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## Rapid modification in the olfactory signal of ants following a change in reproductive status

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**Abstract** In insect societies, the presence and condition of egg-layers can be assessed with pheromones. Exocrine secretions are expected to vary in time in order to give up-to-date information on an individual's reproductive physiology. In the queenless monogynous ant *Streblognathus peetersi*, we allowed a previously infertile high-ranking worker to accede to the alpha rank, thus triggering the onset of her oogenesis (15 replicates). We then studied her interactions with an established egg-layer from the same colony after different durations, ranging from 20 h to several days. Even though her eggs are only ready to be laid after 30 days, the new alpha was recognised within 1–2 days. Detection occurred at a distance of a few millimetres, suggesting the involvement of a pheromone with low volatility, such as cuticular hydrocarbons. When the new alpha had differentiated for >48 h, she was attacked by the established egg-layer. In all cases, low-ranking workers eventually immobilised one of the two alphas: the new alpha was the target if she had differentiated only recently, suggesting that police workers select the dominant worker with the "less fertile" odour. Using the behaviour of ants as our measure, we demonstrate that a dominant's olfactory signal changes rapidly with a modification in her social status, and it occurs well before the onset of egg-laying.

### Introduction

Insect societies consist of only one or a few reproductives, together with a large number of sterile helpers. Recent studies on queenless ants indicate that such extreme reproductive skew is not enforced by the reproductive(s), but results from a consensus among conflicting parties (Monnin and Ratnieks 2001; Cuvillier-Hot et al. 2004a). Helpers compensate for the loss of their direct fitness by contributing to the reproductive success of relatives (Hamilton 1964). However, helpers benefit only if the reproductive's productivity is high enough, and they need honest information to assess this (Keller and Nonacs 1993). Evidence for the chemical signalling of ovarian activity comes from experimentally manipulated ant colonies in which previously infertile workers were induced to start laying eggs; in the presence of gamergates (mated reproductive workers), they were recognised and attacked by infertile nest mates (Gobin et al. 1999; Liebig et al. 1999). Pheromones are also involved in the recognition of ant queens, and experimental studies indicate that workers can assess queen fecundity (Fletcher and Blum 1983; Fletcher and Ross 1985; Keller and Passera 1989; Ortius and Heinze 1999).

In insect societies with totipotent females (many wasps and bees, queenless ants), hierarchies are established on the basis of aggressive interactions, and only the dominant(s) reproduce(s). Once a dominant becomes sufficiently fertile, aggression is replaced by chemical communication (e.g. Cuvillier-Hot et al. 2002). Within a colony of queenless ants, recognition goes beyond the detection of gamergates. In particular, high-ranking ants unambiguously recognise each other prior to interacting aggressively (e.g. Monnin and Peeters 1999). In *Streblognathus peetersi*, nestmates can detect the egg-layer but also high rankers with elevated levels of vitellogenin, a yolk precursor (Cuvillier-Hot et al. 2004b). Chemical communication of fertility is indeed involved in the resolution of reproductive conflicts in different contexts, but it is crucial that the pheromones always convey up-to-date information about individual condition.

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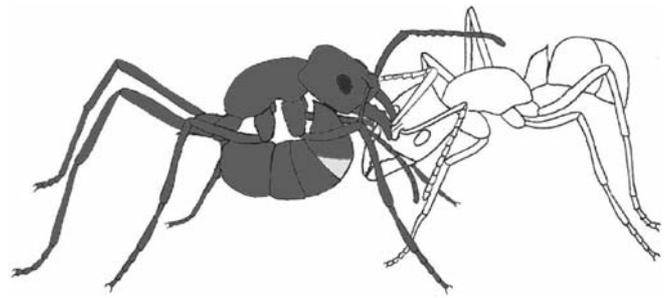
We investigated the speed at which pheromones used for intracolony recognition are modified in the monogynous *S. peetersi*. When the gamergate is removed experimentally, high-ranking workers interact aggressively and one of these (usually beta) fills the vacant top rank and becomes the new alpha (Cuvillier-Hot et al. 2004b). At this time, she has only slightly developed ovaries, and oviposition will begin about 30 days later. However, aggressions drop to a basal level within 6 days (Cuvillier-Hot et al. 2004b). We created a situation in which two related alpha ants were confronted in a colony, to determine how quickly a newly established alpha can be identified. We used the behaviour of both the alphas and nestmate workers to provide us with this information.

## Materials and methods

*Streblognathus peetersi* (Robertson 2002) was collected in South Africa, near Magoebaskloof in Limpopo Province. Seven entire colonies (187±66 workers, range 101–294) were excavated in January and April 2002. In the laboratory, they were reared in plaster nests, at 25°C and 12 h:12 h light/dark cycles. Ants were fed with crickets and mealworm larvae or pupae. Each worker was marked with a small coloured plastic number glued on the thorax (Opalithplättchen designed for *Apis*). We reduced the size of the five biggest colonies; only foragers and low-ranking workers were removed in order not to disturb the existing hierarchy. Accordingly we used seven experimental colonies of 50.5±33.2 workers.

### Determination of the hierarchy

In the monogynous *S. peetersi*, a near-linear hierarchy regulates the reproductive division of labour, and only the alpha mates with a foreign male. The alpha is also the only individual in the nest to have fully active ovaries and lay eggs. Several high-ranking members of the hierarchy compete for the beta position; the beta ant has a high probability of replacing the alpha when the latter becomes senescent. The remaining workers in the colony are low rankers who are rarely involved in dominance interactions (Cuvillier-Hot et al. 2004b). Prior to our manipulations, each colony was observed during 14 bouts of 30 min over 10 days to describe the



**Fig. 1** “Gaster curling” behaviour performed by the alpha worker (grey) on a nestmate of lower social rank (white)

hierarchy. We counted the number of bouts of antennal boxing, and biting performed and received by each worker. In addition, two conspicuous behaviours are characteristic of high rankers: “gaster curling” is performed by the alpha only (she bends the gaster under her thorax, thus exposing the intersegmental membranes of her posterior tergites, and bites the mandibles or the base of a subordinate’s antennae, Fig. 1), and “gaster rise” is performed by the alpha and other high rankers (Cuvillier-Hot et al. 2004b).

### Isolation of alpha

For each replicate, the alpha was isolated from the rest of the colony together with from four to eight nestmate workers (to minimise adverse effects on her fertility). The orphaned colonies were checked regularly to identify the new alpha, which can be clearly recognised when she begins to perform gaster curling behaviour. This time was noted (T<sub>0</sub>). The original alpha was then reintroduced into the colonies after different durations, ranging from T<sub>0</sub>+20 h to T<sub>0</sub>+8 days (Table 1). The behaviour and interactions of both the original and the new alphas were observed directly, and the entire sequences were videotaped. We also noted the reaction of other workers. One colony was used once; five colonies were used twice, and one colony was used 4 times. We thus tested 15 different pairs of alphas.

**Table 1** Outcome of 15 confrontations between two alphas originating from the same colony. *Italics* indicate encounters where the original alpha detected and attacked the new alpha. Decreasing numbers of workers in successive trials reflect mortality

Experimental colony (trial number)	Number of workers	Time to T <sub>0</sub> <sup>a</sup>	Time between T <sub>0</sub> and reintroduction of the original alpha	Which alpha immobilised?
ZH-25 (1)	46	6 h	20 h	New
ZH-23 (1)	14	20 h	25 h	New
ZH-23 (2)	14	1 h	30 h	New
ZH-33 (2)	75	3 h 30	44 h	Original
ZH-30 (2)	55	4 h 30	48 h	Both
Threshold at which the original alpha is able to detect the new alpha				
ZH-32 (2)	50	4 h 30	50 h	Original
ZH-32 (1)	74	2 h	51 h	Original
ZH-31 (2)	41	8 h	62 h	Original
ZH-25 (3)	33	19 h	72 h	New
ZH-31 (1)	88	44 h	75 h	New
ZH-25 (4)	12	18 h	96 h	Original
ZH-30 (1)	120	20 h	5 days	Original
ZH-22	24	30 h	6 days	New
ZH-33 (1)	97	24 h	7 days	Original
ZH-25 (2)	40	4 h	8 days	New

<sup>a</sup> T<sub>0</sub> is the time of the first “gaster curling” performed by the new alpha, and thus *Time to T<sub>0</sub>* is the time elapsed between the removal of the original alpha and the clear emergence of the new alpha

## Measure of the minimum distance of detection

Using the videotapes of each reintroduction of the original alphas, we specifically analysed the sequences during which either of the alphas first detects the other. We considered recognition to occur unambiguously when antennae stretch out suddenly, and/or the ant changes its direction of movement. Images were captured with HyperSnap DX/4 software, and we used Image J to measure the minimum distance required for detection (distance between the extremity of the antennae of the inquiring ant and the closest body part of the other ant).

## Results

### Time needed for the recognition of new reproductive status

The original alpha recognised the new alpha immediately in ten out of the 15 initial encounters (entries in italics, Table 1), and this corresponded to a time threshold of T0+48 h (measure of the association between the two binomial variables “reintroduction of the original alpha before/after 48 h” and “new alpha attacked/not attacked by the original alpha”: Pearson’s contingency coefficient=0.57,  $P=0.017$ ). Upon re-introduction, the original alpha was avoided by her nestmates, which is a normal submissive response. She walked towards the brood chamber where the high-ranking workers (including the new alpha) aggregate. When the new alpha had differentiated >48 h before, recognition occurred in nine cases out of ten (entries in italics below the threshold in Table 1): the original alpha attacked her by “sting smearing”. This behaviour consists of rubbing the sting against the cuticle of the target ant, to deposit Dufour’s gland chemicals that will elicit her immobilisation by other workers (Monnin et al. 2002). The new alpha reacted either by escaping (seven cases out of ten) or by sting smearing directed at the original alpha (three cases out of ten). In the latter case, she appeared more aggressive than the original alpha, staying close to the brood and directing many episodes of gaster curling towards nest mates.

When the new alpha had differentiated 48 h before or less, the original alpha recognised and attacked the new alpha only once out of five times (Table 1). In the four other replicates, the original alpha maintained her status without interacting much with the new alpha, even when the latter behaved quite aggressively.

### Immobilisation of one alpha by the low-ranking ants

In all replicates, either of the two alphas was seized by a few nest mates and immobilised for several hours. In some cases, a successful sting smearing by the other alpha preceded and induced immobilisation; in other cases the low rankers used another cue to decide which alpha to select. Indeed, in the absence of sting smearing, the new alpha ended up immobilised in all cases (non-italics entries in Table 1). In the second trial for colony ZH-30, both alphas were initially immobilised, but the new one

**Table 2** Distance required for either alpha to recognise the other. Measurements were obtained from five colonies

	First encounter	Second encounter
<i>n</i>	8	6
Median	0.13 cm	1.48 cm
Maximum	0.35 cm	2.67 cm
Minimum	0 cm	0 cm

was more aggressive and finally struggled free. In contrast, when the original alpha detected and directed sting smearing at the new alpha (entries in italics in Table 1), the latter ended up immobilised in only three replicates, whereas the former was immobilised in the seven other cases. It appears that new alphas having differentiated for longer are more aggressive and stand a better chance of avoiding immobilisation. The outcome of the confrontations is blurred by the ability of the original alpha to successfully perform sting smearing and by the response of the new alpha. Immobilisation seemed not to be influenced by any other variable (Spearman’s correlation tests based on 14 observations: with time to T0,  $P=0.6$ ; with the delay between T0 and reintroduction of the original alpha,  $P=0.7$ ; with the duration of the original alpha’s isolation,  $P=0.8$ ; with the trial number for the colony,  $P=0.7$ ). Immobilisation of an alpha always led to her removal from the hierarchy.

### Distance of detection

When the two alphas met for the first time, detection occurred only at about 1.3 mm. However, when the same ants met subsequently, they could recognise each other at a longer distance, i.e. >1 cm (Table 2). This “sensitisation” is statistically supported [two samples *t*-test for means with assumed unequal variance,  $t_{8,6}=-3,7$ ,  $P$ -value (two-sided)=0.014] and may reflect an arousal effect of the first contact that lowers the threshold of detection for subsequent encounters.

## Discussion

As a result of dominance interactions and a change in hierarchy rank, an individual ant’s physiology is modified. These modifications are dramatic for a high-ranking worker of *S. peetersi* that accedes to the alpha rank, because her ovaries develop and become able to produce eggs. We demonstrate here that these changes are sufficient to be detected by nestmates within hours, even though eggs can only be laid several weeks later. We experimentally created an unnatural situation in which a healthy fertile alpha was in the presence of a recently established alpha. Depending on how long the former had been isolated, the new alpha will have more or less active ovaries. Our results indicate that her olfactory signal changes quickly. After 20 h, this change was already detected by low rankers, who immobilised the new alpha

upon reintroduction of the original alpha without the latter's intervention. After 48 h, the change was sufficient to trigger attacks by the original alpha. The rapidity of the signal change and the behavioural responses it triggers from other workers indicate that communication of fertility underlies both dominance and policing behaviours in insect societies. Worker policing, which is here manifest as immobilisations, is a collective power exercised by low-ranking workers to enforce their interests (Monnin and Ratnieks 2001)

We used a behavioural marker of the alpha rank (gaster curling) to make an arbitrary decision about the timing of the reintroductions. The time taken for the new alphas to begin gaster curling was very variable (1–44 h; Table 1), which probably reflects different social contexts as well as the age of the new alpha relative to other high rankers (i.e. a callow can accede to the alpha rank quicker). The decision of the original alpha to attack her rival was clearly affected by the time elapsed since the new alpha's establishment. When the original alpha was reintroduced  $\leq 48$  h after T0, she interacted little with the new alpha and, in three cases out of five, quickly regained her monopoly with the help of police workers. In contrast, when she was reintroduced  $>48$  h after T0, she attacked the new alpha in nine cases out of ten. We interpret this as follows: the original alpha attacks her rival only if she detects a strong signal of fertility, otherwise she does nothing, and police workers immobilise whichever alpha has the weaker signal (in one replicate, both alphas were immobilised and the more aggressive alpha freed herself). In the absence of sting smearing, low-ranking workers can assess the physiological condition of the two competitors and immobilise the less fertile one (usually the new alpha). When sting smearing occurs, the outcome is less easy to predict since the interests of each alpha—and their physical abilities—interfere with those of low rankers. Theory predicts that workers should prefer helping the original mated alpha, which is most probably their mother, rather than a virgin sister that may supersede her. Some of the original alphas used for this study were mated, others were virgin, but we noticed no effect of this on the behaviour of low-ranking workers. In another queenless species (*Diacamma ceylonense*, Cuvillier-Hot et al. 2002), workers are unable to detect the mated status of their future reproductive and probably use the “fertility signal” and the general behaviour of their nestmates to assess their social status.

The short range of detection measured in this study suggests that the ants use a pheromone with very low volatility. This is coherent with the hypothesis that long-chained hydrocarbons on the cuticle are used to communicate fertility. In several species, experimental manipulation of social status leads to predictable changes in cuticular profiles, and this provides convincing evidence of the link between ovarian activity and cuticular hydrocarbons (Peeters et al. 1999; Liebig et al. 2000; Cuvillier-Hot et al. 2001, 2004a; Hannonen et al. 2002; Heinze et al. 2002; Dietemann et al. 2003). In *S. peetersi*, high rankers who are unable to lay eggs also have a specific

cuticular profile; this is consistent with their intermediate state of fertility, as measured by vitellogenin levels in the hemolymph (Cuvillier-Hot et al. 2004b). Rather than just ovarian activity, the cuticular hydrocarbon profile reveals the individual hormonal state that underlies dominance and oogenesis.

Our data suggest that there is a rapid turnover of cuticular hydrocarbons. In a study about nestmate recognition, Lenoir et al. (2001) showed that the cuticular profile of workers changes quantitatively after 2 days of isolation from their colony. In *S. peetersi*, changes are perceptible after an even shorter time: 26 h after removal of the original alpha, the new alpha can already be recognised by low rankers. Cuticular hydrocarbons are synthesised by the oenocytes, and transported from the hemolymph to the cuticle (Fan et al. 2003). This synthesis is continuous (Young et al. 1999), presumably throughout adult life. It is well known that ants groom themselves frequently: legs are rubbed against the cuticle, cleaned by the mouthparts, and hydrocarbons thus collected may be transferred to the post-pharyngeal gland. In *S. peetersi*, the alpha spends more time self-grooming than nestmates (Cuvillier-Hot et al. 2004b), and this probably contributes to updating her cuticular profile.

A signal that indicates the presence of a reproductive and her fecundity is evolutionarily stable, because the receivers increase their inclusive fitness by responding to it (Keller and Nonacs 1993). However, receivers benefit only if productivity is assessed correctly, and queenless ant workers should rely on a pheromone that reflects physiological parameters linked with oogenesis, present or future. Our results on *S. peetersi* indicate that a change in social rank produces a rapid modification in some external characteristics of the alpha on which olfactory recognition is based, i.e. a “fertility signal”. Low-ranking workers respond to that signal by policing one of the alphas, while the competing alphas can use it to assess each other.

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