Queen pheromones
The chemical crown governing insect social life

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Group-living species produce signals that alter the behavior and even the physiology of their social partners. Social insects possess especially sophisticated chemical communication systems that govern every aspect of colony life, including the defining feature of eusociality: reproductive division of labor. Current evidence hints at the central importance of queen pheromones, but progress has been hindered by the fact that such pheromones have only been isolated in honeybees. In a pair of papers on the ant Lasius niger, we identified and investigated a queen pheromone regulating worker sterility. The cuticular hydrocarbon 3-methylhentriacontane (3-MeC₃₁) is correlated with queen maturity and fecundity and workers are also more likely to execute surplus queens that have low amounts of this chemical. Experiments with synthetic 3-MeC₃₁ found that it inhibits ovarian development in queenless workers and lowers worker aggression towards objects coated with it. Production of 3-MeC₃₁ by queens was depressed by an experimental immune challenge, and the same chemical was abundant on queen-laid eggs, suggesting that the workers’ responses to the queen are conditional on her health and fecundity. Together with other studies, these results indicate that queen pheromones are honest signals of quality that simultaneously regulate multiple social behaviors.

Key words: social insect, queen pheromone, fertility signal, cuticular hydrocarbon, social physiology, primer pheromone
Submitted: 07/03/10
Accepted: 07/05/10
Previously published online: www.landesbioscience.com/journals/cib/article/12976
DOI: 10.4161/cib.3.6.12976
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Addendum to: Holman L, Dreier S, d’Ettorre P.
Selfish strategies and honest signalling: reproductive conflicts in ant queen associations. Proc R Soc B 2010; 277:2007-15; PMID: 20181562; DOI: 10.1098/rspb.2009.2311 and Holman L, Jørgensen CG, Nielsen J, d’Ettorre P.
Identification of an ant queen pheromone regulating worker sterility. Proc R Soc B 2010; In press.
DOI: 10.1098/rspb.2010.0984

Parsimonious Regulation of Colony Life by Queen Pheromones

In combination with previous results, our new data suggest that queen pheromones can simultaneously regulate multiple aspects of reproductive division of labor and other colony-level processes (Fig. 1). We found that the cuticular hydrocarbon 3-MeC₃₁ is involved in signaling queen fertility, maturity and condition, regulating worker reproduction and preventing worker aggression towards objects bearing the pheromone. Several other functions are more tentatively supported. Our results on the execution of supernumerary queens in founding associations are consistent with selective worker aggression towards the queen(s) with the least 3-MeC₃₁. Workers that cannot directly identify their mother are predicted to attack queens that have produced the fewest workers, maximizing the chance that they are killing an unrelated queen, and 3-MeC₃₁ is strongly correlated with queen productivity.

Queen-like chemicals have also been implicated in the aggressive response to non-preferred reproductives, such as fertile workers and subordinate reproductives, in many other species of ants (especially queenless ants), wasps and bees. Our results and those of Moore and Liebig imply that queen-like hydrocarbons depress worker aggression, which is seemingly incongruous with reports of these same chemicals eliciting aggression in certain contexts, e.g., when expressed by fertile workers or subordinates. This disparity suggests either (A) queen pheromone has a dose-dependent
Perspectives for Future Research

There is now near-unanimous support for the hypothesis that social insect queen pheromones are “honest signals” of fertility or condition, and that the worker response is not counter to their own fitness interests.\(^1\)\(^{2}1^{7}2^{10}2^{1}2^{20}\) I therefore suggest that researchers should focus on the ultimate explanation for this honesty. There are three principal reasons why signals should be honest:\(^18\)\(^{21}\) (1) dishonest signaling is not selected, (2) the signal is a costly “handicap”, such that only high-quality individuals benefit from investing heavily in signaling and (3) the signal is an unfakeable “index” that is inextricably linked to the trait it is signaling. As argued elsewhere,\(^1\)\(^9\) hypothesis 1 is unlikely to be applicable to all social insects, including derived lineages where reproductive conflict is constrained.\(^22\) For example, in \(L.\ niger\), we expect that queens in multi-queen colonies would be equally consistent with the handicap and index hypotheses. In \(L.\ niger\), our immune challenge might have depressed reproductive physiology causing a corresponding drop in pheromone production (index) or reduced condition such that pheromone production was no longer possible (handicap).\(^2\) Intriguingly, treatment with juvenile hormone reduced reproductive activity while slightly augmenting pheromone production in honeybee workers\(^2\)\(^3\) (which appears to falsify the index hypothesis); however, in a comparable experiment in ants both reproduction and putative queen pheromones were suppressed by juvenile hormone.\(^2\)\(^4\)

Determining the underlying genetic architecture, biochemistry and/or fitness costs of pheromone production may be required to definitively discriminate between these hypotheses.

Our study\(^1\) shows how queen primer pheromones may be unambiguously identified, and I believe that it will be fruitful to isolate more in additional taxa. This will allow numerous novel questions to be addressed, e.g., how fast do queen pheromones evolve, are they predominantly single- or multi-component blends, and have similar pheromones evolved independently in phylogenetically-distant taxa? Answering these questions will provide insight into social evolution as a whole. For example, fast-evolving and multi-component signals imply coevolution and possibly conflict.\(^17\)\(^21\) Convergent evolution of homologous queen pheromones would suggest that certain chemicals are particularly suited to the job: they might be particularly costly to produce (handicap hypothesis) or biochemically linked to reproduction (index hypothesis). There is tantalizing evidence that alkanes with a methyl group on the third carbon (like \(3\)-\(MeC\(^3\))\(^3\)\(^1\)\(^3\)\(^1\)\(^6\)\(^1\)\(^6\)\(^1\)\(^6\)\(^1\)\(^6\)) are also queen pheromones in other species of social insects. 3-methyl-alkanes have been correlated with fertility and/or caste in other highly-social formicine ants (\(Camponotus\ floridanus\)\(^2\)\(^5\) and \(Formica\ fusca\))\(^2\)\(^5\) and in more basal ants (\(Myrmecia\ gulosa\),\(^2\)\(^7\) \(Diacamma\ ceylonese\),\(^2\)\(^8\) \(Pachycondyla\ inversa\)\(^2\)\(^9\) and \(Platythyrea\ punctata\)\(^3\)\(^0\)). Even more surprisingly, these compounds are characteristic of reproducives in the distantly-related termites\(^3\)\(^1\) and have been indirectly linked to the

\(\text{Figure 1. Known and hypothesized functions of queen pheromones produced by queens (or other reproductive individuals) and carried on their brood. The numbers give a non-exhaustive list of studies providing evidence consistent with each function.} \)
regulation of worker reproduction in the wasp Ropalidia marginata.12

Acknowledgements

I am grateful to all members of the Centre for Social Evolution, Copenhagen for providing a stimulating work environment. This work was supported by a Marie Curie Intra-European Fellowship (#235403; CHEMDOC).

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