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Sex, age and ovarian activity affect cuticular hydrocarbons in *Diacamma ceylonense*, a queenless ant

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Abstract

In the queenless ant, *Diacamma ceylonense*, the cuticular hydrocarbons (C25–C35) of nestmate workers vary in their proportions according to age and fertility. Newly eclosed adults ('callows') initially have the same cuticular profile, but with time this changes to that typical of foragers. In contrast, workers that begin to produce eggs develop a different cuticular profile. Several substances (*n*-C29 and some methyl C25 and C27) discriminate these different social categories (callows, foragers and egg-layers). In *Diacamma ceylonense*, inter-colony variation of the cuticular hydrocarbons was much lower than intra-colony variation. We also found qualitative differences between the sexes, with males having a clearly different profile with much more alkanes. We discuss these results in the context of physiological models of the relation between ovarian activity and the synthesis of cuticular hydrocarbons. Variations in cuticular profile are a reliable reflection of ovarian activity, and could be used by ants as a fertility signal. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Ponerinae; Reproduction; Gamergate; Age; Cuticular hydrocarbons

1. Introduction

Pheromones play a key role in many aspects of the life of insects, and are generally divided into two kinds: light volatile substances secreted by glands and long hydrocarbonated chains found on the cuticle (reviewed in Howard, 1993). Cuticular hydrocarbons, which also protect insects against desiccation (Wigglesworth, 1964), have been suggested to be involved in colony identification (Blomquist et al., 1998; Lenoir et al., 1999) but have only recently been invoked in studies of the regulation of reproduction in social insects (Peeters et al., 1999; Liebig et al., 2000).

Some social insects have no morphologically specialised queen: all females can potentially reproduce sexually (Wilson, 1971; Peeters, 1993). However, most of the colony members remain virgin and infertile, as a consequence of behavioural regulation among adults (West-Eberhard, 1977; Downing and Jeanne, 1985; Premnath et al., 1996; Monnin and Peeters, 1999). The highly directional nature of dominance interactions suggests that recognition occurs, and this appears to have an olfactory basis. It has been shown experimentally that ant workers can discriminate between nestmates exhibiting varying degrees of ovarian activity (Gobin et al., 1999; Liebig et al., 1999; Monnin and Peeters, 1999). Monnin et al. (1998) previously documented that in the queenless ant, *Dinoponera quadriceps*, egg-laying and infertile workers exhibit characteristic proportions of cuticular hydrocarbons. Similarly, in *Harpegnathos saltator*, egg-layers (both queens and workers) have distinct cuticular profiles from that of infertile queens and workers (Liebig et al., 2000). Further evidence that cuticular hydrocarbons can function as pheromones exists in the bumblebee *Bombus terrestris*, in which workers' ovaries remain inactive in the presence of the cuticular extract of a queen, but develop when exposed to several glandular extracts (Hefetz and Bloch, 1999).

In the queenless ant genus *Diacamma*, reproduction is regulated by the presence of two unique sensory structures on the thorax of workers, the gemmae (Peeters and

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Billen, 1991; Gronenberg and Peeters, 1993). Only one worker per colony retains its gemmae, and is able to mate and thus produce diploid eggs (the 'gamergate': Fukumoto et al., 1989; Peeters and Higashi, 1989). The gamergate bites off the gemmae from each newly-eclosed worker callow, preventing them from mating (Peeters and Higashi, 1989; in one *Diacamma* taxon there is no systematic mutilation, Peeters et al., 1992). Mutilated workers can lay male (haploid) eggs, although this is rarely observed when a gamergate is present in the colony (Fukumoto et al., 1989; Peeters and Higashi, 1989). When physical contact with the gamergate is prevented, workers of *Diacamma* sp. from Japan begin to lay eggs (Tsuji et al., 1999). However, it is not clear whether this effect is due to the absence of behavioural interactions with the gamergate, or to a low-volatility chemical substance such as a cuticular hydrocarbon, or to a combination of the two. In this study we systematically investigate the cuticular hydrocarbon profile of egg-layers and sterile individuals of different ages in *Diacamma ceylonense*, in order to establish whether there is sufficient chemical variation for such substances to act as social signals.

2. Materials and methods

2.1. Ant colonies

Thirteen colonies of *Diacamma ceylonense* (282±93 workers per colony) were collected from Bangalore (southern India) in November 1998, August 1999, December 1999 and April 2000. For laboratory observations, colonies were split into groups of 50–150 individuals, one with the wild-caught gamergate and the others without. Colonies were placed in plaster nests and kept at a constant temperature of 25°C with a 12:12 h light:dark cycle. Each colony was provided with the same food: crickets and mealworm larvae or pupae. Each ant was individually coded with paint. The age of ants that eclosed in the laboratory was noted. Foraging outside the nest is normally a task undertaken by the oldest ants; however, in the laboratory younger ants may sometimes undertake this task. Two groups of foragers were therefore studied: those observed foraging in the laboratory, and those observed outside the nest in the field and foraging in the laboratory. Table 1 describes the different social categories we investigated.

2.2. Dissection

One hundred and thirteen workers were dissected in insect ringer solution and their level of ovarian activity described according to an ad-hoc scale of ovarian development (Fig. 1). We also systematically checked that the spermathecae of unmutilated workers were filled with sperm.

2.3. Chemical analysis

Cuticular hydrocarbon profiles of living ants were measured using solid phase micro extraction (SPME; Arthur and Pawliszyn, 1990). A polydimethylsiloxane SUPELCO fibre (7 μm bonded) was rubbed for 2 min against the intersegmental membranes between the sixth and the eighth abdominal tergites (initial trials showed that this was the part of the body where the cuticular hydrocarbons were most concentrated), following which

Table 1
Description of the *Diacamma ceylonense* social groups used for the chemical analysis of cuticular hydrocarbons profiles

Social Group	Description	Number of individuals measured	Ovarian development (see Fig. 1)
Gamergates (G)	Collected in the field and kept in the laboratory for up to 6 months	13	4–5
Mutilated egg-layers (MutEL)	Mutilated workers, unable to mate, who lay male eggs (reared in the absence of a gamergate)	13	4
C1 (0–4 days old)	Young individuals eclosed in the laboratory and reared in the presence of a gamergate	17	1–2
C2 (5–15 days old)		14	
C3 (6-42 days old)		11	
Laboratory Foragers (F)	Workers active outside laboratory nests	21	1
Field Foragers (F*)	Workers active outside field nests (presumably old)	12	1
Males		8	–

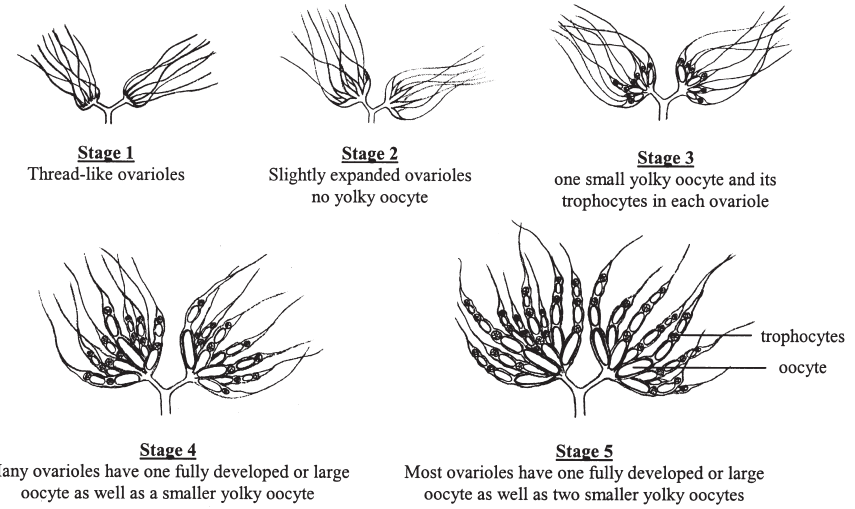

Fig. 1. Different stages of ovarian development in *Diacamma ceylonense*. Each oocyte is connected to its trophocytes. Yellow bodies are only found in stages 4 and 5, but were excluded from this drawing.

the ant was returned to the nest. The fibre was inserted for 5 min into the injector of an HP6890 gas chromatograph fitted with a 30-m-long dimethylsiloxane column (HP-5: 95% dimethylsiloxane, 5% biphenyl polymeric). The temperature of the injector was set at 270°C, and the column followed the programme: 150°C (2 min); 150–240°C (5.5°C/min); 240°C (3 min); 240–270°C (3°C/min); 270°C (2 min); 270–282°C (6°C/min); 282°C (3 min). Chromatograms were integrated using the HP ChemStation software.

Peaks were identified by GC-MS from pentane extracts, using a Varian 3300 gas chromatograph equipped with a Ross injector heated at 280°C (260°C for male extract), 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, non-polar fused-silica capillary column (MDN5-S, Supelco, Bellefonte, PA, USA), using the following programme: 200–300°C at 5°C/min (3°C/min for male extract), 1 min at 300°C, 300–315°C at 10°C/min. Spectral data were obtained with electronic impact (EI, 70 eV, 50–560 amu for female extract, 40–560 amu for male extract). The slight differences in protocol between the two sexes had no effect on peak identification. In case of alkenes, derivitization was performed and did not allow the determination of double-bond position.

2.4. Statistical analysis

Each SPME produced about 40 identifiable peaks. In order to eliminate volatile substances or poison gland secretions that may have been produced by the ant in reaction to the SPME procedure, only those peaks with a retention time >18 min were analysed. Thirty-one such peaks were found. In order to reduce the number of variables, those peaks that represented less than 3% of the sum of the area of the 31 peaks in each group were excluded, leaving 16 peaks. In order to compare the relative proportion of the peaks used in the statistical analysis, percentages were re-calculated with the 16 peaks only (Table 3).

Prior to discriminant analysis with the Statistica software, the area of each peak was corrected using Reyment's formula (Reyment, 1989) for compositional data:

$$Z_{ij} = \ln(Y_{ij}/g(Y_j))$$

where Y_{ij} is the area of peak i for ant j, $g(Y_j)$ is the geometric mean of the areas of all 16 peaks for ant j, and Z_{ij} is the corrected area of peak i for ant j.

The interpretation of the results of the multivariate analyses is mainly grounded on the comparison of the F values and of the squared Mahalanobis distances. The F value gives us information about the strength of the entire discrimination; the squared Mahalanobis distances are calculated between groups, two by two, and enable the distances between the clouds of points to be compared. Percentages of correctly classified individuals are also given in order to evaluate the power of the different cuticular hydrocarbons used as group membership predictors.

3. Results

Cuticular hydrocarbons carry information that could be used at four levels of identification: sex, age, colony and reproductive status.

3.1. Sex

There are substantial sex differences in the cuticular hydrocarbons of this species (Fig. 2; Tables 2 and 3).

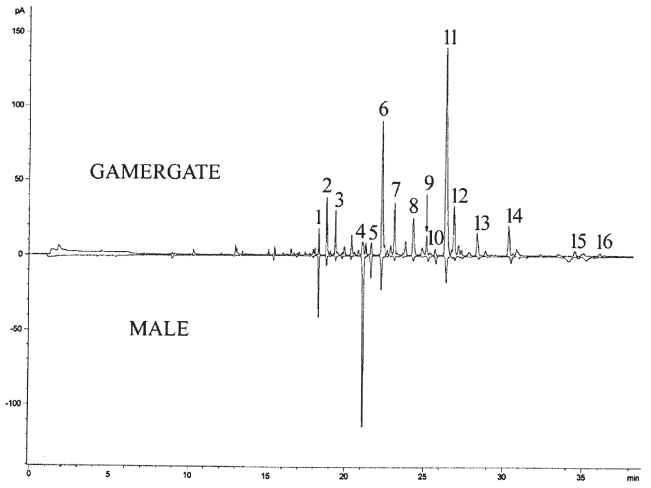

Fig. 2. Mirrored individual chromatograms of cuticular hydrocarbons from a *Diacamma ceylonense* gamergate (top) and male (bottom), sampled by SPME. Peak numbers correspond to the substances identified in Table 2.

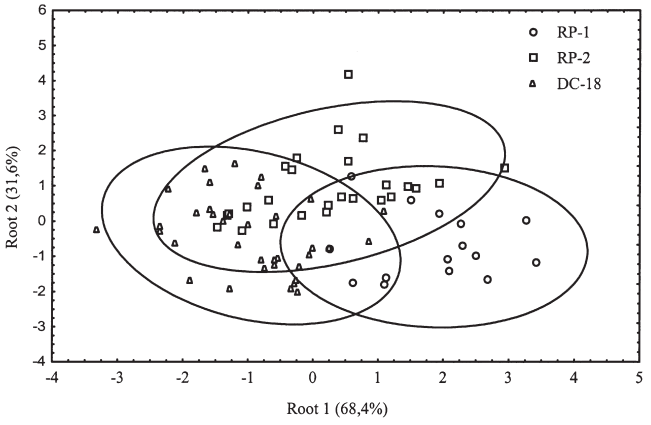

Fig. 3. Discrimination based on colony membership for the three largest colonies. Ellipses correspond to 95% confidence limits. The percentages of variance explained by each of the two main roots are given in parentheses.

Males have proportionately more alkanes (peak 1: *n*-C25; peak 5: *n*-C27) compared with most kinds of workers. The major peak in each case corresponds to a blend of monomethyls but these are heavier in females (peak 11: monomethyls C29, up to 30% of the profile) than in males (peak 4: monomethyls C26, 48% of the profile).

3.2. Colony membership

A discriminant analysis of cuticular hydrocarbons of females segregated according to colony membership was carried out. For this analysis, only individuals belonging to the three largest colonies were included (RP-1, RP-2 and DC-18). The three colonies were significantly discriminated (Fig. 3, $P<0.00001$), but distances between the colonies are small (squared Mahalanobis distances: RP-1/RP-2=5.74; RP-1/DC-18=8.25; RP-2/DC-18=3.51)

Table 2
Chemical composition of the main compounds found on the cuticle of *Diacamma ceylonense*, as determined by GC-MS. Peak numbers are only given for the 16 peaks selected for statistical analysis. Each of them represents more than 3% of the total area of the chromatogram in at least one of the social groups studied. Peaks marked (*) indicate the main compounds found on males (>7%). Bold peaks indicate those that discriminate most between fertile and sterile females. In many cases, a single peak grouped several position isomers, which we were unable to separate

Peak number (cf. Fig. 2)	Components
	n-C19:1
	n-C19
	n-C20:1
	n-C21:1
	n-C21
	n-C22:1
	n-C22
	n-C23:1
	n-C23
	n-C24:1
	3-Me-C23
	n-C24
	8-Me-C24
	n-C25:1
1*	*n*-C25
2	**9-MeC25, 11-MeC25, 13-MeC25**
	7-MeC25
	5-MeC25
3	**3-MeC25**
	n-C26
	8-MeC26
4*	10-MeC26, 11-MeC26, 12-MeC26
	4-MeC26
	n-C27:1
5*	*n*-C27
6*	**5-MeC27, 7-MeC27, 9-MeC27, 11-MeC27, 13-MeC27**
	5-MeC27, 7-MeC27
	11,15-diMeC27
7	**3-MeC27**
	n-C28
	8-MeC28
8	10-MeC28, 11-MeC28, 12-MeC28, 13-MeC28, 14-MeC28
9	4-MeC28
10	***n*-C29**
11*	9-MeC29, 11-MeC29, 13-MeC29, 15-MeC29
	5-MeC29, 7-MeC29
	11,15-diMe-C29, 13,17-diMe-C29
12	3-MeC29
	n-C30
13	10-MeC30, 11-MeC30, 12-MeC30, 13-MeC30, 14-MeC30, 15-MeC30
	n-C31
14	9-MeC31, 11-MeC31, 13-MeC31, 15-MeC31
	11,15-diMeC31, 13,17-diMeC31
15	11-MeC33, 13-MeC33, 15-MeC33
16	11-MeC35, 13-MeC35, 15-MeC35

Table 3
Mean percentages (±SD) of the 16 peaks in fertile females, sterile females and males. Peaks marked (*) indicate the main compounds found on males (>7%). Bold peaks indicate those that discriminate most between fertile and sterile females

Peak number	Gamergates	Mutilated egg-layers	Field foragers	Males
1*	2.37±0.004	3.48±0.011	3.15±0.013	*9.82±0.035
2	**5.92±0.015**	**4.62±0.015**	**2.30±0.006**	1.90±0.004
3	**4.78±0.015**	**5.02±0.009**	**0.99±0.006**	1.23±0.003
4*	4.16±0.049	4.86±0.031	2.66±0.013	*47.56±0.162
5*	1.79±0.003	2.27±0.007	5.80±0.033	*7.14±0.067
6*	**18.92±0.049**	**10.24±0.055**	**5.31±0.015**	*10.63±0.029
7	**6.25±0.013**	**7.59±0.011**	**1.55±0.006**	1.40±0.002
8	5.73±0.012	4.86±0.008	4.88±0.012	1.70±0.002
9	1.41±0.006	2.67±0.016	0.81±0.006	2.26±0.010
10	**2.51±0.047**	**1.84±0.007**	**19.00±0.135**	**3.13±0.038**
11*	28.77±0.071	29.72±0.029	30.29±0.074	*7.89±0.016
12	5.90±0.032	7.84±0.014	12.30±0.031	1.31±0.010
13	2.99±0.007	4.30±0.009	4.12±0.011	1.01±0.005
14	4.67±0.013	6.17±0.017	5.26±0.012	2.28±0.011
15	2.34±0.043	1.65±0.011	1.44±0.002	0.64±0.005
16	1.49±0.030	2.87±0.043	0.13±0.002	0.09±0.002

and the strength of the discrimination is weak ($F(30,120)=3.26$). The statistical model enables 75.3% of individuals to be correctly classified.

3.3. Age

We monitored age-related cuticular hydrocarbon changes in ants that eclosed in the laboratory in the presence of a gamergate and which therefore remained sterile (groups C1–C3). Fig. 4(A) shows the results of a discriminant analysis of gamergates, laboratory foragers, field foragers, two groups of callows of different ages (C1 and C2) and a group of young nurses (C3) (see Table 1). This time, discrimination is both significant and strong ($F(80,326)=8.57$, $P<0.00001$), and the power of prediction of the statistical model is better compared with colony membership discrimination (90.9% of correct classifications).

As the callow ages, its cuticular profile changes from a blend that is common to all newly-eclosed ants (C1) towards the typical forager profile (squared Mahalanobis distances: C1/F=23.43; C3/F=16.95). Field foragers are even more discriminated from callows than are the laboratory foragers (squared Mahalanobis distance C1/F* =50.77). This is probably due to the fact that laboratory foragers may include some younger ants (see Materials and methods).

3.4. Reproductive status

Fig. 4(A) reveals a very good discrimination according to reproductive status. The gamergates group is clearly isolated from the groups of sterile individuals (squared Mahalanobis distances: G/F*=65.98; G/C1=41.43). Plotting the 16 peaks onto the two main roots indicates that all substances are involved to varying degrees in differentiating females of different social statuses (Fig. 4B). However, the five peaks that contribute most to the discrimination between gamergates and non egg-layers (peaks 2, 3, 6, 7 and 10: 3-, 9-, 11-, 13-methyl C25 and C27, and *n*-C29) still provided a significant and even stronger discrimination ($F(25,291)=15.37$, $P<0.00001$), with 73.9% of cases correctly classified (for comparison, discrimination based on colony membership using these five peaks only gives the following results: $F(10,140)=3.09$, $P<0.0014$; 54.5% of cases correctly classified).

Egg production has a significant effect on cuticular hydrocarbon profile. In the absence of the gamergate, some mutilated workers will lay eggs. These mutilated egg-layers (MutEL, 1–3 eggs per day) were compared with the C3 group which corresponds to sterile ants of a similar age, as well as with older sterile ants (merged laboratory foragers and field foragers: F+F*) and with the gamergates (G, 3 eggs per day). The cuticular profile of mutilated egg-layers is intermediate between that of gamergates and that of C3 sterile workers (Fig. 5; $F(48,152)=8.10$, $P<0.00001$; 91.4% of correct classifications). Peaks 2 and 6 (9-, 11-, 13-monomethyls C25 and C27) are mainly responsible for the statistical differentiation between gamergates and mutilated egg-layers, making up over 25% of the cuticular extract for gamergates, as against approximately 15% for mutilated egg-layers (Table 3).

The gamergate in a *Diacamma ceylonense* colony is the only ant that retains its gemmae. These structures are full of exocrine glands (Peeters and Billen, 1991). In order to see whether the cuticular differences observed between gamergates and other ants were due to the former retaining their gemmae, 11 callows (0–4 days) were

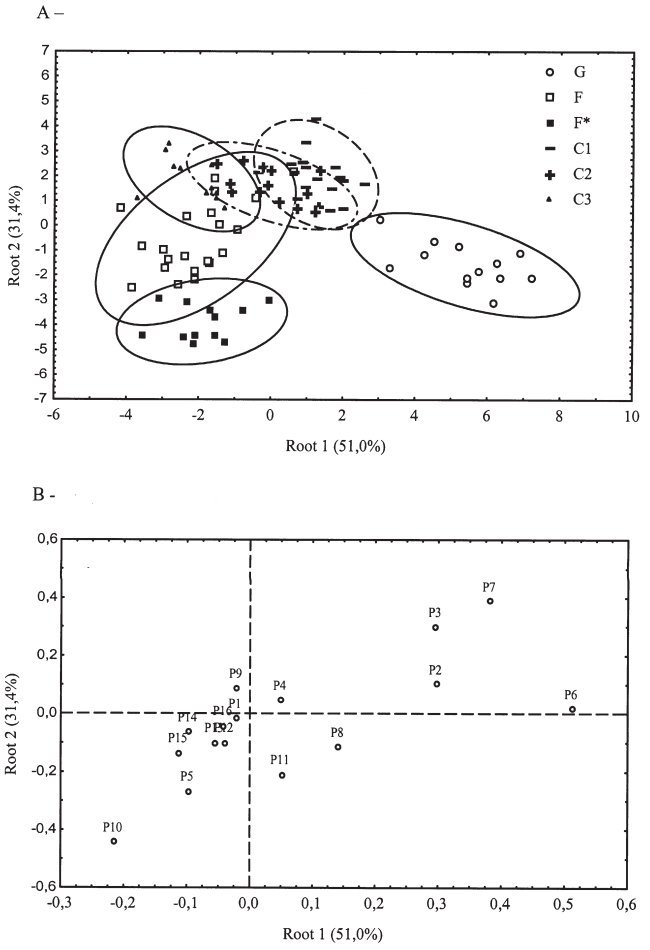

Fig. 4. (A) Relative positions of callows of various ages compared with gamergates (G) and foragers (F, laboratory foragers; F*, field foragers). Discriminant analysis was done on the corrected area of the 16 selected peaks of the cuticular hydrocarbon profile. C1 corresponds to callows from 0 to 4 days old (n=17); C2 corresponds to callows from 5 to 15 days old (n=14); C3 is a group of young workers between 16 and 42 days old (n=11). Ellipses correspond to 95% confidence limits. The percentages of variance explained by each of the two main roots are given in parentheses. (B) Relative contributions of the 16 selected peaks to the first two discriminant roots (factor structure coefficients).

allowed to keep their gemmae and were compared with a control group (n=11) that had been mutilated by the gamergate. No significant differences were found between these two groups for the 16 peaks tested (MANOVA, Exact F=1.77, p=0.248), indicating that gemmae have no effect on the cuticular hydrocarbon profile.

4. Discussion

The cuticular hydrocarbon profile of *Diacamma ceylonense* adults is complex, providing sufficient variation for differences in sex, colony membership and social status to be detected. The expression of several levels of

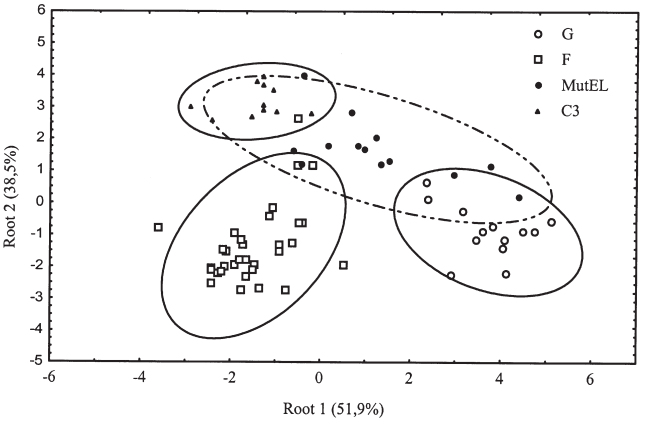

Fig. 5. Discriminant analysis showing the relative positions of gamergates (G, n=13), mutilated egg layers (Mut EL, n=13), sterile workers of approximately the same age as mutilated egg-layers (C3, n=11) and laboratory and field foragers (F+F*, n=33). Data for G, C3, F and F* groups are as in Fig. 4. Ellipses correspond to 95% confidence limits.

information using the same physical basis is yet another example of the potential richness of chemical communication. Previous studies have shown that colony and caste identification might be communicated by cuticular hydrocarbons (Blomquist et al., 1998; Howard et al., 1982; Lenoir et al., 1999). Our aim was not to study the involvement of cuticular hydrocarbons in nestmate recognition, but we note that, among females, inter-colony variation was much lower than intra-colony variation. Although a link between cuticular hydrocarbons and ovarian activity in social insects has been previously suggested (Monnin et al., 1998; Liebig et al., 2000), this is the first time that the three parameters—sex differences, age and fertility—have been investigated in the same species. Our finding that sex differences and age can be communicated by variations in cuticular hydrocarbons is novel.

Male cuticular hydrocarbons have not been previously described in ants, although they have been widely studied in solitary insects and in bees (for a review, see Tillman et al., 1999). It can be assumed that a fundamental role of these hydrocarbons is sex and species identification. A comparison of the clearly different male profile with those of the females suggests that this highly flexible source of information may have been recruited by females for intra-colony communication.

Two interacting factors were found to account for intra-colony variation: age and fertility. Age-related changes in cuticular hydrocarbons have previously been found in solitary and social insects (Trabalon et al., 1992; Nielsen et al., 1999, respectively). They may be a by-product of the hardening of the cuticle as the young insect matures. In three other ponerine ants, *Dinoponera quadriceps*, *Harpegnathos saltator*, and *Streblognathus aethiopicus*, callows (<1 month old) also have a different cuticular profile from older infertile workers (Monnin et al., 1998; Liebig et al., 2000; unpublished

data). Reanalysis of the *Dinoponera quadriceps* data using more homogeneous age groups (1–10 days, 11–20 days, 21–30 days) suggests that there is a gradual change of the young ant's cuticular profile, as in *Diacamma ceylonense* (T. Monnin, personal communication).

If the cuticular hydrocarbon profile is highly correlated with age, it could be used as a reliable marker of the age of a given ant during behavioural interactions. In all queenless ants, young callows are the most likely contenders for the status of egg-layer. In *Dinoponera quadriceps*, callows are frequently victims of aggression in the first few days after eclosion (Monnin and Peeters, 1999); in *Diacamma ceylonense* the only aggression exhibited in the presence of a gamergate is directed at newly-emerged callows. It has been suggested that cuticular hydrocarbons could be a marker involved in the regulation of age-related foraging (Wagner et al., 1998); such an age-marker may also be involved in the regulation of colony fission which requires maintaining a normal age distribution of the resulting colonies.

The effect of fertility on hydrocarbon profile is particularly striking and shows that cuticular hydrocarbons can reflect the endogenous egg-laying capacity. Correlation between ovarian activity and cuticular hydrocarbon profile has been reported in social wasps and bees (*Polistes dominulus*, Bonavita-Cougourdan et al., 1991; *Bombus hypnorum*, Ayasse et al., 1995). In the case of *Diacamma ceylonense*, the main peaks associated with egg-laying consist of closely-related substances (C25 and C27 monomethyls; Table 2). In *Dinoponera quadriceps*, the situation is more straightforward, with only two compounds (9-C31:1 and 9-C33:1) distinguishing egg-layers from sterile individuals (Monnin et al., 1998). In *Harpegnathos saltator*, the cuticular profile of egg-layers is characterised by a shift towards heavier hydrocarbons, and in particular C37 (Liebig et al., 2000). This shows that there are many ways of expressing the same information, and suggests that different species of ants use different patterns of variation in cuticular hydrocarbons.

Future studies may show that, as well as providing binary information—egg-layer or sterile—*Diacamma ceylonense* cuticular hydrocarbons reflect degrees of fertility. Substantial differences in fertility exist between gamergates and mutilated egg-layers. There was also important variability among mutilated egg-layers. More precise measurements of egg-laying rate will be required to test whether there is a relation between cuticular profile and degree of ovarian activity. Finally, it should be noted that the extraction techniques used here do not sample other substances present on the cuticle (e.g. fatty acids, proteins) that could also provide information, either alone or in a blend with the cuticular hydrocarbons described here.

It seems probable that the link between ovarian activity and cuticular hydrocarbons involves gonadotropic hormones. It is generally accepted that cuticular hydrocarbons are synthesised by the oenocytes (Diehl, 1975; Ferveur et al., 1997), cells located near the epidermis or dispersed inside the body (Noirot and Quennedey, 1991). Oenocytes are often associated with fat bodies, a major source of fatty acids that could serve as precursors for hydrocarbon biosynthesis. In adult females of some solitary insects, the oenocytes undergo cyclical activation corresponding to the ovarian cycle (Wigglesworth, 1972). Dillwith et al. (1983) showed that in *Musca domestica*, vitellogenic ovaries were required to initiate but not maintain the synthesis of hydrocarbon pheromones; this argues in favour of an upstream common control for ovaries and oenocytes. Moreover, gonadotropic hormones have a direct effect on oenocyte activity in *Sarcophaga bullata* (20-hydroxyecdysone, Armold and Regnier, 1975; juvenile hormone, Stoppie et al., 1981), and in *Musca domestica* (20-hydroxyecdysone, Blomquist et al., 1995), suggesting that these hormones could be involved in the control of both vitellogenesis and hydrocarbon synthesis.

Whether ants actually use the chemical markers described here as pheromones remains to be determined. However, a number of factors suggest that this may be the case. We observed three colonies of *Diacamma ceylonense*, each of which had a gamergate; in all cases, the gamergate was rarely aggressive towards workers, who were not aggressive towards each other (unpublished data). On the other hand, when the gamergate died, interworker aggression rapidly appeared, and mutilated workers began to lay unfertilised (male) eggs. Finally, similar effects are observed when workers are sequestered apart from the gamergate (Tsuji et al., 1999), emphasising the importance of physical contact with the egg-layer. This suggests that workers are able to detect the presence of a fertile egg layer in the colony through antennation. As a result, their ovaries do not develop. Given that the substances described here appear to directly reflect ovarian function, they are a good candidate for the 'queen signal' (Keller and Nonacs, 1993) which would enable workers to delegate reproduction to a related individual (Grafen, 1990; Keller and Nonacs, 1993; Bourke and Franks, 1995). Further research is necessary to determine whether these substances do act as pheromones.

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References

Armold, M.T., Regnier, F.E., 1975. Stimulation of hydrocarbon biosynthesis by ecdysone in the flesh fly *Sarcophaga bullata*. J. Insect Physiol. 21, 1581–1586.

Arthur, C.L., Pawliszyn, J., 1990. Solid phase microextraction with thermal desorption using fused silica optic fibers. Anal. Chem. 62, 656.

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.

Blomquist, G.J., Tillman, J.A., Reed, J.R., Gu, P., Vanderwel, D., Choi, S., Reitz, R.C., 1995. Regulation of enzymatic activity involved in sex pheromone production in the housefly, *Musca domestica*. Insect Biochem. Mol. Biol. 25 (6), 751–757.

Blomquist, G.J., Tillman, J.A., Mpuru, S., Seybold, S.J., 1998. The cuticle and cuticular hydrocarbons of insects: structure, function, and biochemistry. In: Pheromone Communication in Social Insect. Westview Press, pp. 34–54.

Bonavita-Cougourdan, A., Théraulaz, G., Bagnère, 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. Comp. Biochem. Physiol. 100B, 667–680.

Bourke, A.F.G., Franks, N.R., 1995. Social Evolution in Ants. Princeton University Press, Princeton, NJ, 529 pp.

Diehl, P.A., 1975. Synthesis and release of hydrocarbons by the oenocytes of the desert locust, *Schistocerca gregaria*. J. Insect Physiol. 21, 1237–1246.

Dillwith, J.W., Adams, T.S., Blomquist, G.J., 1983. Correlation of housefly sex pheromone production with ovarian development. J. Insect Physiol. 29 (5), 377–386.

Downing, H.A., Jeanne, R.L., 1985. Communication of status in the social wasp *Polistes fulcatus* (Hymenoptera: Vespidae). Z. Tierpsychol. 67, 78–96.

Ferveur, J.F., Savarit, F., O'Kane, C.J., Sureau, G., Greenspan, R.J., Jallon, J.M., 1997. Genetic feminization of pheromones and its behavioral consequences in *Drosophila* males. Science 276, 1555–1558.

Fukumoto, Y., Abe, T., Taki, A., 1989. A novel form of colony organization in the 'queenless' ant *Diacamma rugosum*. Physiol. Ecol. Jpn. 26, 55–61.

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

Grafen, A., 1990. Biological signals as handicaps. J. Theor. Biol. 144, 517–546.

Gronenberg, W., Peeters, C., 1993. Central projections of the sensory hairs on the gemma of the ant *Diacamma*: substrate for behavioural modulation? Cell Tissue Res. 273, 401–415.

Hefetz, A., Bloch, G., 1999. Reevaluation of the role of mandibular glands in regulation of reproduction in bumblebee colonies. J. Chem. Ecol. 25 (4), 881.

Howard, R.W., 1993. Cuticular hydrocarbons and chemical communication. In: Stanley-Samuelson, D.W., Nelson, D.R. (Eds.), Insect Lipids. University of Nebraska Press, Lincoln, pp. 177–226.

Howard, R.W., McDaniel, C.A., Nelson, D.R., Blomquist, G.J., Gelbaum, L.T., Zalkow, L.H., 1982. Cuticular hydrocarbons of *Reticulitermes virginicus* (Banks) and their role as potential species and caste-recognition cues. J. Chem. Ecol. 8, 1227–1239.

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

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

Liebig, J., Peeters, C., Hölldobler, B., 1999. Worker policing limits the number of reproductives in a ponerine ant. Proc. R. Soc. London, Ser. 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*? PNAS 97 (8), 4124–4131.

Monnin, T., Malosse, C., Peeters, C., 1998. Solid-phase microextraction and cuticular hydrocarbon differences related to reproductive activity in queenless ant *Dinoponera quadriceps*. J. Chem. Ecol. 24 (3), 473–490.

Monnin, T., Peeters, C., 1999. Dominance hierarchy and reproductive conflicts among subordinates in a monogynous queenless ant. Behav. Ecol. 10 (3), 323–332.

Nielsen, J., Boomsma, J.J., Oldham, N.J., Petersen, H.C., Morgan, E.D., 1999. Colony-level and season specific variation in cuticular hydrocarbon profiles of individual workers in the ant *Formica truncorum*. Insectes Sociaux 46, 58–65.

Noirot, C., Quennedey, A., 1991. Glands, gland cells, glandular units: some comments on terminology and classification. Ann. Soc. Entomol. Fr. 27 (2), 123–128.

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

Peeters, C., Billen, J., 1991. A novel exocrine gland inside the thoracic appendages ('gemmae') of the queenless ant *Diacamma australe*. Experientia 47, 229–231.

Peeters, C., Higashi, S., 1989. Reproductive dominance controlled by mutilation in the queenless ant *Diacamma australe*. Naturwissenschaften 76, 177–180.

Peeters, C., Billen, J., Hölldobler, B., 1992. Alternative dominance mechanisms regulating monogyny in the queenless ant genus *Diacamma*. Naturwissenschaften 79, 572–573.

Peeters, C., Monnin, T., Malosse, C., 1999. Cuticular hydrocarbons correlated with reproductive status in a queenless ant. Proc. R. Soc. London 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*). Behav. Ecol. Sociobiol. 39, 125–132.

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

Stoppie, P., Briers, T., Huybrechts, R., De Loof, A., 1981. Moulting hormone, juvenile hormone and the ultrastructure of the fat body of adult *Sarcophaga bullata* (Diptera). Cell and Tissue Research 221, 233–244.

Tillman, J.A., Seybold, S.J., Jurenka, R.A., Blomquist, G.J., 1999. Insect pheromones—an overview of biosynthesis and endocrine regulation. Insect Biochem. Mol. Biol. 29, 481–514.

Trabalon, M., Campan, M., Clément, J.L., Lange, C., Miquel, M.T., 1992. Cuticular hydrocarbons of *Calliphora vomitoria* (Diptera): relation to age and sex. Gen. Comp. Endocrinol. 85 (2), 208–216.

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

Wagner, D., Brown, M.J.F., Broun, P., Cuevas, W., Moses, L.E., Chao, D.L., Gordon, D.M., 1998. Task-related differences in the cuticular hydrocarbon composition of harvester ants, *Pogonomyrmex barbatus*. J. Chem. Ecol. 24 (12), 2021–2037.

West-Eberhard, M.J., 1977. The establishment of reproductive dominance in social wasp colonies. In: Proceedings of the 8th International Congress IUSSI, Wageningen, The Netherlands, pp. 223–227.

Wigglesworth, V.B., 1964. The life of insects. In: Carrington, R. (Ed.),

The World Natural History. The New American Library, New York.
Wigglesworth, V.B., 1972. Principles of Insect Physiology, 7th ed. Chapman and Hall, London.
Wilson, E.O., 1971. The Insect Societies. Harvard University Press, Cambridge, MA.