ANIMAL BEHAVIOUR, 2004, ■■, ■■–■■
doi:10.1016/j.anbehav.2003.11.026

Fertility signalling and reproductive skew in queenless ants

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(Received 27 May 2003; initial acceptance 17 September 2003;
final acceptance 24 November 2003; published online ■ ■ ■; MS. number: A9622)

Social insects often show an extreme reproductive skew. In queenless ants, colonies consist of morphologically identical workers that can all potentially reproduce sexually. Similarly to that in social vertebrates, aggression in these ants functions to select the reproductive(s). We investigated the mechanisms underlying reproductive skew in the monogynous queenless ant *Streblognathus peetersi*. Behavioural observations of disturbed hierarchies were integrated with physiological measures of fertility (vitellogenin titre in the haemolymph) and chemical analysis of cuticular hydrocarbons, which are putative fertility pheromones. This multifaceted approach revealed that the colony reproductive is determined as a result of aggression between high-ranking workers, but once an alpha is established, chemical signalling is enough to maintain reproductive skew. As already reported in several species of ants and also in a social wasp, egg layers have distinct profiles of cuticular hydrocarbons compared with infertile workers. Importantly, 'high rankers' who are unable to lay eggs also have a specific cuticular profile; this is consistent with their intermediate state of fertility indicated by vitellogenin levels. Rather than just ovarian activity, the cuticular hydrocarbon profile thus reveals the individual hormonal state that underlies reproductive activity. We compare the fertility signal in queenless ants with the 'badge of status' reported in various birds. We discuss the evolutionary stability of this communication system and give special emphasis to ecological constraints and the high degree of intracolonial relatedness typical of social insects.

Animal societies vary in the extent to which reproduction is shared among group members. In various social birds and mammals, reproduction is almost equally distributed, whereas reproductive skew is extreme in most insect societies, even in species lacking morphologically specialized queens and workers. Many studies have focused on the role of behavioural interactions in the regulation of reproductive skew in vertebrates (e.g. Hrdy & Hrdy 1976; Creel et al. 1997; Clutton-Brock et al. 2001; Koenig & Haydock 2002). Breeders are selected on the basis of either aggressive encounters or conventions (linked to body size, age or ranks of relatives: e.g. Frank 1986; Creel et al. 1992). In either case, the asymmetry that underlies the dominance relationships is thought to be a good indicator of reproductive potential. Keller & Nonacs (1993) proposed another alternative to aggression in social insects: reproductives can signal their presence and/or reproductive capacity by means of pheromones. Unlike previous explanations based on the inhibition of ovarian activity, honest signals would benefit all the parties (Keller & Nonacs 1993), in line with biological signalling theory (Grafen 1990). This raises the question of whether chemical communication alone is enough to regulate skew, and how such regulation can be evolutionarily stable.

Some ponerine ants have lost the queen caste, and all the morphologically similar workers can potentially mate and reproduce (Peeters 1993). Agonistic interactions between nestmates lead to a dominance hierarchy that regulates reproduction (e.g. Monnin & Peeters 1999): according to species, only one or a few high-ranking workers mate (they are then called 'gamergates') and produce diploid (female) offspring; the other workers remain infertile and are responsible for brood care and nest maintenance. This kind of social organization is comparable to that found in some primitively eusocial wasps (e.g. *Polistes dominulus*: Pardi 1948; Röseler et al. 1986; *Polistes fuscatus*: West-Eberhard 1967; *Parischnogaster nigricans serrei*: Turillazzi 1991; *Ropalidia marginata*: Premnath et al. 1996), as well as some mammals such as naked mole-rats, *Heterocephalus*

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0003–3472/03/$30.00/0

glaber (Jarvis et al. 1994). However, in sharp contrast to these species, in queenless ants, the gamergates cannot breed independently. Accordingly, in a monogynous species, the sole chance for a worker to gain direct fitness is either to reach the top of the hierarchy and replace a senescent gamergate, or wait for opportunities created when the colony divides by fission (Peeters 1991). Aggressive interactions within colonies are then particularly intense. However, as soon as a new alpha is established, the rate of aggression decreases and she begins to lay eggs. This has been found in a number of species: *Pachycondyla sublaevis* (Ito & Higashi 1991); *Amblyopone* sp. (Ito 1993); *Dinoponera quadriceps* (Monnin & Peeters 1999); *Diacamma ceylonense* (Cuvillier-Hot et al. 2002). The presence of a fertile alpha thus seems sufficient to ensure a period of stability during which conflicts are resolved by means other than direct physical confrontations.

In vertebrates, honest signals are mainly used in the context of competition for food or territories; they are seldom involved in the resolution of reproductive conflicts (Rasmussen & Schulte 1998). In contrast, specific traits of insect societies may have allowed the resolution of their conflicts over reproductive sharing to be based on honest olfactory signals. Workers of several species of ants and social wasps can detect the presence of egg layers in the nest, probably through contact pheromones (e.g. for ants: *Gnamptogenys menadensis*: Gobin et al. 1999; *Diacamma* sp.: Kikuta & Tsuji 1999; Tsuji et al. 1999; *Harpegnathos saltator*: Liebig et al. 1999; *Formica fusca*: Hannonen et al. 2002; for wasps: *Polistes dominulus*: Bonavita-Cougourdan et al. 1991; Sledge et al. 2001). A pheromone that reveals fertility could thus be an important component of the regulation of reproduction in such societies. Moreover, within a colony of queenless ants, recognition goes far beyond the detection of egg layers. In particular, high-ranking ants unambiguously recognize each other prior to interacting aggressively. In *Dinoponera quadriceps*, dominance interactions are highly directed (Monnin & Peeters 1999): a worker focuses her aggression towards the few ants just below her in the hierarchy (also in *Polistes* wasps, Pardi 1948). How do workers recognize infertile nestmates having a similar rank in the hierarchy? One hypothesis is that ants advertise not only their fertility as defined by egg production, but also physiological traits that can enable them to reproduce in the immediate future. A high-ranking ant would then be recognized as being physiologically close to an egg layer, even though she may currently be unable to lay eggs. Cuticular hydrocarbons are involved in nestmate recognition in social insects (review by Lenoir et al. 1999), but a growing number of studies show that cuticular hydrocarbon profiles also correlate with ovarian activity in ants (*Dinoponera quadriceps*: Monnin et al. 1998; Peeters et al. 1999; *Harpegnathos saltator*: Liebig et al. 2000; *Diacamma ceylonense*: Cuvillier-Hot et al. 2001; *Formica fusca*: Hannonen et al. 2002; *Pachycondyla* cf. *inversa*: Heinze et al. 2002; *Myrmecia gulosa*: Dietemann et al. 2003) and in other primitively eusocial insect societies (*Bombus hypnorum*: Ayasse et al. 1995; *Polistes dominulus*: Sledge et al. 2001). A distinctive blend of these long-chained molecules could function as the fertility signal, provided that it is a reliable reflection of hormonal parameters associated with dominance and oogenesis.

We studied fertility signalling in *Streblognathus peetersi*, a species whose regulation of reproduction makes it a good model for all queenless ants. Only one gamergate occurs in small colonies of about 100 workers. The different social categories (top ranker (alpha), high ranker, low ranker) are clearly recognized by behavioural observations. The alpha is the only individual to mate and to lay eggs (even when she is still virgin). In *Dinoponera quadriceps*, the alpha mates with one foreign male (Monnin & Peeters 1998), and the closely related *Streblognathus* (Haskins & Zahl 1971) is also very likely to be monandrous. Unlike *D. quadriceps*, high-ranking *S. peetersi* workers are unable to lay eggs. We conducted observations and social manipulations to document the behavioural regulation of reproduction in this species. We measured cuticular hydrocarbons and levels of vitellogenin (used as a fertility index) in workers of known social status to determine whether particular cuticular profiles reflect just egg-laying ability or whether they give additional information about hormonal state and fertility potential. We discuss the importance of balancing the roles of aggression and fertility signalling in societies with totipotent nestmates.

METHODS

Housing Conditions and Behavioural Observations

We collected 22 colonies of *Streblognathus peetersi* (Robertson 2002) near Magoebaskloof (mean ± SD = 94 ± 48 workers, range 32–204), Limpopo Province, South Africa, and three colonies from Vernon Crookes (26, 53 and 123 workers), Kwazulu-Natal Province. In the laboratory, colonies were reared in plaster nests, at 25 °C, under 12:12 h light:dark cycles and were fed daily with mealworm, *Tenebrio molitor*, pupae and crickets, *Gryllus bimaculatus*. Twelve of these colonies were used for behavioural observations, whereas the others provided individuals for physiological and chemical investigations. We individually marked workers with small coloured plastic numbers (Opalithplättchen used for *Apis*) glued onto the thorax and noted the age of workers emerging in the laboratory. Colonies were regularly checked for eggs. Large colonies were split into groups of about 50 ants, taking care to distribute all age classes evenly, to increase the number of alphas studied (a total of 16 experimental groups were thus used).

Behaviour of the alpha worker

Ten small groups (mean = 25 workers, range 11–38) showing a stable reproductive structure (i.e. the alpha had been dominant for several weeks and had started to lay eggs) were each videotaped for 10 h continuously (1400–2400 hours; LD 5:5 h). In three cases, the alpha worker was mated (=gamergate), as revealed by dissection at the end of the experiment. We focused on the behaviour of the alpha: the number of aggressive acts she performed and received, and the time she spent self-grooming. The behaviour of a control subordinate (a randomly chosen low-ranking worker active inside the nest) was also measured for comparison, as was the total number of aggressive acts in the nest (Table 1). Results were compared with exact Wilcoxon tests.

Replacement of the alpha worker

We irregularly observed 10 other groups (mean = 53 workers, range 23–101) showing a stable reproductive structure over several days for a total time of 17.5 h. We counted all agonistic interactions and identified all the ants involved (Table 2). In five groups, we then removed the alpha and conducted a series of 15-min observations for the next 50 days (for each group: 6–10 observation periods in the 50 days before the removal; 3–4 observation periods in the 5 days after the removal; 4–5 observation periods between the 6th and the 50th day after the removal). We counted all aggressive acts performed by the workers during the three periods (day −50–0; day 0–5; day 6–50; Fig. 1a) and by the new alpha during the last two periods (Fig. 1b). For each group of ants and each period, we calculated the mean number of aggressive acts performed per individual and used this value for statistical comparisons.

Titre of the Circulating Vitellogenin

To show a link between cuticular hydrocarbons and social status, we needed detailed information about an ant's capacity for egg production. Following Martinez & Wheeler (1991) and Vargo & Laurel (1994), we estimated fertility using a physiological marker: the circulating level of vitellogenin, a yolk precursor synthesised in the fat body, secreted in the haemolymph and taken into the oocytes by receptor-mediated endocytosis to form vitellins (the major storage proteins in the eggs). The level of vitellogenin in the haemolymph provides a continuous measure of readiness for egg production, irrespective of the current degree of ovary development. Moreover, it gives a more upstream and thus updated measure of the physiological state of the individual, informing about recent changes in social status that will be translated into changes in ovarian activity only several days later. This technique is thus far more sensitive than the traditionally used dissection and visual description of ovarian development, which involves the subjective integration of various parameters (i.e. number and size of oocytes, presence of yolk).

Techniques of haemolymph storage and antibody production were mostly derived from Wheeler et al. (1999). Ants of known social status were sacrificed to measure their levels of circulating vitellogenin. Haemolymph was collected by decapitation of cooled ants and centrifugation of the body, and immediately mixed with 15 μl of a cocktail of antiproteases (Sigma-Aldrich, Lyon, France) after measuring the volume. Samples were then stored at −80 °C until quantification. The vitellin (yolk) in *S. peetersi* eggs was first characterized by electrophoresis in denaturing conditions. The equivalent of seven freshly laid eggs

Table 1. Aggressive behaviours of gamergates or unmated alphas compared to control subordinates in 10 colonies of *Streblognathus peetersi* that were each observed continuously for 10 h

	% Aggressions performed				% Aggressions received				Mean number of agonistic interactions per hour in the colony			
Focal individual	AB	B	GR	GC	AB	B	GR	GC	AB	B	GR	GC
Gamergates (N=3)	20.4 ± 0.06^a	34.3 ± 0.3^a	98.3 ± 0.03^a	100 ± 0^a	0 ± 0^a	0 ± 0^a	0 ± 0^a	0 ± 0^a	13.3±8.8	3.8±3.5	4.1±3.7	0.6±0.8
Unmated alphas (N=7)	44.5 ± 0.29^a	59.7 ± 0.3^a	82.3 ± 0.30^a	100 ± 0^a	0.4 ± 0.0^a	0.1 ± 0.0^a	0 ± 0^a	0 ± 0^a	17.7±13.8	9.3±8.9	7.2±3.4	3.6±5.4
Control subordinates (N=10)	3.3 ± 0.03^b	0.9 ± 0.02^b	0 ± 0^b	0 ± 0^b	5.6 ± 0.0^b	5.1 ± 0.0^b	6.9 ± 0.07^a	3.6 ± 0.12^a	16.4±12.2	7.7±7.9	6.2±3.6	2.7±4.7

AB = antennal boxing; B = biting; GR = gaster rise; GC = gaster curling. Values are given as mean values ± SD. Statistical tests: exact Wilcoxon two-sample tests; significance level: $P < 0.05$. Pairwise differences between groups are indicated by different superscript letters.

Table 2. Distribution of the aggression in 10 groups of *Streblognathus peetersi* that were each observed 0.5–3.5 h, with all aggressive acts monitored and the identity of participants noted

Status of the dominant	Number of workers in group	Mean frequency of alpha aggressions (per hour)	Mean % aggressions performed by alpha	Median number of high-ranking workers* (range)	Mean % aggressions received by the high-ranking workers	Mean % aggressions of alpha on high-ranking workers	Mean % GC+GR of alpha on high-ranking workers
Gamergate	29–67	2.6	16.6	8 (2–13)	33.8	54.5	76.7
Unmated alpha	23–101	43.0	49.7	7 (1–12)	36.1	45.3	58.9

GC = gaster curling; GR = gaster rise.

*High-ranking workers were defined as workers that performed together more than 75% of the total aggressions in the nest, after excluding the behaviours performed by the alpha ant.

crushed in antiprotease solution was allowed to migrate in 10% SDS-PAGE to determine the number of subunits and their molecular weight (MW Standard-BioRad). An appropriate amount of vitellin was then isolated by migration of pooled extracts of freshly laid eggs in nondenaturing conditions (23 and 25 eggs on two 6% polyacrylamide gels). On both gels, the biggest band revealed by 30-s staining in Coomassie Blue was cut out, following 24 h of decoloration in distilled water (4 °C), and lyophilized (37 °C, 3.5 h). An antigenic solution was prepared by crushing the lyophilized band and resolving it in phosphate buffered solution (PBS). Five mice and two rats received two injections of antigen; 15 days elapsed between each injection. All animals were bled 15 days after the second boost. The sera obtained were pooled separately for mice and rats, and stored in small aliquots at −20 °C. The sera were used to perform enzyme-linked immunosorbent assay (ELISA) measurements of the vitellogenin level in haemolymph extracts of individual ants: microplates were coated with the mouse serum, and the haemolymph content of vitellogenin was revealed by the rat serum detected by peroxidase-conjugated mouse anti-rat secondary antibodies (Interchim). Absorbance was read at 492 nm with a model 550 Microplate Reader (Bio-Rad). Because no standardized solution of *S. peetersi* vitellogenin exists, we used a reference solution to compare the concentration of our different samples. This reference solution, which comprised 48 fresh eggs crushed in PBS and antiproteases, was used to build the standard curve. All results are thus relative and given in arbitrary units (AU) referring to the reference solution. *Streblognathus peetersi* has the advantage of having very large individuals, about 2 cm long. This allows the collection of a sufficient quantity of haemolymph from individual ants without the need to pool samples. Results are given as mean amounts for each social category: egg-laying alphas ($N = 10$), high-ranking ants (beta and gamma, $N = 14$), low-ranking ants ($N = 15$), foragers ($N = 8$) and callows (0–4 days old, $N = 12$). Amounts were compared with nonparametric two-sample Wilcoxon signed-ranks tests.

Cuticular Hydrocarbon Measurements

Cuticular hydrocarbons (CH) were collected with the nondestructive technique of solid-phase microextraction: a polydimethylsiloxane SUPELCO fibre (7 μm bonded)

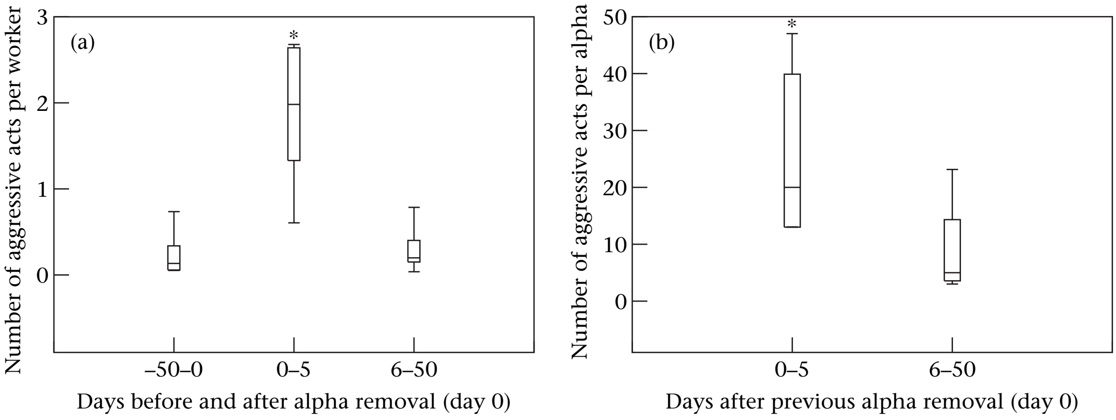

Figure 1. Changes in the number of aggressive acts (antennal boxing, biting, gaster rise and gaster curling) after the experimental removal of the alpha ant in five colonies. (a) Behaviours performed by infertile workers. The numbers of aggressive acts were counted during 15-min observation periods before and after alpha removal (on day 0). Results are given as means and percentiles (10th, 25th, 75th and 90th) of aggressive acts per worker. The behaviours performed by the alpha ants (the previously established alpha, or the new alpha) are excluded. (b) Behaviours performed by the new alpha after removal of the previously established alpha. Results are given as mean numbers. The colonies and the conditions of observation are the same as in (a) for the periods 0–5 days and 6–50 days. In (a) and (b), an asterisk marks a significant difference between groups (one-sided Wilcoxon signed-ranks test: $Wx = 15$, $N = 5$, $P = 0.03$).

was rubbed for 2 min on the gaster of live ants and in particular on the intersegmental membranes of the posterior tergites. The fibre was then inserted for 5 min into the injector of an HP6890 gas chromatograph, fitted with a 30-m long HP-5 column (95% dimethylsiloxane, 5% biphenyl polymeric). The temperature of the injector was set at 270°C, and the oven temperature programme was: 60°C (5 min); 60–230°C (50°C/min); 230°C (1 min); 230–280°C (2°C/min); 280–300°C (6°C/min); 300°C (10 min). Chromatograms were integrated with the HP Chemstation Software (Agilent Technologies, Palo Alto, California, U.S.A.). The chemical nature of the CH present on the cuticle was determined by gas chromatography-mass spectroscopy from pentane extracts, using a Varian 3300 gas chromatograph equipped with a Ross injector heated at 280°C and linked to a Nermag R10-10C quadrupole mass analyser. Compounds were eluted on a 30 m × 0.32-mm ID, 0.5-μm film-phase, nonpolar fused-silica capillary column (MDN5-S, Supelco, Bellefonte, Pennsylvania, U.S.A.) using the following programme: 200 to 300°C at 5°C/min, 1 min at 300°C, 300 to 315°C at 10°C/min. Spectral data were obtained with electronic impact (EI, 70 eV, 50–560 atomic mass units).

For the statistical analysis, the areas under the peaks of the chromatograms were first transformed according to Reyment's formula ($Z_{i,j} = \ln[A_{i,j}/g(A_j)]$, where $A_{i,j}$ is the area of the peak i in the ant j, $g(A_j)$ is the geometric mean of the areas of all peaks for ant j, and $Z_{i,j}$ is the corrected area of the peak i for the ant j; Reyment 1989) to correct for the high dependence of this type of compositional data. To reduce the number of variables, the corrected values were examined in a principal component analysis (PCA): only the 17 peaks that had the biggest factorial weight on the first two roots were selected. The corrected areas of these peaks were recalculated, taking into account the reduced data set, and then used to perform a discriminant analysis with the same social categories as for vitellogenin measures: egg-laying alphas ($N = 15$), high-ranking ants (beta and gamma, $N = 21$), foragers ($N = 26$) and callows (0–4 days old, $N = 22$). New alphas (i.e. recently acceded to the top rank and not ovipositing yet, $N = 13$) were included to study intermediate stages of the cuticular profiles. Additional ants were measured both for their cuticular hydrocarbons and for their levels of vitellogenin in the haemolymph (nine egg-laying alphas/gamergates, five high-ranking ants, six low-ranking ants, five foragers and six callows). They were not included in the discriminant analysis described above but were treated as illustrative individuals to examine the relationship between their cuticular hydrocarbon profiles and their levels of circulating vitellogenin.

Dissections

Several workers from each social category were dissected in insect Ringer's solution under a microscope to determine the degree of development of their ovaries: 39 alphas (including eight gamergates), 28 high rankers, 51 low rankers, 28 foragers and 12 callows. These numbers include all the ants used for vitellogenin measurements.

RESULTS

Behaviour of the Alpha and Organization of Stable Hierarchies

We identified four agonistic behaviours in *Streblognathus peetersi*.

(1) Gaster curling: a dominant worker bends her gaster under her thorax, thus exposing the intersegmental membranes of her posterior tergites, while biting the mandibles or the base of a subordinate's antennae. This behaviour is strikingly similar to the 'gaster rubbing' performed by alphas of *D. quadriceps* (Monnin & Peeters 1999).

(2) Gaster rise: a dominant worker holds her gaster high, the intersegmental membranes exposed, and turns around, placing her gaster in front of a subordinate worker's head.

(3) Biting: a dominant worker quickly bites a target worker, grasping any part of her body.

(4) Antennal boxing: a dominant worker repetitively hits the head or the dorsal part of a subordinate with her antennae.

Several weeks after acceding to the top rank, the alpha ants remained aggressive, but at a very low level: they performed few gaster curling and gaster rise behaviours and were responsible for less than 35% of the total antennal boxing and biting (Table 1: two-tailed binomial test: $P = 0.05$). Colonies with a gamergate showed lower basal levels of aggression than colonies with an unmated alpha (Table 2; permutation test with Monte Carlo resampling approximation: 10 000 tables, $T = 12.85$, $P = 0.0062$). The most striking result was the restriction of gaster curling to the alpha worker, whether mated or virgin (Table 1: Wilcoxon two-sample exact tests: $W = 3.35$, $N = 13$, $P = 0.0008$ for gamergates versus control subordinates; $W = 3.94$, $N = 17$, $P = 0.0001$ for unmated alphas versus control subordinates). Gaster curling can thus be considered as a marker of the alpha rank, allowing the rapid and reliable identification of the alpha ant in a colony. Similarly, gaster rise was generally restricted to the alpha (Wilcoxon two-sample exact tests: $W = 3.32$, $N = 13$, $P = 0.0009$ for gamergates versus control subordinates; $W = 3.80$, $N = 17$, $P = 0.0001$ for unmated alphas versus control subordinates), but high rankers and especially betas also performed this behaviour (data not shown). Compared with control subordinates, alpha ants spent more time self-grooming (mean ± SD: 4927 ± 2204 s versus 3286 ± 1099 s during the 10-h observation period; two-sample t test: $t_{18} = 2.12$, $P < 0.05$).

Only a few colony members were involved in agonistic encounters (Table 2): 4–23% of the workers were responsible for 75% or more of the aggressive acts performed (alphas excluded). We termed these workers high rankers; in contrast, low rankers were submissive and almost never involved in aggressive interactions. Representing a mean of 13% of the total number of workers, the high rankers received more than 30% of all the aggressive acts (Table 2; two-tailed binomial test: $P = 0.003$). The behaviour of the alpha ants towards them was unequivocal: although the alpha ants interacted with many nestmates, more than

70% of their gaster curling and gaster rise behaviours were directed at high rankers (Table 2; two-tailed binomial test: $P = 0.023$). A previous study on *D. quadriceps* showed that it is not possible to detect a strict limit between low and high rankers (Monnin & Ratnieks 1999). In *S. peetersi*, the gaster rise behaviour was only performed by alpha and high-ranking ants. It can thus help us to identify high rankers, but its infrequent occurrence makes it suitable only to detect beta, gamma and delta ants. Accordingly, we used an arbitrary threshold (75% of the total aggressions in a colony, excluding those performed by alphas) to define the class of high rankers.

Our observations contrast with the report of Ware et al. (1990) on the lack of dominance behaviour in the closely related species, *Streblognathus aethiopicus*. We suspect that they observed colonies with a long-established gamergate, in which overt aggression had almost totally disappeared.

Experimental Removal of the Alpha

In the first 5 days following the removal of an alpha, the level of aggression increased (Fig. 1a; Friedman test: $Fr = 7.6$, $N = 5$, $P = 0.02$). Then aggression decreased back to the initial level within 2 weeks. This peak of aggression reflected the intense competition between high rankers for the top rank. Within a few hours after the removal of an alpha, one ant (beta in three out of five cases, gamma or delta in the other two cases) began to perform gaster curling. This individual then initiated most of the attacks. However, within 5 days of becoming the new alpha, the frequency of her aggressive acts significantly decreased (Fig. 1b; one-tailed Wilcoxon signed-ranks exact test: $T = 15$, $N = 5$, $P = 0.029$).

Ovarian Development and Vitellogenin Level

The alpha was always the only individual in the colony with ovaries sufficiently developed to produce eggs. No conspicuous differences were observed between the ovaries of gamergates and virgin established alphas. High rankers and especially betas can have slightly developed ovaries with clearly differentiated follicles, sometimes with traces of yolk (=vitellin), but none of them had mature oocytes. Low-ranking ants, foragers and callows had undeveloped ovaries. These dissection data support our behavioural observations that the alpha is the sole egg layer in a colony. Monitoring of egg production indicated that it took on average 30 days for a new alpha to start oviposition (mean ± SD = 29.5 ± 20.6 days; range 5–81; $N = 39$ alphas). The alphas showed a cyclic egg-laying activity: they layed batches of about 30 eggs within a period of approximately 3 weeks (mean ± SD = 25.9 ± 17.4 days, range 8–67, $N = 14$ periods), then stopped for several weeks (mean ± SD = 64.4 ± 33.0 days, range 24–117, $N = 8$) before initiating a new cycle.

The eggs of *S. peetersi* contain vitellin composed of two subunits of 160 and 46 kD. The titres of circulating vitellogenin revealed decreasing mean levels in accordance with the social hierarchy (Fig. 2). As expected, alpha workers had a high level of circulating vitellogenin whereas low-ranking ants, foragers and callows showed almost no vitellogenin. Importantly, high-ranking ants showed significant medium levels of this protein (permutation tests: egg layers/high ranks: $N_1 = 10$, $N_2 = 14$, $P = 0.008$; high ranks/low ranks: $N_1 = 14$, $N_2 = 15$, $P = 0.003$; high ranks/foragers: $N_1 = 14$, $N_2 = 8$, $P = 0.022$; high ranks/callows: $N_1 = 14$, $N_2 = 12$, $P = 0.012$). Their fertility can thus be qualified as intermediate, even though their slightly developed ovaries cannot produce mature oocytes.

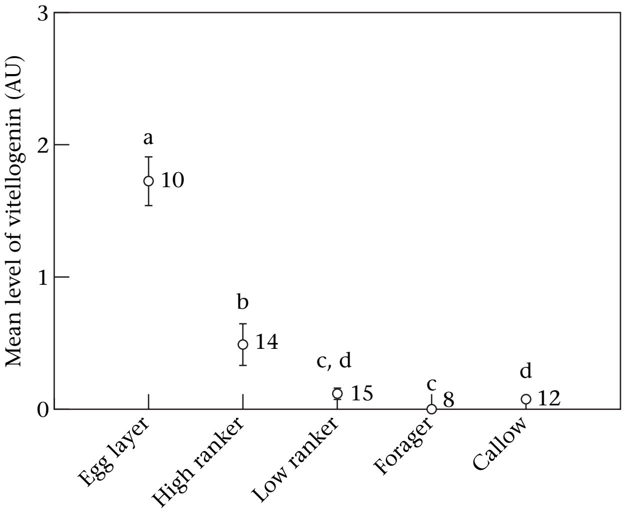

Figure 2. Comparison of the mean ± SE amounts of circulating vitellogenin in the haemolymph of individual ants grouped according to their social status. Sample sizes are indicated beside each data point. Pairwise differences between groups (exact permutation tests with general scores) are indicated by different letters ($P < 0.05$).

Variation in Cuticular Hydrocarbon Profiles

The cuticular hydrocarbons of fertile and infertile individuals vary in their relative proportions (Fig. 3). A stepwise discriminant analysis was performed on five categories of ants (egg-laying alphas, new alphas, high rankers (beta and gamma), foragers and callows) using the 17 peaks selected by PCA. This generated four significant roots explaining all the variance (Fig. 4a; global model: Wilk's $\lambda = 0.008$, $F = 12.5$, $P < 0.0001$). This function led to 91.8% correct classification of the individuals within the five social categories. The distribution of the ants based on their cuticular hydrocarbon profiles showed that egg layers and new alphas (i.e. not yet egg laying) are clearly distinct from infertile individuals (foragers and callows). The first root of the discriminant analysis completely segregated these categories (canonical $R = 0.92$, Wilk's $\lambda = 0.008$, $\chi^2_{60} = 415.8$, $P < 0.000001$). High-ranking ants had an intermediate position on the first root and were clearly separated from the foragers and the callows by the second (canonical $R = 0.90$, Wilk's $\lambda = 0.051$, $\chi^2_{42} = 256.1$, $P < 0.000001$) and the third root (not represented here: canonical $R = 0.73$, Wilk's $\lambda = 0.270$, $\chi^2_{26} = 112.6$, $P < 0.000001$; the overlapping of the ellipses in Fig. 4a is an effect of the two-dimensional

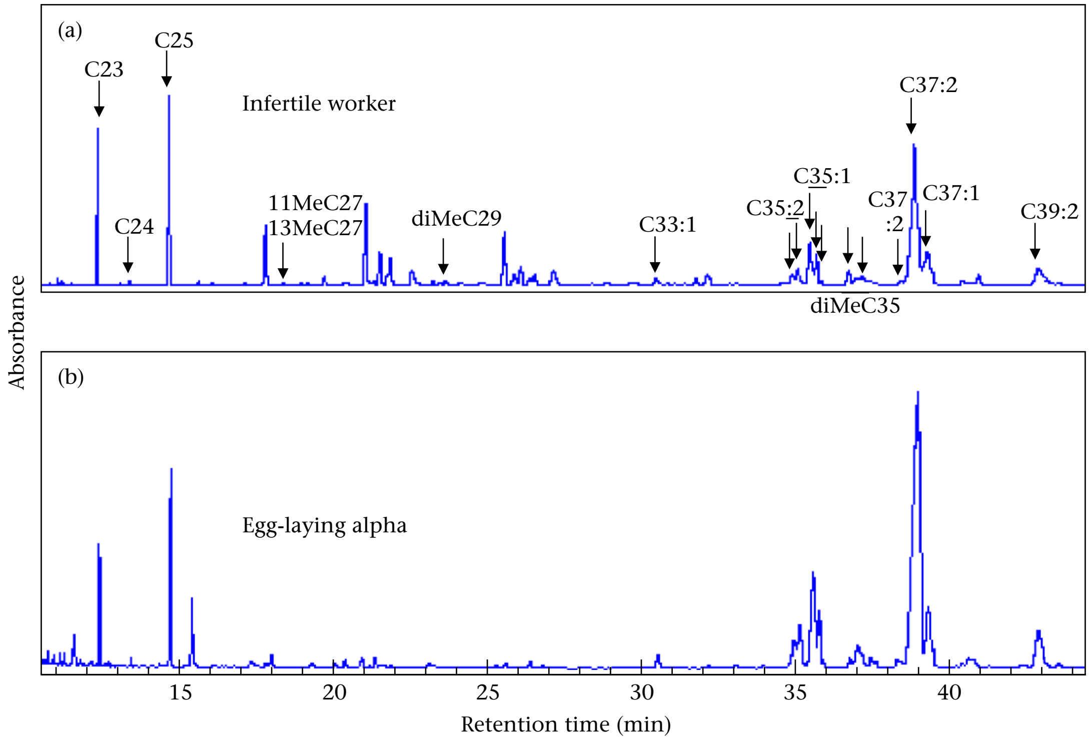

Figure 3. Chromatograms showing differences in the cuticular hydrocarbons of (a) an infertile worker and (b) an egg-laying alpha of *S. peetersi*. Arrows indicate the 17 peaks selected by principal component analysis and used as variables in the discriminant analysis. Chemical identification of these peaks is given (the positions of unsaturations in alkenes were not determined; a few peaks correspond to different alkenes with the same number of double bonds).

projection). Egg-laying alphas, new alphas and high-ranking ants were roughly distributed along an apparent ‘axis of fertility’. This axis represents the change that occurs in the cuticular profile of a high ranker when she accedes to the alpha position and then begins to lay eggs.

We investigated the correlation between cuticular profiles and our physiological measures of fertility. Additional workers with known vitellogenin levels were added to the statistical model in Fig. 4a, using its discriminant function. Their blends of cuticular hydrocarbons matched both their dominance status and their levels of circulating vitellogenin (Fig. 4b). Workers with no or only trace levels of vitellogenin remained close to infertile foragers and callows; workers that expressed vitellogenin were clearly separate, closer to the egg layers and new alphas. Moreover, the more vitellogenin a worker had in her haemolymph, the further along the axis of fertility her cuticular profile occurred (except for two mismatches). The cuticular hydrocarbon signature thus provides reliable information not only about current fertility, but also about the reproductive potential of high rankers.

DISCUSSION

Colonies of queenless ants are potentially immortal and gamergate replacement is a crucial event given that nestmates compete to be the next reproductive. The behavioural events associated with this transition allow us to investigate which modalities are used by ants to resolve their reproductive conflicts. Two distinct phases can be detected in the colony life cycle of *S. peetersi*. Stable phases are characterized by few acts of aggression and high fertility of the alpha ant. In contrast, just after a dominance turnover, the new alpha has only slightly developed ovaries and uses aggression to enforce her position. Aggression is thus the mechanism by which the dominance hierarchy is formed, but it is not involved in the maintenance of the reproductive skew. In *S. peetersi*, the alpha ant has two unique characteristics: she lays eggs and she performs gaster curling. The latter behaviour is visually very conspicuous but it is unlikely to convey information in itself. Indeed, all these social interactions take place in the darkness of the underground nest, where visual signals are totally ineffective. Because gaster curling uncovers posterior intersegmental membranes (as does gaster rise), which are areas especially rich in cuticular hydrocarbons (Monnin et al. 1998), this behaviour could help to release these molecules.

Alpha and the high-ranking ants of *S. peetersi* can be clearly identified on the basis of their cuticular profiles, which encode sufficient and graded information to label not only egg layers but also workers with intermediate reproductive potential (Fig. 4a, b). This is also true in *D. quadriceps* (Monnin et al. 1998) and *Diacamma ceylonense* (Cuvillier-Hot et al. 2002). Moreover, a new alpha of *S. peetersi* can be detected by nestmates many days before the onset of her oviposition (unpublished data), which suggests that the CH profile reflects the hormonal state of a worker more than its current egg-laying rate. This

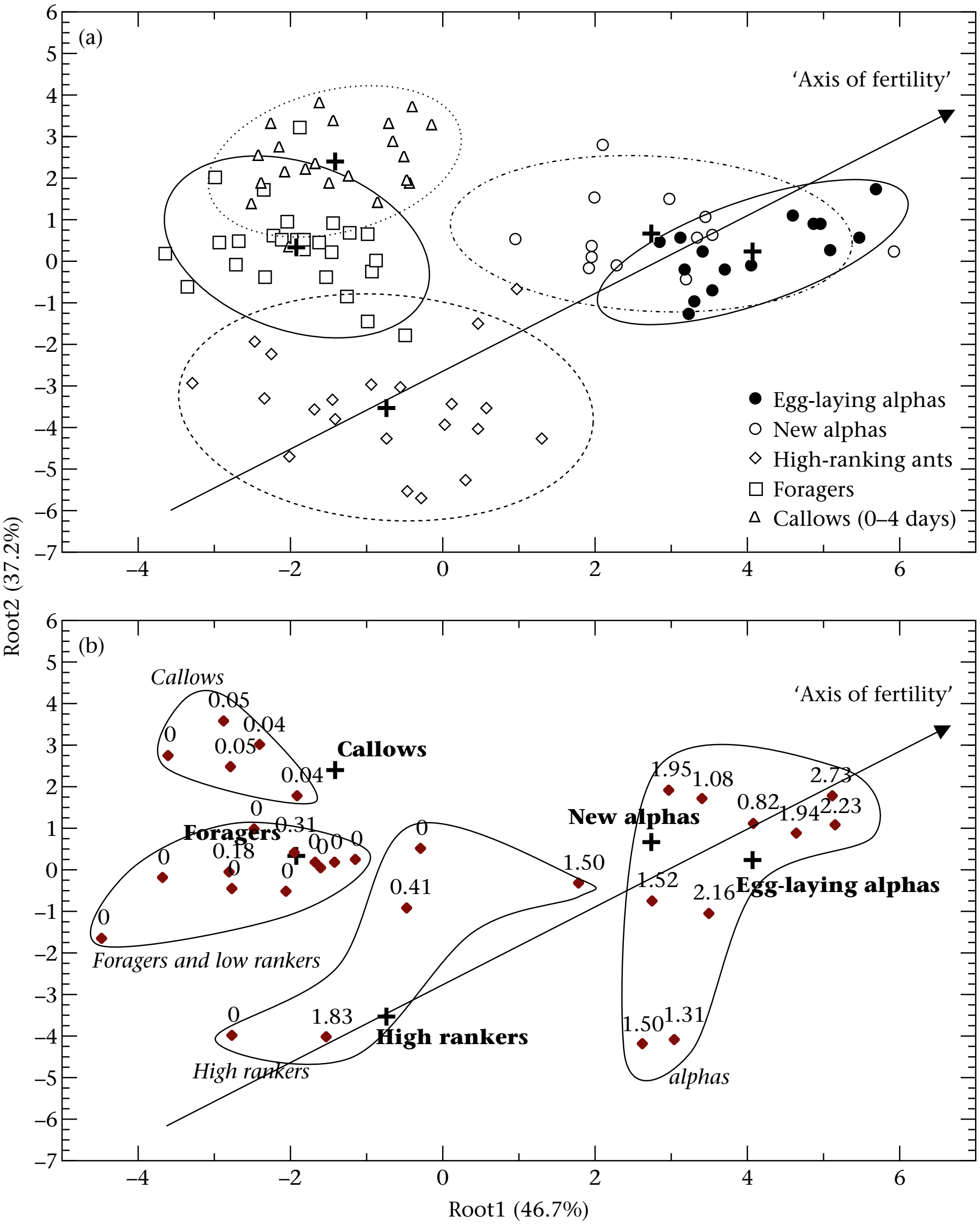

Figure 4. (a) Discriminant analysis of the cuticular hydrocarbon profiles of individuals classified into five social categories (egg-laying alphas, $N = 15$; new alphas, $N = 13$; high-ranking ants, $N = 21$; foragers, $N = 26$; callows of less than 4 days old, $N = 22$). Crosses correspond to the centroids of the categories and ellipses are the 90% confidence limits. (b) Cuticular hydrocarbon profiles of additional ants of known social status and vitellogenin levels were positioned using the discriminant function produced from the data set used in (a). A cross shows the centroid of each of the five social categories, with the corresponding name in bold. The number above each diamond gives the vitellogenin level associated with the cuticular profile of a particular individual. These additional individuals are encircled arbitrarily according to their social status (in italics).

hormonal link is further supported by our observation that cyclic egg-laying activity seems not to affect the odour of the reproductive. Indeed, periods without egg laying did not trigger challenges from high rankers. There is recent evidence of a tight link between yolk production by fat bodies and the activity of oenocytes (Jensen & Børgesen 2000), which are the cells that synthesize cuticular hydrocarbons (Fan et al. 2003).

We suggest that the ants use variations in cuticular hydrocarbons to communicate their fertility. This is supported by the cuticle-presenting behaviours of dominants and the submissive responses of their targets. As soon as an alpha is reproductively active, her odour signal replaces aggressive interactions as the mechanism for regulating reproduction within the colony. The high frequency of self-grooming shown by an alpha may function to update her cuticular hydrocarbons, which are synthesized continuously (Young et al. 1999). In support of Keller & Nonacs' (1993) claim, such a fertility signal communicates information that maximizes the recipients' fitness, and it does not have an inhibitory function. Aggression and signalling by cuticular hydrocarbons are thus two modalities used in alternation to regulate reproduction in queenless ants. They are both reliable indicators of reproductive capacity (for aggression, this point has already been discussed in vertebrates: Hrdy & Hrdy 1976; and in insects: West-Eberhard 1967). Similar to the conventions used in other social animals (e.g. Seppä et al. 2002, in wasps), the replacement of aggression by fertility signalling may have been favoured because of the scarcity of reproductive alternatives for subordinate workers (Nonacs 2001), and for productivity reasons because it minimizes the turnover time during which the colony does not produce any brood. Provided that she shows an adequate fertility signal, the new alpha is soon helped by the low-ranking ants, allowing her to reallocate resources from confrontation to egg production. Fertility signalling thus increases low rankers' fitness as well as that of the alpha.

Nestmates respond to the fertility signal according to their own reproductive potential. It is likely that workers are physiologically able to reproduce only when young, as suggested by experimental failures to induce old workers to reproduce (*Pachycondyla sublaevis*: Ito & Higashi 1991; *Ropalidia marginata*: Gadagkar et al. 1988). Indeed, callows often quickly try to enter the circle of high rankers, displacing the established ones and causing occasional bursts of aggression (in *D. quadriceps*, more than half of 64 callows studied reached ranks 1–4 within 2 weeks, and 94% had ranks 1–10; Monnin & Peeters 1999). Consequently, the high-ranking workers are most frequently the youngest individuals of the colony. In contrast, the low-ranking workers are too old to reproduce but they benefit from retaining a fertile gamergate, who is most likely their mother. Indeed, over-frequent changes of the reproductive has negative consequences on colony productivity and decreases the relatedness between nestmates, because the new alpha will mate with a foreign male. Consequently, low-ranking ants should favour stability as long as the reproductive is sufficiently fertile. It is thus very risky for a high ranker to try to replace a fertile alpha, because she will be punished via immobilization (policing: Monnin & Ratnieks 2001; Monnin et al. 2002; Cuvillier-Hot et al. 2004). The value of the contested resource (i.e. monopoly of reproduction) is evidently not high enough compared to the risks (i.e. loss of rank) associated with a challenge. High-ranking ants would thus benefit from relying on a fertility signal, such as that encoded in the cuticular profile, to measure the quality of the alpha and to wait for her decline. In contrast, when the gamergate dies or when her fertility decreases, the vacant alpha position is a high-value resource for high rankers, and low-ranking ants no longer benefit from policing challengers. In this situation, the risks for high rankers are lower and the stakes are sufficiently high to justify the potential costs of direct physical confrontation needed to accede to the alpha position. This will last until the new alpha advertises a sufficiently high fertility signal to benefit from the 'protection' of low-ranking ants.

The fertility signal in queenless ants appears analogous to the 'badge of dominance' used in vertebrates to settle peacefully contests over resources (Maynard Smith 1982; Maynard Smith & Harper 1988; Johnstone & Norris 1993). For example, in male house sparrows, *Passer domesticus*, the size of the black throat feathers is a reliable signal of competitive ability (Møller 1987; Gonzalez et al. 2002). Such dominance signalling in vertebrates has often been described in the case of competition over food, territories or sexual mates (e.g. Rower 1975; Bradbury & Vehrencamp 1998), but almost never for the allocation of intragroup reproduction. One exception may be the reproductive suppression of female naked mole-rats, whose societies have many similarities with eusocial insects including a high intracolonial relatedness as a consequence of inbreeding (Faulkes & Bennett 2001). In queenless ants, the high level of relatedness between competitors, the short life span of individuals and the inability of workers to start a new colony on their own reduce the difference in the fitness benefits between the reproductive and the infertile nestmates that raise her brood. In other words, a helper insect does not give up that much compared with most social vertebrates. This may explain why a chemical signal can be efficient to regulate reproduction in ants. According to the terminology defined for animal signalling in vertebrates (Maynard Smith & Harper 1995), the fertility pheromone in queenless ants is an 'evaluation signal', meaning that it provides information about some property of the signaller (=self-reporting signal). Similar to the badge of status in birds (e.g. Johnstone & Norris 1993; Gonzalez et al. 2002), honesty of the fertility signal is ensured by the cost linked to the dominant behaviours required to sport it (=indirect cost) and by the risk of social punishment for cheaters (immobilization = incidental cost). Aggression is thus essential to the system as it is a quality check that ensures that the best worker is selected as the future reproductive. Johnstone & Norris (1993) argued that an additional contest-independent cost was necessary to guarantee signal honesty. Otherwise, a hypothetical 'cryptic egg layer' (i.e. an egg layer that does not reveal her fertility) could easily invade the population (Maynard Smith & Harper 1988; Bradbury & Vehrencamp 1998). Although further investigations are needed to identify such costs in the cuticular signalling of fertility, there is growing evidence of a tight link between the dynamics of cuticle formation and immunity defence, especially through shared enzymes (i.e. phenoloxidase, Ashida & Brey 1998). Considering fertility signals as a badge of status integrates chemical communication in ants into the more general framework of animal signalling and illustrates how the high intracolonial relatedness and ecological constraints typical of invertebrate societies affect the mechanisms responsible for reproductive skew.

Acknowledgments

The authors especially thank J. De Oliveira for providing some of the ants, C. Haussy, D. Wheeler and N. Buck for technical help and S. Mousset for computing assistance. Thanks to M. Beekman, M. Cobb, T. Monnin, P. Nonacs and B. Oldroyd who provided very useful comments on the manuscript. The Department of Water Affairs and Forestry (Limpopo Province) and the KwaZulu-Natal

Nature Conservation Service are acknowledged for providing collecting permits. This work was supported by travel grants from Centre National de la Recherche Scientifique (CNRS) and National Research Foundation (South Africa), as well as the Groupement de Recherche CNRS 2155 ('Écologie Comportementale').

References

Ashida, M. & Brey, P. T. 1998. Recent advances in research on the insect prophenoloxidase cascade. In: *Molecular Mechanisms of Immune Responses in Insects* (Ed. by P. T. Brey & D. Hultmark), pp. 135–172. London: Chapman & Hall.

Ayasse, M., Maelovits, T., Tengö, J., Taghizadeh, T. & Francke, W. 1995. Are there pheromonal dominance signals in the bumblebee *Bombus hypnorum* L. (Hymenoptera, Apidae). *Apidologie*, **26**, 163–180.

Bonavita-Cougourdan, A., Théraulaz, G., Bagnères, A. G., Roux, M., Pratte, M., Provost, E. & Clément, J.-L. 1991. Cuticular hydrocarbons, social organisation and ovarian development in a polistine wasp: *Polistes dominulus* Christ. *Comparative Biochemistry and Physiology B*, **100**, 667–680.

Bradbury, J. W. & Vehrencamp, S. L. 1998. *Principles of Animal Communication*. Sunderland, Massachusetts: Sinauer.

Clutton-Brock, T. H., Brotherton, P. N. M., Russel, A. F., O'Riain, M. J., Gaynor, D., Kansky, R., Griffin, A., Manser, M., Sharpe, L., McIlrath, G. M., Small, T., Moss, A. & Monfort, S. 2001. Cooperation, control, and concession in meerkat groups. *Science*, **291**, 478–481.

Creel, S., Creel, N. M., Mills, M. G. L. & Monfort, S. L. 1997. Rank and reproduction in cooperatively breeding African wild dogs: behavioral and endocrine correlates. *Behavioral Ecology*, **8**, 298–306.

Creel, S. R., Creel, N. M., Wildt, D. E. & Monfort, S. L. 1992. Behavioral and endocrine mechanisms of reproductive suppression in Serengeti dwarf mongooses. *Animal Behaviour*, **43**, 231–245.

Cuvillier-Hot, V., Cobb, M., Malosse, C. & Peeters, C. 2001. Sex, age and ovarian activity affect cuticular hydrocarbons in *Diacamma ceylonense*, a queenless ant. *Journal of Insect Physiology*, **47**, 485–493.

Cuvillier-Hot, V., Gadagkar, R., Peeters, C. & Cobb, M. 2002. Regulation of reproduction in a queenless ant: aggression, pheromone and reduction in conflict. *Proceedings of the Royal Society of London, Series B*, **269**, 1295–1300.

Cuvillier-Hot, Y., Lenior, A. & Peeters, C. 2004. Reproductive monopoly enforced by sterile police workers in a queenless ant. *Behavioral Ecology*, **15**, 970–975.

Dietemann, V., Peeters, C., Liebig, J., Thivet, V. & Hölldobler, B. 2003. Cuticular hydrocarbons mediate discrimination of reproductives and nonreproductives in the ant *Myrmecia gulosa*. *Proceedings of the National Academy of Sciences, U.S.A.*, **100**, 10341–10346.

Fan, Y., Zurek, L., Dykstra, M. J. & Schal, C. 2003. Hydrocarbon synthesis by enzymatically dissociated oenocytes of the abdominal integument of the german cockroch, *Blatella germanica*. *Naturwissenschaften*, **90**, 121–126.

Faulkes, C. G. & Bennett, N. C. 2001. Family values: group dynamics and social control of reproduction in African mole-rats. *Trends in Ecology and Evolution*, **16**, 184–190.

Frank, L. G. 1986. Social organization of the spotted hyena *Crocuta crocuta*. II. Dominance and reproduction. *Animal Behaviour*, **34**, 1510–1527.

Gadagkar, R., Vinutha, C., Shanubhogue, A. & Gore, A. P. 1988. Pre-imaginal biasing of caste in a primitively eusocial insect. *Proceedings of the Royal Society of London, Series B*, **233**, 175–189.

Gobin, B., Billen, J. & Peeters, C. 1999. Policing behaviour towards virgin egg layers in a polygynous ponerine ant. *Animal Behaviour*, **58**, 1117–1122.

Gonzalez, G., Sorci, G., Smith, L. C. & de Lope, F. 2002. Social control and physiological cost of cheating in status signalling male house sparrows (*Passer domesticus*). *Ethology*, **108**, 289–302.

Grafen, A. 1990. Biological signals as handicaps. *Journal of Theoretical Biology*, **144**, 517–546.

Hannonen, M., Sledge, M. F., Turillazzi, S. & Sundström, L. 2002. Queen reproduction, chemical signalling and worker behaviour in polygyne colonies of the ant *Formica fusca*. *Animal Behaviour*, **64**, 477–485.

Haskins, C. P. & Zahl, P. A. 1971. The reproductive pattern of *Dinoponera grandis* Roger (Hymenoptera, Ponerinae) with notes on the ethology of the species. *Psyche*, **78**, 1–11.

Heinze, J., Stengl, B. & Sledge, M. F. 2002. Worker rank, reproductive status and cuticular hydrocarbon signature in the ant, *Pachycondyla* cf. *inversa*. *Behavioral Ecology and Sociobiology*, **52**, 59–65.

Hrdy, S. B. & Hrdy, D. B. 1976. Hierarchical relations among female *Hanuman langurs* (Primates: Colobinae, *Presbytis entellus*). *Science*, **193**, 913–915.

Ito, F. 1993. Social organization in a primitive ponerine ant: queenless reproduction, dominance hierarchy and functional polygyny in *Amblyopone* sp. (reclinata group) (Hymenoptera: Formicidae: Ponerinae). *Journal of Natural History*, **27**, 1315–1324.

Ito, F. & Higashi, S. 1991. A linear dominance hierarchy regulating reproduction and polyethism of the queenless ant *Pachycondyla sublaevis*. *Naturwissenschaften*, **78**, 80–82.

Jarvis, J. U. M., O'Riain, M. J., Bennett, N. C. & Sherman, P. W. 1994. Mammalian eusociality: a family affair. *Trends in Ecology and Evolution*, **9**, 47–51.

Jensen, P. V. & Børgesen, L. W. 2000. Regional and functional differentiation in the fat body of pharaoh's ant queens, *Monomorium pharaonis* (L.). *Arthropod Structure and Development*, **29**, 171–184.

Johnstone, R. A. & Norris, K. 1993. Badges of status and the cost of aggression. *Behavioral Ecology and Sociobiology*, **32**, 127–134.

Keller, L. & Nonacs, P. 1993. The role of queen pheromones in social insects: queen control or queen signal? *Animal Behaviour*, **45**, 787–794.

Kikuta, N. & Tsuji, K. 1999. Queen and worker policing in the monogynous and monoandrous ant, *Diacamma* sp. *Behavioral Ecology and Sociobiology*, **46**, 180–189.

Koenig, W. D. & Haydock, J. 2002. Dividing up the kids. *Science*, **291**, 442–443.

Lenoir, A., Fresneau, D., Errard, C. & Hefetz, A. 1999. Individuality and colonial identity in ants: the emergence of the social representation concept. In: *Information Processing in Social Insects* (Ed. by C. Detrain, J. L. Deneubourg & J. M. Pasteels), pp. 219–237. Basel: Birkhauser Verlag.

Liebig, J., Peeters, C. & Hölldobler, B. 1999. Worker policing limits the number of reproductives in a ponerine ant. *Proceedings of the Royal Society of London, Series B*, **266**, 1865–1870.

Liebig, J., Peeters, C., Oldham, N. J., Markstädter, C. & Hölldobler, B. 2000. Are variations in cuticular hydrocarbons of queens and workers a reliable signal of fertility in the ant *Harpegnathos saltator*? *Proceedings of the National Academy of Sciences, U.S.A.*, **97**, 4124–4131.

Martinez, T. & Wheeler, D. E. 1991. Effect of the queen, brood and worker caste on haemolymph vitellogenin titer in *Camponotus festinatus* workers. *Journal of Insect Physiology*, **37**, 347–352.

Maynard Smith, J. 1982. *Evolution and the Theory of Games*. Cambridge: Cambridge University Press.

Maynard Smith, J. & Harper, D. G. C. 1988. The evolution of aggression: can selection generate variability? *Philosophical Transactions of the Royal Society of London, Series B*, **319**, 557–570.

Maynard Smith, J. & Harper, D. G. C. 1995. Animal signals: models and terminology. *Journal of Theoretical Biology*, **177**, 305–311.

Møller, A. P. 1987. Variation in badge size in male house sparrows *Passer domesticus*: evidence for status signalling. *Animal Behaviour*, **35**, 1637–1644.

Monnin, T. & Peeters, C. 1998. Monogyny and regulation of worker mating in the queenless ant *Dinoponera quadriceps*. *Animal Behaviour*, **55**, 299–306.

Monnin, T. & Peeters, C. 1999. Dominance hierarchy and reproductive conflicts among subordinates in a monogynous queenless ant. *Behavioral Ecology*, **10**, 323–332.

Monnin, T. & Ratnieks, F. L. 1999. Reproduction versus work in queenless ants: when to join a hierarchy of hopeful reproductives? *Behavioral Ecology and Sociobiology*, **46**, 413–422.

Monnin, T. & Ratnieks, F. L. 2001. Policing in queenless ponerine ants. *Behavioral Ecology and Sociobiology*, **50**, 97–108.

Monnin, T., Malosse, C. & Peeters, C. 1998. Solid-phase microextraction and cuticular hydrocarbon differences related to reproductive activity in queenless ant *Dinoponera quadriceps*. *Journal of Chemical Ecology*, **24**, 473–490.

Monnin, T., Ratnieks, F. L. W., Jones, G. & Beard, R. 2002. Pretender punishment induced by chemical signalling in a queenless ant. *Nature*, **419**, 61–65.

Nonacs, P. 2001. A life-history approach to group living and social contracts between individuals. *Annales Zoologici Fennici*, **38**, 239–254.

Pardi, L. 1948. Dominance order in *Polistes* wasps. *Physiological Zoology*, **21**, 1–13.

Peeters, C. 1991. The occurrence of sexual reproduction among ant workers. *Biological Journal of the Linnean Society*, **44**, 141–152.

Peeters, C. 1993. Monogyny and polygyny in ponerine ants with or without queens. In: *Queen Number and Sociality in Insects* (Ed. by L. Keller), pp. 234–261. Oxford: Oxford University Press.

Peeters, C., Monnin, T. & Malosse, C. 1999. Cuticular hydrocarbons correlated with reproductive status in a queenless ant. *Proceedings of the Royal Society of London, Series B*, **266**, 1323–1327.

Premnath, S., Sinha, A. & Gadagkar, R. 1996. Dominance relationship in the establishment of reproductive division of labour in a primitively eusocial wasp (*Ropalidia marginata*). *Behavioral Ecology and Sociobiology*, **39**, 125–132.

Rasmussen, L. E. L. & Schulte, B. A. 1998. Chemical signals in the reproduction of Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants. *Animal Reproduction Science*, **53**, 19–34.

Reyment, R. 1989. Compositional data analysis. *Terra Review*, **1**, 29–34.

Robertson, H. G. 2002. Revision of the ant genus *Streblognathus* (Hymenoptera: Formicidae: Ponerinae). *Zootaxa*, **97**, 1–16.

Röseler, P. F., Röseler, I. & Strambi, A. 1986. Studies of the dominance hierarchy in the paper wasp, *Polistes gallicus* (L.) (Hymenoptera, Vespidae). *Monitore zoologico italiano*, **20**, 283–290.

Rower, S. 1975. The social significance of avian winter plumage variability. *Evolution*, **29**, 593–610.

Seppä, P., Queller, D. C. & Strassmann, J. E. 2002. Reproduction in foundress associations of the social wasp, *Polistes carolina*: conventions, competition and skew. *Behavioral Ecology*, **13**, 531–542.

Sledge, M. F., Boscaro, F. & Turillazzi, S. 2001. Cuticular hydrocarbons and reproductive status in the social wasp *Polistes dominulus*. *Behavioral Ecology and Sociobiology*, **49**, 401–409.

Tsuji, K., Egashira, K. & Hölldobler, B. 1999. Regulation of worker reproduction by direct physical contacts in the ant *Diacamma* sp. from Japan. *Animal Behaviour*, **58**, 337–343.

Turillazzi, S. 1991. The Stenogastrinae. In: *The Social Biology of Wasps* (Ed. by K. Ross & R. Matthews), pp. 74–98. Ithaca, New York: Cornell University Press.

Vargo, E. L. & Laurel, M. 1994. Studies on the mode of action of a queen primer pheromone of the fire ant *Solenopsis invicta*. *Journal of Insect Physiology*, **40**, 601–610.

Ware, A. B., Compon, S. G. & Robertson, H. G. 1990. Gamergate reproduction in the ant *Streblognathus aethiopicus* Smith (Hymenoptera: Formicidae: Ponerinae). *Insectes Sociaux*, **37**, 189–199.

West-Eberhard, M. J. 1967. Foundress associations in polistine wasps: dominance hierarchies and the evolution of social behavior. *Science*, **157**, 1584–1585.

Wheeler, D. E., Liebig, J. & Hölldobler, B. 1999. Atypical vitellins in ponerine ants (Formicidae: Hymenoptera). *Journal of Insect Physiology*, **45**, 287–293.

Young, H. P., Bachmann, J. A. S., Sevala, V. & Schal, C. 1999. Site of synthesis, tissue distribution, and lipophorin transport of hydrocarbons in *Blattella germanica* (L.) nymphs. *Journal of Insect Physiology*, **45**, 305–315.