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# Selfish strategies and honest signalling: reproductive conflicts in ant queen associations

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Social insects offer unique opportunities to test predictions regarding the evolution of cooperation, life histories and communication. Colony founding by groups of unrelated queens, some of which are later killed, may select for selfish reproductive strategies, honest signalling and punishment. Here, we use a brood transfer experiment to test whether cofounding queens of the ant *Lasius niger* ‘selfishly’ adjust their productivity when sharing the nest with future competitors. We simultaneously analysed queen cuticular hydrocarbon (CHC) profiles to investigate whether queens honestly signal their reproductive output or produce dishonest, manipulative signals, providing a novel test of the evolutionary significance of queen pheromones. Queens produced fewer workers when their colony contained ample brood, but only in the presence of competitors, suggesting selfish conservation of resources. Several CHCs correlated with reproductive maturation, and to a lesser extent with productivity; the same hydrocarbons were more abundant on queens that were not killed, suggesting that workers select productive queens using these chemical cues. Our results highlight the role of honest signalling in the evolution of cooperation: whenever cheaters can be reliably identified, they may incur sanctions that reduce the incentive to be selfish.

**Keywords:** social insect; queen pheromone; trade-off; cooperation; coercion; sanctions

## 1. INTRODUCTION

A key concept in the evolution of communication and life histories is that trade-offs exist between different traits (Roff & Fairbairn 2007). For example, signalling incurs life history costs (Gustafsson *et al.* 1995; Hunt *et al.* 2004), and early- and late-life fitness are negatively related (Stearns 1992). The eusocial insects have sophisticated communication and derived life histories, and, therefore, provide unique opportunities to study trade-offs. Social insect queens may live for decades and produce millions of offspring (Hölldobler & Wilson 1990), ultimately because trade-offs between reproduction and longevity are relaxed by the actions of the workers. Sociality also places additional demands on communication. For example, social animals often possess signals that allow them to be recognized as group members or individuals, or as being dominant versus submissive (Maynard Smith & Harper 2003; Tibbetts & Dale 2007). Increasing the specificity of these signals may help to exclude enemies and increase efficiency, but could lead to rejection errors (Reeve 1989; Ratnieks 1991).

Social insects rely heavily on the chemical channel to maintain group cohesion, identify enemies, coordinate colony-level activities and signal their status (e.g. Hölldobler 1995; Le Conte & Hefetz 2008). Chemical recognition in social insects is primarily based on cuticular hydrocarbons (CHCs; Lenoir *et al.* 1999; d’Ettorre &

Moore 2008). Intraspecific recognition using CHCs occurs at three or more hierarchical levels (d’Ettorre 2008): between members of different colonies (e.g. Bonavita-Cougourdan *et al.* 1987; Lenoir *et al.* 1999; Ozaki *et al.* 2005), between different groups within colonies (Greene & Gordon 2003; Hefetz 2007) and between specific individuals (d’Ettorre & Heinze 2005; Dreier *et al.* 2007).

Reproductive social insects typically have a distinct CHC profile from workers, which may also covary with ovarian activation and productivity, suggesting that CHCs signal caste and/or reproductive potential (e.g. Monnin *et al.* 1998; Peeters *et al.* 1999; Liebig *et al.* 2000; Sledge *et al.* 2001; Heinze *et al.* 2002; Dietemann *et al.* 2003; d’Ettorre *et al.* 2004; Smith *et al.* 2009). Chemicals specific to fertile individuals are potential queen pheromones, which we define here as a chemical or blend of chemicals that (i) characterizes the reproductive(s) and (ii) induces behavioural and/or physiological responses in other colony members; a well-studied example is that of the honeybee (Slessor *et al.* 2005; Le Conte & Hefetz 2008). The ultimate function of queen pheromones is debated (see Heinze & d’Ettorre 2009); they may either be a form of manipulation, causing the receivers to increase the senders’ fitness at a cost to themselves, or an honest signal of reproductive value to which it benefits the receivers to respond. Keller & Nonacs (1993) introduced the terms ‘queen control’ (manipulation) and ‘queen signal’ (honest signal) to describe these ideas. Assuming pheromone quantity affects the response of receivers (Hoover *et al.* 2005), the queen control hypothesis implicitly predicts that pheromone production should increase with the number of receivers

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that need to be controlled, regardless of whether the receivers are the offspring of the focal reproductive. By contrast, the queen signal hypothesis predicts that pheromone production should reliably indicate offspring production and/or ovarian activation, but should otherwise be independent of the number of receivers.

Chemical cues such as queen pheromones are also likely to be involved in situations where the colony members must choose which queen(s) should lead the colony and which should be eliminated. Queen elimination occurs routinely in several ant species that have cooperative colony foundation by two or more reproductives (pleometrosis); the first clutch of workers kills all but one queen when the benefits of multiple queens cease to outweigh the costs (Rissing & Pollock 1988; Bernasconi & Strassmann 1999). Several studies have attempted to discover the factors that determine which queen survives, with emphasis on testing whether workers assist their mother (e.g. Sommer & Hölldobler 1995; Bernasconi & Keller 1996; Adams & Balas 1999; Aron *et al.* 2009), although to date such nepotism has not been clearly identified. If workers are incapable of recognizing their mother, they are predicted to favour the most productive queen because she is statistically the most likely to be their mother (Balas 2005). Workers would probably assess queen productivity via the CHC profile, but there is currently little direct evidence that chemical cues inform worker decisions in queen elimination following pleometrosis (but see Cuvillier-Hot *et al.* 2004a, 2005).

As well as producing pheromones that win the support of the workers, queens in pleometrotic associations could increase their chances of surviving to lead the colony by reserving some of their energy for the queen elimination phase. Queen ants may lose 20 to 40 per cent of their body weight rearing the first workers (Cahan 2001; Aron *et al.* 2009), so there are likely to be trade-offs between early reproduction and the ability to fight or resist worker aggression. Cofounding queens that anticipate queen elimination may therefore invest less in reproduction than singly founding conspecifics, a prediction that has found empirical support (Bernasconi & Strassmann 1999; Cahan 2001). However, abstaining from reproduction is costly to all individuals in the colony because the survival probability of incipient colonies is positively related to the number of workers (Adams & Tschinkel 1995; Sommer & Hölldobler 1995; Fjerdingstad & Keller 2004). We therefore expect that queens may be able to (i) assess the quantity of developing brood and the number of cohabiting queens, and (ii) adjust their reproductive effort according to the amount of brood, but only when competing queens are present. Strategic reproduction by cofounding queens could be described as selfish because it lowers the fitness of the other queens and their offspring (Bernasconi & Strassmann 1999).

In the present study, we tested for risk-sensitive, potentially selfish reproductive strategies by queens in incipient colonies of the black garden ant *Lasius niger* by experimentally controlling the number of queens and brood. We also used chemical analysis to measure how queens' CHC profiles related to (i) their productivity and (ii) the number of workers in the colony. We predict that under the queen signal hypothesis, changes in

productivity will be accompanied by shifts in queens' chemical profiles. By contrast, under the queen control hypothesis, we expect that the expression of chemical cues will be positively correlated with the number of workers present in the colony. In this species, all but one of the queens are invariably killed soon after the first workers eclose (Sommer & Hölldobler 1995), and we tested whether a queen's likelihood of survival was related to her CHC profile. We also examined the development of the chemical profile in the months following colony establishment to determine which hydrocarbons are temporally associated with ovarian activation and the appearance of workers, and are thus potential signals or manipulations, respectively. Finally, we observed colonies before and after worker eclosion to study aggression and queen elimination behaviour.

## 2. MATERIAL AND METHODS

### (a) *Ant collection and housing*

Recently mated *L. niger* queens were collected following a mating flight in Copenhagen, Denmark, on 5 July 2008; all queens had shed their wings and were searching for a nest site. Queens were housed in plaster nests (8 × 6 × 5 cm) on the day of collection, either alone or in groups of two or three; queens often establish nests cooperatively in the wild in groups of two to three (Sommer & Hölldobler 1995). Nests were kept at room temperature (range 18–26°C) under a natural photocycle. Queens were only given water until the first workers had eclosed (day 36), at which point they were also supplied with *ad libitum* honey and *Tenebrio molitor* larvae. This regime mimics natural 'claustral' colony foundation, in which queens have a fixed energy reserve until workers begin to forage.

### (b) *Brood transfer experiment*

We experimentally altered the size of the brood pile by either adding or removing cocoons prior to the eclosion of the first workers. First, we randomly paired up incipient colonies on day 1 of the experiment; colony pairs contained one, two or three queens per colony. In each pair, one colony was randomly assigned to the 'cocoon removal' treatment (abbreviated C-) and one to the 'cocoon supplementation' treatment (C+). We checked the colonies daily for the appearance of cocoons and used soft forceps to gently move them from the C- colonies to the corresponding C+ colonies. No rejection behaviour was observed, and colonies naturally practise intraspecific brood raiding (Sommer & Hölldobler 1995). Our results are unlikely to be confounded by differences in worker genetic diversity among our treatments because (i) workers do not appear to be able to identify their mother (Sommer & Hölldobler 1995; Aron *et al.* 2009) and (ii) there is no clear reason why queens would adjust their productivity based on their relatedness to the brood. We transferred all cocoons that appeared from day 23 (when cocoons were first seen) to day 34, which corresponded to  $20 \pm 1.6$  cocoons (range: 16–25) in the one-queen colony pairs,  $33 \pm 3.2$  (21–41) in the two-queen pairs and  $62 \pm 2.9$  (52–73) in the three-queen pairs. In addition to the C+ and C- colonies, we set up control colonies with one to three queens, in which cocoons were not transferred.

The C+ and control two- and three-queen colonies were maintained until all but one queen was killed. The colony

Table 1. The minimum adequate model of the parameters that affected the productivity of colonies in the brood transfer experiment. The dependent variable is colony productivity, measured by the number of adult workers and cocoons at the end of the experiment. The predictor 'number of cocoons moved' is positive for C+ colonies, negative for C- colonies and zero for controls. The results are contrasts from a GLM with Poisson errors (d.f. = 54).

independent variable	contrast	coefficient	<i>z</i> -value	<i>p</i> -value
number of cocoons moved	—	-0.018	-3.58	0.0003
number of queens	2 versus 1	-0.57	-8.98	<0.0001
	2 versus 3	0.38	7.64	<0.0001
treatment	control versus C+	0.55	3.12	0.0018
	control versus C-	-0.65	-3.58	0.0003
number of cocoons moved × number of queens	2 versus 1	-0.0099	-1.97	0.048
	2 versus 3	0.015	1.916	0.055

was then frozen and the queen placed in an individual glass vial for storage at  $-20^{\circ}\text{C}$ . Queens that had been killed were removed and frozen upon discovery (colonies were checked at least every 24 h). Whenever a C+ colony was frozen, we also froze its paired C- colony; the C- colonies typically had no adult workers at this stage (one colony had one worker, another had two), and queen elimination was never observed in the C- treatment (see also Sommer & Hölldobler 1995). The one-queen colonies were all frozen on day 45, when approximately 50 per cent of the two- and three-queen colonies had been frozen; as expected, queens were never killed by workers in the one-queen colonies. Productivity was scored for all colonies by counting the number of adult workers plus the number of cocoons. For the non-control colonies, the cocoons that had been transferred were counted towards the productivity of the C- colony, not the C+ colony. The final sample size was 55 colonies (one-queen colonies: 9 control, 5 C+ and 5 C-; two-queen colonies: 5 control, 6 C+ and 6 C-; three-queen colonies: 7 control, 6 C+ and 6 C-). We analysed the CHCs of all queens used in this experiment (see electronic supplementary material for details of the chemical and statistical analyses).

#### (c) *Maturation of the queen cuticular hydrocarbon profile*

To measure how queen CHC profiles changed as queens aged and began to reproduce, we individually housed an additional 140 queens in plastic cylinders ( $28 \times 37$  mm). We froze a random subset of the colonies at various intervals after colony establishment, namely days 9 ( $n = 20$ ), 23 ( $n = 20$ ), 39 ( $n = 19$ ), 65 ( $n = 20$ ), 94 ( $n = 19$ ) and 113 ( $n = 13$ ; all the surviving colonies), and subsequently analysed the queens' CHCs. We also analysed virgin queens ( $n = 21$ ) that were caught and frozen during the same mating flight; the wings were removed before processing for consistency with the mated queens.

#### (d) *Measurement of body size and behavioural observations*

We quantified body size of all queens used in the brood transfer experiment by measuring head width. The head was photographed at  $32\times$  magnification, and the distance between the eyes was quantified with LEICA APPLICATION SUITE 2.5.0 software (averaging three measurements).

We also conducted behavioural scan sampling on another set of colonies not involved in the main experiment, which were established as part of a pilot study using queens from a mating flight on 15 July 2007. These colonies again contained

one ( $n = 11$ ), two ( $n = 13$ ) or three ( $n = 7$ ) queens; in the multi-queen colonies, the queens were individually marked with dots of paint on the gaster. The colonies were observed one to three times a day on days 9–52 of the experiment to quantify the behaviour of queens and workers (once the latter had eclosed). Conspicuous interactions between queens prior to worker eclosion were never observed, but we recorded the positions of the queens in order to test for evidence of more subtle behavioural dominance in incipient colonies using the following scores: on the brood pile (score: 1), within 1 cm of the brood pile (0.5) and away from the brood pile (0). During the elimination phase, we observed colonies daily to determine whether queens were more often killed by workers or other queens.

### 3. RESULTS

#### (a) *Effects of queen number and brood transfer treatment on productivity*

Altering the size of the brood pile had a significant effect on queen productivity in the three-queen colonies; the C+ colonies became 25 per cent less productive, whereas the C- colonies were 18 per cent more productive, relative to controls (table 1 and figure 1*a*). However, in one- and two-queen colonies the treatment had little effect on colony productivity. The change in productivity associated with the C+ and C- treatments was correlated with the number of cocoons that had been added or removed, but this relationship depended on queen number (table 1). The interaction between cocoon number and queen number suggested that two- and three-queen colonies responded to treatment in the same direction, and significantly differently to one-queen colonies (table 1). However, this did not translate into a clear productivity difference between treatments in the two-queen colonies (figure 1*a*). The mean head size of queens in a colony was not correlated with colony productivity in the overall model, although there was a relationship within the one-queen colonies (GLM:  $t_{18} = 2.00$ ,  $p = 0.045$ ).

#### (b) *Effects of the queen number and brood transfer on queen cuticular hydrocarbons*

We selected 30 gas chromatography–mass spectrometry peaks that were consistently present in all samples for the analysis (figure S1 and table S1, electronic supplementary material;  $n = 96$ ). We first performed a discriminant analysis using 11 principal components (PCs; 86% of the variance), which indicated that

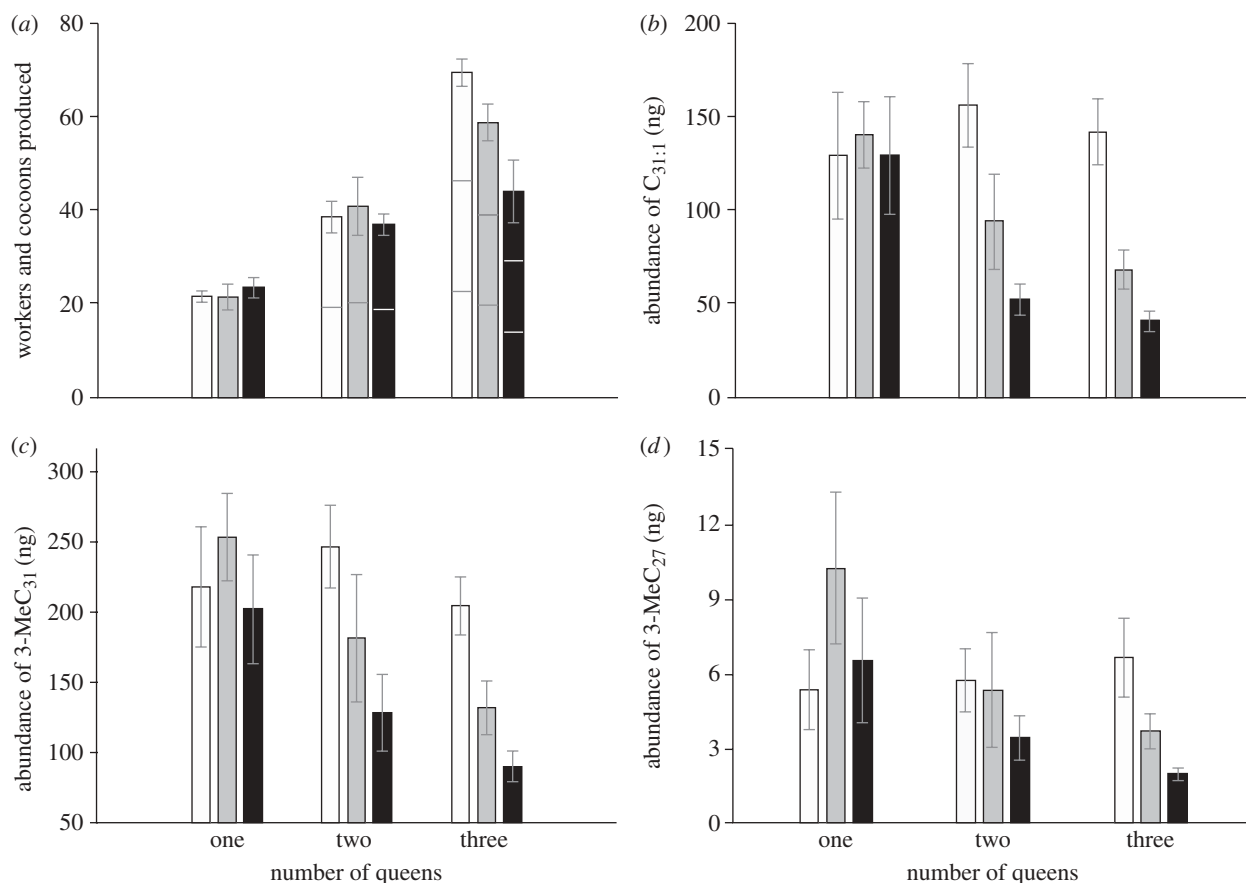


Figure 1. The number of queens in a colony and the brood transfer treatment had interacting effects on both colony productivity and the abundance of some CHCs. (a) The effect of queen number and treatment on the number of cocoons and workers produced by a colony; brood transfer had a pronounced effect in three-queen colonies. The lines on the bars of the two- and three-queen colonies illustrate the mean productivity per queen. The changes in productivity were accompanied by similar changes in the abundance of several CHCs; shown here are (b) C<sub>31:1</sub>, (c) 3-MeC<sub>31</sub> and (d) 3-MeC<sub>27</sub> that covaried to some extent with the shifts in productivity shown in (a) (see also figure S3, electronic supplementary material). Bars show mean  $\pm$  s.e. White bar, C-; grey bar, control; black bar, C+.

differences existed in the hydrocarbon profiles of queens from different treatment groups (Wilk's  $\lambda = 0.58$ ,  $F_{22,166} = 2.34$ ,  $p = 0.0012$ ), particularly between the C+ and C- queens (figure S2, electronic supplementary material). To further investigate these differences, we fitted three generalized linear mixed models (GLMMs) using PC1 (27% of the variance), PC2 (15%) and PC3 (12%) as dependent variables.

PC1 was unrelated to the number of queens, treatment, number of cocoons moved, head size and all interaction terms. However, it was correlated with mean queen productivity (i.e. colony productivity divided by queen number;  $t_{52} = 2.05$ ,  $p = 0.045$ ). We analysed the seven peaks that strongly affected PC1 (loading  $> 0.66$ ) and found that five of these were significantly affected by both treatment and queen number, with a significant or marginally significant interaction (table 2; figure 1b-d; figure S3, electronic supplementary material). In the three-queen colonies, the abundance was highest in C- queens, lowest in C+ queens and the controls were intermediate. This pattern was similar in the two-queen colonies, and in one-queen colonies there was no difference between treatments (table 2; figure S3, electronic supplementary material). The fact that neither productivity nor CHCs were affected by treatment in the one-queen colonies shows that worker

number does not affect queen CHCs directly. The abundances of the hydrocarbons C<sub>31:1</sub>, 3-MeC<sub>31</sub> and 3-MeC<sub>27</sub> were most strongly affected by treatment and covaried quite closely with the differences in productivity that were induced by treatment and queen number (figure 1). Queen number and treatment also interacted in their effects on PC2 ( $t_{48} = 2.03$ ,  $p = 0.047$ ). We therefore also tested the peaks that loaded most strongly onto PC2; three peaks containing long-chain dimethylalkanes were the only ones significantly related to treatment or queen number (table 2). These peaks were significantly more abundant on queens in the C- treatment relative to the controls, but only in two- and three-queen colonies (table 2). PC3 was not significantly related to any measured variables.

To verify whether any of the measured chemicals were expressed more in the presence of workers (which might indicate a function in 'queen control'; Keller & Nonacs 1993), we inspected plots of all 30 peaks for all combinations of treatment and queen number (figure S3, electronic supplementary material). In two- and three-queen colonies, the mean abundance was always highest in the C- queens, which typically never encountered workers. In one-queen colonies, the abundance of all peaks displayed no consistent pattern among the three treatments.

Table 2. List of CHCs that correlated with productivity, predicted survival in queen elimination and/or increased with age. The peak numbers correspond to those in figure S1 and table S1, electronic supplementary material. The paired columns show statistics from GLMMs, each with the abundance of one of the peaks as the response variable. Queen number, treatment and their interaction were fitted as predictors, and the  $p$ -values shown are from contrasts of the interaction term. For all of these peaks, abundance was greatest in the C- treatment, lowest in the C+ treatment and intermediate in the control in the multi-queen colonies, but variable in the one-queen colonies (figure S3, electronic supplementary material). These statistics test whether the effect of treatment became significantly more pronounced as queen number increased (d.f. = 48, two-tailed), as did the data on productivity. The fourth column shows the difference in the mean mass of the chemical on the cuticle of surviving and killed queens, expressed as a percentage. The rightmost column shows the slope of the relationship between time since colony foundation (in days) and the percentage of that hydrocarbon in the cuticular profile (binomial GLMs, d.f. = 130).

chemical	peak	$p$ -value of treatment $\times$ queen number interaction		difference between surviving and killed queens (%)	mean change in % per day
		C+ versus control	C- versus control		
C <sub>27</sub>	1	0.51	0.32	+110 ( $p = 0.028$ ) <sup>a</sup>	+0.0005 ( $p = 0.75$ )
C <sub>29</sub>	3	0.45	0.50	+83 ( $p = 0.015$ ) <sup>a</sup>	+0.001 ( $p = 0.82$ )
C <sub>31:1</sub>	12	0.85	0.053 <sup>b</sup>	+71 ( $p = 0.023$ ) <sup>a</sup>	+0.04 ( $p < 0.0001$ ) <sup>a</sup>
C <sub>33:1</sub>	23	0.34	0.15	+22 ( $p = 0.33$ )	+0.02 ( $p < 0.0001$ ) <sup>a</sup>
3-MeC <sub>27</sub>	2	0.27	0.015 <sup>a</sup>	+77 ( $p = 0.12$ )	0 ( $p = 0.97$ )
3-MeC <sub>29</sub>	7	0.46	0.083 <sup>b</sup>	+76 ( $p = 0.025$ ) <sup>a</sup>	+0.01 ( $p = 0.006$ ) <sup>a</sup>
3-MeC <sub>31</sub>	17	0.81	0.10 <sup>b</sup>	+84 ( $p = 0.009$ ) <sup>a</sup>	+0.06 ( $p < 0.0001$ ) <sup>a</sup>
diMeC <sub>31S</sub>	16	0.091 <sup>b</sup>	0.0006 <sup>a</sup>	-13 ( $p = 0.47$ )	-0.02 ( $p = 0.004$ ) <sup>a</sup>
diMeC <sub>33S</sub>	25	0.82	0.0044 <sup>a</sup>	-10 ( $p = 0.58$ )	+0.01 ( $p < 0.0001$ ) <sup>a</sup>
diMeC <sub>33S</sub>	26	0.79	0.0011 <sup>a</sup>	-7 ( $p = 0.68$ )	+0.04 ( $p < 0.0001$ ) <sup>a</sup>
diMeC <sub>35S</sub>	30	0.57	0.015 <sup>a</sup>	8 ( $p = 0.71$ )	+0.01 ( $p = 0.0006$ ) <sup>a</sup>

<sup>a</sup>Results significant at the 95% confidence level.

<sup>b</sup>Non-significant trend ( $p \leq 0.1$ ).

### (c) Relationship between queen cuticular hydrocarbons and queen elimination

Apart from being affected by experimental brood transfer, queen CHC profiles also differed between queens that survived and those that were eliminated. We analysed the first three PCs with GLMMs, using a reduced dataset containing all the individuals from the two- and three-queen colonies that could be identified as surviving or eliminated queens ( $n = 45$ ). PC1 was significantly higher in queens that survived queen elimination relative to those that were killed ( $t_{23} = 2.10$ ,  $p = 0.046$ ), whereas PC2 and PC3 were not significantly associated with survival. We tested the compounds that loaded strongly onto PC1 and found five that significantly predicted queen survival (table 2; figure 2).

There was a non-significant trend for surviving queens to have a larger head width than queens that were killed (GLMM:  $t_9 = 1.83$ ,  $p = 0.10$ ), and we found that body size was correlated with the quantity of C<sub>31:1</sub> ( $t_{91} = 2.65$ ,  $p = 0.009$ ) and C<sub>29</sub> ( $t_{91} = 2.27$ ,  $p = 0.026$ ) on the cuticle. However, all five of the compounds in figure 2 remained significant predictors of queen survival if body size was left in the model, indicating that body size alone cannot explain the observed chemical differences between surviving and killed queens. All other CHCs that were linked to productivity, survival and/or age (listed in table 2) were not significantly related to body size ( $p_{91} > 0.12$ ).

### (d) Maturation of the queen cuticular hydrocarbon profile

There were pronounced changes in the composition of the queen CHC profile in the first 113 days following

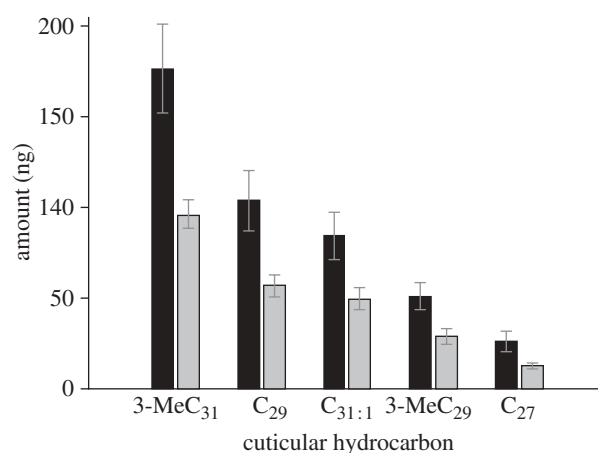


Figure 2. Queens that survived the queen elimination phase had significantly more 3-MeC<sub>31</sub>, C<sub>29</sub>, C<sub>31:1</sub>, 3-MeC<sub>29</sub> and C<sub>27</sub> in their CHC profiles than queens that were killed (bars show mean  $\pm$  s.e.).  $n = 20$  surviving queens and 25 killed queens. Black bar, surviving queens; grey bar, killed queens.

the mating flight (figure 3). There was a large increase in the total mass of CHCs ( $t_{130} = 5.82$ ,  $p < 0.0001$ ) and in their average chain length (measured by mean weighted retention time; van Zweden *et al.* 2009;  $t_{130} = 4.61$ ,  $p < 0.0001$ ) with time. Additionally, seven hydrocarbon peaks made up a significantly larger proportion of the profile as queens aged (table 2). All of these hydrocarbons were also identified as potential correlates of productivity by the brood transfer experiment, with the exception of C<sub>33:1</sub>. However, the abundance of C<sub>33:1</sub> did closely follow the changes in productivity

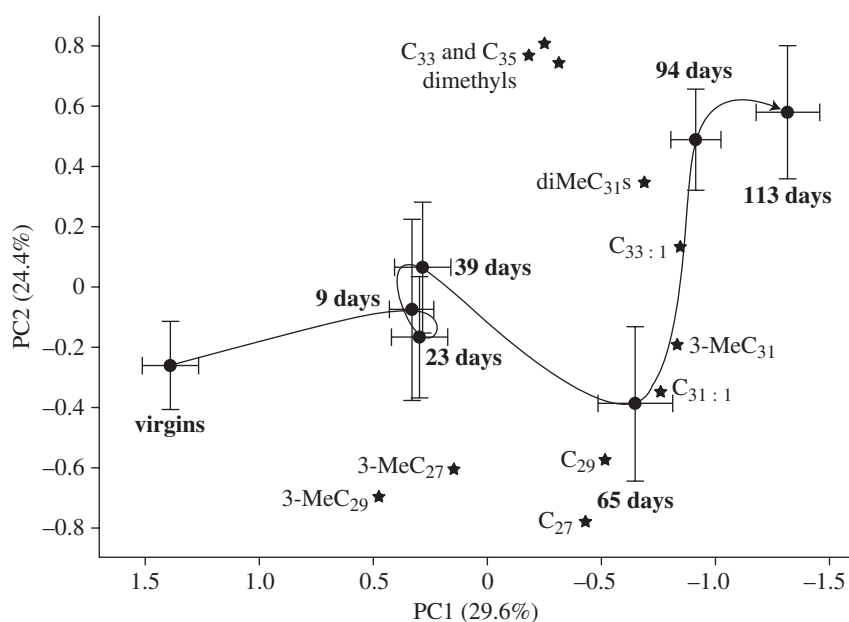


Figure 3. Principal component analysis showing how queen CHC profiles change with age ( $n = 132$ ). Each data point shows the mean  $\pm$  s.e. value of PC1 and PC2 for one age class of queens (days since mating colony foundation written in bold); the further apart the two points are, the more different are the chemical profiles. The stars show the loadings of the 11 hydrocarbon peaks listed in table 2. If a starred compound is close to a data point, then the proportion of that chemical in the profile is high relative to other data points; for example, queens sampled at 65 days after colony foundation have a higher proportion of  $C_{31:1}$  and  $3\text{-Me}C_{31}$  than virgins. Chemicals for which stars are close together are correlated.

shown in figure 1*a* (figure S3, electronic supplementary material), despite a lack of statistical significance (table 2). The most rapid change in the CHC profile was observed in the first 9 days after mating (figure 3), which coincides with ovary activation and the onset of oviposition. The queens that were sampled 39 days after colony foundation were the youngest in our sample that had adult workers, but their chemical profiles overlapped with those of 9- and 23-day-old queens (figure 3) that had only pre-imaginal brood.

#### (e) Behavioural observations

Although aggression was never observed between queens prior to worker emergence, queens in multi-queen colonies were often observed away from the brood pile, while singly housed queens spent almost all their time there (figure S4, electronic supplementary material). Moreover, queens that eventually survived the queen elimination phase spent more time on the brood pile than those that were killed in two-queen ( $t_{12} = 2.78$ ,  $p = 0.010$ ) and three-queen colonies (survivors versus queens killed first:  $t_{12} = 2.83$ ,  $p = 0.011$ ). There was no significant difference between queens that were killed first or second in the three-queen colonies ( $t_{12} = 0.62$ ,  $p = 0.54$ ). Taken together, these results imply that queens prefer to be on the brood pile and may compete for this position without conspicuous aggression.

The queen elimination phase took place reliably in all multi-queen colonies within a relatively short time frame ( $44 \pm 0.9$  days after colony foundation, range = 38–60), meaning that all colonies were alive for a similar amount of time. In the three-queen colonies, the queen killed second was always killed within 0–3 days of the first. Queen age or colony age were never significant predictors in the other analyses ( $p > 0.31$ ) and so were not included in the minimum adequate models. Queens typically died

as a result of a sustained attack by all the mature workers in the colony; queens were also seen attacking each other, but such fights were observed only twice, and neither was fatal. Queens usually ran from workers rather than fighting back, although they could easily kill workers.

#### 4. DISCUSSION

The present study experimentally demonstrates that *L. niger* queens are sensitive to the number of brood and other queens in their colony, and adjust their investment in worker production accordingly. In our experiment, queens housed in groups of three reproduced at a rate inversely proportional to the amount of brood, whereas the productivity of queens in one- or two-queen colonies was largely independent of the brood transfer treatment. These results provide support for the prediction that cohabiting queens conserve resources for the elimination stage, assuming that the colony has sufficient brood. This reproductive abstinence could be described as selfish, assuming there is a positive relationship between worker number and colony fitness (Bernasconi & Strassmann 1999). There are two reasons (not mutually exclusive) why queens could benefit from lowering their productivity only when brood is abundant. First, 'selfish' reproductive abstinence could have a less detrimental effect on colony survival if the colony already has many brood, increasing the net gains of being selfish. Secondly, the size of the brood pile is a potential cue indicating the number and/or quality of cohabiting queens (Liebig *et al.* 2005); queens in the C+ treatment may therefore have perceived that they were about to face strong competition in the elimination stage and, consequently, conserved extra resources.

A proximate question posed by these results is how queens are able to adjust their productivity to the

number of cocoons in the brood pile. Social insects modulate a range of behaviours based on the rate at which they encounter conspecifics (Gordon *et al.* 1993; Gordon & Mehdiabadi 1999; Passera 2008), brood (Liebig *et al.* 2005) and CHCs (Greene & Gordon 2007), so it seems most parsimonious that queens adjust their productivity based on the rate at which they encounter chemical and/or tactile cues associated with cocoons. For example, chemicals on the brood may act as primer pheromones that depress queen productivity. We observed that queens spent most of their time on the brood pile and frequently moved cocoons around, and analysis of *L. niger* cocoons confirmed that they are coated with hydrocarbons (see electronic supplementary material).

A remaining ultimate question is whether queens altered their productivity in order to have a better chance of surviving the queen elimination phase. In support of this, a study of the ant *M. pergandei* found that queens from a population that is typically pleometrotic invested less in reproduction, lost less weight and were more likely to win fights when founding a colony with queens from a non-pleometrotic population (Cahan 2001). However, a study of *L. niger* found no evidence that weight loss or productivity during colony growth predict queen survival (Aron *et al.* 2009), while another found that reducing a queen's oviposition rate using juvenile hormone injection did not affect her chances of winning (Sommer & Hölldobler 1995). We suspect that resource depletion during worker production does reduce the probability of surviving queen elimination, but that this cost is partially offset by other factors; for example, productive queens receive more attention from workers (Sommer & Hölldobler 1995; Hannonen *et al.* 2002). Such differential care is likely to be based on CHC profile differences and could therefore have contributed to the relationship we observed between CHCs and queen survival, but this remains to be tested experimentally.

Our chemical analyses suggested that queen CHCs provide cues to both productivity and reproductive maturity. The shifts in reproductive investment in the three-queen colonies induced by experimental cocoon transfer were matched very closely by changes in the amounts of several CHCs. However, in the two-queen colonies, some of the hydrocarbons responded to treatment similarly to the three-queen colonies, but productivity was not affected. In other ants, changes in productivity are preceded by changes in the CHC profile (Cuvillier-Hot *et al.* 2005), so it is possible that we would have observed a treatment effect on the two-queen colony productivity if queens had lived long enough. The CHCs that appeared to covary with productivity comprised alkenes, branched alkanes and hydrocarbons with comparatively long chain lengths. Previous studies of other ants have found similar correlations between these types of CHCs and caste or reproductive activity (reviewed in Cuvillier-Hot *et al.* 2004b; Monnin 2006; Hefetz 2007). We also found differences in the CHC profiles of queens that survived queen elimination and those that were killed; surviving queens had more of two linear alkanes, an alkene and two methylalkanes. These chemicals could be cues that contain information about a queen's overall condition or competitive ability, and/or

pheromones that affect the amount of aggression and care received from workers.

Some of the CHCs identified here as correlates of productivity, survival and maturity—particularly those that were repeatedly implicated—such as 3-MeC<sub>31</sub> and C<sub>31:1</sub>, are likely to be queen pheromones (i.e. queen-produced chemicals that affect the behaviour and/or physiology of nestmates). If these chemicals are indeed pheromones, then our results are most consistent with the 'queen signal' hypothesis (Keller & Nonacs 1993; Heinze & d'Ettorre 2009). As predicted by this hypothesis, the putative pheromones were positively correlated with ovarian activation, maturity and experimentally induced changes in productivity. Under the competing 'queen control' hypothesis, we would expect a positive relationship between some CHCs and the number of workers (independent of queen productivity), but this was never observed. Additionally, queens with newly eclosed workers had similar chemical profiles to queens with pre-imaginal brood, providing no evidence that queens begin to produce novel blends of chemicals in response to worker eclosion.

The fact that 3-MeC<sub>31</sub>, C<sub>31:1</sub> and 3-MeC<sub>29</sub> are related to productivity, maturity and the outcome of queen elimination suggests two non-exclusive possibilities: (1) workers might be using these chemicals as cues to selectively kill the least productive or mature queen as predicted (Balas 2005); and (2) queen productivity, CHC profile and competitive ability are correlated because of a shared dependence on overall condition. In either case, the present result is most consistent with the 'queen signal' hypothesis. In case 1, the workers' choice is predicted to increase their inclusive fitness (Balas 2005); in case 2, the chemical cues are correlates of both fertility and condition (i.e. traits that affect worker fitness).

We suggest that if unproductive queens are reliably identifiable and receive aggression, the selective advantage of selfishly conserving resources will be significantly reduced. Worker attacks against unproductive queens could represent 'sanctions' (Kiers *et al.* 2003; Lehmann & Keller 2006; Wenseleers & Ratnieks 2006; Herre *et al.* 2008) that impose extra costs against queens that under-invest in worker production and select for greater cooperation in queen associations. Once sanctions are in place, cheaters that can escape punishment are predicted to evolve (Lehmann & Keller 2006); in our case, these cheaters would be queens that over-produce the pheromone without being very productive. Such 'dishonest' queen pheromones have yet to be conclusively identified (Heinze & d'Ettorre 2009), implying that their evolution is impossible, or at least impractical. The ultimate reasons for this dishonesty remain largely speculative (Heinze & d'Ettorre 2009), but pheromone production may be (i) inextricably linked to reproduction (e.g. by shared underlying physiology), or (ii) costly, such that only high-quality individuals can maintain it (indexes and handicaps; Maynard Smith & Harper 2003).

Our behavioural observations revealed that queens that eventually survived elimination spent more time on the brood pile than those that were killed. Queens excluded from the brood were more likely to be killed in *Solenopsis invicta* (Adams & Balas 1999), and proximity to the brood is associated with behavioural dominance by *Odontomachus brunneus* (Powell & Tschinkel 1999). Our



result could therefore indicate that certain queens are physically dominant throughout colony foundation and exclude weaker queens, despite a lack of conspicuous aggression prior to worker emergence. We also observed that queens usually ran from workers that were attacking them rather than fighting back, although they can easily kill workers; this may be because colonies in which the first cohort of workers has been killed are unlikely to survive.

In summary, the present study demonstrates that queens adjust their productivity in a risk-sensitive manner according to the presence of rivals and brood. We identified several candidate queen pheromones encoded in the CHCs that are correlated with maturity, with survival in queen elimination and, in three-queen colonies, with productivity. Our results suggest a model in which investment in worker production is the product of multiple trade-offs: productive queens have less energy for competition in the elimination phase and somatic investment, but 'selfish' unproductive queens reduce the survival probability of their colony (and thereby themselves) and are more likely to be attacked. The present study highlights how the evolution of cooperation among unrelated individuals may be promoted by honest signals that allow identification of cheaters. Punishment and honest signalling may be universally important in the evolution of cooperation, particularly where the actors have widely divergent interests.

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