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Research article

Effects of social isolation on hydrocarbon pattern and nestmate recognition in the ant *Aphaenogaster senilis* (Hymenoptera, Formicidae)

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Summary. In the non-trophallacting ant *Aphaenogaster senilis*, a change over time was observed in cuticular and postpharyngeal gland hydrocarbon profiles. A change was also observed after individual social isolation. Short periods of isolation induced amicable reaction, such as allogrooming, which may have facilitated re-integration of the isolated ants into their mother colonies. Longer periods of isolation, on the other hand, caused overt aggression towards the isolated ants when reintroduced into their mother colonies, and also resulted in higher changes in cuticular hydrocarbon profile. This correlation suggests a link between these two phenomena. We suggest that in *A. senilis,* in the absence of cue transfer by trophallaxis: a) colony odour constitutes a gestalt, and b) the major means of cue transfer is allogrooming. The possible evolution of allogrooming and trophallaxis as cue transfer modalities is discussed.

Key words: Aphaenogaster senilis, social isolation, hydrocarbons, postpharyngeal gland, nestmate recognition, allogrooming.

Introduction

In social insects, cuticular chemical cues vary according to species, colony and castes and are considered to play a key role in nestmate recognition. Many correlative studies have suggested that, at least in ants and wasps, cuticular hydrocarbons constitute the signal of colonial identity (reviewed in Lorenzi et al., 1996; Singer, 1998; Vander Meer and Morel, 1998; Lenoir et al., 1999). Recently, experiments with *Cataglyphis niger* and the meat ant *Iridomyrmex purpureus,* indicated that it is only the hydrocarbons' fraction (HCs) of the cuticular lipids that allows nestmate recognition (Lahav et al., 1999; Thomas et al., 1999). Nevertheless, in other species more polar lipids may also be involved, as indicated by the role of fatty acids in nestmate recognition in *Leptothorax acervorum* (Franks et al., 1990a).

Several behavioural and chemical (based on hydrocarbon profiles) studies (see above-mentioned reviews) showed that all the individuals within a colony share their own recognition cues to form a "gestalt colony odour" as postulated by Crozier and Dix (Crozier and Dix, 1979; Crozier, 1987). But, within-colony variation in hydrocarbon composition was also observed according to the castes (Bonavita-Cougourdan et al., 1993; Wagner et al., 1998; Kaib et al., 2000) or the reproductive status (Dahbi and Lenoir, 1998a; Peeters et al., 1999; Liebig et al., 2000). It has been further demonstrated that the postpharyngeal gland (PPG), a unique gland of Formicidae, is a reservoir of the colonial odour and its content acts as modifier of aggressive behaviour (Soroker et al., 1994; Hefetz et al., 1996).

Despite its relative uniformity, colony odour is dynamic and may change over time, or according to the season. These are quantitative changes in relative proportions of the different cuticular HC classes (Vander Meer et al., 1989; Provost et al., 1993; Dahbi and Lenoir, 1998b; Nielsen et al., 1999). It is predicted from the gestalt hypothesis that workers have to continually exchange HCs in order to conform with the Gestalt and be well integrated into their colony. In the very few studies that tested this hypothesis, all used split colonies in which the separated ants were still kept as groups (Provost, 1989; Crosland, 1990; Dahbi and Lenoir, 1998a; Lahav et al., in press.). Although HC-profile changes were demonstrated in these studies, they were characteristic of the group since the isolated ants could still exchange HCs within the group. Indeed, as predicted from the gestalt model, when these groups were reunited with the mother colony the incidences of inter-group trophallaxis were higher than intra-group trophallaxis (Dahbi et al., 1999). These experiments, however, could not test whether individual composition changes over time or how it relates to the colony gestalt. Such experiments were also hampered by the difficulties in maintaining ant workers alive in complete social isolation. Recently, such

a study was successfully performed in the formicine species *Camponotus fellah* demonstrating a relationship between isolation-induced changes in HCs profiles and behaviours characteristic to nestmate recognition (Boulay et al., 1999). After 3 days of isolation, workers showed a slight HC divergence, and when reintroduced into their mother colony they were treated amicably and provoked frequent trophallactic solicitation. On the other hand, after 20-days of isolation the HC profile of the isolated ants diverged considerably. When reintroduced into their colony, they were generally aggressed by the resident workers (Boulay et al., 2000). These results clearly support the existence of a gestalt colony odour in *C. fellah* and meet the prediction that since individual hydrocarbons production is dynamic, workers are obliged to continually exchange hydrocarbons (mainly by trophallaxis) in order to maintain full integration in the colony gestalt.

In the formicine ants *C. niger, C. iberica* and *C. fellah,* trophallaxis and allogrooming constitute the major behavioural modalities for sharing the colonial odour (Soroker et al., 1995; Dahbi et al., 1999; Boulay et al., 2000). Although trophallaxis seems to be the predominant modality for obtaining a gestalt, the latter also occurs in species that do not perform trophallaxis. Gestalt was reported for the ponerine *Pachycondyla apicalis*, in which the use of radioactive tracers revealed that allogrooming enables the sharing of colonial odour. This mode of transfer seems to be sufficient for a gestalt formation in this species, which has small colonies of less than 100 individuals (Soroker et al., 1998). The objective of the present study was to investigate HC dynamics in *Aphaenogaster senilis* (Myrmicinae), a member of a genus that does not trophallax but forms large and monogynous colonies (Delage and Jaisson, 1969; Ledoux, 1971). We first characterised the cuticular and PPG (as the reservoir of colonial odour) hydrocarbon profiles in queenright workers and observed whether they change with time. We then investigated HC dynamics in individually isolated workers and how it relates to the reaction of their nestmates when reintroduced into their mother colony.

Material and methods

Seven colonies of *A. senilis* were collected in Doñana National Park, Andalusia (sea level, Huelva Province, SW Spain). They were mature queenright colonies with at least 800–1000 workers. Colonies were maintained in the laboratory in artificial nests made of plaster to maintain a constant humidity, and covered with a glass plate that was gently removed to collect ants from the nest after sedation with CO_2. Food (honey, *Tenebrio* larvae and various seeds) was supplied twice a week in a foraging arena (30 × 30 × 10 cm) connected to the nest. Individually isolated workers were kept in test tubes (18 × 0.18 cm) with water and food *ad libitum* until the day of the experiment.

Experiment 1: Chemical analysis

Prior to dissection, the workers were sacrificed by freezing. The PPGs were dissected and immersed in 1 ml of pentane for 24 hours. To extract cuticular HCs the whole body was immersed individually in 1 ml of pentane for 5 min. The extracts were stored at –20 °C until analysis.

The identity of cuticular and PPG HCs was first determined by gas chromatography coupled to mass spectrometry (VGM250Q) using a DB-5 fused silica capillary column (Temperature program: 60 °C–270 °C at 5 °C/min; hold at 270 °C for 30 min and then heated to 295 °C at 20 °C/min). The eluting HCs were identified by their fragmentation pattern.

Comparative quantitative analyses of cuticular and PPG HC profiles of control and isolated workers were achieved by a Varian on-column GC, equipped with a DB-5 fused silica capillary column (Temperature program: 80 °C to 180 °C at 10 °C/min, 180 °C to 280 °C at 5 °C/min, hold 10 min at 280 °C). Before analyses, the samples were evaporated to dryness and re-dissolved in 50 µl of pentane containing pentadecane as an internal standard, of which 1 µl was then injected into the gas chromatograph.

In experiment 1a, we quantified the modifications in the composition of cuticular and PPG hydrocarbon in members of two colonies (colonies 1 and 2) after 20 days (analyses at D0 and D20 respectively). Six workers were used for each set.

In experiment 1b, we compared the profiles of 5 isolated workers at 2, 4, 6, 8, 10, 15 and 20 days (I2 to I20), with that of control ants from the mother colonies at 0, 10 and 20 days (T0, T10 and T20). Colonies 3 and 4 were used for this experiment.

Experiment 2: Behavioural analysis

We established seven sets of ten isolated workers (2 workers per colony, colonies 3 to 7) corresponding to the same isolation periods: 2, 4, 6, 8, 10, 15 and 20 days (I2 to I20). At the end of the respective social isolation period, the test tubes containing the isolated ants were connected individually to their respective mother colony's nest entrance. Thereafter, we recorded for 30 min all selfgrooming, allogrooming and aggression (threat and sizing with mandibles) acts. For the last two behaviours, we separated active (introduced ant performing) from passive (introduced ant submissive) acts. When two or more nestmates were active at the same time (for example the introduced ant being immobilised by several nestmates), we summed the duration of their respective acts. The results are expressed as the total duration during the observation period. Two control groups were constructed: C1 (non-manipulated), constituting ants observed inside the nest without any manipulation, and C2 (manipulated) constituting ants kept individually in test tubes for only 10 min (the time necessary to calm down) before being reintroduced to their mother nest.

Statistics

Data are presented as mean ± SD. The total amounts of HCs as well as the respective quantities of the different HCs classes were compared by Manova, with Degree (Arcsin (Sqrt(p)) transformation when the data were percentages. The evolution of the HC profiles was analysed by a discriminant analysis performed on quantifiable peaks. We also compared the homogeneity of HC profiles between workers in the different groups and between groups using the squared Mahalanobis distances and an ANOVA.

Since behavioural variables did not follow a normal distribution (tested with the Shapiro-Wilk W test), we used the non-parametric Kruskal-Wallis test for global comparisons. When global analysis gave a significant effect, inter-group comparisons were then tested with the non-parametric Mann-Whitney *U* test.

All statistics were made with Statistica software.

Results

Experiment 1: Chemical analysis

Cuticular washes and PPG extracts of *A. senilis* workers revealed the presence of 37 peaks comprising 45 identifiable HCs (Table 1 and Fig. 1). The HC profile was composed of 6 alkanes (C_{25} to C_{30}), traces of 1 alkene ($C_{25:1}$), 21 monomethylalkanes, 17 dimethylalkanes and 2 trimethylalkanes.

Table 1. Cuticular and PPG hydrocarbons of *Aphaenogaster senilis* in relative proportions (mean ± SD). CUT = cuticle and PPG = post-pharyngeal gland. n = 33 for CUT and 23 for PPG. MANOVA for the 4 classes after Arcsin (Sqrt (p)) transformation in degrees: $P < 0.001$. All the differences for the classes are significant between CUT and PPG ($P = 0.049$ for DiMeAlkanes; $P < 0.001$ for other classes). Data pooled for two colonies

Peak	Name	CUT		PPG	
		Mean	SD	Mean	SD
1	C25:1	t		t	
2	C25	0.69	1.86	1.38	2.59
7	C26	0.31	0.25	0.06	1.30
13	**C27**	**8.10**	2.25	**1.63**	1.97
19	C28	5.95	1.73	0.62	2.78
27	**C29**	**8.44**	2.14	1.13	1.21
34	C30	t		t	
	Total n-alkanes	*23.49*	*6.17*	*4.83*	*4.00*
3	11+13MeC25	0.23	0.36	0.44	1.23
4	7MeC25	0.28	0.38	0.27	0.56
6	3MeC25	0.10	0.18	0.13	0.16
8	12MeC26	1.39	0.54	2.92	1.05
9	8MeC26	0.62	0.39	1.27	1.09
15	**9+11+13 MeC27**	**12.91**	2.63	**24.79**	10.79
18	**3MeC27**	**19.98**	4.05	**7.85**	3.61
21	**10MeC28**	**9.82**	2.37	**15.41**	7.57
22	6MeC28	0.37	0.51	0.20	4.06
29	**11MeC29**	**5.51**	1.28	**8.31**	4.02
30	9+7MeC29	t		t	
31	5MeC29	1.94	0.34	0.42	0.36
33	3MeC29	**4.97**	1.35	0.64	0.54
35	10+12MeC30	t		t	
36	11MeC31	t		t	
	Total Me alkanes	*58.09*	*4.36*	*62.65*	*7.50*
5	7,9DiMe C25	t		t	
10	8,12DiMeC26	0.73	0.89	1.90	1.52
11	6,12DiMeC26	0.32	0.47	0.39	0.52
12	4,8DimeC26	0.74	0.31	0.99	0.94
16	9,11+9,17DiMeC27	2.25	1.32	2.81	1.71
17	9,13DiMeC27	0.36	0.42	0.49	3.42
20	3,7+3,9DiMeC27	1.34	1.31	3.63	5.74
23/24	4MeC28+8,12DiMeC28	3.24	2.74	**7.44**	3.04
25	6,10DiMeC28	1.88	0.44	2.69	0.68
26	4,8DiMeC28	2.52	0.74	3.32	1.20
32	Mix 5,x DiMeC29	t		t	
37	11,19DiMeC31	t		t	
	Total Di Me alkanes	*13.24*	*6.08*	*23.65*	*5.17*
14	4,8,12TMeC27	3.22	1.15	5.84	8.35
28	4,8,12TMeC28	1.95	0.90	3.04	2.69
	Total Tri Me alkanes	*5.17*	*2.03*	*8.87*	*1.25*

The major compounds were in the C_{27} series composing roughly 45% of the total amount. The total amounts of HC on the cuticle and PPG did not differ significantly, amounting to 3400 ± 1871 ng/ant and 2900 ± 1942 ng/ant in the cuticle and PPG respectively (Anova, $P = 0.396$, NS). Analysis of the different HC classes indicated a significant difference in the total amount of n-alkanes (783 ± 411 and 135 ± 103 ng/ant respectively for cuticle and PPG, Anova, $P < 0.001$). In the other classes of HCs the differences were not significant (Anova, $P > 0.17$), indicating some accumulation of n-alkanes on the cuticle (Fig. 2). We did not observe significant differences between the two colonies either in the proportions of the different classes or in the total amounts, therefore we pooled the data. There were differences in the relative proportions of specific substances between PPG and cuticle. The two major cuticular peaks were 3 methylheptacosane (peak 18, 20 ± 4%) and the complex of 9 + 11 + 13 methylheptacosanes (peak 15, 12.9 ± 2.6%). In the PPG, on the other hand, the latter complex of methylheptacosanes was the major peak (24.8 ± 10.8%), accompanied by a second large peak of 10 methyloctacosane (peak 21, 15.4 ± 7.6%). In the PPG the 3 methylheptacosane was only 7.8 ± 3.6% of the total secretion (Table 1; all the differences in relative proportions were significant; Manova after Arcsin(sqrt) transformation).

Experiment 1a: Changes of HC profile over time

Determination of the Nei index between individual HC profiles revealed a weak, but significant, difference between the intracolonial and the intercolonial variation, indicating that the colonies have different profiles (intracolonial and intercolonial variations: Nei index = 0.970 ± 0.018, n = 30 and 0.955 ± 0.054, n = 36, $P = 0.05$). However, a discriminant analysis performed to estimate the cuticle and PPG HC-profile divergence as a function of time, over 20 days, and that included the colony identity and the origin of HCs (PPG or cuticle) as variables, showed that the two colonies are not discriminated significantly. This indicates that they possess HC profiles that are more similar than the divergence induced by isolation. We therefore pooled the results in subsequent discriminant analyses. There was a highly significant change in HC profiles in the 4 groups examined (C0 = 0 days and C20 = 20d for cuticle and P0 = 0d and P20 = 20d for PPG; $P < 0.001$; Fig. 3). The first root represented 89.8% of the variance, discriminating the cuticle from the PPG, and confirming the data in Table 1 and Fig. 2. The time dependent change of the profile that appears on root 2 was lower than the differences between cuticle and PPG, representing only 7.9% of the variance. Nevertheless the change is significant both for cuticle (C0d/C20d, $P = 0.007$) and PPG (P0d/P20d, $P = 0.015$).

For all groups, the different within-group points were well clustered. A comparison of the within-group Mahalanobis distances of each individual, as well as the centre of its centroid group, for the cuticle and PPG showed that they were not different (c/centroid d = 25.3 ± 6.05, n = 21 vs. p/centroid d = 25.7 ± 7.16, n = 23; Anova, $P = 0.84$, NS). This indicates that the dispersion around the gestalt is identical both for cuticle and PPG. We also compared the between-group distances between 0 and 20 days for the cuticle and the PPG. This proved to be non-significant, indicating that the changes of the cuticular and PPG profiles were of similar magnitude (T0/T20d = 75.98 ± 19.61, n = 12 vs. T0/T20d = 67.99 ± 17.53, n = 11; Anova, $P = 0.27$).

Experiment 1b: Effect of social isolation on HC profile

The total quantities of HCs on the cuticle and PPG in the isolated ants (after 10 and 20 days) were not different from

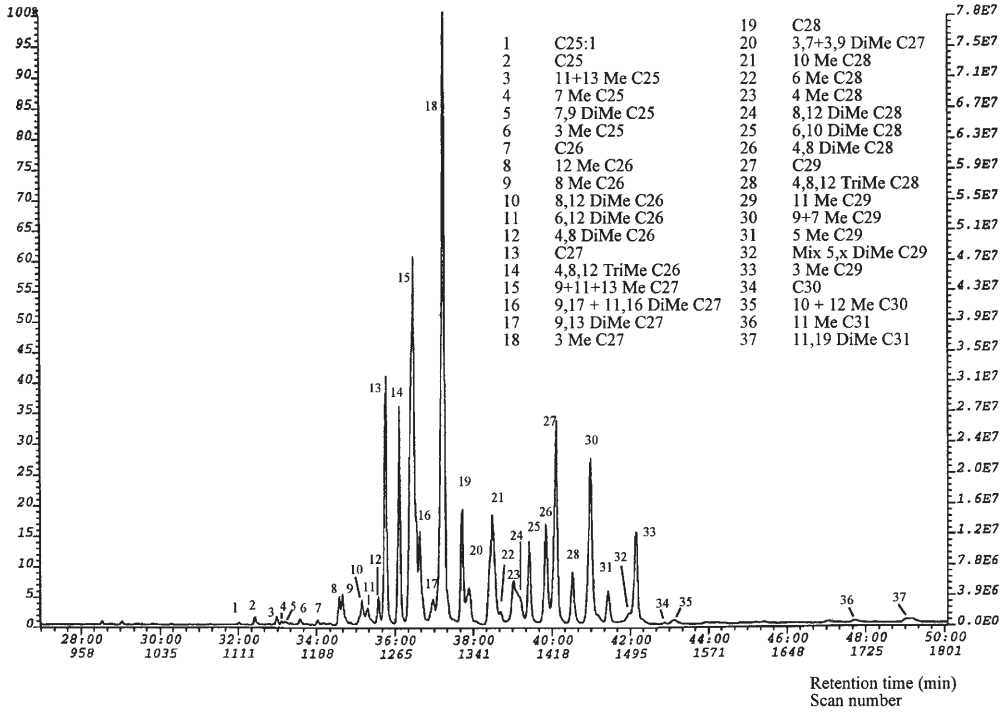


Figure 1. Gas chromatogram of a postpharyngeal gland of *A. senilis*

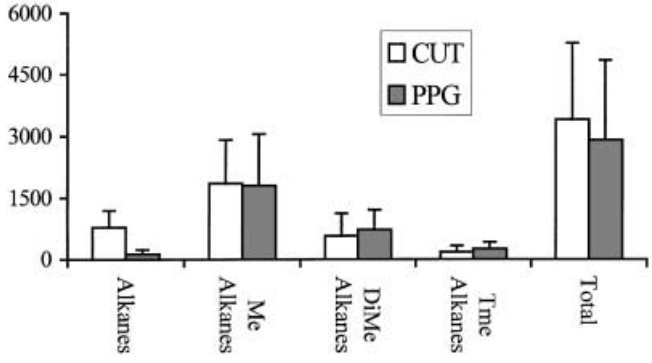


Figure 2. Quantities of HC classes in cuticle (CUT) and postpharyngeal gland (PPG) of *A. senilis* workers in ng/ant (mean ± SD). MANOVA: alkanes $P < 0.001$; Me Alkanes $P = 0.854$; DM Alkanes $P = 0.384$; TM Alkanes $P = 0.172$; Total $P = 0.396$; $n = 21$ for CUT and $n = 23$ for PPG (Data pooled for 2 colonies)

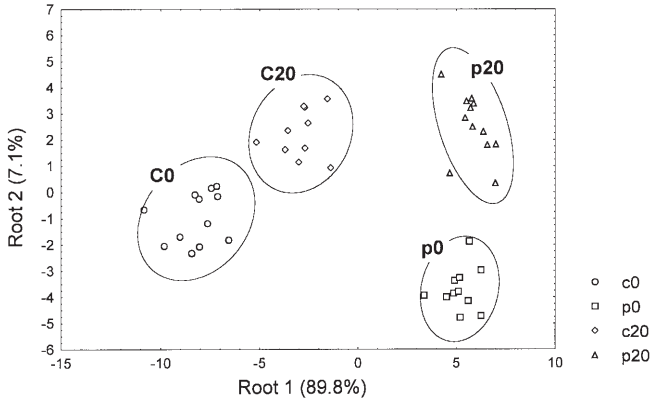


Figure 3. Discriminant analysis of cuticular and PPG hydrocarbon profile over 20 days ($P < 0.001$). c = cuticle, p = PPG, 0 and 20 days (2 colonies pooled) – Ellipsoids = 95% IC c vs. p: $P < 0.001$; c0 vs. c20: $P = 0.007$; p0 vs. p20: $P = 0.015$

those in control ants, indicating that HCs biosynthesis was not perturbed by isolation.

Isolation, however, did result in quantitative profile changes. Figure 4a shows the results of a discriminant analysis for the cuticular profiles. Analyses of the control ants taken from the colony confirmed the change of the HC profile already after 10 days (T0 vs. T10, $P < 0.001$). However the pattern did not change during the 10 following days (T10 vs. T20, $P = 0.59$, NS). A more detailed study of the HC profile change is presented in the socially isolated ants (Fig. 4a). As early as 2 days post isolation (I2) a significant modification of the HC profile was revealed between the isolated and the control ants (T0 vs. I2, $P < 0.001$). Among the isolated ants the differences were small and non-significant between 2, 4 and 6 days (I2 vs. I4 and I6, $P > 0.15$, NS) but became significant from 8 days onwards (I2 vs. I8, $P = 0.001$). After this isolation period the differences were not significant, indicating an important change mainly in the first 2 days, and after 6–8 days.

Figure 4b depicts the compositional change in the PPG. In the control ants there was a significant change between t = 10, t = 10d and t = 20d (T0 vs. T10, $P = 0.015$ and T10 vs. T20, $P = 0.005$). In the isolated ants the change in the PPG was also significant, but it progressed slower than for the

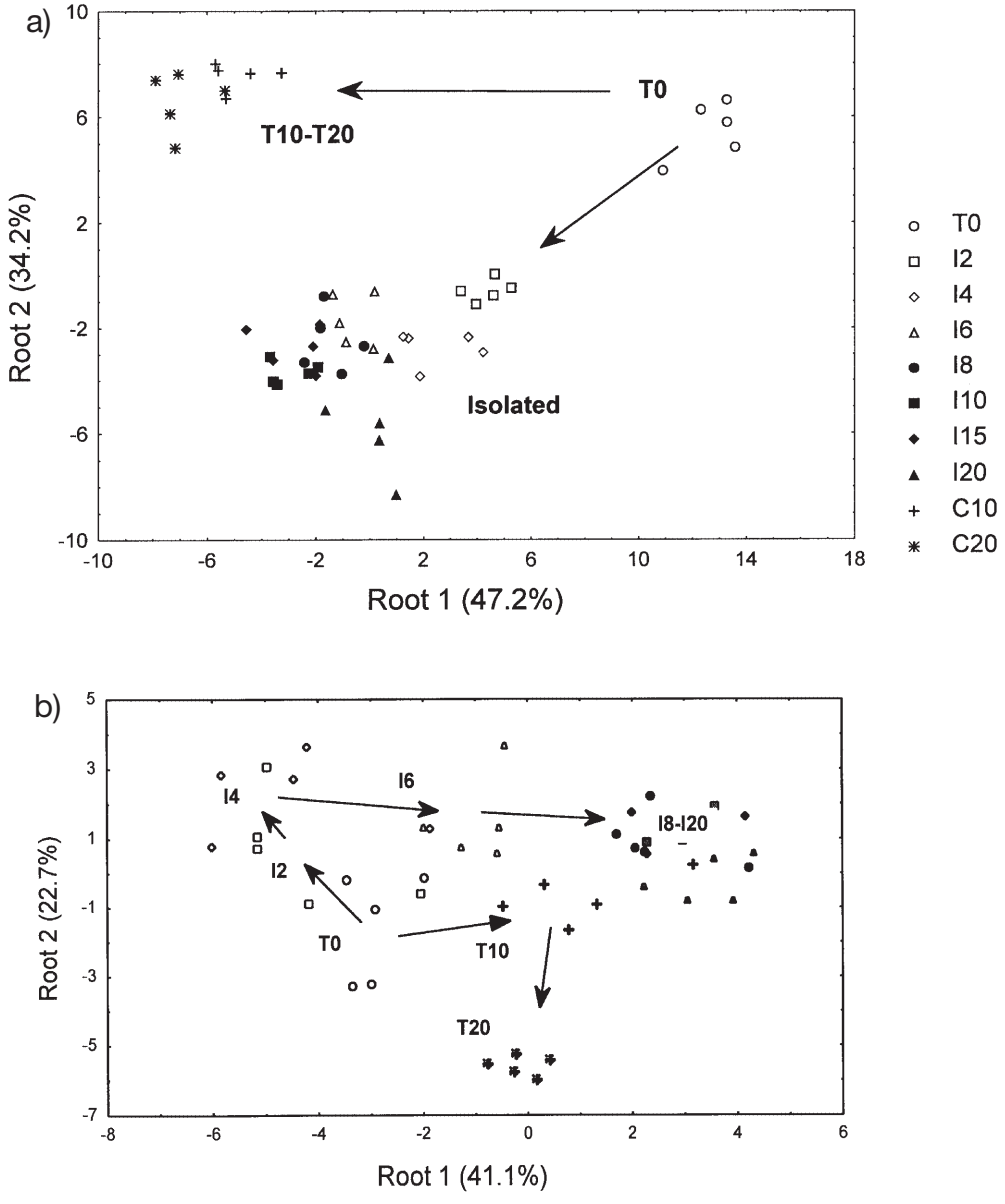


Figure 4. Discriminant analysis of cuticular (a) and PPG (b) hydrocarbon profile of control and isolated workers over 20 days ($P < 0.001$) a) T0 vs. I2: $P < 0.001$; vs. other I: $P < 0.001$ T0 vs. T10: and T20: $P < 0.001$; T10 vs. T20: $P = 0.59$ I2 vs. other I: NS. b) T0 vs. T10: $P = 0.015$; vs. T20: $P = 0.005$; T10 vs. T20: $P = 0.005$ T0 vs. I2: $P = 0.273$; vs. I4: $P = 0.03$; and vs. longer isolation periods $P < 0.001$ I2 vs. I4: $P = 0.95$; vs. I6: $P = 0.34$; vs. I8: $P = 0.003$ and id I4 vs. I6: $P = 0.21$; vs. I8: $P = 0.002$ and id I8/I10/I15/I20: $P > 0.19$ but I10 vs. I20: $P = 0.009$

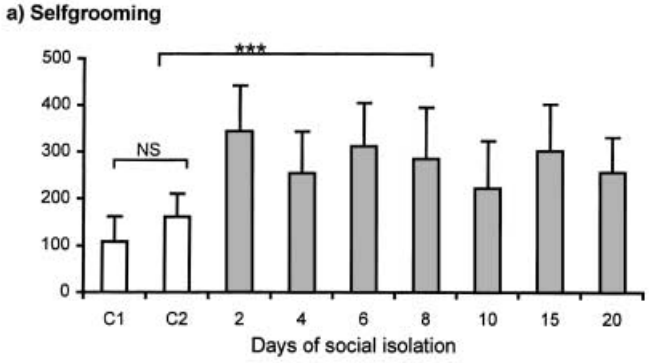
cuticle. The change from the t = 0 ants became significant only after 4 days (T0 vs. I2, P = 0.273, NS, vs. I4, P = 0.03), and continued to change up to 8 days (I2 vs. I8, P = 0.002). Among ants isolated for 8, 10, 15 and 20 days only the change between 10 and 20 days was significant (I10 vs. I20, P = 0.009).

Experiment 2: Behavioural analyses

We did not observe either trophallactic-like behaviour or mouth to mouth contacts, confirming the absence of trophallaxis in *A. senilis*.

Selfgrooming was spontaneously performed for 100 to 150 s (about 300s observation) by the non-manipulated and manipulated control ants (C1 vs. C2; Mann-Whitney U test, NS). After 2-days of isolation selfgrooming became significantly higher (by a factor of 2) than in the control (Mann-Whitney, $P < 0.001$). However, it did not increase further despite the prolongation of the isolation period for 18 more days. Thus there were no significant differences in selfgrooming between I2 to I20 isolated ants (Fig. 5a).

Allogrooming was observed with respect to the introduced ant, or the observed individual in case of the non-manipulated control. In non-manipulated controls allogrooming was performed for 100 s (i.e. 5.6% of the time) and was half-passive (the observed ant is groomed) and half-active (the observed ant grooms) (Fig. 5b). For the manipulated ants, control and isolated ants alike, active allogrooming was minor and did not change for either manipulated control ants or for all the isolated workers. In contrast, passive allogrooming was very extensive. The differences in passive allogrooming between the non-manipulated and manipulated control ants reflect the stress induced by the manipulation



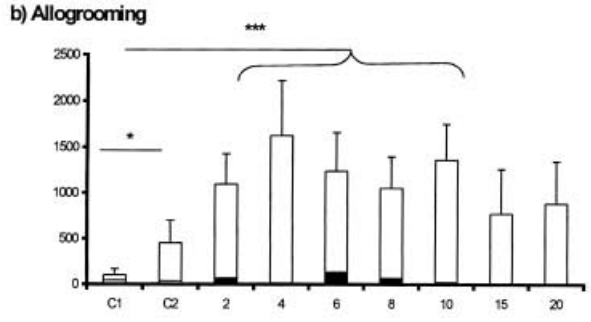


Fig 5c see at the end of the paper

Figure 5. Selfgrooming (a), allogrooming (b) and aggressive (c) behaviours in control and isolated workers over 20 days (mean in s ± SD); C1 = non-manipulated control, C2 = manipulated control, 2 to 20d isolation. n = 10 for each value (5 colonies). a) Kruskal-Wallis test: P = 0.002. C1 vs. C2: P = 0.059; between I2, I4 to I20 no significant differences; C2 vs. all I: P = 0.003 (Mann-Whitney U test). b) Active allogrooming (black): Kruskal-Wallis test P = 0.689 Passive allogrooming (white): Kruskal-Wallis test P < 0.001 (C1 vs. C2: P < 0.001, C1 vs. all I: P < 0.001, no significant differences between all isolated durations – Mann-Whitney U test). c) Active aggression (black): Kruskal-Wallis test P < 0.001 as a global effect (no significant differences between groups). Passive aggression (white): Kruskal-Wallis test P < 0.001 (C1, C2, I2 to I10 vs. I15: P = 0.004; vs. I20: P = 0.003, Mann-Whitney U-test)

(C1 vs. C2; Mann-Whitney, P < 0.001). Passive allogrooming increased even further for the 2-days isolated workers as compared to the manipulated control ants (C2 vs. I2; Mann-Whitney, P < 0.001). However it did not increase further for longer times of isolation.

We never observed aggression among the resident ants (C1) or towards the introduced control ants (C2; Fig. 5c). Ants isolated for 4 days and reintroduced into the nest attacked, and were sporadically attacked by their nestmates for short periods, but the differences were not significant due to rarity of these events. However, if introduced after 15 or 20 days of isolation, the workers were seriously aggressed (roughly 400 sec), generally immobilized by the residents, in a similar fashion to that observed in intercolonial encounters. Only a few ants performed these attacks while their nestmates could be observed simultaneously licking the intruder.

Discussion

Aphaenogaster senilis ants have a cuticular chemical pattern composed of long chain-saturated hydrocarbons, most of which have at least 27 carbons (mainly heptacosane series). The HC content in the PPG is roughly 3 µg/ant and that on the cuticle is 3.5 µg/ant, indicating that the ants can replace the total HC bouquet on the cuticle while self or allogrooming. The qualitative congruency between PPG and cuticular HC composition has been demonstrated in several species (Bagnères and Morgan, 1991; Do Nascimento et al., 1993; Soroker et al., 1995; Soroker et al., 1998), and was also confirmed in *A. senilis*. While the congruency in *A. senilis* is qualitative, there were some important quantitative differences: the cuticle had more n-alkanes in the range C27 to C29 than the PPG (23.5 vs. 4.8%). Similar results were reported for *Myrmica rubra* (Bagnères and Morgan, 1991), *C. niger* (Soroker and Hefetz, 2000) and *Pachycondyla apicalis* (Hefetz et al., 2001). It is not a general rule, as higher abundance of n-alkanes on the cuticle were not observed in other ants, like *Camponotus vagus, C. lateralis, Manica rubida* and *Formica selysi* (Bagnères and Morgan, 1991). The extra alkanes may originate from sources other than the PPG, for example the tarsal gland, and may be an adaptation to hot climates as long chain n-alkanes have a higher transition temperature than their branched counterparts (Gibbs, 1998). In *Pogonomyrmex barbatus*, foragers have 20% more n-alkanes on the cuticle than inside-nest workers. This may be due to environmental conditions experienced by foragers and may enhance their desiccation resistance to the desert environment (Wagner et al., 1998).

In contrast to other social insects investigated i.e., other ant and wasp species (Howard, 1993; Lorenzi et al., 1996; Singer, 1998; Vander Meer and Morel, 1998), HCs composition in *A. senilis* show only weak colony specificity. Since these colonies were kept under constant laboratory conditions for 3–5 months between collection and analyses, we cannot exclude the possibility that this phenomenon reflects the effect of environmental conditions about the HCs. Unfortunately the lack of knowledge about colony differences in the field or in the laboratory under variable conditions does not allow to draw a definitive conclusion on the relative contribution of genetics and environment to HC composition. An alternative explanation relates to the biology of the species. Colonies of *A. senilis* are always monogynous and reproduce by budding (Ledoux, 1971; Ledoux, 1973; confirmed by X. Cerdá, pers. comm.). Since the two colonies analysed were only 20 m apart they could represent two related colonies. A similar relationship between budding and HC similarity

was reported for *Cataglyphis cursor* (Lenoir et al., 1988; Nowbahari et al., 1990).

Temporal changes in the relative cuticular HCs composition demonstrated for *A senilis* were also found in field colonies of *Solenopsis invicta* (Vander Meer et al., 1989), *Formica truncorum* (Nielsen et al., 1999) and *Camponotus japonicus* (Liu et al., 1998), as well as in laboratory-maintained colonies of *Leptothorax lichtensteini* (Provost et al., 1993) and *C. fellah* (Boulay et al., 2000). If we regard HCs as a model for the dynamics of recognition cues, our current findings confirm that the colony gestalt is dynamic and in permanent change. Ten days are generally sufficient to observe profile modification of any specific colony. This change may be related to various environmental factors such as change in diet, or seasonality and internal factors like the changes in physiology with age and the demography of the colony.

Social isolation was followed by a progressive change in cuticular and PPG HC profiles. The fact that we could not detect differences in the amounts of hydrocarbons after 10 or 20-days isolation seems to rule out the possibility that this stressing situation perturbed HC biosynthesis. The change in cuticular profile expressed by *A. senilis* was already apparent after a short (2-days) isolation, indicating: a) rapid turnover of cuticular HCs, enabling the individual HC expression in the isolated ants, and b) the high efficacy of the mechanisms that maintain the gestalt in the intact colony despite each individual expressing its own HC composition. Comparable results were obtained in *C. fellah* (Boulay et al., 2000) in which HCs transfer is extensive and trophallaxis-mediated. This suggests that allogrooming may be equally effective in the maintenance of the gestalt in the non-trophallacting *A. senilis*. Observations of non-perturbed workers inside the nest indicate that allogrooming represents 6% of the time budget (pers. obs.). Experiments on the non-trophallacting ant *P. apicalis* showed that allogrooming is able to maintain a uniform colony odour with a frequency of allogrooming representing 3% of the ant's time behavioural budget (Soroker et al., 1998). These differences in allogrooming may be related to the size of the colony. Colonies of *P. apicalis* are composed of only a few dozen individuals and the 3% time-budget allocated for allogrooming may be sufficient. We suggest that the higher incidences of allogrooming exhibited in *A. senilis,* are adjustments for rapid achievement of a uniform colony odour in these more populous colonies. Interestingly, the divergence of the PPG profiles showed a "zigzag pattern" while that of the cuticle showed a "linear pattern", which indicates that they follow different time patterns.

The behaviour towards the isolated ants changed after only 2 days of isolation, manifested mostly by extensive allogrooming by nestmates. This is nicely correlated with the demonstrated change in cuticular hydrocarbon profile, suggesting that these changes in profile were sufficient to induce interest of nestmates, but not aggression. From 2 to 10 days of isolation, cuticular profile changed progressively and sporadic incidences of aggression by nestmates appeared. After 15 days of isolation the changes in cuticular profile became more profound, with a concomitant significant rise in aggression. The fact that allogrooming and aggression could occur concomitantly suggests that odour changes in reintroduced ants may induce ambiguous behaviours from resident ants with different threshold sensitivities. Similar results were obtained for two species of *Camponotus*: *C. fellah* and *C. acvapimensis* (Boulay et al., 2000; Cybulska et al., 2000) increased their trophallaxis after a short isolation period, changing to overt aggression when the isolation period was prolonged. In other ant species, group separation induced investigative behaviour such as long antennation or even trophallaxis, but did not escalate to overt aggression even after several months of separation (Vander Meer et al., 1989; Provost et al., 1993; Dahbi and Lenoir, 1998a). It was earlier hypothesised that the use of PPG as a reservoir of the gestalt odour may have evolved early and that in some myrmicine and formicine ants trophallaxis evolved as a means of cue transfer. The role of the PPG as a gestalt organ was also demonstrated in the non-trophallactic ponerine ant, *Pachycondyla apicalis*, where observation showed that physical contact as well as allogrooming are sufficient modes of cue transfer to create a uniform colony odour (Soroker et al., 1998; Lenoir et al., 1999). The present study suggests that allogrooming is an important behaviour for effective cue transfer in the non-trophallactic, yet more populous, *A. senilis*. Recent reports demonstrated that allogrooming is efficient in protection against pathogens in damp wood termites, effectively counterbalancing the cost of disease transmission in insect societies due to the close proximity of individuals in the nest (Rosengaus et al., 1998). This is corroborated by the fact that foragers of *Leptothorax acervorum* are intensively groomed when re-entering the nest (2.2% vs. 0.43% for re-entering foragers and in-nest workers respectively (Franks et al., 1990b). Although this function may provide a strong selection pressure favouring allogrooming evolution, we argue that obtaining a uniform colony odour in non-trophallactic species may also have been a simultaneous driving force for allogrooming evolution. When the colonies became more populous, trophallaxis evolved as a more efficient mode of cue transfer (3% transmission of HCs for 24 h in *P. apicalis* vs. 13–25% in three other trophallacting myrmicine and formicine species) (Soroker et al., 1998). Allogrooming therefore represents an intermediate step in the evolution of cue transfer modalities, and may have remained as an ancestor character in the myrmicine species, which did not evolve trophallaxis. In conclusion, regarding cuticular and PPG HCs as a model for the dynamics of recognition cues, our results support the existence of a gestalt colony odour in the myrmicine *Aphaenogaster senilis*. As predicted, it is essential for nestmates to frequently homogenise their HCs in order to create a unique and uniform colony-specific profile, and in the non-trophallacting *A. senilis* allogrooming seems to be an efficient way to achieve this.

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Erratum

In the article "Effects of social isolation on hydrocarbon pattern and nestmate recognition in the ant *Aphaenogaster senilis* (Hymenoptera, Formicidae)" by A. Lenoir, D. Cuisset and A. Hefetz, published in issue 48/2 of Insectes Sociaux (pp. 101–109), an error appeared in Figure 5 on p. 106.

We here print the correct version of this figure, and very much apologize for this error.

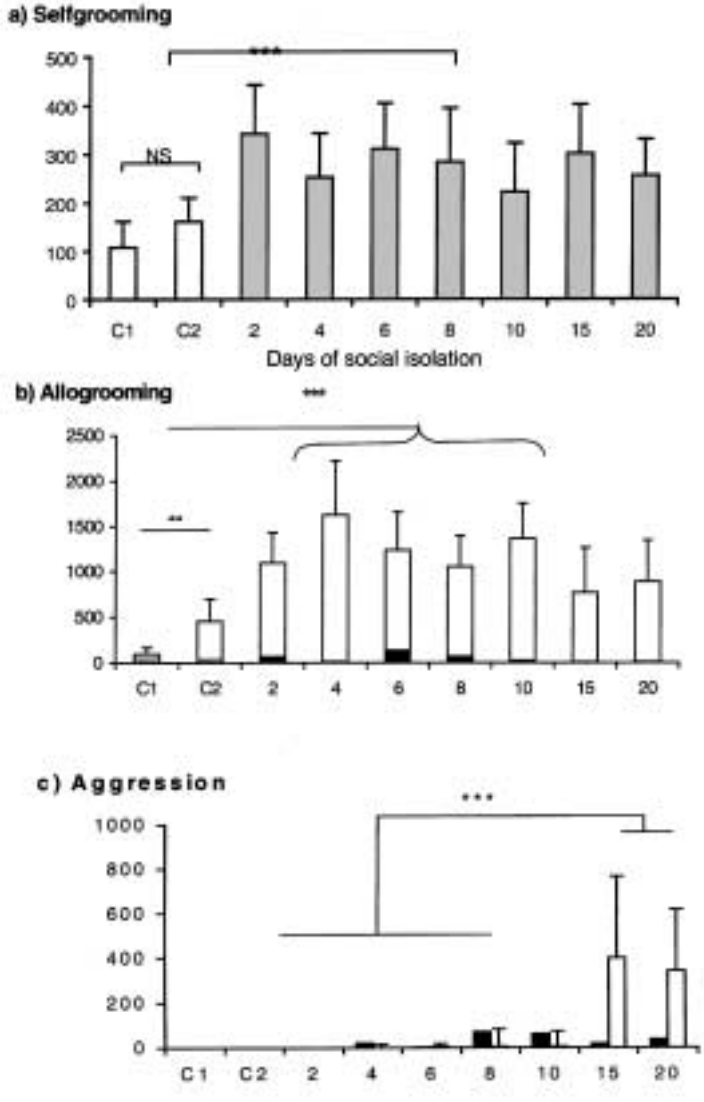

Figure 5. Selfgrooming (a), allogrooming (b) and aggressive (c) behaviours in control and isolated workers over 20 days (mean in s ± SD); C1 = non-manipulated control, C2 = manipulated control, 2 to 20d isolation. n = 10 for each value (5 colonies). a) Kruskal-Wallis test: P = 0.002. C1 vs. C2: P = 0.059; between I2, I4 to I20 no significant differences; C2 vs. all I: P = 0.003 (Mann-Whitney U test). b) Active allogrooming (black): Kruskal-Wallis test P = 0.689 Passive allogrooming (white): Kruskal-Wallis test P < 0.001 (C1 vs. C2: P < 0.001, C1 vs. all I: P < 0.001, no significant differences between all isolated duration – Mann-Whitney U test). c) Active aggression (black): Kruskal-Wallis test P < 0.001 as a global effect (but no significant differences between groups). Passive aggression (white): Kruskal-Wallis test P < 0.001 (C1, C2, I2 to I10 vs. I15: P = 0.004; vs. I20: P = 0.003, Mann-Whitney U test)