to a worker-like state and contribute to colony

tasks. As a requirement, workers rely on queen pheromones that signal the presence and identity of the true queen.

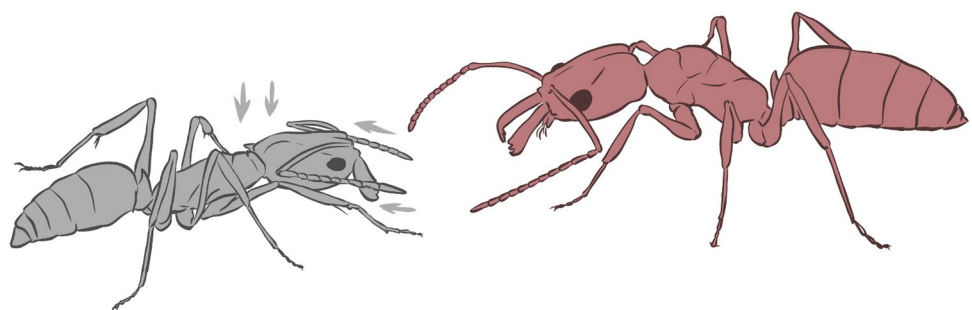
Monogyne *Solenopsis invicta* workers imprint on the pheromonal signature of their mother queen, killing any other dealate (wingless reproductive) queens presented to the colony (Fletcher and Blum 1983a; Gotzek and Ross 2007). Only when a colony is rendered queenless for a few days will it accept an unrelated queen, and the longer the colony stays queenless, the more accepting of a foreign queen it becomes (Fletcher 1986; Vander Meer and Alonso 2002). Additionally, when presented with multiple reproductive queens, such hopelessly queenless workers usually select the most physogastric one, which might be due to a higher amount of fertility signal produced by such queens (Fletcher and Blum 1983a).

In *Aphaenogaster senilis*, workers attack supernumerary gynes and only the oldest gyne ascends to become the sole reproductive queen (Chéron et al. 2009). In Argentine ants, *Linepithema humile*, queenless colonies show lower aggression towards intruder queens compared to queenright colonies, which usually kill intruder queens within 24 h (Vásquez and Silverman 2008). Here, adoption decisions were not influenced by fecundity, but by similarity of CHC profile to the nestmate queens (Vásquez and Silverman 2008; Vásquez et al. 2008). This was contrary to *Camponotus floridanus*, where a more fertile queen would be accepted into a queenright colony while a less fertile queen would not (Moore and Liebig 2010). Thus, there appears to be variable linkages between queen pheromones involved in nestmate recognition, fertility signaling, and regulation of reproduction in ants.

Regulation of colony social structure

An archetypical colony of social insects is composed of a single family, headed by a single queen and her offspring workers. It is less known that multiple-queen colonies occur in many ant taxa and such variation of colony social structure, being either monogyne (single-queen) or polygyne (polygyne), exist both within species and across species. Phylogenetic analysis suggested that eusociality of ants

Fig. 3 Workers (gray) of *Odontomachus* species display typical submissive gestures (crouching their bodies and retracting their antennae) upon detecting a queen (red) or queen pheromones



evolved under the monogyne condition, while polygyne forms subsequently evolved independently in many ant taxa (Ross and Carpenter 1991; Hughes et al. 2008). In a general sense, the evolution of social structure (from monogyny to polygyny) and the evolution of eusociality in ants, raised similar problems as to why individuals are willing to forgo personal reproductive success for the benefit of group reproductive output.

Queen pheromones are also involved in the regulation of such variation in colony social structure, an important but often overlooked class of function. Although this function of queen pheromones may be ubiquitous in diverse ant taxa (Hölldobler and Carlin 1985; Evison et al. 2012; Abril and Gómez 2019), the only such case that has received careful study to date is the regulation of colony social form in *S. invicta* (Box 1, Q6).

In stark contrast to the single-queen, monogyne form, the polygyne form houses multiple reproductive queens, as many as a few hundred, in a colony. The two social forms are distinct from each other in many other natural history traits, such as nest density in the wild, the average weights of alate gynes and reproductive queens, colony founding mode, and worker size distributions (Keller and Ross 1995; Gotzek and Ross 2007; Tschinkel 2013; Huang and Wang 2014). The genetic underpinning of this social form polymorphism in *S. invicta* and several congeners is an inversion-based selfish genetic element termed the *Social b* (*Sb*) supergene. The element spans a large portion of chromosome 16, comprising three adjacent inversions and encompassing over 500 described genes (Yan et al. 2020; Stolle et al. 2022; Helleu et al. 2022).

In monogyne colonies, all female members are homozygous for alternate, wild-type haplotype (*SB*), and only one *SB/SB* reproductive queen is tolerated. In polygyne colonies, all reproductive queens and over half of the worker population are heterozygous at the supergene locus. Polygyne workers enforce this striking genotype composition of their queens in a green-beard fashion, accepting additional *Sb*-carrying queens but executing *SB/SB* queens, including nestmate *SB/SB* gynes shortly after they emerge as adults (Ross and Keller 1998; Keller and Ross 1998).

The pheromonal basis of the supergene genotype signal was first demonstrated by the findings that polygyne workers rubbed against *SB/SB* queens were attacked by their nestmate workers (Keller and Ross 1998). Specific cuticular hydrocarbons were found to be uniquely present on the cuticle of *SB/Sb* queens, the abundance of which increased as the fertility of the queen increased (Eliyahu et al. 2011). Tribble and Ross (2016) showed that polygyne workers showed strong preferences toward polygyne queen extracts over monogyne queen extracts, confirming the presence of a supergene pheromone. Zeng et al. (2022) then showed that a complex blend of unsaturated CHCs functioned as this

signal of queen supergene status to workers (Box 1, Q7). However, beyond the recognition of supergene status, the precise mechanisms by which multiple *Sb*-carrying queens are permitted in polygyne colonies remain elusive. A potential general explanation may be that *Sb* queens are perceived as identical individuals, despite substantial variation in their fertility status and material apportionment (Ross 1988).

Discussion

Ant queen pheromones exhibit a variety of functional properties revealed by experimental analysis (summarized in Fig. 1), many of which are observed in other social insects including non-hymenopteran groups (e.g., termites), illustrating that convergent functions of queen pheromones arose across multiple independent origins of eusociality (Bortolotti and Costa 2014; Oi et al. 2015b; Funaro et al. 2018; Mitaka and Akino 2021). In essence, queen pheromones convey her presence to adult members of the colony, while signaling (1) her fertility and health condition, and at times (2) her individual identity and genotype status. The informed colony members then develop or behave accordingly to optimize the efficiency and productivity of the colony, or to adjust the colony social structure. It is reasonable to predict similar fundamental roles of queen signals in the ontogeny of other social animal colonies. Importantly, such signals may not be confined to chemical compounds but could involve multiple sensory modalities, which again necessitate a thorough understanding of their natural history to make useful predictions (Orlova and Amsalem 2021).

Several functions or properties of queen pheromones described in this review are hypothetical, because the chemical nature of the putative signals was not demonstrated experimentally. In other words, the inferred functions were associated with the presence of the queen without direct causal mechanisms or links to isolated chemicals or even crude solvent extracts from queens having been established. These studies serve as useful starting points to conceive functional frameworks for identifying specific compounds, and can provide guidance to future studies. Nonetheless, one must ask why, after decades of research focused on the problem, only very few compounds have been identified and explicitly shown to induce one or more of the discussed pheromonal effects.

The successful identification of the honeybee queen mandibular pheromones reveals that characterization of a complete blend of queen pheromones requires a combination of reliable bioassays and sophisticated chemical approaches (Butler and Fairey 1964; Butler et al. 1997), the lack of which, perhaps, is slowing progress in ants. As reviewed in the previous sections, the effects of queen pheromones are often manifested through worker behaviors. Thus, evaluating worker response behaviors to candidate pheromonal compounds can be an effective

approach to designing informative bioassays. In behavioral assays, it is valuable to practice blind experiments when possible to reduce observational bias and enhance validity of data. However, it is not always straightforward to devise an assay scoring system that is highly informative while rooted in a firm understanding of the natural history of the species and the natural context of the behavior. It cannot be overstated how important it is that investigators become intimately familiar with the social biology of the focal species in order to design and implement the most informative and meaningful bioassays.

Assigning a single numeric score to measure complex behavioral traits in a social context is difficult but can be achieved based on a sufficiently large dataset and validation of the biological meaning of the score through back-testing (Wild et al. 2021). Alternatively, it is often efficient to take measurements of distinct actions that are reliably quantifiable and use these directly, such as the number of antennation inspections by workers towards a treated glass slide (Dietemann et al. 2003). Another way to design an informative bioassay is by observing and quantifying behaviors unique to the study species. For instance, workers of *Odontomachus brunneus* display submissive gestures when a queen or other dominant individual is nearby (Fig. 3) (Medeiros et al. 1992), which was used as an indicator for screening pheromone compounds (Smith et al. 2012b, 2013, 2015).

A few characteristics of queen pheromones are helpful for the identification of their specific components. Candidate compounds are often uniquely present in the queen caste in species with pronounced caste dimorphism, and the amount of candidate compounds are usually correlated with fertility status (Holman et al. 2010, 2013). Consequently, a more fertile queen should exert a stronger pheromonal effect (Fletcher and Blum 1983a; Willer and Fletcher 1986; Ortius and Heinze 1999; Oi et al. 2015a). However, as exceptions are common in biology, these apparent general characteristics of queen pheromones do not always hold true. For instance, piperidine molecules, despite their abundance and a positive correlation with fertility, did not signal queen fertility in *S. invicta*; instead, trace polar compounds displayed the expected pheromonal effects (Eliyahu et al. 2011; Zeng et al. 2022).

Chemical ecologists often aspire to identify a single molecule with extensive, if not the complete array of, effects comprising the focal behavioral or other trait released or primed by the putative pheromone (Jacobson 2012; Ebrahim et al. 2023). That being said, such reductionist thinking often oversimplifies the complex chemical composition, multiple glandular and tissue origins, and variable functions of most insect pheromones, especially social insect queen pheromones exemplified by the well-studied honey bee queen pheromones (Keeling et al. 2003; Slessor et al. 2005; Symonds and Elgar 2008; Princen et al. 2019). Thus far, known glandular and cellular sources of pheromonal components in ants include the oenocytes, poison

sac, postpharyngeal gland, metapleural gland, and Dufour's gland (Vargo 1997; Vargo and Hulsey 2000; Yek and Mueller 2011; Kocher and Grozinger 2011). Multiple glandular sources of queen pheromones affecting a singular behavioral response have been demonstrated clearly in *S. invicta* (Vargo and Hulsey 2000), and we can expect the same for other ant species.

Many recent attempts to identify queen pheromones have focused on the CHCs (Van Oystaeyen et al. 2014; Oi et al. 2015b, a; Smith and Liebig 2017; Holman 2018) (Box 1, Q8), which is grounded in their essential signaling roles in the colony social life and the relative ease of their quantification and identification (Martin and Drijfhout 2009; Kroiss et al. 2011). While studies on CHCs remain important, more studies are starting to highlight other molecule classes playing roles of queen pheromones (Smith et al. 2016; Villalta et al. 2018; Steitz and Ayasse 2020). Once we obtain a holistic blend of identified pheromonal compounds of the proper ratio that encapsulates the full range of functional properties in even a few model ant species, with advances in the neurophysiology of odor reception and genetic basis of reproductive division of labor, we will grasp more fully how queen pheromones work to regulate colony social life of ants (Yan and Liebig 2021) (Box 1, Q9).

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Data Availability There is no data in the sense of any generated dataset. All the info is present in the text and supplementary information.

Declarations

Competing interests The author declares no competing interests.

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