Review article

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The critical role of primer pheromones in maintaining insect sociality

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Abstract: Primer pheromones play a pivotal role in the biology and social organization of insect societies. Despite their importance, they have been less studied because of the complexity of the required bioassays and, consequently, only a few of them have been chemically identified to date. The major primer pheromones are that of the queen pheromones that regulate reproductive skew and maintain colony cohesion and function. From a theoretical viewpoint, several features regarding the chemistry of queen pheromones can be predicted. They should be generally nonvolatile in order to avoid saturation of the colony space, which might otherwise hamper their perception because of sensory habituation. Accordingly, they should be actively dispersed throughout the colony by workers. The queen pheromone should also be caste-specific, qualitatively different from any worker pheromone, and preferably multicomponent, to allow unequivocal identification of the queen. The bipotency of the female larvae in social Hymenoptera to become queen or worker necessitates strict regulation over pheromone production. Indeed, in the honeybee, the biosynthetic pathways as well as the genomic expressions are completely disparate between queens and workers. Future advances in chemical analyses, transcriptomics, proteomics, and metabolomics will enrich our understanding of the chemistry, mechanisms, and crucial role that primer pheromones play in social evolution.

Keywords: queen pheromones; social behavior; social insects.

1 Introduction

Social insects are endowed with exocrine glands that produce a dazzling array of chemicals that serve as pheromones, which play a pivotal role in almost every aspect of their biology. Generally, pheromones can be classified into two categories: releaser pheromones that elicit an immediate behavioral response and primer pheromones that affect physiological processes that generally culminate in behavioral changes. A special type of primer pheromone in social insects is that of the queen pheromones, the presence of which is essential for maintaining colony cohesion and proper worker behavior. Despite their importance, only a handful of queen pheromones have been characterized to date. The present review engages with some theoretical aspects of the nature of queen pheromones and provides examples of the systems that have been investigated so far.

2 The raison d'être of a queen primer pheromone

The hallmark of insect sociality is their harmonious self-organization system, whereby a coordinated global colony behavior emerges from the behavior of individuals that is based on their perception of local information. This cooperation, accompanied by a division of labor, enables the performance of concurrent multitasking, leading to colony prosperity and propagation. However, social insects are also renowned for their extreme reproductive division of labor, in which only one or at most a few individuals reproduce, while all other colony members forego reproduction and engage in performing all other required colony duties. Relinquishing reproduction is evolutionarily enigmatic and raises potential evolutionary conflicts over who is going to reproduce. In the Hymenoptera, phylogenetic evidence indicates that in the ancestor species, at the evolutionary root of sociality, colonies were composed of a single queen that was singly inseminated. This, coupled with the haplo-diploid sex determination system, created an unusual within-colony relatedness that through kin selection enabled the propagation of social traits in the population. Once sociality was established, social cooperation as well as reproductive division of labor became adaptive, thus stabilizing the evolution of sociality.

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At the core of advanced self-organization systems is sophisticated communication, which in the social insects is mostly chemical, i.e. pheromones. Elaborate social communication systems enable individuals to convey rapidly and reliably knowledge of their status and condition, so that local knowledge becomes global, an essential component of self-organized behavior. Indeed, in social insects, almost every aspect of individual or colony behavior is mediated through pheromones. Pheromones comprise two types: *grosso modo*, releasers and primers. Releasers are notable for eliciting an immediate behavioral change and therefore are easier to characterize both behaviorally and chemically. Primer pheromones, on the contrary affect first physiological processes that may or may not culminate in overt changes in behavior. Therefore, their characterization, or even providing proof of their existence, is more difficult. A major primer pheromone in social insects is that involved in delineation of the reproductive and the sterile castes, and as it is seemingly produced by the queen (the reproductive caste), it has been named the "queen pheromone."

The evolution as well as maintenance of worker sterility in social insects remains unresolved. There are two prevailing hypotheses for its evolution [1]. The first postulates coercive control by the queen: that is, the queen, via her pheromone(s), affects workers directly through an as yet unknown mechanism that causes sterility. According to this so-called "queen control" hypothesis, the queen pheromone is a true primer pheromone. The alternative hypothesis, the "queen signal" (also termed "worker control") hypothesis, posits that coercive control is evolutionarily unstable as it promotes the evolution of countermeasures by workers, such as becoming pheromone-insensitive and reproducing despite the presence of the queen. It is further argued according to this hypothesis that worker reproductive self-restrain is driven by kin selection as well as by higher colony reproductive success. This hypothesis, therefore, requires a means for workers to evaluate the queen's quality, which results in the evolution of a queen signal advertising the queen's quality. Thus, although both hypotheses posit the evolution of a queen-specific pheromone, its classification as primer or releaser pheromone depends on its mode of action. If it directly regulates worker reproduction (queen-control hypothesis), it can be classified as a primer pheromone. If, on the contrary, the pheromone constitutes a queen signal, it can be classified as a releaser.

Although these two mechanisms appear mutually exclusive, they need not necessarily be so. Therefore, I suggest a combined mechanism affecting worker sterility: the queen produces a primer pheromone that directly affects worker physiology culminating in ovary inactivation (queen control). I further argue that any countermeasure taken by workers, for example, through evolving insensitivity to the queen pheromone and therefore initiating reproduction, will be selected against because it will hamper colony reproduction because of within-colony worker conflicts over reproduction and the breakdown of the adaptive division of labor (worker control).

3 The effect of the presence/absence of the queen on colony cohesion

Observation of a queenright colony reveals harmonious cooperation and perfectly self-organized division of labor. The queen can be clearly detected because of the presence of retinue workers around her that feed and groom her extensively [2, 3; Figure 1]. However, nothing in the queen behavior reveals that her presence

Figure 1: Retinue behavior in the desert ant *Cataglyphis niger*.

is necessary for colony cohesion. In the absence of the queen, in contrast, there is a sharp change in worker behavior, manifested as intra-nest competition and chaos. In the honeybee, the removal of the queen is noticed by the workers after about 10 h and results in rapid attempts to requeen the colony [4]. If, however, we render the colony hopelessly queenless by removing all nondetermined larvae (younger than 3 days), the colony enters a chaotic phase in which workers initiate ovary activation and become internally aggressive [5]. This shift in worker behavior results in reduced foraging efforts as well as reduced brood care by the workers, resulting in colony degradation [6, 7]. Queenlessness can be remedied by introducing an extract of the queen mandibular gland pheromone (QMP), emphasizing the importance of this pheromone [8–10]. Noteworthy is the finding that the activity of QMP is synergized by several cephalic substances, glandular origin undetermined, highlighting the magnitude of complexity that this pheromone reaches [2].

Advertising the queen's presence by chemical signaling seems to be the rule in the species with large populous colonies, in which the queen cannot attain physical contact with each worker. Moreover, to produce a pheromone with low volatility seems to be adaptive as it avoids pheromone vapor saturation in the nest, which could cause sensory habituation, which in turn may result in workers not responding to the pheromone. Dispersion of the pheromone, therefore, is mediated by retinue workers that lick off the pheromone from the queen and pass it on to other workers in the hive. This was demonstrated in the honeybee both by behavioral observations [4, 11, 12] and a study of the within-hive distribution of radiolabeled 9-oxo-2-decenoic acid (9-ODA) [13].

In the ant *Aphaenogaster senilis*, gyne (future reproductive queens) caste determination is also regulated by a queen pheromone. The species reproduces by colony fission, and gyne production is timed to when the colony is very large and ready to split [14]. Under queenless conditions, however, gyne production is immediately initiated. In this case, too, the postulated queen pheromone does not appear to be volatile because separating workers and brood from the queen by a double screen results in raising gynes. Unfortunately, there is no knowledge of the pheromone chemistry or its glandular source. Chemical analysis of two major glandular sources, the postpharyngeal (that stores cuticular hydrocarbon) and Dufour's gland, have revealed differences between queens and workers, but neither the secretion nor its components have been tested [14]. Although it is difficult to generalize from the few studied cases, the hypothesis that the queen primer pheromone should not be volatile but be transmitted via the workers seems sound.

4 The dispersion of primer pheromones in the colony

Another requirement of an effective queen pheromone is that of dispersion within the colony, so that each worker senses the pheromone in order to be affected by it. Assuming that the pheromone is not volatile, it follows that it should be transmitted to workers throughout the nest by means of an intermediary messenger. In the honeybee, it was shown, both by behavioral observations and the use of radiolabeled pheromone, that the retinue workers acquire the queen pheromone by constantly licking her and assuming the role of a messenger bee to transfer pheromone bouts to neighboring workers [4, 13]. These messenger bees become attractive to other workers, which lick them and in turn transmit the pheromone they have acquired to additional workers. Attraction to attendant bees lasts for 40 min, presumably because of the fade out of the pheromone at this time either through volatilization or its transfer to other workers [11]. Following this dynamics, we can assume that the queen has to constantly secrete the pheromone, and in large colonies, she should accordingly produce copious amounts of pheromone. In the honeybee, these requirements are met, as the mandibular glands of the queen are hypertrophied. The mode of pheromone transmission to the attendant workers, however, is still elusive. The evidence so far indicates that upon secretion, the pheromone becomes smeared on the queen's body surface, from where it is picked up by the retinue workers, mostly through licking.

I would like to suggest also that the pheromone can also be transmitted from queen to workers and among workers directly, through trophallaxis. Measurements of the frequency of trophallaxis acts as well as the direction of material transferred in the honeybee suggest that in addition to food transfer it also serves as a communication mode [15]. In ants it was shown empirically that trophallaxis is the means of hydrocarbon exchange between the postpharyngeal glands of the interacting individuals [16] and regulatory molecules such as enzymes, hormones, and RNA [17]. Transfer of the queen pheromone by trophallaxis can both increase dispersion effectiveness and enable its internalization, thus directly affecting internal tissue, e.g. trigger ovary activation.

Another means of effective pheromone dispersion is by depositing it on the queen-born eggs. This is effective in species (ants and termites) in which the eggs are located in masses at different locations within the nest and/or are carried by workers. Egg-marking was demonstrated in the ants *Camponotus floridanus* [18], *Dinoponera quadriceps* [19], *Solenopsis invicta* [20], and *Gnamptogenys striatula* [21] and in the termite *Reticulitermes speratus* [22]. While egg-marking by the queen seems less effective for pheromone dispersal in species in which eggs are deposited individually within a cell (bees and wasps), worker policing in honeybees (whereby workers destroy worker but not the queen eggs) indicates that the latter are marked, but neither the source nor the chemistry of the pheromone are known [23, 24].

5 The chemistry of queen primer pheromones: the importance of blends and chemical idiosyncrasy

To date, the chemistry of only a handful of pheromones that meet the definition of a queen primer pheromone is known (Table 1). While the small number of identified pheromones does not allow us to generalize rules regarding their optimal chemistry, two questions regarding their chemical nature can still be raised: Should the pheromone be a blend; and should it be distinctively idiosyncratic?

Table 1: Chemically known queen primer pheromones in social insects.

Species	Chemical composition	Reference
Apis mellifera	*R*-(–)-9-Hydroxy-(*E*)-2-decenoic acid *S*-(+)-9-Hydroxy-(*E*)-2-decenoic acid 9-Oxo-(*E*)-2-decenoic acid Methyl *p*-hydroxy benzoate homovanillyl alcohol	[3, 25, 26]
Bombus terrestris	*n*-Pentacosane	[27]
Vespula vulgaris	*n*-Heptacosane *n*-Octacosane *n*-Nonacosane 3-Methyl nonacosane	[27]
Dolichovespula saxonica	Mix of cuticular hydrocarbons	[28]
Dinoponera quadriceps	9-Hentriacontene	[29]
Cataglyphis iberica	*n*-Heptacosane 3-Methyl heptacosane *n*-Nonacosane 3-Methyl nonacosane	[27]
Silvestritermes minutus	10-Pentyl-3,4,5,8,9,10-hexahydro-2*H*-oxecin-2-one	[30]
Reticulitermes speratus	*n*-Butyl-*n*-butyrate 2-Methyl-1-butanol	[22]
Nasutitermes takasagoensis	Phenylethanol	[31]
Cryptotermes secundus	Mix of cuticular hydrocarbons	[32]
Reticulitermes flavipes	Heneicosane	[33]

5.1 Why should a queen pheromone be a blend?

Screening the chemical nature of insect pheromones reveals that these are generally ubiquitous natural products and many are commonly used by a variety of organisms. In particular, there is a considerable overlap between plant and insect natural products, increasing the probability of an insect misidentifying the source of the substance (for example, identifying the wrong species) and consequently responding erroneously. One solution to this problem was to increase the number of pheromonal components, which considerably reduces the probability of overlap between different species. This created a selective pressure that resulted in the evolution of pheromones as blends of substances. Once a pheromone evolved as a blend, it further enabled specificity as well as the encoding for more information. Social insects are not exceptional in that many of their pheromones have been shown to be multicomponent. Moreover, the need for both specificity and the conveying of complex information is even more acute in regard to maintaining social cohesion. For example, pheromone-mediated nestmate recognition entails chemical distinction between colonies within a species, which, given the limited genetic diversity within species, is possible only by employing a complex blend of chemicals while relying on variation in the relative abundance of its components. Likewise, multicomponent trail pheromones encode for multiple behaviors, such as foraging initiation, recruitment of new foragers, trail following, and trail marking reinforcement [e.g. *S. invicta*; 34–36]. Increased specificity can also be obtained by employing two sets of chemicals from two different sources [e.g. *Lasius japonicus*; 37, 38].

Should the queen primer pheromone also be multicomponent? The queen pheromone in social insects conceivably meets all the above criteria and in all likelihood evolved as a blend. Although confined to a nest and, therefore, overlap in composition with other organisms is rare, the need for specificity is still present: for example,

to ensure against the invasion of queens of social parasites, or in multiple queen colonies, to ensure distinction among individual queens. In the honeybee, the queen primer pheromone comprises six components, all of which is necessary for proper activity (inhibition of gyne rearing), albeit not as effective as a fully functional queen [39], suggesting that the presence of additional queen signals is needed for full activity. Supporting this latter hypothesis is the discovery of substances, in addition to QMP, which synergize the retinue behavior eliciting of QMP [2]. Likewise, the primer queen pheromone of the termite *R. speratus* is a two-component pheromone, which are essential for full activity (suppressing the differentiation of new neonetics) [22]. In the termite *Cryptotermes secundus*, a complex queen-specific multicomponent mixture of cuticular hydrocarbons acts as a fertility signal [32]. A gene responsible for these differences, *neofeme4*, is overexpressed in queens than in workers [40]. Silencing this gene, and thus changing the queen odor to that of a worker, resulted in a worker butting behavior, indicative of the onset of reproductive replacement differentiation. Thus, although not explicitly tested as a primer queen pheromone, these results imply a primer pheromone effect [41].

5.2 Caste chemical idiosyncrasy

One of the requirements of a queen pheromone is uniqueness, so that its perception by workers unequivocally signals "I am the queen," thus avoiding a mistaken response to "false queens," in species in which workers are not irreversibly sterile. Producing a queen-specific set of compounds in the Hymenoptera is particularly challenging because female larvae are totipotent and can develop either to queen or to worker. Moreover, even at the adult stage, workers that normally do not possess the queen primer pheromone start to produce it concomitant with their ovary activation. What, then, prevents workers from producing the pheromone and becoming "false queens"? The need for absolute caste specificity has also been selected for the evolution of strict regulation at both the biosynthetic and genomic levels in order to ensure the unique production of the pheromone by the queen (see below for details). Mutations occur, such as in the cape honeybee, *Apis mellifera capensis*, in which workers are able to produce the queen mandibular pheromone even in the presence of the queen. This feature enables them to successfully invade nests of another honeybee race, *Apis mellifera scutellata*, and to become a false queen. This emphasizes the power of the queen pheromone and why caste specificity is so important.

In view of the absolute requirement for caste specificity, it would also seem maladaptive to have a system whereby the queen and workers produce the same pheromone compound(s), but the queen possesses far greater amounts of the pheromone. A recent report tends to negate this, claiming that in several taxonomically disparate social insect species, the pheromonal differences between queens and workers are not qualitative (the presence of unique substances) but quantitative: that is, the compound that is present in greater amounts in the queen compared with workers is responsible for the inhibition of worker ovary activation [27]. Those authors further argue that the common use of hydrocarbons by disparate taxa indicates that it constitutes an evolutionary conservative mechanism for regulating reproductive skew in the social Hymenoptera.

I would like to argue here against the generality of these assumptions:

5.2.1 The pheromone is common both to queens and workers but is present in greatest quantities in queens

As described previously, one of the prerequisites of an effective queen pheromone is its rapid dispersal among colony members. Considering the hypothesis that the use of a nonvolatile pheromone is adaptive, this requires, on the one hand, the production of copious pheromone amounts and, on the other hand, an effective means of its dispersal in order to reach each worker within a reasonable time frame. We can also assume that in populous colonies comprising thousands of workers, the queen cannot encounter each worker at sufficient frequencies to convey her presence. In these cases, pheromone dispersion by messenger workers, as in the case of the honeybee [42], seems to be an evolutionarily sensible solution. Given the above assumption, it follows that the queen pheromone should be composed of substances that qualitatively differ from those present in workers. For illustration, let us consider a case in which the pheromone is composed of a single component that is present in both queens and workers, but its proportion in queens is several times higher than in workers, and the colony contains several thousand workers [e.g. *Lasius niger* queen pheromone; 43]. If we adopt the dynamics of pheromone dispersion found in honeybee, the amount of pheromone, because of dilution, at the *n*th worker may be as low as the level normally found in workers, thus becoming ineffective. The *n*th workers, therefore, which did not receive the information that a queen is present, are hypothesized to behave as if queenless. In large societies that are composed of hundreds of thousands of workers, the

probability of encountering the queen or a worker that has just met the queen is very low. Consequently, in such societies, we should find many workers that have initiated ovary activation (in species in which worker sterility is not irreversible). Although rare, some experimental results indicate such an effect of pheromone dilution on worker behavior. Experiments with the honeybee have shown that workers that are confined to part of the hive without access to the queen and are also remote from workers that do have access to the queen behave selfishly as if being hopelessly queenless, and many of them activate their ovaries [44]. In the ant *A. senilis*, gyne production in the presence of the queen is possible only in large colonies, presumably because of the dilution of the queen pheromone to the point below perception threshold, which eventually leads to colony fission [14].

5.2.2 Hydrocarbons constitute a conserved class of queen pheromones

"What we see depends mainly on what (and where; A.H) we look for" (John Lubbock), or 'the streetlight effect' – looking under a lamppost for a key that was lost on the other side of the street."

In recent years, much attention has been focused on the role of cuticular hydrocarbons as queen pheromones. Hydrocarbons constitute a major component of the epicuticular lipids of insects, and in social insects, ants in particular, they compose complex blends often comprising several dozens of compounds. Their initial function is assumed to be that of creating a waterproof lining to reduce water loss through evaporation, but the abundance of components, in particular that of alkenes and branched hydrocarbons, suggests that they may also have a role in communication [45 and references therein]. The ease of extraction and structural analysis by gas chromatography/mass spectrometry has made the cuticular hydrocarbons a favorite study subject. Their assignment as queen pheromones relies in most cases on a comparison between queen and worker profiles, but a few studies have also tested specific hydrocarbons that were more abundant in queens than in workers [27, 43, 46]. What is surprising is that none of the exocrine glands, the usual source of pheromones, has been examined in any of these species. Social insects are endowed with exocrine glands, some of which are major glands with a visible reservoir while others constitute a tissue composed of a few cells only. In ants, for example, over 20 glandular sources of putative pheromones have been described [47], producing a plethora of compounds [48–50]. In the honeybee, the mandibular glands of the queen are immensely hypertrophied and produce the queen pheromone [3]. Likewise, Dufour's gland of the queen is 10-fold larger in queens than in workers, and it too produces queen-specific compounds [51]. Should some of these multiple glands not be considered as a possible source of a queen pheromone? Unfortunately, none has been examined in depth in the published studies that have pinpointed cuticular hydrocarbons as queen pheromones. Finally, several independent studies in ants have shown that cuticular hydrocarbons specifically act as nestmate recognition cues. There is an inherent problem in these cases, however, as the queen cuticular hydrocarbons, if acting as a queen pheromone, should differ from those of workers, yet the queen is not (and cannot be) identified as an alien ant [45, 52].

In sum, although cuticular hydrocarbons may play a certain role in signaling the queen's presence, it is clearly not the whole story. It is not inconceivable that additional glandular sources as well as other natural products should be considered in the search for the identification of queen primer pheromones.

6 The tight regulation of pheromone biosynthesis in queens and workers: the case of the honeybee queen mandibular pheromone

The immense importance of the queen pheromone in regulating proper colony function imposes great selective pressure to ensure its distinctiveness from any worker pheromone. An added complexity is that in most social Hymenoptera, the diploid, female larva is totipotent and able to develop into either queen or worker, and furthermore, multiple studies have shown that workers with activated ovaries also produce the queen pheromone. Thus, the genetic composition as well as the biosynthetic mechanisms for both the queen and worker chemical idiosyncrasy exists in every female. An even more intriguing point is that perception of the queen pheromone inhibits the production of this very same pheromone in the perceiving individual. It thus follows that regulation of the pheromone biosynthesis will be tight and carried out in multiple stages of the pheromone biosynthesis.

The well-studied honeybee QMP exemplifies this nicely. The pheromone is composed of five components: two enantiomers of 9-hydroxy-2-decenoic acid (9-HDA), 9-ODA, which are the major components, and methyl *p*-hydroxy

benzoate and 4-hydroxy-3 methoxyphenylethanol (homovanillyl alcohol; HVA), which are the minor components [3, 6]. Workers, in contrast, possess 10-hydroxy-2-decenoic acid in their mandibular gland. This switch in the hydroxyl position is critical as the 9-hydroxy is the precursor of 9-ODA, reported to be a crucial pheromone component for proper activity. In an elegant study, Plettner et al. [53] showed that the hydroxylating step (ω carbon versus ω-1 in workers and queens, respectively) is indeed the key to the differences in pheromone composition between the two castes. A recent study of gene expression in the mandibular gland revealed an even tighter regulation, comprising at least four regulatory steps in the biosynthesis of the acid pheromone components [54, 55]. The production of stearic acid constitutes regulatory step 1. Although stearic acid is a common precursor of both queen and worker acid components, the implicated enzymes, fatty acid synthase and long chain fatty acid synthase, are controlled by two different gene sets, one expressed mainly in workers and the other in queens. Moreover, gene expression in egg-laying workers shifts toward the queen pathway, in line with the initiation of QMP production in such workers [5, 56, 57]. Regulatory step 2 involves genes encoding for the hydroxylating enzymes of the cytochrome P450 family, namely *CYP4AA1* for the ω hydroxylation (worker pathway) and *CYP18A1* for the ω-1 hydroxylation (queen pathway). Thereafter, gene expression is caste-specific in accordance with the caste-specific biosynthetic intermediate. A final step in the biosynthesis in queens is the conversion of 9-HDA to 9-ODA. Indeed, queens, but not workers, exhibit high expression of the gene coding for alcohol dehydrogenase, the presumed enzyme that catalyzes this reaction.

Unfortunately, the honeybee is the only social insect to date for which such in-depth study has been performed. However, given the importance of the queen pheromone in the regulation of colony normal self-organized behavior, complex and fine-tuning regulation is also predicted to have evolved in other social insects that utilize queen primer pheromones.

7 Social and evolutionary perspective of queen pheromones

7.1 Honest signaling – the cost of pheromone production vs. social cost

Honest signaling is a hallmark of animal communication, but what makes a signal honest is still mostly elusive. The Index Theory, for example, posits that the signal is directly correlated to a measurable quality, e.g. size, height jump, and depth of voice [58]. In terms of chemical communication, aposematic coloration can serve as an indexed signal (it is correlated with the individual's toxicity, but nonetheless prone to cheating, i.e. Betsian mimicry). The Handicap Theory, conversely, suggests that an honest signal should be costly to a degree that signal manifestation is correlated to the emitter's quality. Simply put, low-quality individuals cannot afford, in terms of survival or fitness, to produce a signal with the same intensity, if at all, compared with high-quality individuals [59]. In terms of chemical communication, it is postulated that either the biosynthesis is highly costly or, alternatively, the chemical used for signaling may have a toxic effect at concentrations that low-quality individuals cannot sustain. To date, there is no information on whether any of the known primer pheromones is costly to produce or has a toxic effect at a particular physiological concentration. This is not entirely surprising because in social insects there may be a social cost, rendering other possible costs redundant. There are several examples where the uncalled-for production of the queen pheromone elicits antagonism in other members of the society. In queenright colonies, workers that attempt to reproduce are aggressed by nestmates [*D. quadriceps* (ponerinae) 60, honeybees (Apidae) 61]. Under hopeless queenless conditions, honeybee workers engage in aggressive competition over reproduction [5, 57]. Worker policing, whereby under queenright conditions worker-born eggs are devoured by nestmate workers, is another possible cost paid by workers that attempt to lay eggs [62, 63]. Worker policing makes these reproductive attempts futile, and if fitness gain through cooperation and reproductive self-restraint surpasses that of selfish behavior, it is likely to be selected for. In all of the above, the production of the queen-like pheromone seems to be the signal that rival workers perceive and to which they react aggressively.

7.2 Redundancy of pheromones and pheromone glands – an evolutionary enigma

When examining the wealth of chemicals employed as social pheromones and the myriad of glandular sources in the Hymenoptera, it becomes apparent that one cannot assign either certain groups of chemicals or glandular sources to a specific pheromonal function. It would seem that in the course of evolution an existing set of biosynthetic pathways and a set of exocrine glands that were present in the solitary ancestors were employed

fortuitously, as the need arose. In addition, many ants possess multiple novel glands that probably evolved after sociality became established. We have evidence of this from trail and alarm pheromones of ants, and it is feasible that it also holds true for the queen primer pheromones. The adoption of existing natural products and their glandular origin for social functions has also enabled redundancy in their use. A good example of this is the redundancy of pheromones of the queen honeybee signaling reproductive dominance. At least three sources of the pheromones have been described including the mandibular glands [64] characterized by hydroxy- and oxo- acids and aromatic compounds, tergal glands [65] characterized by hydrocarbons, and Dufour's gland [23] characterized by long chain esters. A similar system, for example, is found in the alarm-defense of ants, e.g. *Acanthomyops claviger* [66], which uses terpenoids from the mandibular glands, formic acid from the poison gland, and hydrocarbons from Dufour's gland, all of which separately or combined elicit an alarm response. What might be the explanation for such redundancy? One possibility is piggyback adaptation. Undecane, the major Dufour's hydrocarbon, serves also as a wetting agent to facilitate the permeation of the highly polar formic acid through the hydrophobic epicuticle. Being of lower volatility than formic acid, it persisted in the environment and may have been adopted as an additional alarm pheromone during evolution. Another explanation could be that of assurance that the message will be conveyed quickly and efficiently, irrespective of the body part present (head or gaster in the case of the ants). In the case of the primer pheromones, it is possible that each glandular secretion conveys slightly different information, and it is the complement that provides the full effect. An interesting hypothesis is that multiple primer queen pheromones reflect a queen-worker arms race [67]. If the primer pheromone affects worker behavior coercively (that is, it forces the workers to behave in contrast to their fitness gain), it is expected that the workers will evolve a countermeasure, e.g. pheromone insensitivity, eventually leading to a stalemate in the arms race. When workers became resistant to one pheromone, the queen therefore evolved another pheromone and so on.

8 Concluding remarks

The establishment of insect sociality constituted a major evolutionary transition that required the concomitant evolution of many traits that facilitated the complex behavior and self-organization systems exhibited by these societies. A hallmark of this is the evolution of an elaborate means of communication that enables the performance of coordinated behavior, particularly in the very large colonies that typify most social insect species. It is also very clear from the abundant research over the past five decades that chemical communication is the major means of communication in social insects. Although the study of pheromone releasers of social behavior over the years has been extensive, revealing a plethora of chemical structures, that of primer pheromones is wanted, and only a handful of chemicals have been unequivocally identified. A major primer pheromone category in social insects is that of the queen pheromone, which affects the physiology (mainly the inhibition of reproduction) of either workers or other queens. It is not entirely surprising that only a few such pheromones have been unequivocally identified, given the difficulties and the time-consuming nature of constructing bioassays to test specific chemicals for such effects. Moreover, if the queen pheromone does not produce a true primer effect but merely functions as a queen signal [1], a direct bioassay will be hard to design. One possibility to circumvent this problem is to search for queen pheromones that elicit a retinue behavior (i.e. operate as releasers), while assuming that these may also function as primer pheromones [68 and Figure 1].

There are several open questions regarding the primer function of queen pheromones:

1. *Does the primer queen pheromone inhibit its own production?* As found in the honeybee and several ant species, workers are not devoid of the ability to produce the queen pheromone and in fact do so under queenless conditions. It follows that the presence of the pheromone inhibits its own production in all the individuals that perceive it, except for the emitter. Moreover, this presumed immunity appears to be a unique feature of the queen because, under queenless conditions, there is no apparent sign of mutual inhibition among workers, several of which can simultaneously produce the queen pheromone. This suggests that the queen may have an additional pheromone or additional components to the existing queen pheromone that is permanently blocked in workers and therefore may be linked to the developmental processes that determine larval caste determination.
2. *What is the mode of action of the queen primer pheromones?* Classically, it is accepted that pheromones are perceived through the olfactory system and affect the receiver in a top-down manner, from the brain to the responsive tissues or organs, either neurally or humorally. However, there is also a possibility that the pheromone is internalized and affects the respective tissues

or organs directly. We do not have evidence for this pathway yet, but we can draw an analogy from studies with *Drosophila*, in which males manipulate the female behavior by transferring seminal proteins to her reproductive tract, some of which also reach the circulatory system [69, see also remark in 70], supporting this possibility. For example, one of the components of the honeybee QMP is HVA, which is structurally very similar to dopamine, a biogenic amine that is implicated in the regulation of reproduction in this species [71, 72]. As QMP is transferred by contact, it is not inconceivable that HVA is absorbed in the recipient bee and directly affects its reproductive physiology.

3. *How can genomics facilitate the identification of queen pheromones?* The vast advances in genomics, transcriptomics, and proteomics in the social insects open great avenues for discovering new queen primer pheromones as well as underpinning the effect of gene expression on pheromone production. Microarray and RNAseq studies that compare queens and workers or reproductive versus nonreproductive workers have already revealed differences in gene expression. For example, in the fire ant, the differences in the transcriptome between forager and nonforager workers are dependent on queen presence (presumably via her primer pheromone) [73]. When the pheromone source is known, comparing its tissue gene expression may result in more specific expression differences and facilitate the study of target genes involved in the pheromone biosynthesis, e.g. the honeybee mandibular glands [54, 55]. However, a caveat in such comparative studies is that gene annotation relies mostly on their orthologs in a model system, i.e. the *Drosophila* genome. First, it is not certain that they function in a similar way, and second, it is unhelpful in cases in which the genes are specific to the organism in question, e.g. a social hymenopteran [74]. It is also important to realize that the queen primer pheromone, as powerful as it is, does not operate in an empty environment and synergism and antagonism may modulate its function considerably. For example, in the bumblebee *Bombus terrestris*, the pheromone is potent only in young colonies, while in old colonies workers seem to ignore it and initiate reproduction despite the presence of the queen [reviewed in 75]. Likewise, when we examine the genomics of primer pheromone production and function, we have to consider the importance of indirect gene effects [74]. Perception of the primer pheromone not only affects gene expression in the receiver's brain [76] but also initiates a cascade of reactions, including gene expression in other target tissues. This creates a complex network of interactions involving gene expression of social traits that loop back to the pheromone emitter and forward again to the receiver. Moreover, being a self-organization system, it involves many colony members, each of which performs diverse tasks, creating a genuine sociogenomic system [77].

Future advances in chemical analyses, transcriptomics, proteomics, and metabolmics will enrich our understanding of the mechanisms and the crucial role that primer pheromones play in social evolution.

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