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Research Article

Chemical Integration of Myrmecophilous Guests in *Aphaenogaster* Ant Nests

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Social insect nests provide a safe and favourable shelter to many guests and parasites. In *Aphaenogaster senilis* nests many guests are tolerated. Among them we studied the chemical integration of two myrmecophile beetles, *Sternocoelis hispanus* (Coleoptera: Histeridae) and *Chitosa nigrita* (Coleoptera: Staphylinidae), and a silverfish. Silverfishes bear low quantities of the host hydrocarbons (chemical insignificance), acquired probably passively, and they do not match the colony odour. Both beetle species use chemical mimicry to be accepted; they have the same specific cuticular hydrocarbon profile as their host. They also match the ant colony odour, but they keep some specificity and can be recognised by the ants as a different element. *Sternocoelis* are always adopted in other conspecific colonies of *A. senilis* with different delays. They are adopted in the twin species *A. iberica* but never in *A. simonellii* or *A. subterranea*. They are readopted easily into their mother colony after an isolation of different durations until one month. After isolation they keep their hydrocarbons quantity, showing that they are able to synthesize them. Nevertheless, their profile diverges from the host colony, indicating that they adjust it in contact with the hosts. This had never been demonstrated before in myrmecophile beetles. We suggest that the chemical mimicry of *Sternocoelis* is the result of a coevolution with *A. senilis* with a possible cleaning symbiosis.

1. Introduction

Ant colonies often host microcosms of myrmecophile guests, mostly arthropods that take advantage of ant nest favourable environment and food resources [1–3]. The largest known association is the army ant *Eciton burchellii* with more than 300 guest species [4]. Interactions with ants range from true predators, commensals that live on ant food remains, mutualists, and parasites [3, 5, 6]. In order to get accepted they must break the ant colony "fortress" which is based on a chemical recognition system by which ant workers are able to recognize and exclude aliens. More precisely, a colony-specific mixture of cuticular hydrocarbons has been shown to constitute the recognition pheromone of most ant species [7, 8]. Several strategies have been described for the chemical integration of myrmecophiles into ant colonies. Chemical mimicry is achieved either in a few cases by biosynthesising the same hydrocarbons as their host (but this is rare) or more generally by acquiring them through cuticular contacts and/or grooming (e.g., the guest actively licks the host's cuticle; see reviews by [9–11]). Myrmecophiles like woodlice, mites, phorid flies, and snails can also be "chemically insignificant," that is, their cuticle bears very small amounts of hydrocarbons as it has been shown in *Leptogenys* [6]. Similarly, callow ants are chemically insignificant which allows them to get accepted in alien colonies during the first hours after emergence (see [12]). Another possibility of integration has been discovered recently in social insects: guests and parasites can be chemically "transparent" if they have only saturated hydrocarbons, which are not involved

in recognition [13]. Nevertheless, some myrmecophiles like *Pella* in *Lasius fuliginosus* colonies do not present chemical mimicry, simply escaping from the ants or using appeasement or repelling behaviour [14].

In the present study we conducted a survey of all arthropods living in the nest of the gipsy ant *Aphaenogaster senilis* in southern Spain. Then, we compared the chemical integration of two myrmecophiles beetles (*Sternocoelis hispanus* and *Chitosa nigrita*) with that of an undetermined silverfish. We hypothesized that guests specialized with only one host (like *Sternocoelis*) have coevolved with it and biosynthesize the hydrocarbons while host-generalists like silverfish would mimic passively their hosts and can shift easily to different host species. To test for host specificity and relate it to chemical distance, we designed adoption experiments with *Sternocoelis* in conspecific colonies and congeneric species. We then analysed the mechanisms of chemical mimicry looking at the effects of separation of the beetles from their host. After two weeks, the exogenous hydrocarbons of the myrmecophile beetle *Myrmecaphodius* begin to disappear [15]. Therefore, after one-month isolation, we supposed that all exogenous hydrocarbons acquired by contact with ants had disappeared. As *Sternocoelis* was frequently observed licking the ant larvae, we investigated possible roles of these beetles in larval predation or prophylaxis. If the beetles fed on larvae by piercing the cuticle (haemolymph feeding on larvae by ant workers is known in *Amblyopone* [16]), larvae were supposed to decline. On the contrary, if the beetles fed only by licking the cuticle, larvae will maintain their wellbeing.

2. Material and Methods

2.1. Inventory of Guests in A. senilis Colonies. We completely excavated 57 nests between February 2008 and December 2009 on the banks of Guadalquivir near Sanlúcar de Barrameda to list and count all the guests, mites, silverfish, sowbugs, staphylinids, and histerid beetles.

2.2. The Guest Studied

2.2.1. Sternocoelis (Coleoptera: Histeridae). This genus regroups myrmecophile beetles that live in ant nests of several species within the genera *Aphaenogaster, Cataglyphis*, *Formica* and *Messor* [17]. They are frequently found on the brood pile on which they were thought to feed (Figure 1(h)). According to Lewis [18] *Sternocoelis* feed on larvae and dead adult ants. Otherwise, little is known on their biology and reproduction. Larvae and pupae are unknown [17]. *Sternocoelis hispanus* (Figures 1(a) and 1(b)) occurs in central and southern Portugal and Spain, as well as in northern and central Morocco (see [19] for details). In the Iberian Peninsula it has been found living only in *A. senilis* colonies [17, 19]. On the other hand, in Morocco it was found in at least four different species of *Aphaenogaster* with more than 30 specimens in some nests (Lackner, unpublished). After exposing the colony by turning the stone under which they live, some *S. hispanus* immediately headed for the security of the nest searching for the nearest gallery, whereas the other attempted to "hitch a ride" by clinging onto the ants (Figures 1(c) and 1(e)). The histerids, rather than the ants (as is the case in *Chennium bituberculatum* observed in eastern Slovakia; Lackner, unpublished), always actively seek out the ants in order to be transported into the nest. This phenomenon of *Sternocoelis* riding the ants has so far been observed only in four *Sternocoelis* species: *S. hispanus*, *S. slaoui*, *S. arachnoids,* and *S. espadaler* (Lackner, unpublished). As very few is known on *S. hispanus* biology, we measured the length, width, and weight of the *Sternocoelis* to search for sex differences.

2.2.2. Chitosa (Coleoptera: Staphylinidae) and Silverfish (Thysanura). They are very active insects moving rapidly into the nest. Very little is known on their biology. Since they are associated with various ant species, they are apparently host generalists. *Chitosa nigrita* is a rare myrmecophilous species known only from Spain and Morocco [20] (Figure 2). We collected *C. nigrita* in two colonies of *A. senilis* (1 and 4). From colony 4 we also collected two silverfish. Silverfish are known to move freely within the entire nest [6]. *Chitosa* and silverfish were only used for chemical analyses.

2.3. Ant Colonies. In November 2008, 43 *S. hispanus* beetles were discovered in an *A. senilis* colony (hereafter, colony 1) in Andalusia, Doñana National Park (Las Beles, 36°58.53′N, 6°29.11′W, sea level). Three other colonies were collected, colony 2 and 4, just a few meters from colony 1, and a fourth one (colony 3) collected 60 km apart, near Aznalcazar in a pine forest (37°14.77′N, 6°12.17′W, 36 m). For adoption experiments we used four colonies of different *Aphaenogaster* species: one colony of *A. simonellii* (Egine's island, Greece; 37°45.22′N, 23°31.46′E, 580 m), one colony of *A. subterranea* (Cévennes, France; 44°02.57′N, 3°49.68′E, 370 m) and two colonies of *A. iberica* (Sierra Nevada, Spain; 37°08.42′N, 3°28.34′E, 1370 m). Like *A. senilis* and *A. simonellii, A. iberica* belongs to the subgenus *Aphaenogaster*, while *A. subterranea* belongs to a different subgenus (*Attomyrma*), suggesting it is phylogenetically more distant from *A. senilis* than the other two.

Colonies were maintained in the laboratory in large plastic boxes and fed at libitum with live maggots, pieces of orange, sliced *Tenebrio* larvae, and a commercial solution for bumblebees (Beehappy).

2.4. Behaviour of Sternocoelis. We performed a behavioural repertoire of the beetles using scan sampling method: during 3 days, we recorded during 50 sequences the behaviour of all beetles that were visible in colony 1 (total number of observations 741). Behaviours were the followings: isolated in the colony (either immobile or moving), on larvae, on prey, on a worker, licked by a worker (see Figure 1).

As the *Sternocoelis* were observed frequently on the ant larvae, we made small nests with 6 *A. senilis* nurse worker ants, 6 beetles, and 6 larvae of different developmental stages. The behaviour of the beetles and the number and aspect of larvae were observed during 30 days.

2.5. Adoption Experiments. We observed the behaviour of the *Sternocoelis* beetles and examined whether the beetles can be

Figure 1: *Sternocoelis* beetles (Coleoptera: Histeridae). (a, b): *Sternocoelis hispanus* morphology, sex unknown (photo (b) by Martin Švarc and Peter Koniar); (c): two *Sternocoelis slaoui* riding on an *Aphaenogaster* worker (Photo Martin Švarc and Peter Koniar, Larache, Morocco, February 2010); (d): *Sternocoelis hispanus* beetles feeding on mealworm larvae; (e): *S. hispanus* jumping on an ant worker; (f, g): aggressive behaviour against allocolonial *S. hispanus* in the foraging arena-transport by an *Aphaenogaster* worker (f): aggression; (g): *S. hispanus* cleaning ant larvae. All photos unless (b) and (c) by Alain Lenoir.

Figure 2: *Chitosa nigrita* (Coleoptera: Staphylinidae) (photo by Alain Lenoir). Determination by Munetoshi Maruyama.

adopted by another *A. senilis* colony or a colony of another species. Adoption tests were conducted on small colony fragments ($n = 6$ for *A. senilis*) containing 120 workers and a brood kept in small flat plaster nests covered with a glass. Observations were realized through a red plastic sheet. The nest communicated with a foraging arena, made of a plastic box where the beetles were introduced. These experimental colonies have been acclimated in the experimental setup for at least 24 h before the adoption experiments were conducted. Consecutive experiments were separated by at least one week.

One or two beetles were introduced in the foraging arena of each *Aphaenogaster* experimental colony. We then measured the following variables:

(i) Latency to the first Contact with the beetle (LC);

(ii) Total time of Contact between the beetle and ants in the External area (TCE);

(iii) total time of TRansport of the beetle by ants into the nest (TR);

(iv) total time of Contact between the beetle and ants In the Nest (CIN);

(v) the sum of these four durations, the Total Time until Adoption (TTA). In some cases the beetle was again aggressed inside the nest and we added this duration to the first TTA.

If the beetle was left in the foraging arena during one week and always neglected, it was considered not adopted and returned to its original nest. *Sternocoelis* beetles were introduced either into a fragment of their own colony (controls, colony 1; $n = 10$ beetles), a different colony of *A. senilis* (colony 2 and 3; $n = 10$ beetles per colony), a colony of *A. simonellii* ($n = 4$ beetles), a colony of *A. iberica* ($n = 12$ beetles), or a colony of *A. subterranea* ($n = 4$ beetles).

In order to evaluate the chemical integration of *Sternocoelis* into colonies of *A. iberica*, we made 5 more trials: 3 were adopted and used for chemical analysis, 2 disappeared, probably killed by foragers.

2.6. Isolation Experiments. To observe the effect of separation from the *Aphaenogaster* hosts, groups of 5 *Sternocoelis* were isolated in a small glass tube with water and food. Individual isolation was not possible as the beetles died rapidly. They were reintroduced into their original nest after 1, 3-4, 5-6, 7-8, and 30 days of isolation ($n = 4$ for each and $n = 6$ for 30 days) and we measured the readoption time. These data were compared to controls retrieved directly into the host nest ($n = 8$). We performed chemical analysis of the hydrocarbons on the 8 controls, 5 beetles isolated for 4 and 8 days, and 6 beetles isolated 30 days (see Section 2.7).

2.7. Chemical Analyses. In a first step we used the whole ants and the whole myrmecophiles. The animals were frozen at $-18°$C and immersed in 200 μL of pentane during one hour, and the extract stored at $-18°$C until analysis. Substances were identified by combined gas chromatography/mass spectrometry (Turbomass system, Perkin-Elmer, Norwalk, CT, USA, operating at 70 eV) using a nonpolar DB-5HT apolar fused silica capillary column (length: 25 m; ID: 0.25 mm; film thickness: 0.25 μm). Samples injections were performed in splitless mode for 1 minute, a temperature program from 100°C (2 min initial hold) to 320°C at 6°C min^{-1} with 5 min of final hold. A mixture of 10 linear hydrocarbon standards (from C20 to C40) was injected at regular time intervals in order to recalibrate retention times. To analyze the effects of social isolation, as we had only a few beetles, we used SPME: the live beetle was held in forceps and rubbed gently on the dorsal and lateral surfaces with a polydimethylsiloxane (PDMS, 7 μ fused silica/SS, Supelco, color code green) fiber for 3 minutes. The fiber was immediately desorbed in the GC-MS in the same conditions of pentane extracts. It has been shown that the profiles obtained with SPME and classical solvent extraction are qualitatively identical [21, 22], but a precise quantitative analysis showed that the proportions of compounds are slightly different [23], so we made also SPME controls for ants. SPME were made on 4, 8, and 30 days of isolation. An internal standard (eicosane) was added to the extract or deposited on the fiber to measure the hydrocarbon quantities. Hydrocarbons of *A. senilis* were previously identified [24, 25] and we added some new compounds present in very small quantities.

2.8. Statistics. ANOVA was performed on behavioural data for adoption experiments, Kruskall-Wallis on hydrocarbon quantities of isolated beetles.

Statistical analysis of the chemical profiles was done using all peaks that were identified. To determine the level of similarity of the CHC profile of the beetles and their hosts, isolated beetles and between species we used hierarchical cluster analysis (Euclidean distances, Ward's method) to construct a single-linkage dendrogram (see, e.g., [26, 27]). The Nei index of similarity was used to compare the chemical profiles of the species when large qualitative differences are observed (see, e.g., [27–29]).

3. Results

3.1. Inventory of Guests in Aphaenogaster Nests. Figure 3 shows the frequency of nests in relation to the number of guests. Many nests did not contain any guest; for example, 51% of nest were free of mites, 98% of histerids beetles. Out of the 57 nests excavated, 23 contained at least one individual of the staphylinid *Chitosa nigrita* (mean ± SE: 1.1 ± 0.3; range: 0–9). Silverfish were present in 14 colonies (mean ± SE: 0.9 ± 0.3; range: 0–14) while sowbugs were found in 15 colonies (mean ± SE: 0.8 ± 0.3; range: 0–13). In Doñana National Park *Sternocoelis* beetles were rare. With the exception of two colonies that contained 43 and 30 individuals (resp., in November 2008 and July 2011), only five *Sternocoelis* beetles were found in four different colonies among more than 300 colonies. The mean length of *Sternocoelis* was 2.01 mm (SE = 0.01; min 1.79, max 2.24, $n = 42$) and the width 1.29 mm (SE = 0.01, min 1.06, max 1.39, $n = 42$). The weigh was 2.25 mg (SE = 0.18, min 1.5,

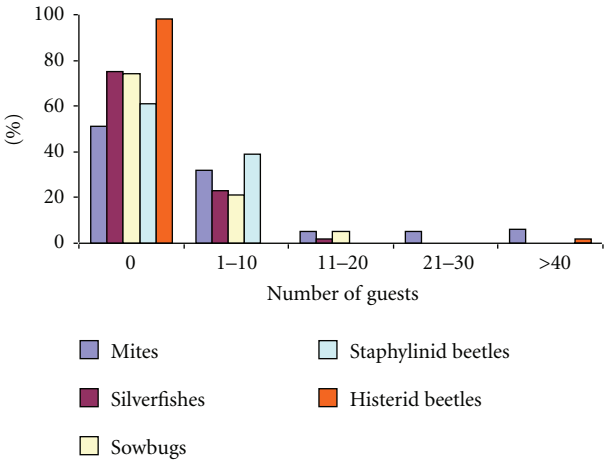

Figure 3: Frequency of colonies containing 0, 1 to 10, 11–20, 21–30, and > 40 guests in *A. senilis* colonies.

max 2.80, $n = 8$). The distributions were unimodal, and therefore no sexual dimorphism appeared.

3.2. Behaviour and Longevity. Beetles stayed isolated or moved freely inside the host nest (28% of observations; it is a raw indication of the time budget of the beetles). From time to time they clutched to a worker's leg, jump on it, and stayed there (34.55% of observations) (Beetle on the ant body: Figures 1(c) and 1(e)). As observed by Yélamos [17], they were frequently found near or on the larvae (31.6%) (Figure 1(h)) and fed directly on pieces of *Tenebrio* (4.3%) (Figure 1(d)). They were occasionally licked by a worker (1.35%). It is possible that in the nests there is competition for food. Beetles had a rather long life, since one year later 18 of them were still alive. We never observed any sexual behaviour nor found any *Sternocoelis* larvae, so we do not know how these beetles reproduce.

In the small nest experiments with larvae we always observed at least 3 beetles on the ant larvae while the others were moving around searching for food. On the larvae they were either immobile or licking the cuticle. After one month, the observations were stopped because we did not observe any larval mortality and the larvae appeared to maintain normally. We never observed any brown spot, which would indicate a piercing of the cuticle.

We did not quantify the behaviour of the other guests, but observations indicated that *Chitosa* beetles and silverfish had a very different behaviour compared to *Sternocoelis*: they had very few interactions with the host, moving frequently in the nest. Silverfish were very fragile and died in less than 24 hours in the laboratory nests.

3.3. Adoption Tests in Alien Colonies

Behaviour

(i) when deposited into the foraging arena of the alien colony, the beetle spent some time without contact with ants either because they did not meet it or because they did not perceive their presence. They also simply stopped and inspected the ants, and continued thier way;

(ii) at their first contact with the beetle, ants behaved aggressively. They seized them in their mandibles, maintained them on the ground and inspected them with their antennae. They made short attacks with their mandibles (Figure 1(g)). The beetles are difficult to seize with the mandibles in account of their hard, smooth and rounded surface [17, 18] (Figure 1(f)). Some ants stopped after this initial inspection and continued their way;

(iii) thence, the ants grasped the beetles by their legs. They were transported either inside the nest, or, on the contrary, farther from the nest. Sometimes the beetle held a prey and it was therefore more difficult to seize. It could also cling to the legs and thus be transported passively. Alternatively, it could cling to the antennae of the ant, which would try to shake it off;

(iv) when the beetle reached the nest, it was maintained by ants and received a mixture of aggression and grooming. Sometimes it was transported again into the foraging arena, which indicates a rejection, at least provisory. The adoption was considered successful when the beetle was neglected and moved freely. Once adopted inside the nest, it searched rapidly for the chambers with larvae.

In *A. senilis* alien colonies adoption was almost systematically a success except for colony 2 which rejected one beetle (=5% total rejection). All but 2 beetles introduced in *A. iberica* were adopted, 2 were rejected and died ($n = 17$, = 11%). The duration of the adoption phases is given on Table 1. The latency without contact (LC) and duration of contacts (TCE) in the foraging arena, the transport times (TR) are longer in colony 2. The total adoption times (TTA) is 20 minutes in controls, more than one hour in colony 3 and very long in colony 2 where it attains 38 hours as the beetle is seized and aggressed many times. In *A. iberica* the duration of contacts in the nest is longer, the total adoption time is also longer (3 hours) but the difference is not significant (Table 1). When the adopted beetles were reintroduced into their *A. senilis* mother colony, they were aggressed but readopted rapidly.

In *A. simonellii* ($n = 4$) and *A. subterranea* ($n = 4$), the beetles were maintained always in the foraging arena and no adoptions occurred.

3.4. Adoption of Isolated Beetles. When reintroduced into their mother colony, isolated beetles were rapidly readopted in half an hour versus 22 minutes in controls. There is a small tendency to increase the adoption time with the isolation duration but none of the measures were significant (ANOVA, $n = 30$, Lambda de Wilk = 0.242, $F_{(25,\,86.943)} = 1.615$, $P = 0.054$—details not shown). At 30 days, 2 about 10 (i.e., 20%) died during the isolation.

3.5. Chemical Profile of the Beetles and Hosts. *Sternocoelis*, *Chitosa* and silverfish had the same hydrocarbons as their host *Aphaenogaster senilis* (Figure 4 and Table 2).

Table 1: Duration of different behavioural phases of adoption of *Sternocoelis* beetles (mean ± SE). LC: latency of the first contact with ants in the external area; TCE: time contacts in the external area; TR: time of transport of the beetle into the nest; CIN: time of contact with ants in the nest; TTA: total time of the adoption. *A. iber*: *A. iberica*.

	LC		TCE		TR		CIN		TTA	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Col 1 (control)	6.5	2.0	2.0	0.8	2.2	0.3	12.3	3.4	22.9	3.1
Col 2	**73.3**	42.5	**154.9**	33	**44.7**	16.3	73.4	17.3	**2491.1**	479.1
Col 3	20.6	8	23.7	14.6	7	3.7	19.6	3.5	70.8	22.6
A. iber	6	2	12.7	2.6	2.2	0.5	**158.2**	21.3	179.3	20.5

ANOVA, Wilk = 0.006, $F = 28.45$, df = 15, $P < 0.00001$; in bold significant differences for each column with post hoc Neumann-Keuls ($P < 0.001$, all other with $P > 0.15$).

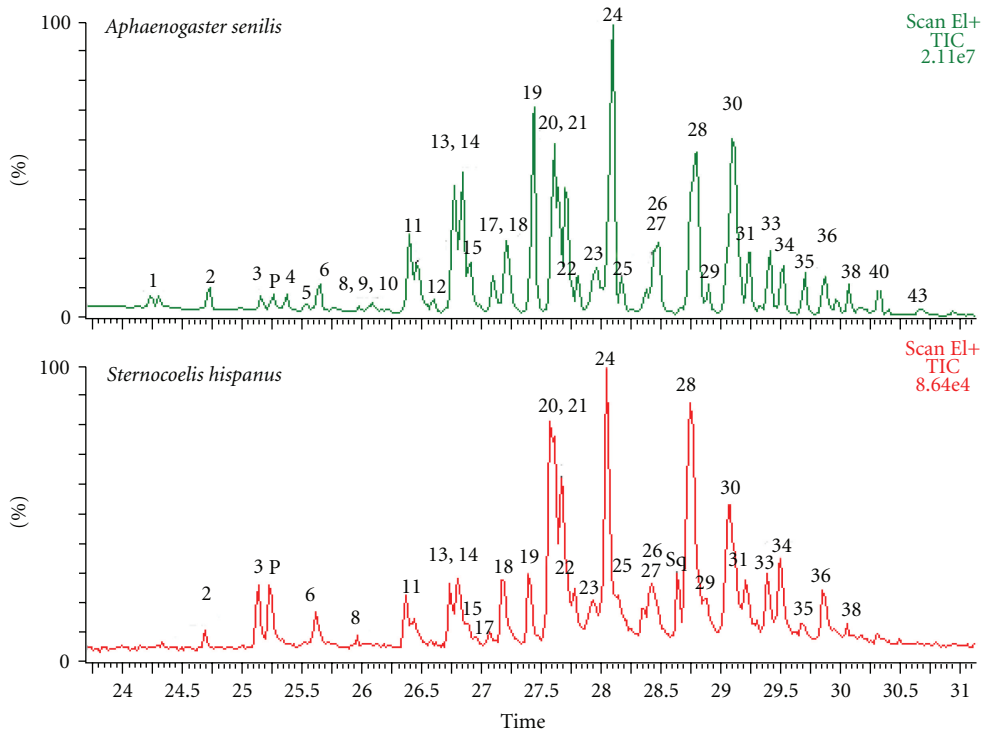

Figure 4: Chromatograms of *Aphaenogaster senilis* and *Sternocoelis hispanus*. Numbers refer to hydrocarbons in Table 2. P: phthalate and Sq: squalene pollutants.

Aphaenogaster iberica and *A. simonellii* also had the same hydrocarbons as *A. senilis* (Nei indexes were close: *A. senilis*/*A. iberica* = 0.75; *A. senilis*/*A. simonellii* = 0.65; *A. iberica*/*A. simonellii* = 0.88). *A. subterranea* had a very different profile with very small quantities of hydrocarbons (using total peak areas) and 20% of unsaturated alkanes which were absent in all other species. Surprisingly, it has also a lot of heavy hydrocarbons (25.8% had more than 32 carbons) that were not found in other species. This species is mostly subterranean and lacks saturated hydrocarbons protecting against desiccation. The Nei index between *A. subterranea* and the other *Aphaenogaster* species is very low (0.211), indicating a high chemical disparity. Therefore, this ant species has not been included in the following analyses.

In the first analysis we constructed a dendrogram of chemical distances between the guests and their *Aphaenogaster* host. It appeared clearly that the four *A. senilis* colonies had different profiles (Figure 5), confirming previous analyses [30]. All the beetles, both *Sternocoelis* and *Chitosa*, were grouped with their host colony, indicating a chemical mimicry fitting the colonial signature. Nevertheless, beetles aggregated distinctly from their host. The chemical distance between colonies did not depend on their geographical distance, and was not linked to the beetle adoption time. Colonies 2 and 3 were equally chemically distant to colony 1 but accepted the beetles more or less rapidly. *Aphaenogaster iberica* and *A. simonellii* were close to *A. senilis* colonies 2 and 3 (data not shown) but the first species accepted the beetles whereas the second did not (but only 4 adoption trials). On the contrary, the silverfish did not match the host colony. Interestingly, *Sternocoelis* adopted in *A. iberica* were close to their new host but did not match completely to the new colony. *A. subterranea* is very different and as expected never adopted the beetles.

Table 2: Hydrocarbon quantities (mean ± SE) in *Aphaenogaster simonellii, A. subterranea, A. senilis, A. iberica, Sternocoelis hispanus, Chitosa nigrita,* and a silverfish. Blanks indicate the absence of the substance or that it is present only as not quantifiable traces.

Peakno.	Name	*Aph simonelli*		*Aph subterranea*		*Aph senilis*		*Aph iberica*		*Sternocoelis*		*Chitosa*		Silverfish	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	C25:1	0.19	0.07			0.33	0.08	0.07	0.04	0.77	0.32	1.10	0.52	1.08	0.20
2	C25	1.43	0.20	19.86	3.47	0.29	0.04	1.38	0.31	0.81	0.13	0.67	0.19	4.26	0.37
3	11+13C25 (+7C25)	1.38	0.34	1.04	0.40	1.08	0.17	6.51	1.26	4.70	1.21	0.57	0.16	0.98	0.02
4	5C25	0.33	0.06	0.15	0.15	0.18	0.06	1.41	0.84	0.46	0.08	0.20	0.07	0.83	0.05
5	9,15C25					0.04	0.02			1.21	0.58	1.39	0.52		
6	3C25	0.92	0.11	2.26	0.40	1.01	0.18	1.30	0.21	5.05	1.63	1.22	0.38	3.72	0.25
7	5,9C25					0.14	0.02	0.82	0.23	0.49	0.07	0.17	0.07		
8	C26	4.31	0.91	2.45	0.49	0.16	0.04	5.04	0.62	0.24	0.07	0.10	0.03	0.75	0.01
9	4,6C25					0.12	0.05			0.85	0.33	0.08	0.02		
10	10+12C26	10.67	2.03	0.49	0.13	2.21	0.20	6.48	0.71	2.39	0.27	1.34	0.34	4.91	0.78
11	6+8C26	1.93	0.35			1.76	0.19	0.41	0.12	1.65	0.21	1.06	0.11	0.45	0.17
12	4C26	0.41	0.07	0.29	0.19	1.06	0.18	0.59	0.11	0.36	0.05	0.31	0.07	2.82	0.22
13	10,14C26	2.14	0.28	0.11	0.11	4.49	1.19	1.91	0.30	2.44	0.35	3.23	0.98	5.36	0.10
14	8,12C26					2.07	0.57	0.42	0.09	4.17	0.52	3.23	0.72		
15	C27:1			1.46	0.45										
16	6,10C26					0.65	0.14	0.05	0.05	1.03	0.19	0.21	0.07		
17	4,8C26					0.22	0.12			1.11	0.19	0.94	0.31		
18	C27	15.59	3.19	11.27	2.19	3.82	0.37	13.05	1.38	2.83	0.16	0.76	0.04	6.47	0.19
19	4,8,12C26					5.77	0.79	0.20	0.09	2.97	0.30	6.98	0.60		
20	9+11+13C27	8.36	1.78	1.43	0.21	18.84	2.08	17.04	1.08	11.01	0.49	9.00	1.84	5.84	0.14
21	7C27	1.15	0.46	0.07	0.07	3.65	0.49	1.03	0.19	3.64	1.02	4.64	1.37	2.12	0.06
22	5C27	2.29	0.39	0.18	0.08	1.50	0.34	0.87	0.22	1.48	0.39	1.83	0.58	1.10	0.05
23	9,13C27					0.43	0.24			3.12	0.20	2.43	0.80		
24	3C27	24.55	2.82	1.81	0.54	15.64	1.50	17.53	2.05	7.21	1.15	10.02	0.90	38.77	0.51
25	5,9C27					1.07	0.16			1.96	0.41	0.87	0.22		
26	C28	6.17	1.37	2.48	0.21	1.00	0.32			0.67	0.24	0.11	0.04	1.07	0.03
27	3,7+3,9+3,11C27	6.09	0.97			1.32	0.47	7.30	0.65	2.47	0.83	5.35	0.28	3.53	0.04
28	10+12C28	5.70	1.56	1.29	0.74	7.32	0.70	3.40	0.22	10.32	1.57	8.56	0.56	3.33	0.09
29	6C28	1.21	0.29			0.61	0.05			1.02	0.28	0.75	0.06	0.57	0.08
30	4C28+10,14C28	1.08	0.12	0.44	0.21	6.11	0.96	2.32	0.31	8.41	0.50	10.31	0.65	4.79	0.15
31	6,10C28	0.64	0.17			2.01	0.16	0.52	0.24	2.67	0.47	3.40	0.18	1.16	0.07
32	C29:1			1.06	0.40										
33	4,8+4,10C28	0.11	0.08			1.89	0.15	0.27	0.06	2.76	0.26	6.01	0.68	0.82	0.05
34	C29	1.14	0.21	9.43	1.09	2.39	0.35	0.48	0.08	3.22	0.51	1.27	0.06	1.78	0.05
35	TM C28					1.38	0.08			1.33	0.04	3.32	0.36		
36	11C29	0.79	0.23	2.86	0.65	3.90	0.49	4.12	0.53	2.24	0.30	1.95	0.14	0.49	0.03
37	7C29	0.19	0.05			2.04	0.49	1.65	0.16	0.45	0.12	0.62	0.20	1.00	0.15
38	5C29	0.86	0.12			1.79	0.34	0.32	0.08	0.48	0.24	1.55	0.35	2.03	0.01
39	11,15C29					0.14	0.06			0.52	0.20	0.26	0.06		
40	7,xC29					0.18	0.10			0.76	0.13	1.80	0.64		
41	3C29			2.36	0.25										
42	5,9C29	0.26	0.02	0.00	0.00	0.17	0.02	0.72	0.24	0.16	0.06	0.42	0.08		
43	C30	0.13	0.05	1.14	0.13	0.08	0.03	0.00	0.00	0.24	0.09	1.07	0.47		
44	10+12C30					0.11	0.02	1.14	0.42	0.17	0.07	0.43	0.24		
45	10,14+10,16+12,14C30					0.17	0.04	0.86	0.16	0.17	0.08	0.25	0.08		
46	C31:1			3.32	0.58										
47	4,8+4,10+4,12C30					0.01	0.01	0.03	0.02			0.01	0.00		
48	C31			1.15	0.06										
49	?			0.00	0.00	0.05	0.04					0.01	0.01		
50	11+13C31			2.54	0.46	0.20	0.07	0.19	0.09			0.10	0.09		

Table 2: Continued.

Peakno.	Name	*Aph simonelli*		*Aph subterranea*		*Aph senilis*		*Aph iberica*		*Sternocoelis*		*Chitosa*		Silverfish	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
51	11,15+13,xC31			3.23	0.63	0.58	0.20	0.31	0.07			0.08	0.05		
52	C32														
53	10C32			2.19	0.44			0.26	0.06						
54	11C33			2.17	0.54										
55	11,15C33			3.23	0.75										
56	12C34			3.16	0.72										
57	11+12C35:1			9.08	2.87										
58	C35:1			6.01	1.68										
	Total	100		100		100		100		100		100		100	
	$n =$	5		5		16		11		4		9		2	

11+13C25: 11-MethylC25 + 13-MethylC25; 9,15C25: 9,15-DiMethylC25; TMC28: TriMethylC28.

In the second analysis, we constructed the dendrogram of isolated *Sternocoelis* beetles (Figure 6). It revealed that they did not match completely the colony odour in a few days compared to controls maintained in their host colony. Some beetles after 4 or 8 days had always their colony profile—in a red ellipse on Figure 6—indicating a progressive change. Nevertheless, these changes were not sufficient to induce the rejection of the beetle.

We also measured the quantities of hydrocarbons on the cuticles. In the pentane rinses, the hydrocarbon quantities of *Aphaenogaster senilis* workers were 1099 ng/worker (± 860, $n = 5$), 446 ng (± 542, $n = 5$) for *Sternocoelis*, and 1567 ng (± 1270, $n = 5$) for *Chitosa*. These beetles were not chemically insignificant. On the contrary, silverfish had only 34 ng (30.1 and 37.7; $n = 2$) indicating that these insects are insignificant and not protected against desiccation and explains why they die very rapidly after collection. For isolated *Sternocoelis* we retrieved by SPME only a very small quantity of hydrocarbons (1 to 5 ng/beetle, see medians in Figure 7), but the profile was comparable to liquid extracts. There were no differences between 4, 8, and 30 days isolated beetles compared to the controls (Kruskal-Wallis Chi-square = 3.91, df = 3, $P = 0.27$) . It shows that the beetles maintained their hydrocarbons quantities independently of their host.

4. Discussion

The three guest species mimic chemically their host: they have the same hydrocarbons (chemical mimicry sensu lato). This explains why they are tolerated inside the nest without being aggressed the ants and they have the host colony odour. This was predictable for *Sternocoelis*, which lives intimately with brood in the colony, but it was more surprising for *Chitosa* which has very few interactions with the host workers. Nevertheless, both species maintain some chemical specificity into the host colony (Figure 5), they are probably recognised as a different though tolerated element. This can be compared to social parasites that also keep their own identity into the host colony [11, 31]. It indicates that chemical mimicry is not sensu stricto, it means that ant workers have a double template: they must know and recognize both their nestmates and their guests. The queen also has a slightly different chemical profile and is recognized by workers (see reviews [7, 21, 27]). Recently, Vantaux et al. [27] described chemical mimicry between predatory larvae of a *Diomus* coccinellid and the little fire ant *Wasmannia auropunctata*. The myrmecophile larvae of *Diomus* also segregate separately in the clusters made on hydrocarbons, indicating that it may be general [27]. On the contrary, silverfish while matching the host hydrocarbons also, do not have an *A. senilis* colony-specific odour. They escape host aggression by avoiding contacts. They probably get their hydrocarbons directly by transfer from the host ant as it has been demonstrated in *Malayatelura* silverfish and their host *Leptogenys* using radioisotopes [32].

Adoptions of *Sternocoelis* are possible in all colonies of *A. senilis* with different delays. *Aphaenogaster* colonies are not completely closed [30], favouring the adoption of beetles that bear the same hydrocarbons in different proportions. The differences in adoption times can probably be explained by the fact that *A. senilis* colonies are very different in aggression levels [30]. Differences between *A. iberica* (acceptance) and *A. simonellii* (rejection) are difficult to explain. They may be due at least partly to the chemical distances with *A. senilis* being more important to *A. simonellii* (0.65) compared to *A. iberica* (0.75). We can also hypothesize that *A. iberica* are less aggressive, they do not have alkaloids in the venom gland, which may repel the beetles [33]. It may also simply not representative as we had only one small colony of *A. simonellii* and 4 adoption trials.

We discovered that *Sternocoelis* beetles kept their hydrocarbon quantities even after one-month isolation. Therefore, chemical mimicry by biosynthesis, rather than camouflage, may explain the host tolerance. If the hydrocarbons were transferred from the host passively, they should have disappeared in a few days because of the rapid turnover of these substances on the cuticle. For example, *Myrmecaphodius* isolated from their *Solenopsis* host colony lose their hydrocarbon profile in two weeks [15] and silverfish *Malayatelura*

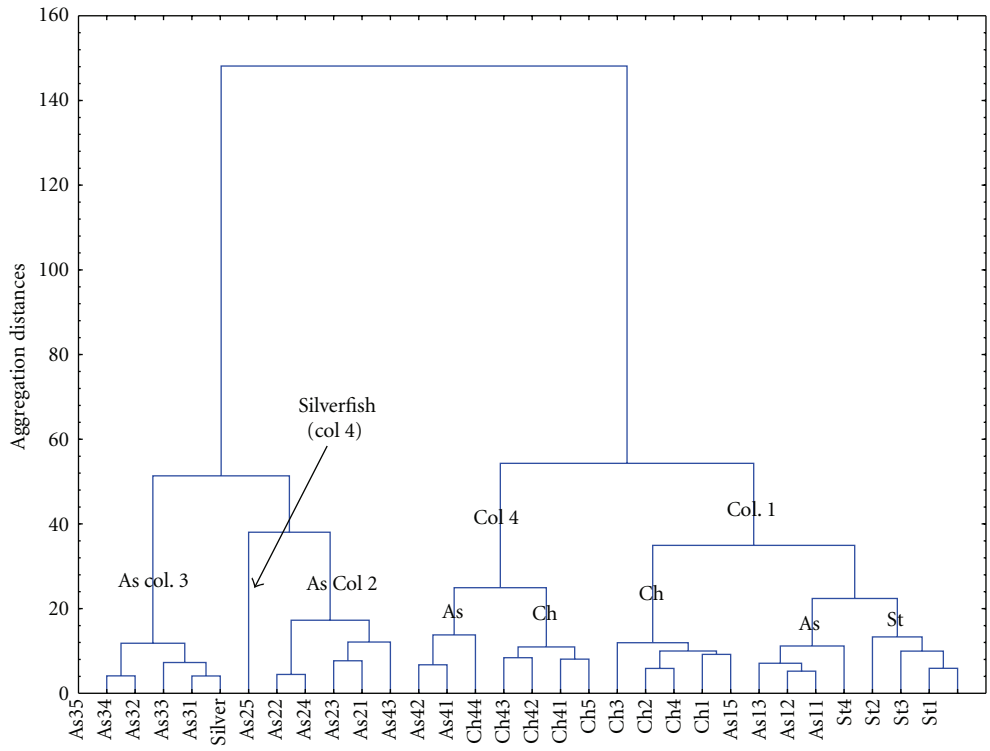

Figure 5: Dendrogram of chemical distances (Ward method, Euclidian distances). As: *A. senilis* colonies (colony 1 As11 to As15; col. 2 As21 to 24; col. 3 As31 to 35; col. 4 As 41 to 43). Ch: *Chitosa nigrita* (colony 1 Ch1 to 4; col. 4 Ch41 to 44). St: *Sternocoelis hispanus* (col 1 St1 to St4). Silverfish form col. 4 (mean of 2 individuals).

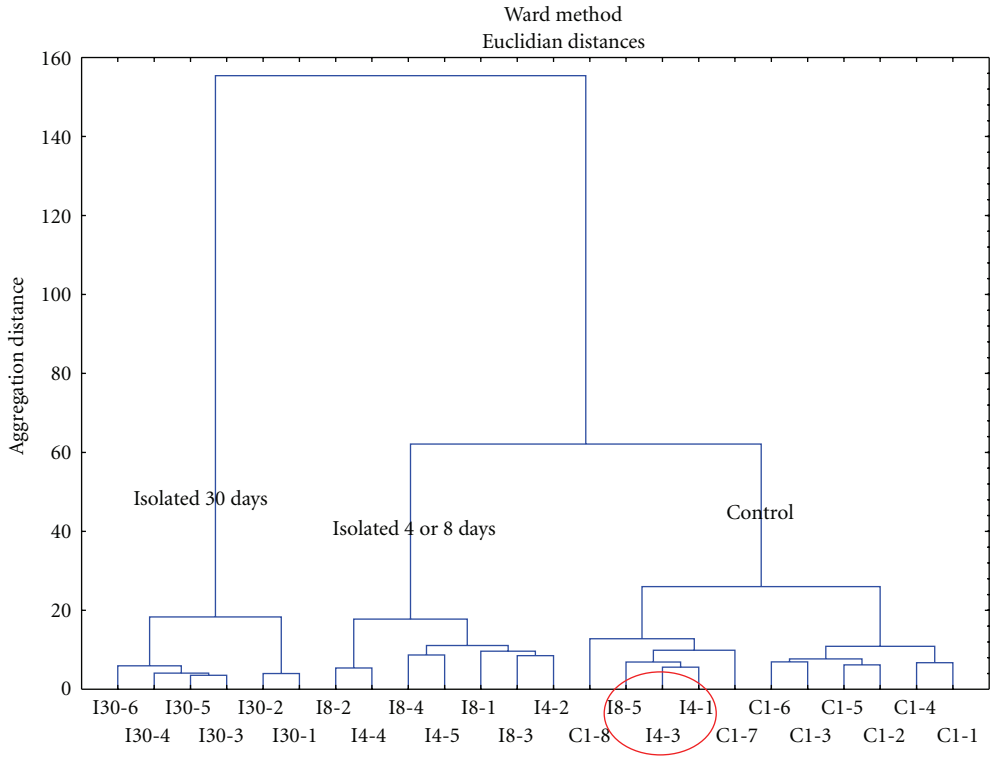

Figure 6: Dendrogram of the isolated *Sternocoelis* beetles (Ward method, Euclidian distances). Controls: C1 ($n = 8$); I4 ($n = 5$), I8 ($n = 5$), I30 ($n = 6$) Isolated during 4, 8, and 30 days. In the red ellipse, isolated individuals having kept completely their colony odour.

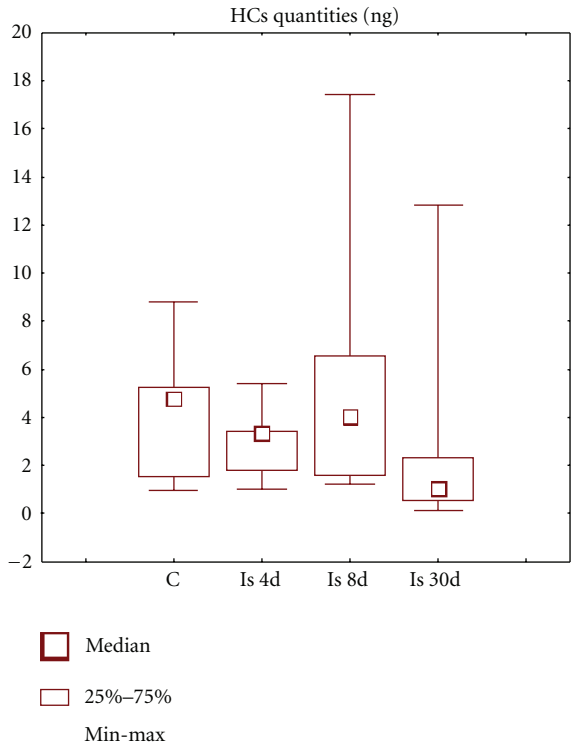

Figure 7: Hydrocarbon quantities (ng/individual; median, quartiles, Min-Max) retrieved by SPME on *Sternocoelis* beetles. C = Controls (beetles in the host nest, $n = 8$), I4 ($n = 5$), I8 ($n = 7$), and I30 ($n = 17$): beetles isolated from the hosts during 4, 8, and 30 days.

after six or nine days separation from their host *Leptogenys* showed reduced chemical host resemblance and received more aggression [32]. This suggests that *Sternocoelis* beetles are able to biosynthesize the host hydrocarbons and adjust their profile to the host colony by contacts. It explains why they change a little their profile after isolation, but are always accepted. This is an indication of a coevolution with the host, with a species-specificity of the association. We could not determine whether the chemical mimicry of the *Chitosa* staphylinid is an active or passive camouflage, but it is probably the latter as the beetle has very few direct interactions with the host and these beetles are not species-specific (Maruyama, pers. comm.). The silverfish have very few hydrocarbon quantities and are chemically insignificant, as observed in a species living in *Aenictus* colonies (but no details are given in the paper, [34]). Nevertheless they also have the host odour, probably acquired simply by contact with the nest material (see above V. Witte pers. comm.), but it is not colonyspecific. It is interesting to note that the inside nest material odour is not colony specific as shown in *Lasius niger* [23]. This may explain why silverfish are killed in *Leptogenys* experimental colonies [6].

Chemical mimicry has been studied only in a few beetles in social insects and all the situations are possible. Biosynthesis has been demonstrated using radio-labelling 14C-acetate. It was shown to occur in two species of thermitophile Staphylinidae with their host *Reticulitermes* [35, 36]. Hydrocarbons are also biosynthesized by the larvae of the fly *Microdon* that are transported in the ant nest [37, 38]. The larvae of the butterfly *Maculinea rebeli* use a double mechanism: they first synthesize the hydrocarbons of the ant brood and later acquire additional hydrocarbons from the ants enhancing the mimicry [39]. Concerning the association of larvae of *Diomus* coccinellid and the little fire ant the authors suggested mimicry by biosynthesis, but they do not prove it [27]. In all the other cases studied, the myrmecophile mimics passively its host (see [9, 10]).

Is the presence of *Sternocoelis* beetles costly for the ant colony? In the army ant *Leptogenys distinguenda* workers are able to recognize and kill the intruders (and possibly eat them) to various degrees, which is the mark of a counterstrategy of the ant [6]. Nevertheless, *Leptogenys* are nomadic ants without a permanent nest, and the situation is different in ants that build a nest and mark it with the colony odour. Inside the nest, all individuals including guests are considered as friends as it was first hypothesized by Jaisson [40] and chemically explained in *Lasius niger* [23]. Apparently, the cost of *Sternocoelis* is insignificant for the host, but some competition for food is possible as beetles and ant larvae feed in the same chambers and beetles can be very numerous. On the opposite, the beetles licking the ant larvae may benefit if they protect them against parasites and infection. We suggest that this may mean a cleaning symbiosis as known in vertebrates, for example, between a cleaner fish and a client [41]. This symbiosis is probably weak as the *Sternocoelis hispanus* cleaners are rare, at least in some places. Therefore, our data suggest that *Sternocoelis* beetles cannot be considered as parasites and that an arms race with the ants will not occur like *Maculinea* larvae with their host ants [42].

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