Report

Current Biology

Dual Effect of Wasp Queen Pheromone in Regulating Insect Sociality

Highlights

- Specific queen pheromones induce sterility in social insect workers
- One of these queen pheromones is shown to also signal egg maternity
- The pheromone enables workers to recognize or "police" eggs laid by cheater workers
- This shows that queen pheromones regulate insect sociality in several distinct ways

Authors

Cintia A. Oi, Annette Van Oystaeyen, Ricardo Caliari Oliveira, ..., Kevin J. Verstrepen, Jelle S. van Zweden, Tom Wenseleers

Correspondence

cintiaakemi.oi@bio.kuleuven.be (C.A.O.), tom.wenseleers@bio.kuleuven.be (T.W.)

In Brief

Social insect queens use pheromones to stop the workers from reproducing. Oi et al. show that in wasps, one of these sterility-inducing pheromones is also used by the queen to mark her eggs and enable the workers to recognize and "police" eggs laid by other workers. This shows that queen pheromones regulate insect sociality in several distinct ways.

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Oi et al., 2015, Current Biology *25*, 1638–1640

http://dx.doi.org/10.1016/j.cub.2015.04.040

CellPress

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Dual Effect of Wasp Queen Pheromone in Regulating Insect Sociality

Cintia A. Oi,[1,*] Annette Van Oystaeyen,[1] Ricardo Caliari Oliveira,[1] Jocelyn G. Millar,[2] Kevin J. Verstrepen,[3,4] Jelle S. van Zweden,[1] and Tom Wenseleers[1,*]
[1]Laboratory of Socioecology and Social Evolution, KU Leuven, Naamsestraat 59, 3000 Leuven, Belgium
[2]Department of Entomology and Department of Chemistry, University of California, Riverside, Riverside, CA 92521, USA
[3]VIB Laboratory for Systems Biology, KU Leuven, Gaston Geenslaan 1, 3001 Leuven, Belgium
[4]CMPG Laboratory for Genetics and Genomics, KU Leuven, Gaston Geenslaan 1, 3001 Leuven, Belgium
*Correspondence: cintiaakemi.oi@bio.kuleuven.be (C.A.O.), tom.wenseleers@bio.kuleuven.be (T.W.)
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SUMMARY

Eusocial insects exhibit a remarkable reproductive division of labor between queens and largely sterile workers [1, 2]. Recently, it was shown that queens of diverse groups of social insects employ specific, evolutionarily conserved cuticular hydrocarbons to signal their presence and inhibit worker reproduction [3]. Workers also recognize and discriminate between eggs laid by the queen and those laid by workers, with the latter being destroyed by workers in a process known as "policing" [4, 5]. Worker policing represents a classic example of a conflict-reducing mechanism, in which the reproductive monopoly of the queen is maintained through the selective destruction of worker-laid eggs [5, 6]. However, the exact signals used in worker policing have thus far remained elusive [5, 7]. Here, we show that in the common wasp, *Vespula vulgaris*, the pheromone that signals egg maternity and enables the workers to selectively destroy worker-laid eggs is in fact the same as one of the sterility-inducing queen signals that we identified earlier [3]. These results imply that queen pheromones regulate insect sociality in two distinct and complementary ways, i.e., by signaling the queen's presence and inhibiting worker reproduction, and by facilitating the recognition and policing of worker-laid eggs.

RESULTS AND DISCUSSION

Even though social insects may appear to work together in total harmony, social insect colonies are in fact rife with conflict [1, 5]. A case in point is the queen-worker conflict over male production, observed in many species, whereby rogue workers will surreptitiously lay unfertilized eggs, destined to become males, instead of working for the benefit of the colony [5]. To keep such transgressing workers at bay, many species have evolved a "policing" system in which eggs laid by workers are selectively detected and cannibalized [5, 8]. First discovered in the honeybee a quarter of a century ago [4], this phenomenon of worker policing has since been reported in more than a dozen social insect species, including bees, ants, and wasps [8]. It has long been hypothesized that the workers' ability to discriminate between queen-laid and worker-laid eggs might be aided by a pheromone mark left on the queen's eggs [5]. Such a queen egg-marking pheromone should be evolutionarily stable because the pheromone would benefit both the queen, by increasing her share of the colony's reproduction, and the workers, by reducing the chance of them accidentally destroying the queen's eggs and thereby reducing their inclusive fitness [5, 8–10]. However, despite decades of research, no social insect queen egg-marking pheromone has been unambiguously identified [5, 7, 11].

Cuticular hydrocarbons provide a waxy protective layer on the cuticles of all insect life stages, including eggs [12, 13]. The profiles of cuticular chemicals are known to show pronounced differences between castes [3, 11] and have acquired important signaling functions in social insects, including as queen pheromones [3]. We hypothesized that the same hydrocarbons that function as queen pheromones might also play a role in signaling egg maternity. That is, based on evolutionary parsimony, we predicted that the co-option and repurposing of an existing maternal signal might be easier than evolving an entirely new signaling system from scratch. To test this hypothesis, we carried out experiments with the common wasp, *Vespula vulgaris* (Figure 1A), from which we recently identified several sterility-inducing queen pheromones [3], and in which the workers, as in the honeybee, police each other's eggs [14]. To shortlist possible egg-marking pheromones, we first carried out a detailed comparison of the hydrocarbon profiles of queen-laid and worker-laid eggs ([15]; Figure S1 and Supplemental Experimental Procedures). Our analysis showed that two compounds were consistently more abundant on the surface of queen-laid eggs than on the surface of worker-laid eggs, namely 3-methylnonacosane (3-MeC$_{29}$), which we earlier found to act as a sterility-inducing queen signal [3], and 3-methylheptacosane (3-MeC$_{27}$), which shows weaker caste differences on the cuticle but is highly characteristic of queen-laid eggs (Figure S1).

Subsequently, we collected six colonies of the common wasp, from which we isolated about half of the worker force from their mother queen to stimulate egg laying. We then treated worker-laid eggs with synthetic versions of the queen-characteristic pheromones 3-MeC$_{27}$ and 3-MeC$_{29}$, either individually or as a blend ("mix," Figure 1B), dissolved in acetone. These treatments

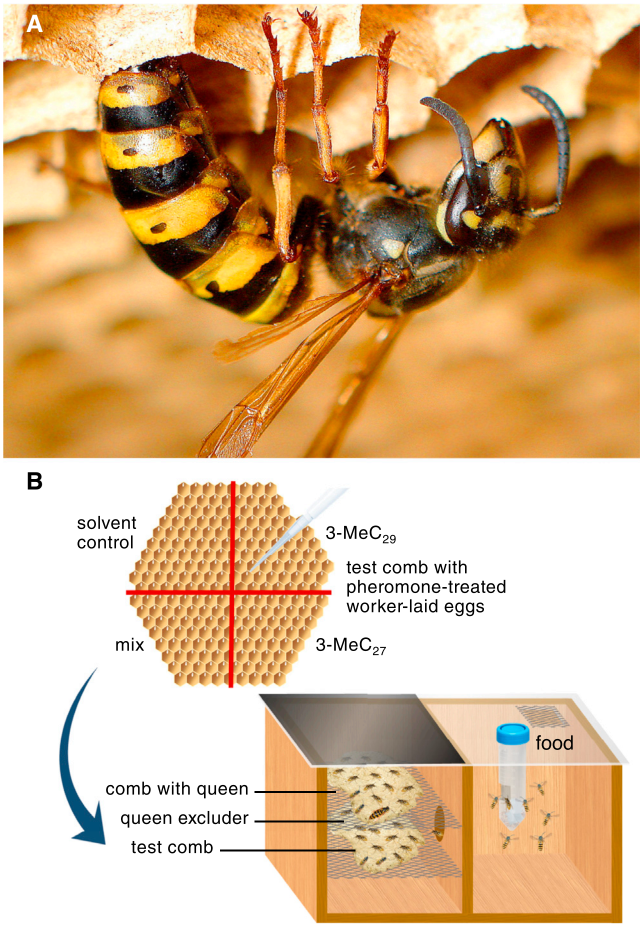

Figure 1. Experimental Setup Used to Test the Role of Queen Pheromones in Signaling Egg Maternity during Worker Policing in the Common Wasp

(A) To test the hypothesis that hydrocarbon queen pheromones, which stop workers from reproducing [3], could also play a role in signaling queen egg maternity, we performed experiments with the common wasp *Vespula vulgaris*. As in most social insects, common wasp queens have a near monopoly over reproduction [14]. This reproductive monopoly is enforced partly via a mechanism of "worker policing," whereby workers selectively detect and destroy eggs laid by transgressing workers [14].

(B) In bioassays, we determined whether treatment with an acetone solution of the hydrocarbon 3-methylnonacosane (3-MeC$_{29}$), one of the main sterility-inducing queen signals in this species [3]; 3-methylheptacosane (3-MeC$_{27}$), which is characteristic of queen-laid eggs; or a mix of both caused worker-laid eggs to become more "queen-like" and thus acceptable to the workers upon reintroduction into their mother colony.

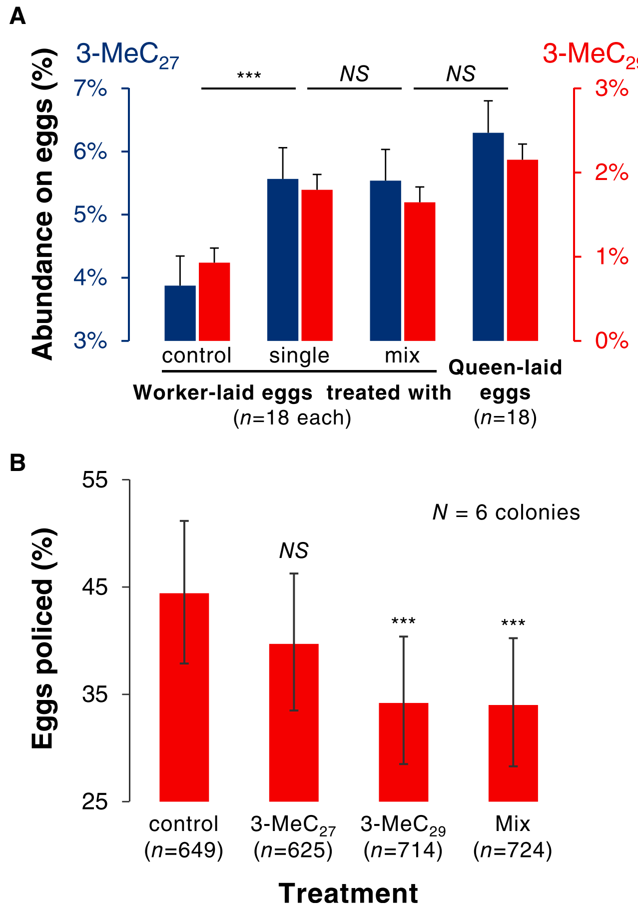

Figure 2. A Sterility-Inducing Queen Pheromone Also Protects the Queen's Eggs against Worker Policing in the Common Wasp

(A) Treatment of worker-laid eggs with acetone solutions of the queen egg characteristic compounds 3-MeC$_{29}$ and 3-MeC$_{27}$, either as single compounds ("single") or as a blend of the two compounds ("mix"), caused their relative abundance to significantly increase relative to the control and to approach those found naturally on queen-laid eggs (mean ± SEM, Tukey's post hoc tests, n = 18 eggs each per egg type and treatment from N = 6 colonies each; Table S1). Compound concentrations were compared separately but significance levels are presented only once, given that the results were the same for both compounds (***$p < 0.001$; NS, not significant).

(B) Our bioassays confirmed that 3-MeC$_{29}$ also signals egg maternity, because the percentage of eggs that were policed was significantly reduced when 3-MeC$_{29}$ was applied onto worker-laid eggs compared to the solvent-only control (mean ± 95% confidence level, binomial mixed model, Table S1; N = 6 replicate colonies, n = 625–724 eggs per treatment; ***$p < 0.001$; NS, not significant).

rendered worker-laid eggs chemically more similar to queen-laid eggs (Figure 2A; Table S1), and our hypothesis was that application of these compounds should therefore reduce the rate at which these eggs were policed. By reintroducing the chemically manipulated eggs into the mother colony (Figure 1B), we confirmed this hypothesis. Specifically, the bioassays confirmed that 3-MeC$_{29}$ was strongly correlated with signaling egg maternity because it significantly reduced the rate at which worker-laid eggs were policed compared to solvent-treated controls (Figure 2B; Table S1). By contrast, 3-MeC$_{27}$, which is present in significantly higher amounts on queen-laid eggs than on worker-laid eggs, did not inhibit policing, and the blend of both compounds was not significantly different from 3-MeC$_{29}$ used alone. The fact that applying the compound 3-MeC$_{29}$ onto worker-laid eggs only partially protected them against policing, however, indicates that our treatments were only partially effective at mimicking the complete profiles of queen-laid eggs. This could be explained either by small concentration mismatches (Figure 2A) or by the fact that worker-laid eggs also contain some specific cues that give away their maternal origin, and which we could not experimentally remove. Indeed, our chemical analysis reveals several short-chain mono- and dimethyl alkanes that are specific to worker-laid eggs (Figure S1) and might be produced as some intrinsic side effect of worker-specific physiological processes [16]. Hence, 3-MeC$_{29}$ forms an important part of the queen signal, but an additional contribution of other queen- or worker-specific compounds cannot be entirely excluded. In fact, a recent study has shown that in ants, a hydrocarbon fertility signal is maximally effective only when perceived within its full chemical context [17]. It is likely that the same is true for the queen egg-marking signal that we have documented here.

Overall, our study is the first to unambiguously identify a social insect queen egg-marking pheromone used in worker policing. Furthermore, our results show that the egg-marking pheromone appears to be the same as one of the main sterility-inducing queen signals in this species [3]. From an evolutionary perspective, these findings make sense because a queen pheromone has all the necessary attributes to be a marker of maternal origin and could easily be co-opted into a valuable secondary function as an egg-marking pheromone. A dual function of queen pheromones in inducing sterility and egg marking had been suggested previously for volatile queen pheromone components in the termite *Reticulitermes speratus* [18, 19], as well as for non-volatile hydrocarbons in the ants *Camponotus floridanus* [20] and *Pachycondyla inversa* [11]. In the latter two cases, particular long-chain hydrocarbons are highly specific for queens and their eggs, but a dual function in communication and regulation of sociality was not confirmed with defined blends of pure compounds, causing the functional roles of these compounds to remain speculative in these cases. Nonetheless, these results together with ours clearly suggest that hydrocarbon queen pheromones have a central role in regulating the spectacular reproductive division of labor of diverse lineages of hymenopteran social insects.

SUPPLEMENTAL INFORMATION

Supplemental Information includes one figure, one table, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.04.040.

AUTHOR CONTRIBUTIONS

C.A.O., J.S.v.Z., and T.W. designed and contributed to all aspects of this study. A.V.O. first conceived the experiments and provided some of the bioassay results. R.O.C. helped to collect nests and perform bioassays. J.G.M. synthetized the methyl-branched hydrocarbons. K.J.V. provided the GC-MS instrument used to analyze the chemical profiles of eggs. C.A.O., J.S.v.Z., and T.W. wrote the manuscript.

ACKNOWLEDGMENTS

This study was supported by Research Foundation Flanders grant G.0A51.15 and KU Leuven Centre of Excellence grant PF/2010/007 (to T.W.), postdoctoral fellowship 12Q7615N of the Research Foundation Flanders (to J.S.v.Z.), CNPq-Brazil scholarships 201959/2012-7 and 238127/2012-5 (to C.A.O. and R.C.O.), and Hatch project CA-R*ENT-5181-H (to J.G.M.). We are grateful to A. Vollet-Neto and A. Vandoren for assistance with fieldwork.

Received: March 7, 2015
Revised: April 20, 2015
Accepted: April 20, 2015
Published: May 7, 2015

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