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Modifications of the Chemical Profile of Hosts after Parasitism Allow Parasitoid Females to Assess the Time Elapsed Since the First Attack

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Abstract In solitary parasitoids, only one adult can emerge from a given host. In some of these species, when several eggs are laid on the same host, supernumerary individuals are eliminated by lethal larval fights. In the solitary parasitoid *Anisopteromalus calandrae*, the probability of a second larva winning the fight depends on the time elapsed since the first oviposition. The older the first egg is at the moment a second egg is laid, the less chance the second egg has of winning the competition. As a consequence, females of this species lay their eggs preferentially on recently parasitized hosts rather than on hosts parasitized by an egg about to hatch. *Anisopteromalus calandrae* females parasitize bruchid larvae located in cowpea seeds. In a series of choice test experiments using an artificial seed system, we demonstrated that the cue that allows parasitoid females to differentiate between hosts parasitized for different lengths of time comes from the host and not from the artificial seed or the previously laid egg. This cue is perceived at short range, indicating that the chemicals involved are probably partly volatile. Interestingly, although parasitism stops host development, cuticular profiles continue to evolve, but in a different way from those of unparasitized hosts. This difference in the host's cuticular profile after parasitism, therefore, probably underlies the parasitoid female's discrimination.

Key Words Cuticular hydrocarbons · Semio-chemicals · Kairomone · Oviposition strategies · Solitary parasitoids · *Anisopteromalus calandrae*

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Introduction

Parasitoid females have to localize hosts to lay their eggs. This localization often is mediated by semiochemicals that can have different origins, such as volatiles released by plants when attacked by herbivores, chemical markers left by host females when they oviposit, or host sex or aggregative pheromones (for a review see Hilker and McNeil 2008). For example, the aphid parasitoid *Aphidius ervi* (Hymenoptera: Braconidae) is attracted by volatiles emitted by the blackcurrant when attacked by aphids (Birkett et al. 2000). Similarly, the Pteromalid wasps *Dinarmus basalis* and *Anisopteromalus calandrae* can localize their bruchid larvae hosts in cowpea seeds due to chemical markers deposited by the bruchid females after oviposition (Kumazaki et al. 2000; Onodera et al. 2002).

After locating their hosts, females have to select which host to parasitize. Indeed, all hosts are not of equal quality, and one of the important parameters influencing this quality is the host's parasitized status. Hosts that have been parasitized already have a lower quality than those that are unparasitized because of the competition among larvae resulting from several clutches laid on the same host (Godfray 1994). In some solitary parasitoids, when several eggs are laid in or on the same host, larvae fight until only one remains. Only one adult thus can emerge from a given host in these species (Hubbard et al. 1987). Parasitoid females generally are able to discriminate between these two categories of hosts (Gauthier et al. 1996; Weber et al. 1996; Darrouzet et al. 2007; Lebreton et al. 2009a). These discrimination capacities may be based on perception of different chemical markers deposited by the first parasitoid during parasitization (Völkl and Mackauer 1990; Jaloux et al. 2005; Stelinski et al. 2007) as well as on alarm

pheromones produced by the host itself after having been stung the first time (Outreman et al. 2001). In other species, host discrimination can be based on the interaction of several protagonists. In the ectoparasitoid *D. basalis* for example, the cue is located on the host, but a transfer of molecules from the parasitoid egg to the host is needed over an extended period (Gauthier and Monge 1999).

It would appear disadvantageous for a parasitoid female to lay several eggs on the same host or to lay eggs on already parasitized hosts. This behavior, called superparasitism, has, however, been demonstrated to be adaptive under certain circumstances, particularly when unparasitized hosts are scarce or distances between oviposition patches are long (van Alphen and Visser 1990). When females are confronted with several parasitized hosts, the survival probability of their offspring can vary from one host to another depending on different factors. Among these, the time elapsed between the two ovipositions is one of the more important (Visser et al. 1992; van Baaren and Nénon 1996; Goubault et al. 2003; Lebreton et al. 2009a); the older the first egg, the less chance the second egg has of surviving, because the previously laid egg hatches first and the larva kills the second egg before it can hatch (Godfray 1994). In a previous study with the solitary ectoparasitoid *Anisopteromalus calandrae*, we demonstrated that survival probability of a second egg decreases strongly when the time interval between two ovipositions increases (Lebreton et al. 2009a). On the other hand, two eggs laid at the same time have about the same probability of winning the competition, while the survival probability of the second egg decreases to 15% when it is laid shortly before the hatching of the first egg. As a consequence, females lay their eggs preferentially on recently parasitized hosts rather than on those parasitized by an egg about to hatch (Lebreton et al. 2009a).

The present study aimed to identify the cue responsible for this discrimination capacity. *Anisopteromalus calandrae* females parasitize bruchid larvae contained in cowpea seeds. When a female encounters a seed containing a host, she taps the seed surface with her antennae to locate the exact position of the host, and then she inserts her ovipositor into the seed in order to probe the host. The cue perceived by females thus could come from a quantitative or qualitative change in an external marker deposited by the first female after oviposition. It also could be due to an internal marker that originates from the host in response to the first sting, which could evolve with time, or from modifications to the egg itself that probably take place during its development. In some species, consecutive cues of different origins inform females about the parasitized status of a host (Islam and Copland 2000; Outreman et al. 2001). Perception of one or more of these cues can inform parasitoid females of the time elapsed since the first parasitism. However, to our knowledge, the few studies that have dealt with this discrimination capacity have never focused on the chemical signals involved.

In the present study, we aimed to investigate whether the cue perceived by females and involved in discrimination between hosts parasitized for different lengths of time originates from the seed, the host, or the egg. For this purpose, by using an artificial seed system, we performed a series of choice tests, in which we presented females with either parasitized seeds, previously laid eggs, or parasitized hosts. After locating the cue, we investigated which compounds could be involved by using gas chromatography and mass spectrometry.

Methods and Materials

Insect Rearing *Anisopteromalus calandrae* were reared in the laboratory on larvae and pupae of the bruchid *Callosobruchus maculatus* (Coleoptera: Bruchidae). Both *C. maculatus* and *A. calandrae* originated from Ivory Coast (collected in 2000) and were mass-reared under conditions close to that of their area of origin: 12 hr light at 29°C, 12 hr dark at 22°C, and 65% r.h.

Behavioral Analysis To obtain parasitized hosts, we used an artificial seed system consisting of gelatine capsules (1.5 cm long x 0.6 cm diam) containing a bruchid larva (Gauthier and Monge 1999; Darrouzet et al. 2003). The bruchid L4 larvae were placed inside the capsules after removal from seeds by dissection and selection by size. Five capsules were proposed to a group of 5 virgin females during a 2 hr period in a climatic chamber (29°C, 65% r.h.). Because Hymenopteran parasitoids reproduce by arrhenotokous parthenogenesis, virgin females were able to lay only male eggs, which avoids a bias due to the sex of the eggs during the choice test. At the end of the exposure period, eggs on parasitized hosts were located with a dissecting microscope, and parasitized artificial seeds were selected for choice tests.

Choice tests consisted of two artificial seeds each containing one host, one which had been parasitized for 2 hr (recently parasitized capsule, P 2 hr) and the other for 28 hr (parasitized by an egg about to hatch, P 28 hr), placed in a Petri dish (3.5 cm diam x 1 cm high) (Lebreton et al. 2009a). The two capsules were separated by 0.5 cm. To determine whether the cue perceived by females was located on the capsule, on the egg, or on the host, three choice tests were performed, each offering a different part of the artificial seed system to the females. In test 1, capsules were presented that had contained hosts previously parasitized for 2 hr and 28 hr, but now contained an unparasitized host. In test 2, 2- and 28-h-old eggs were

offered in a clean capsule, on an unparasitized host. Finally, in test 3, hosts parasitized for 2 and 28 hr, from which eggs had been removed, were offered in clean capsules.

In a previous study, we demonstrated that mated females distinguish between 2 and 28 hr parasitized artificial seeds (Lebreton et al. 2009a). In the present study, to avoid a bias due to the mating status of females, mated females also carried out these choice tests. The whole oviposition sequence was observed (first capsule visited, capsule with oviposition). The tests ended as soon as females laid an egg but lasted no more than 2 hr to avoid the first egg hatching during the test. After the test, females were removed from the Petri dish. The first capsules visited and the selected capsules were noted. Statistical analyses were performed with a χ^2-*test*.

Chemical Analysis of the Hosts' Cuticular Profile We quantified and identified the chemical compounds present on the host cuticle. For this purpose, samples from one bruchid larva were extracted in 10 µl of pentane, to which 2 µl of an internal standard (C20) were added for 1 min. Samples were analyzed with a Perkin-Elmer Autosystem XL GC (Perkin-Elmer, Wellesley, MA, USA) equipped with a flame ionization detector (FID) and interfaced with Turbochrom workstation software. Each sample was dried to 2 µl. These 2 µl then were injected into the GC-FID injector heated to 220°C in splitless mode, and analyzed by using a BP1 capillary column, which was temperature programmed from 50°C (2 min hold) to 310°C at 7°C/min with a final hold of 10 min. Five categories of host were analyzed: larvae parasitized for 2 hr (P 2 hr, N=6) or 28 hr (P 28 hr, N=14) and unparasitized larvae stored in the same conditions as 2 hr (UnP 2 hr, N=9) or 28 hr parasitized hosts (UnP 28 hr, N=9). As some unparasitized larvae develop to a pre-pupa stage after 28 hr, unparasitized pre-pupae (UnP Pre-pupae, N=6) also were analyzed. The components were identified by GC-MS analysis, performed using a Hewlett-Packard 5890 GC system coupled to a 5989A MS, controlled by HP chemstation software. Hydrocarbons were identified tentatively by their mass spectra (Lockey 1988; Blomquist 2010) and corroborated by their ECL indices. Individual samples and pools of 2 hr and 28 hr parasitized hosts were injected into the GC/MS injector following the same procedure as described above. To analyze whether there was a difference in the cuticular profile of the different categories of host, we performed a discriminant analysis based on the relative amount of the 30 major peaks.

Bioassays with Chemical Extracts from the Host To confirm that compounds observed in GC-MS were responsible for the parasitoid females' capacity to perceive the time elapsed since the first oviposition, we performed another series of choice tests. In these, mated females (N=42) were presented with two capsules, each containing an unparasitized host on which an extract of parasitized hosts had been deposited. These extracts were obtained by immersing 100 hosts parasitized for 2 hr or 28 hr in 200 µl of pentane for one min. Then, 2 µl of each extract (2 or 28 hr; amount corresponding to one host) were deposited on an unparasitized host previously rinsed in pentane to remove its own chemical compounds. After rinsing, we checked that hosts were still alive. One host of each category (with the 2 hr or 28 hr parasitized extract) was used for the choice tests, which took place in a climatic chamber as described above. The first capsule visited and the one selected for oviposition were noted. The proportion of each category of capsules visited first and those on which females oviposited then was calculated. Statistical analyses were performed with a χ^2-*test*. All statistical analyses were performed using Statistica 6.0 software (Statsoft, Inc.).

Results

Behavioral Analysis In tests 1 and 2, parasitoid females first visited each category of capsules equally (test 1, χ^2 = 0.03, df = 1, P=0.87; test 2, χ^2 = 0.61, df = 1, P=0.43) and laid the same number of eggs in each category of capsules (Fig. 1; test 1, χ^2 = 1.58, df = 1, P=0.21; test 2, χ^2 = 0.03, df = 1, P=0.85). In test 3, most females (69.2%) first visited the capsules containing the 2 hr parasitized host rather than the 28 hr parasitized host (χ^2 = 7.69, df = 1, P=0.006). Moreover, most (72.7%) selected the 2 hr parasitized hosts to lay their eggs (Fig. 1; χ^2 = 6.82, df = 1, P=0.009).

Chemical Analysis of the Hosts' Cuticular Profile The GC-MS analysis showed 44 major peaks (Fig. 2) and 71 identified components (Table 1) on hosts. These included a series of *n*-alkanes and methyl-branched alkanes (C_{25}-C_{35}; Table 1). The same compounds were found on each category of host but in different proportions (Table 1).

The discriminant analysis revealed a difference between UnP Pre-pupae and UnP Larvae (UnP 2 hr: F=3.53, df = 30.1, P=0.020; UnP 28 hr: F=3.35, df = 30.1, P=0.023), but no statistical differences were found between the two categories of UnP Larvae (F=1.21, df = 30.1, P=0.39; Fig. 3). P 2 hr were not different from UnP 2 hr (F=1.28, df = 30.1, P=0.30), whereas P 28 hr were different from P 2 hr (F=3.64, df = 30.1, P=0.018) and had a tendency to diverge from UnP 28 hr (F=2.39, df = 30.1, P=0.073; Fig. 3). P 28 hr also were different from UnP Pre-pupae (F=3.12, df = 30.1, P=0.031; Fig. 3).

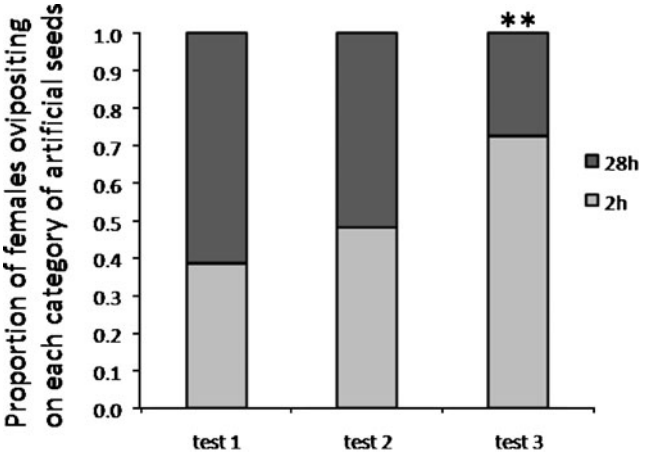

Fig. 1 Proportion of *Anisopteromalus calandrae* females selecting each category of artificial seed on which to lay their eggs (2 hr or 28 hr parasitized) when confronted with each test condition: capsules that previously contained parasitized hosts, but which were presented with an unparasitized host inside for the test (test 1), eggs on an unparasitized host in a clean capsule (test 2), and parasitized hosts on which eggs had previously been removed, in a clean capsule (test 3). Marks show significant differences between proportions observed and a random distribution of 50:50 ** $P<0.01$

UnP Larvae and P 2 hr could be distinguished from P 28 hr and UnP Pre-pupae on the basis of the first discriminant variable and were characterized by the occurrence of *n*-C_{28} (peak 15) and *n*-C_{29} (peak 22). In contrast, P 28 hr and UnP Pre-pupae were characterized by a higher proportion of 3-MeC_{25} (peak 2), *n*-C_{26} (peak 3), MeC_{27} (peaks 10, 11, 12, 14), $DiMeC_{27}$ (peaks 13, 14), MeC_{28} (peaks 17, 19, 21), and MeC_{29} (peaks 23, 24). The P 28 hr and UnP Pre-pupae differed in the second discriminant variable. P 28 hr were characterized by the occurrence of 3-MeC_{28} (peak 21), MeC_{29} (peak 23), 9,13-$DiMeC_{29}$ (peak 25), and MeC_{31} (peak 32), whereas UnP Pre-pupae were more particularly characterized by the presence of *n*-C_{26} (peak 3), an unidentified compound (peak 35), and 3,9,13-TriMe-C_{31}.

Bioassays with Cchemical Extracts from Hosts Most females (70.7%) visited the capsule containing the 2 hr parasitized extract first (χ^2 = 7.04, *df* = 1, P=0.008). Ovipositing females tended to lay more eggs on these hosts (64.3% vs. 35.7% on hosts with the 28 hr parasitized extract), but the difference was not significant (χ^2 = 2.29, *df* = 1, P=0.13).

Discussion

Semio-chemicals are known to play a crucial role in host localization in parasitoids (Afsheen et al. 2008). Host cues can have many different origins such as oviposition markers (Kumazaki et al. 2000; Onodera et al. 2002), chemical residues left by adult hosts on the substrate (Colazza et al. 1999, 2007; Peri et al. 2006), chemicals originally from host feces (Meiners et al. 1997; Steidle et al. 2003; Steiner et al. 2007; Inoue and Endo 2008), or possibly even carbon dioxide (Hilker and McNeil 2008). Some of these compounds arise from the adult's activity, and often are used by immature parasitoids (eggs or larva stage) to locate their hosts. However, other chemicals could come directly from larvae, and these compounds could give information about both the presence of a host and its parasitized status (Outreman et al. 2001; Lizé et al. 2006). In our study, we demonstrated a new level of information: semiochemicals inform the parasitoid about the time elapsed since the first parasitism. With a series of choice tests, we demonstrated that this cue is not located on the seed, nor produced by the egg, but is found on the host itself.

Our behavioral results show that most of the tested females visited the capsule containing the 2 hr parasitized host first (test 3, 70%), thus indicating that compounds involved in this discrimination capacity are detected not by direct contact but from a short distance. In fact, when females were introduced in the choice test, they came within a few millimetres of the capsules with their antennae but without touching them before finally making their choice (Lebreton, personal observation).

When females were confronted with only the chemical compounds (experimentally deposited on an unparasitized host), they also more often visited the host exhibiting the "most recently parasitized" profile first. However, although they tended to lay their eggs on these hosts, this choice was not as marked as for actually parasitized hosts (Lebreton et al. 2009a). This result suggests that several cues could in fact be necessary for a female to assess host quality accurately. During the first parasitism, *A. calandrae* females also paralyze their hosts by injecting venom (Lebreton et al. 2009b). In the present experiment (bioassays of chemical extracts from hosts), hosts were unparasitized and therefore not paralyzed. It is possible that initially females perceive hosts as "recently parasitized", but on coming closer detect that they are not in fact parasitized because they are not paralyzed.

In a previous study, Outreman et al. (2001) showed that an alarm pheromone produced by aphids after being parasitized allows parasitoid females to determine whether a host is already parasitized or not. They correlated an antennal rejection of the parasitized host with the presence of the pheromone in the hours following the first parasitism. As the pheromone disappeared, the antennal rejection was replaced by a sting rejection. This result indicates that several cues can give the same information.

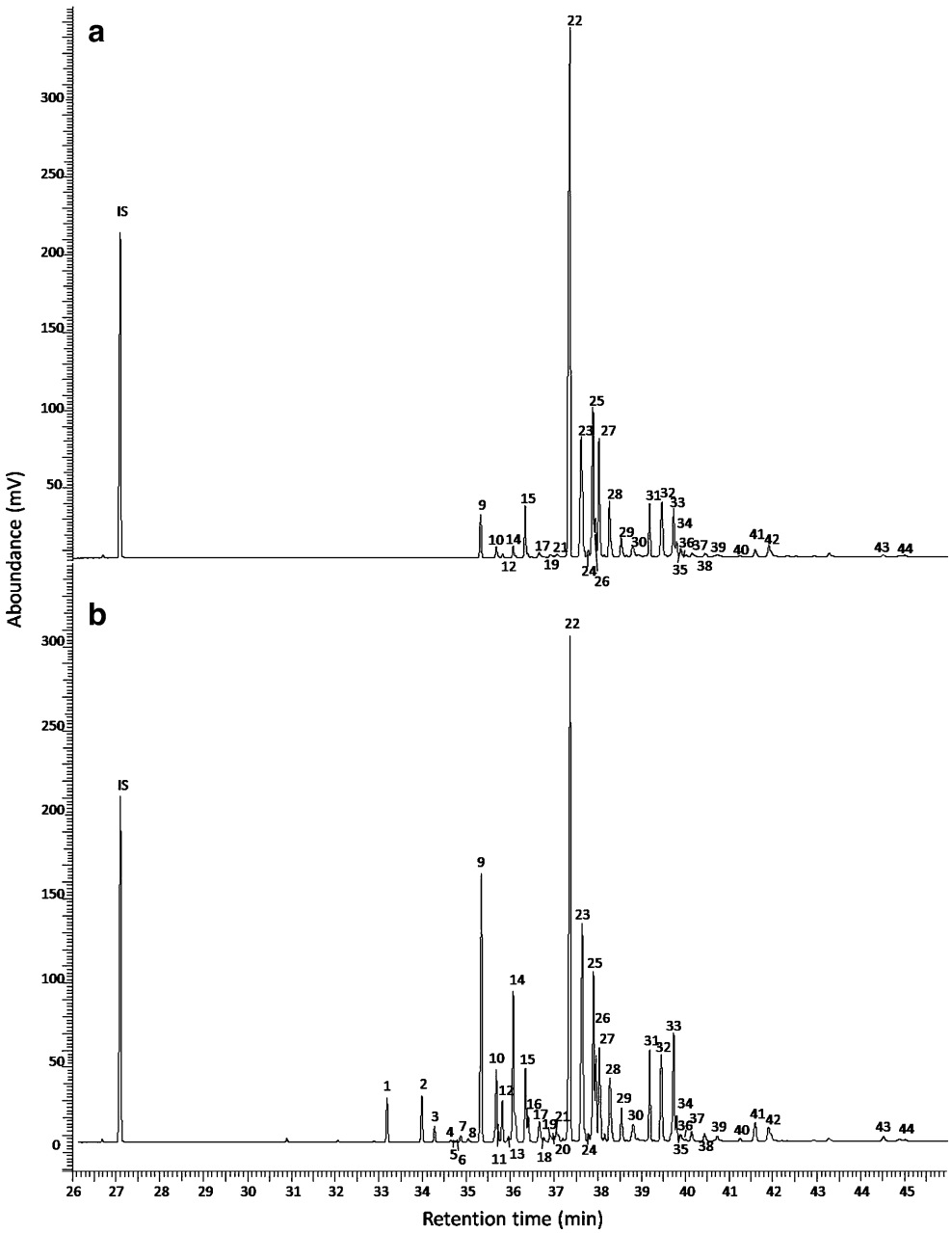

Fig. 2 Gas chromatogram of the cuticular pentane extract of hosts parasitized for 2 hr (**a**) and 28 hr (**b**). IS = internal standard. Peak numbers correspond to those listed in Table 1

In our study, the difference in the cuticular profile of hosts was visible mainly after 28 hr. It is thus possible that this cue does not inform parasitoids about the parasitized status of hosts just after parasitism, whereas other cues, such as oviposition markers deposited by parasitoids, give this information for shorter periods. When *A. calandrae* females oviposit on unparasitized hosts, generally they deposit a substance with their ovipositors after laying (Lebreton, personal observation). Moreover, when confronted with parasitized capsules (test 1), females tend to select 28 hr parasitized capsules slightly more, even if this difference is not statistically significant from a random choice. This observation may support the hypothesis of an oviposition marker that is more detectable shortly after its deposit. Oviposition markers generally are produced in the Dufour gland. In *A. calandrae*, the Dufour gland contains a mixture of *n*-alkanes (C_{30} to C_{39})(Howard and Baker 2003). Such marking-pheromones commonly are used by parasitoids to inform competitors about previous ovipositions and thus avoid competition (Hoffmeister 2000; Rosi et al. 2001; Stelinski et al. 2007). They also seem to be used by females to discriminate between hosts parasitized by themselves and those parasitized by other parasitoids (van Dijken et al. 1992). More recently, it also has been shown

Table 1 Tentatively identified cuticular hydrocarbons present on the host cuticle, with their relative amount for each host category

Peak	Hydrocarbons	ECL[a]	CN[b]	Relative amount (% of total)				
				UnP 2hr	UnP 28hr	P 2hr	P 28hr	UnP pre-pupae
1	*n*-C25	25.00	25	0.23	0.25	0.21	0.55	0.59
2	3-MeC25	25.68	26	0.07	0.10	0.14	0.40	0.44
3	*n*-C26	26.00	26	0.10	0.22	0.17	0.30	0.46
4	9MeC26	26.30	27	0.00	0.01	0.03	0.06	0.04
5	6-MeC26	26.47	27	0.00	0.00	0.00	0.03	0.00
6	5-MeC26	26.49	27	0.03	0.01	0.01	0.02	0.05
7	4-MeC26	26.51	27	0.01	0.03	0.03	0.10	0.10
8	3-MeC26	26.67	27	0.02	0.03	0.04	0.13	0.13
9	*n*-C27	27.00	27	4.88	9.99	6.29	8.58	10.19
10	9-MeC27	27.30	28	0.75	0.83	1.45	2.63	2.18
11	7-MeC27	27.32	28					
12	5-MeC27	27.43	28	0.11	0.23	0.35	0.65	0.57
13	9,15-DiMeC27	27.59	29	0.11	0.19	0.18	0.32	0.25
14	3-MeC27 +	27.69	28	0.78	1.54	1.62	3.54	3.36
	5,9-DiMeC27	27.72	29					
15	*n*-C28	28.00	28	4.00	4.77	4.05	3.46	3.70
16	3,9-DiMeC27	28.05	29	0.21	0.20	0.15	0.09	0.24
17	10-MeC28	28.26	29	0.27	0.32	0.46	0.77	0.65
18	6-MeC28	28.30	29	0.01	0.04	0.03	0.14	0.06
19	4-MeC28	28.50	29	0.11	0.20	0.16	0.32	0.28
20	2-MeC28	28.66	29	0.03	0.07	0.09	0.10	0.04
21	3-MeC28	28.67	29	0.07	0.26	0.29	0.51	0.41
22	*n*-C29	29.00	29	56.95	48.43	47.55	34.47	36.66
23	15-MeC29 +	29.28	30	5.25	4.79	7.32	10.44	7.52
	13-MeC29 +	29.28	30					
	11-MeC29 +	29.28	30					
	9-MeC29 +	29.30	30					
	7-MeC29	29.32	30					
24	5-MeC29	29.39	30	0.14	0.15	0.26	0.33	0.27
25	9,13-DiMeC29	29.49	31	5.26	5.45	5.72	7.06	6.05
26	7,11-DiMeC29 +	29.60	31	0.80	1.45	1.11	1.45	1.93
	7,13-DiMeC29	29.60	31					
27	3-MeC29	29.68	30	2.44	4.14	3.75	4.08	4.08
28	*n*-C30 +	30.00	30	4.02	3.32	3.44	3.20	3.67
	3,9-DiMeC29 +	30.05	31					
	3,11-DiMeC29 +	30.05	31					
	3,13-DiMeC29	30.05	31					
29	14-MeC30 +	30.28	31	0.80	0.82	1.05	1.23	1.11
	12-MeC30	30.28	31					
30	10,14-DiMeC30 +	30.48	32	0.62	0.79	0.84	0.92	0.95
	9,13-DiMeC30 +	30.48	32					
	8,12-iMeC30 +	30.48	32					
	8,18-DiMeC30	30.48	32					
31	*n*-C31	31.00	31	4.96	4.03	3.86	3.65	3.76
32	15-MeC31 +	31.28	32	2.27	1.98	2.97	3.34	2.47
	13-MeC31 +	31.28	32					
	11-MeC31 +	31.28	32					
	9-MeC31 +	31.30	32					
	7-MeC31	31.30	32					
33	9,13-DiMeC31	31.46	33	2.43	2.81	3.23	3.48	3.75
34	7,11-DiMeC31 +	31.59	33	0.44	0.24	0.39	0.49	0.52

Table 1 (continued)

Peak	Hydrocarbons	ECL[a]	CN[b]	Relative amount (% of total)				
				UnP 2hr	UnP 28hr	P 2hr	P 28hr	UnP pre-pupae
	7,13-DiMeC31 +	31.59	33					
	3-MeC31 +	31.66	32					
	5,13DiMe-C31	31.64	33					
35	*unknown*			0.30	0.38	0.38	0.33	0.51
36	*n*-C32	32.00	32	0.04	0.08	0.06	0.06	0.15
37	3,9,13-TriMeC31	32.11	34	0.25	0.29	0.30	0.34	0.46
38	14-MeC32 +	32.28	33	0.18	0.20	0.26	0.28	0.30
	12-MeC32	32.28	33					
39	10,14-DiMeC32	32.46	34	0.11	0.15	0.21	0.20	0.16
40	*n*-C33	33.00	33	0.13	0.13	0.10	0.15	0.29
41	15-MeC33 +	33.28	34	0.31	0.30	0.48	0.59	0.50
	13-MeC33	33.28	34					
42	11,15-DiMeC33 +	33.48	35	0.48	0.64	0.75	0.89	0.94
	9,13-DiMeC33 +	33.48	35					
	7,11-DiMeC33	33.60	35					
43	*n*-C35 +	35.00	35	0.04	0.09	0.13	0.15	0.09
	13-MeC35	35.29	36					
44	13,17-DiMeC35 +	35.46	37	0.02	0.06	0.12	0.17	0.09
	11,15-DiMeC35 +	35.48	37					
	9,13-DiMeC35	35.48	37					

[a] ECL = equivalent chain length

[b] CN = carbon number

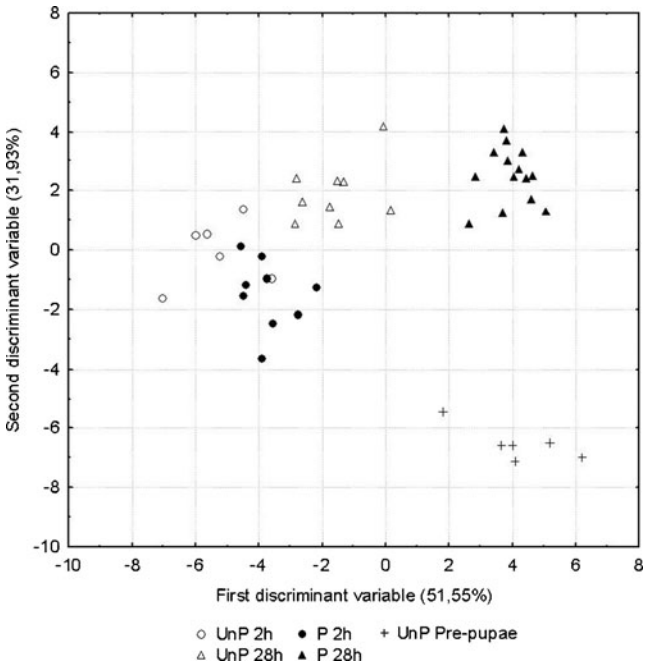

Fig. 3 Discriminant analysis based on the relative amount of the 30 major peaks of the cuticular profile of the different host categories (L4 larvae parasitized for 2 hr (P 2 hr) or 28 hr (P 28 hr), unparasitized L4 larvae stored under the same conditions as 2 hr (UnP 2 hr) and 28 hr (UnP 28 hr) parasitized hosts, and unparasitized pre-pupae (UnP Pre-pupae))

that oviposition markers deposited by larval parasitoids can be perceived by adult hosts, and thus avoid laying their eggs on unsuitable substrate (Stelinski et al. 2009).

Our results show differences in the cuticular profiles of the hosts analyzed. First, the profiles of the unparasitized hosts depend on their developmental stage, with larvae and pre-pupae exhibiting different profiles. The profile of parasitized hosts also changes with time, with hosts parasitized for 2 hr and 28 hr showing different profiles. However, it evolves differently in unparasitized hosts. Although unparasitized pre-pupae and 28 hr parasitized host profiles were similar in terms of the first discriminant variable, they diverged from each other in regard to the second discriminant variable. This result suggests that parasitism affects the evolution of the host profile. The compounds identified (a series of linear and methyl alkanes) were in accordance with those previously identified by Howard (2001). Although these consist of heavy long-chain hydrocarbons (C_{25} to C_{35}), and generally are considered as being detected through direct contact, in our choice test experiments, parasitoid females were able to detect the time elapsed since the first oviposition at a short distance (a few millimetres), i.e., outside the capsule. Nevertheless, considering the high temperature in our experimental procedure (29°C, the mean temperature of the area of origin of these species), some of these

compounds may be semi-volatile. A previous study demonstrated that cuticular hydrocarbons from C_{25} to C_{27} can be volatile and perceived at a distance at 25°C (Saïd et al. 2005). It is possible that only the lighter components play a role in this discrimination. This hypothesis is in accordance with our results, which show that the major difference between 2 hr and 28 hr parasitized hosts is related to these compounds (more particularly from C_{25} to C_{29}). This remains to be tested. Another possibility is the spontaneous oxidation of heavy cuticular lipids to volatile aldehydes or alcohols, as previously observed in other insect species (Bartlelt and Jones 1983; Bartlelt et al. 2002). However, the compounds involved in the discrimination observed in our experiment were pentane-soluble (obtained by immersing bruchids in a pentane solution), and only alkanes were found in these pentane extracts. Further studies are necessary to determine precisely which compounds are involved in this discrimination. The use of selected fractions of the hosts cuticular profiles or the use of blends of synthetic compounds would give us some indications. Moreover, by using olfactometry experiments, we would be able to differentiate between an attractant and an arrestment effect of these compounds. It also would be possible to know whether the profile of 2 hr parasitized hosts could be attractive for parasitoid females or whether that of hosts parasitized for 28 hr could be repellent.

Our results also raise an interesting question about the action of parasitoid venom on host physiology. When females inject their venom into hosts, the latter are paralyzed and their development is stopped. Their cuticular profile, however, continues to evolve. This indicates that some elements of host physiology still operate after venom injection. However, the evolution of the cuticular profile of a parasitized host is perceptibly different from that of an unparasitized host. The parasitoid venom thus appears to disturb the metabolism of hydrocarbons, interfering either with their production or with their dynamic release at the cuticle level. In a previous study, we observed that parasitism disturbs lipid metabolism by decreasing the amount of total lipids present in the host haemolymph (Lebreton et al. 2009b). Studies with other species also have demonstrated that modifications in the composition of the host haemolymph occur after venom injection (Rivers and Denlinger 1995; Nakamatsu and Tanaka 2003). However, in most of these species, the amount of lipids increases after parasitism, probably due to the lysis of fat body cells by the components of the venom (Nakamatsu and Tanaka 2004). The opposite result found in our study suggests other mechanisms. This result raises new questions about the affect of parasitism on a host's lipid metabolism. Identifying the components of venom could shed light on the way parasitoid venom acts on the host physiology.

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