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Chemical and tactile communication between the root aphid parasitoid *Paralipsis enervis* and trophobiotic ants: consequences for parasitoid survival

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Abstract. Females of the aphid parasitoid *Paralipsis enervis* received liquid food by regurgitation (trophallaxis) from workers of the ant species *Lasius niger*, but were not fed by workers of *Myrmica laevinodis* and *Tetramorium caespitum*. While *P. enervis* was not treated aggressively by workers of any of these species, *Lasius flavus* workers killed the parasitoid. This different ant behaviour resulted in a different parasitoid longevity. While *P. enervis* survived for only 10 min in the presence of *L. flavus* (due to ant aggression) or for approximately one day in the presence of *T. caespitum* and *M. laevinodis* (due to lack of trophallaxis), survival increased significantly to more than five days in the presence of *L. niger*, which provided food regularly to the parasitoids. Our study suggests that *P. enervis* mimics behavioural signals of *L. niger*, as well as odor cues of its host aphid *Anoecia corni*, to avoid aggression by *L. niger*.

Key words. Aphidiidae; ant-parasitoid interactions; specialization; chemical mimicry; trophallaxis; parasitoid longevity.

Introduction

A variety of arthropod species belonging to various taxa live temporarily or permanently in close association with ants[1–3]. These myrmecophiles have evolved a number of mechanisms to avoid detection and/or aggression by ants. Common strategies are chemical mimicry or chemical camouflage[4–7], behavioural mimicry of the ants' communication signals[1,8], and the supply of honeydew or nutritive gland secretions[9–13]. Members of the last group, the so-called trophobionts, benefit from their association with ants by being protected against natural enemies[9,13–16]. Both parasitoids and the predators of trophobionts need mechanisms to prevent ant aggression. For example, larvae of the chrysopid species *Chrysopa slossonae* and *Ceraeochrysa cincta* (Neuroptera: Chrysopidae) coat themselves with waxes obtained from their homopterous prey to fool attending ants, thus following the 'wolf-in-sheep's clothing' strategy[17,18].

The aphid parasitoids *Lysiphlebus cardui* and *L. hirticornis* (Hymenoptera: Aphidiidae), and larvae of the coccinellid beetle *Platynaspis luteorubra* (Coleoptera: Coccinellidae), use chemical mimicry to prevent ant aggression[19–22]. However, these predators/parasitoids are not specifically associated with particular ant species, but have been recorded in coexistence with a variety of ant species. Furthermore, they also commonly develop in unattended situations, although they benefit significantly from ant attendance by increased larval survival[21,23]. By contrast, *Paralipsis enervis* (Nees), an aphidiid parasitoid (Hymenoptera: Aphidiidae) of root-feeding aphid species of the genera *Anoecia*, *Forda*, *Tetraneura*, *Anuraphis*, *Dysaphis* and *Aphis*[24,25], was recorded up to now exclusively in association with the ant species *Lasius niger* L.[26–29], although its hosts are regularly attended by various ant species[30]. *P. enervis* shows some behavioural adaptations for coexistence with *L. niger*. Females regularly display a mutual antennating with *L. niger* workers and solicit regurgitated liquid food during this tactile communication; i.e., they engage in trophallaxis[26,29].

In the present study, we have tried to find explanations for the close association between *P. enervis* and *L. niger*. First, we tested whether different ant species differ in their aggressiveness when encountering *P. enervis* females. Such ant-specific aggression was shown for the closely related Japanese species *Paralipsis eikoae* Takada & Shiga, which is tolerated by *L. niger* but attacked by *Pheidole fervida* Fr. Smith[31]. We used four ant species which commonly attend root aphids[30], i.e. *L. niger*, *L. flavus* Deg., *Myrmica laevinodis* Nyl. and *Tetramorium caespitum* L. We hypothesized that *P. enervis* females prevent aggressive behaviour of *L. niger* by mimicking *L. niger*-specific tactile behavioural patterns during antennal communication, and that this kind of communication might fail with other ant species. We also assumed that *P. enervis* additionally mimics the epicuticular hydrocarbon pattern of either *L. niger* or of its aphid host, to prevent recognition as a non-nestmate

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before and during trophallaxis. Second, we examined the effect of trophallaxis on the longevity of *P. enervis* and tested whether this parasitoid species depends obligatorily on the food which it receives from the ant.

Materials and methods

Insect source. *P. enervis*-parasitised mummies were collected in a garden in the vicinity of Bayreuth/Germany in August 1993 from *Anoecia* cf. *corni* Koch colonies. The aphids were feeding on the roots of sown wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.). The numerous *A.* cf. *corni* colonies at the collection site (which covered approx. 150 m^2) were heavily tended by the four ant species *L. niger*, *L. flavus*, *M. laevinodis* and *T. caespitum*, but *P. enervis*-parasitised mummies were found only in association with *L. niger*.

Mummies were kept together with the wheat roots in small cages at 15 °C and checked daily for emerged adults at two-hour intervals. During the night, they were stored at 5 °C to prevent adult parasitoid eclosion.

Ant-parasitoid interactions. We studied the interactions between *P. enervis* and ants in a set of eight experiments (table 1). To assess any possible influence of the wasps' previous contacts with ants[22,31], we used three kinds of parasitoids which differed in their pre-experimental experience. Unconditioned wasps had no prior contact with ants or aphids and were used within two hours after emergence. Conditioned wasps had prior contact to aphids and ants, the latter belonging either to the same species as the one used in the trials or to a different species. These wasps were between 5 and 36 h old, and had no ant contact for 3 h before being used in the experiments.

We released single females of *P. enervis* into a small Petri dish (diam. 8 cm, ht 2 cm) containing wheat roots, 10 *A.* cf. *corni* and 5 ants, and observed all ant-parasitoid interactions continuously for 20 min (except in experiment 8, which was finished after the parasitoid females were killed). When encountering each other, ants and parasitoids displayed one of the following behavioural patterns: (a) Ignoring – the ant and the parasitoid made physical contact but otherwise did not respond to each other. (b) Antennal tapping – the ant or the parasitoid made physical contact and tapped the partner at least twice with the antennae; the partner could either respond with antennating and with contact of the forelegs, as is typical for ants engaging in trophallaxis[2], or abandon the contact. If both partners engaged in antennal tapping, this behaviour could either result in (c) trophallaxis – ant and parasitoid had mouth-to-mouth contact, and the ant regurgitated liquid food and fed the parasitoid, or (d) no trophallaxis – ant and parasitoid did not succeed in mouth-to-mouth contact. (e) Attacking – the ant responded aggressively and seized the parasitoid with its mandibles; the parasitoid was either killed or could escape by flight.

Adult parasitoid longevity under different conditions. Longevity of *P. enervis* adults was measured in a climate chamber at 20 °C, 75% RH, 2000 Lux and 16:8 L:D. Single newly emerged parasitoid females (age <2 h) were transferred to small gauze-covered cages (diam. 2.5 cm, ht 8 cm) which were generally equipped with the roots of two wheat plants and some soil. Cages were covered with paper boxes to simulate underground light conditions and controlled every four hours during the day. We set up seven different experimental designs (table 2) to analyse parasitoid longevity under different conditions.

Analysis of epicuticular hydrocarbon patterns. The cuticular lipids of newly emerged *P. enervis* females (n = 5 individuals), *Anoecia* cf. *corni* (n = 50), and workers of the ant species *L. niger*, *L. flavus* and *M. laevinodis* (n = 15 for each species) were recovered by extracting the insects twice in hexane for 60 s at room temperature. The hexane extracts were unified and concentrated under a gentle stream of nitrogen. The samples were either immediately analysed or stored at −50 °C.

Gas chromatography was performed for *P. enervis* and *A. corni*. GC was carried out on a Carlo Erba GC 6000

Table 1. Experimental design for the analysis of interactions between females of the aphid parasitoid *Paralipsis enervis* and four species of ants, and the average number of *P. enervis*-ant contacts (mean ± SD; means sharing the same letter do not differ at $p < 0.05$; Tukey's test) in the different experiments.

Expt no.	n	Prior conditioning of *P. enervis* females	Ant species	Mean no. of *P. enervis*-ant contacts/20 min
1	12	Uc	*T. caespitum*	8.3 ± 1.9^a
2	10	c with *L. niger* colony	*T. caespitum*	9.5 ± 4.3^a
3	14	Uc	*M. laevinodis* (v)	9.1 ± 2.8^a
4	15	c with *L. niger* colony	*M. laevinodis* (v)	9.1 ± 3.1^a
5	15	Uc	*L. niger* (v)	15.5 ± 7.6^b
6	17	c with different *L. niger* colony	*L. niger* (v)	14.2 ± 5.8^b
7	14	c with *M. laevinodis* colony	*L. niger* (v)	17.4 ± 9.4^b
8	14	Uc	*L. flavus*	4.1 ± 2.0^c

Uc = unconditioned (wasps had no contact after adult emergence with ants and aphids), c = conditioned (wasps had prior contact with ants and aphids), n = number of tested parasitoid females, v = data were analysed using video film shot with a Minolta camera system equipped with a 270 mm macro lens.

Table 2. Experimental design for the analysis of *P. enervis* longevity under different conditions. n = number of replicates.

Expt no.	n	design
9	11	+plants
10	12	+plants + 10 *A. corni*[a]
11	11	+plants + 10 *A. corni* + 3 *T. caespitum*[b]
12	11	+plants + 10 *A. corni* + 3 *M. laevinodis*[b]
13	14	+plants + 10 *A. corni* + 3 *L. niger* for 6 h
14	11	+plants + 10 *A. corni* + 3 *L. niger* continuously; aphids were not changed during a trial
15	13	+plants + 10 *A. corni* + 3 *L. niger* continuously; 10 new aphids were added every two days, while old aphids were removed

[a]5 adult aphids + 5 larvae in each trial, [b]ants were always workers.

Vega Series 2 equipped with a split-splitless injector (injector temperature: 230 °C) and a flame ionization detector. A fused silica capillary column (30 m × 0.32 mm) with a chemically bonded DB-1 stationary phase of 0.10-µm film thickness was used for the analysis. Helium was the carrier gas with a flow rate of 1.5 ml/min. The oven temperature was kept at 140 °C for 10 min, increased to 250 °C with a rate of 15 °C/min, then further increased with 6 °C/min to the final temperature of 325 °C which was kept constant for 10 min. An *n*-alkane standard from C20 to C36 was injected for a comparison of retention times (RT). Additionally, known substances of equivalent chain lengths from other aphid parasitoid species were used for identification purposes.

GC/MS could be performed for the three ant species. The analyses were carried out on a Finnigan MAT 95 connected with a Varian 3700 chromatograph that was equipped with a fused silica capillary column (DB-1, 30 m × 0.3 mm, FD = 0.25 µm). Helium was used as carrier gas. The oven temperature was programmed from 180 °C to 310 °C at a rate of 3 °C/min. The final temperature was kept constant for 20 min. The mass spectra were obtained at an electron energy of 70 eV. The substances were identified by their characteristic mass spectral fragmentation patterns and retention times. We could not identify cuticular hydrocarbons of parasitoids and host aphids by GC/MS, since the amount of available material was too small. Therefore, we did not carry out similarity analysis of the epicuticular hydrocarbon patterns, as was done in other studies[32,33].

Results

Ant-parasitoid interactions. The average number of ant-parasitoid contacts per 20 min differed significantly between ant species, but showed no relation to the wasps' prior experience with a given ant species (table 1). The nature of interactions also differed significantly between ant species.

Encounters between *P. enervis* and *T. caespitum* (expts 1, 2) resulted generally in a mutual ignoring, and neither ant nor parasitoid showed any response to the other species. This result was independent of the parasitoids' prior conditioning.

M. laevinodis workers also did not attack *P. enervis*, independently of the wasps' prior conditioning (expts 3, 4). Most encounters resulted in a mutual ignoring, but *M. laevinodis* workers tapped the parasitoid with the antennae in 28% of the observed cases. Even if *P. enervis* females responded to this contact by antennating, they never succeeded in soliciting regurgitation of liquid food (fig. 1A). We also observed that the 'antennal strike frequency' differed considerably between *M. laevinodis* (mean ± S.D: 4.09 ± 1.27 strikes/s; n = 22) and *P. enervis* (8.94 ± 1.48 strikes/s; n = 52) ($t = 13.37$, $p < 0.001$).

In encounters between *P. enervis* and *L. niger* (expts 5–7), 70.7% resulted in a mutual antennating of parasitoid and ant, and the partner responded in the same way (fig. 1B). The frequency of this behaviour was independent of the wasps' prior conditioning (likelihood chi-square ratio = 2.618, df = 4, $p = 0.624$). *L. niger* and *P. enervis* stroked their antennae at approximately the same frequency (*L. niger*: 9.13 ± 1.27 strikes/s; *P. enervis*: 8.94 ± 1.48 strikes/s; $t = 0.60$, $n_1 = 29$, $n_2 = 52$, $p = 0.553$). In all, 19.8% of all encounters resulted in trophallaxis (fig. 1B), and *P. enervis* females succeeded on average 2.8 ± 0.7 times per 20 min in soliciting regurgitation. In contrast to observations with *P. eikoae*[31], we could not observe that *P. enervis* females which had had previous contact with other ant colonies tried to rub their legs on the ant's body. Furthermore, there was no mutilation of the parasitoid's wings by *L. niger*, as was assumed by Maneval[26] and Stary[28]. However, we did frequently observe that the tiny wings were rubbed off when parasitoids were kept together with plants and hosts in wet soil.

L. flavus workers (expt 8) ignored *P. enervis* workers as long as the parasitoid did not try to come into mutual antennal contact. When contacted by *P. enervis*, *L. flavus* first responded in a friendly way and also engaged in antennating. The antennal strike frequency between *P. enervis* and *L. flavus* did not differ (*P. enervis*, 9.71 ± 1.77 strikes/s; *L. flavus*, 10.50 ± 1.87, strikes/s; $t = 1.14$, $n_1 = 14$; $n_2 = 14$, $p = 0.264$). However, this mutualistic behaviour changed drastically after 11.9 ± 1.4 s (range: 5–25 s; n = 14). *L. flavus* workers obviously discovered that they were communicating with a 'non-nestmate' individual and began to attack the parasitoid heavily. After a few seconds, all the neighbouring workers joined in and participated in these attacks. Thirty percent of the parasitoids were killed by *L. flavus* during the first attack. The other individuals were heavily injured, e.g. on legs and antennae, before they could escape. Nevertheless, these individuals did not leave the

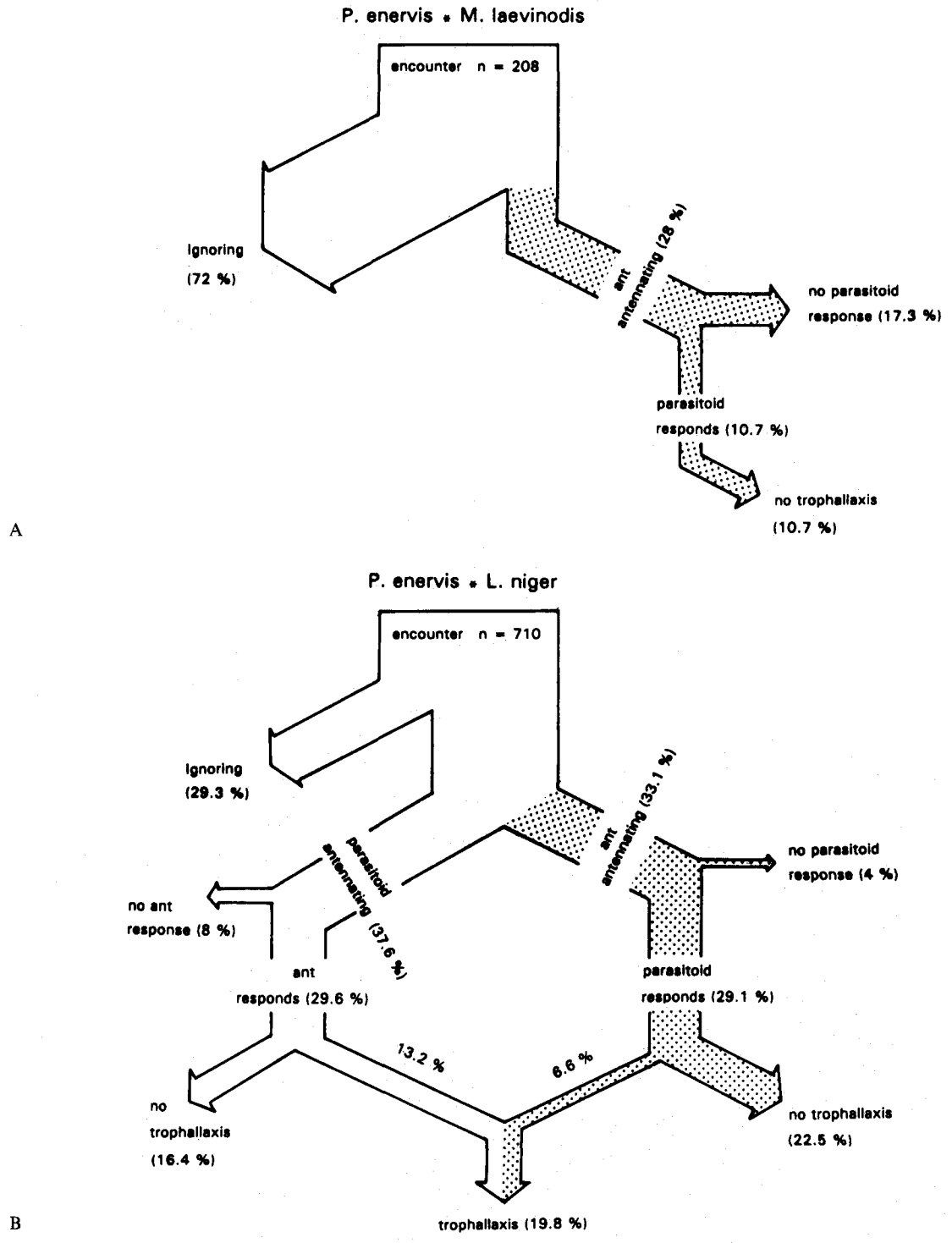

Figure 1. Behavioural interactions between the parasitoid *P. enervis* and (*A*) *M. laevinodis* and (*B*) *L. niger*. All observations from expts 3 and 4 (interactions between *P. enervis* and *M. laevinodis*) and 5–7 (interactions between *P. enervis* and *L. niger*) were combined. For details, see 'Material and methods'.

vicinity of the *L. flavus* workers, and remained unmolested until they repeated to beg for food. This next trial also ended with ant aggression. Interestingly, the second attack – which was always mortal – happened significantly quicker than the first one (6.6 ± 1.0 s, $n = 10$; Wilcoxon test for paired samples: $p = 0.006$). On average, *P. enervis* survived for 10.3 ± 1.9 min in the presence of *L. flavus*.

Adult parasitoid longevity under different conditions. Newly emerged females which were kept only with root material (expt 9) or together with hosts (expt 10) hardly survived for longer than one day (fig. 2). A similar

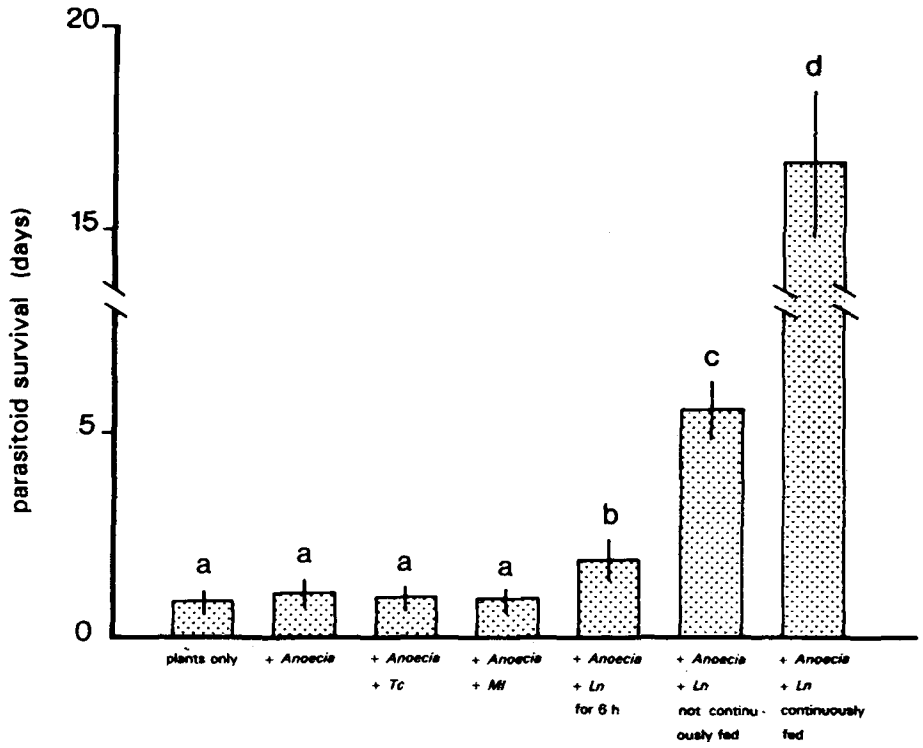

Figure 2. Average longevity (mean ± 95% C.I.) of *P. enervis* females under different conditions (expts 9–15). Means sharing the same letter do not differ at $p < 0.05$ (Tukey's test). Tc = *T. caespitum*, Ml = *M. laevinodis*, Ln = *L. niger*. For details, see table 1 in 'Material and methods'.

longevity was found for individuals which were kept together with hosts and *T. caespitum* (expt 11) or *M. laevinodis* (expt 12), respectively. If *L. niger* was present, newly emerged *P. enervis* began to beg for food within one hour after emergence. If these parasitoids were removed from their ant colony after 6 h and kept only with hosts (expt 13), they survived significantly longer than in expts 9–12. Survival time was longest if parasitoids were continuously kept together with *L. niger*. However, there was a significant difference in parasitoid longevity according to whether aphids were not changed during the experiment (expt 14) or if new aphids were added every two days (expt 15) (fig. 2). In expt 9-f, the aphid honeydew production stopped after 2–3 days. Thus, the honeydew source for *L. niger* workers dried up, and the ants suffered from a lack of honeydew after this time interval. If new honeydew sources for *L. niger* workers were added continuously, the ants were also able to provide food regularly for the parasitoids.

Epicuticular hydrocarbon patterns. The gas chromatogram of the hexane extract of the parasitoid *P. enervis* showed a total of about 24 peaks in the mass range between C20 and C30 (fig. 3A). A comparison of the RT of parasitoid compounds identified substances from the extracts of the ants and other parasitoid species, and an *n*-alkane standard revealed that *P. enervis* possessed *n*-alkanes from C21 to C27 (peak no. 1, 2, 4, 6, 9, 13, 16) and C29 (21). Moreover, methyl-branched hydrocarbons, both terminally (peak no. 3, 5, 7) and internally (10, 11, 14, 17) substituted monomethyl alkanes, as well as dimethyl alkanes (12, 18), might also be present on the parasitoid's cuticle. The lipid profile of the aphid species *A. corni* corresponded to some extent to the profile of *P. enervis*. A total of 12 components (see numbers in fig. 3A) were identical in both species when RT were compared (fig. 3A and B). All substances in the lower molecular weight range (< C26) and especially the dominant peaks (4, 9) were also present in higher amounts on the parasitoid. The prevalent component of *P. enervis*, identified by comparison of RT as 2-methyl tetracosane (7), could also be detected on the host aphid but only in minor amounts. In the higher molecular weight range (> C26) three trace compounds (15, 20, 21) could be found in both species. The host aphid possessed four species-specific substances (a, b, c, d) that were present in rather large amounts. Presumably, these substances might belong to the wax ester fraction.

The comparison of the chromatograms of *P. enervis* and the ant species (fig. 3C–E) revealed that *M. laevinodis* had a total of 12 components in common with the parasitoid (see numbers in fig. 3C). Most of the substances that could be identified by GC/MS in the hexane extract of the ant belonged to the *n*-alkane fraction in the range from C22 to C29 (peak nos 2, 4, 6, 9, 13,

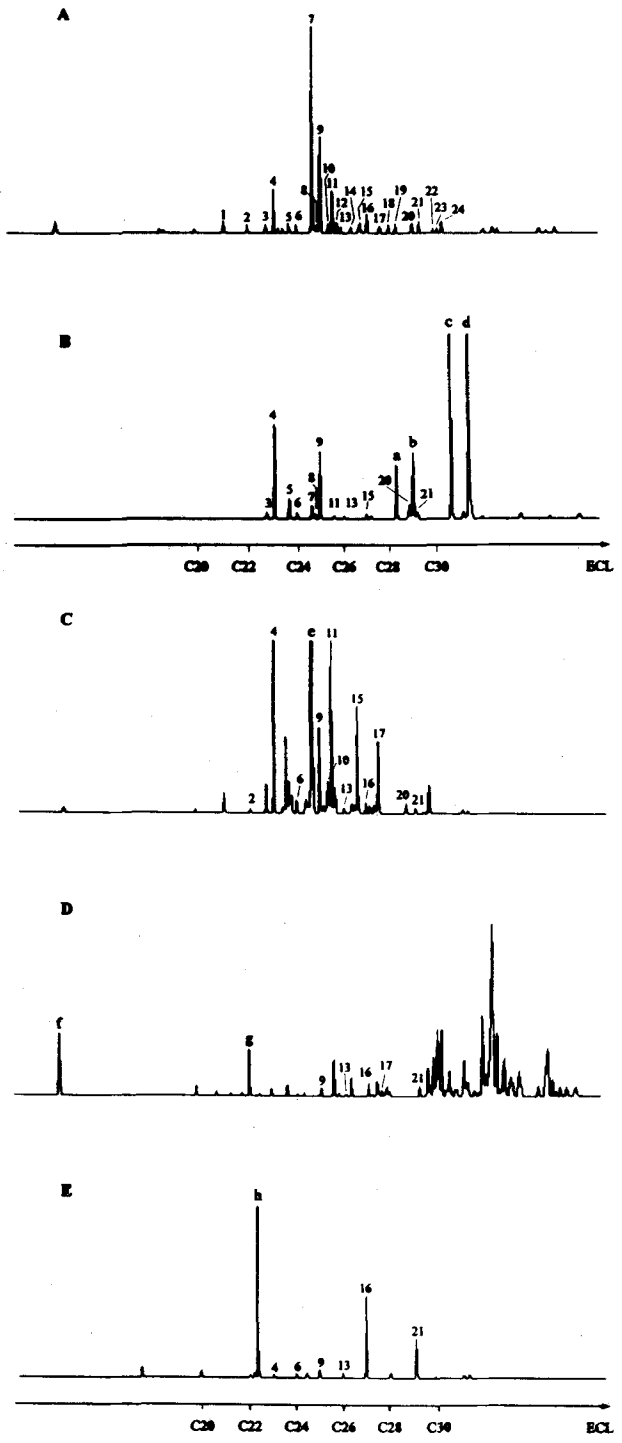

Figure 3. Gas chromatograms of hexane extracts from (*A*) *P. enervis*, (*B*) *A.* cf. *corni*, (*C*) *M. laevinodis*, (*D*) *L. niger* and (*E*) *L. flavus*. The numbers in the chromatograms mark peaks that have retention times identical to those of *P. enervis* extracts. Peaks marked with a letter denote prevalent species-specific compounds. ECL = equivalent chain length.

16, 21). The remaining substances were internally branched monomethyl alkanes, with the branching position at C11 (10) and C5 (11, 17). The compounds 15 and 20 that were present on both parasitoid and ant cuticle could be identified in the *M. laevinodis* extract as heptacosene and nonacosene. The dominant substance on the cuticle of *M. laevinodis* was also an unsaturated hydrocarbon, namely pentacosene (e). In contrast, the cuticular lipid profiles of *L. niger* and *L. flavus* corresponded only in a few components with that of the parasitoid *P. enervis* (fig. 3D and E). Comparison to the RT of the *n*-alkane standard and to the RT of the identified substances of the *Lasius* species showed that most of the peaks corresponding between *L. niger* and the parasitoid were *n*-alkanes (9, 13, 16, 21), only peak 17 belonged to the monomethyl alkanes (5-meC27). In the case of *L. flavus* all corresponding compounds were *n*-alkanes from C23 to C27 (4, 6, 9, 13, 16) and C29 (21). In general, *L. flavus* was characterized by a rather simple cuticular lipid profile (fig. 3E). The prevalent compound h could not be identified so far, but its mass spectral fragmentation pattern indicated an alcohol or an aldehyde structure. *L. niger* possessed species-specific compounds in the lower (f: heptadecane; g: octadecyl acetate) molecular weight range and particularly in the range beyond C29, mainly acetates and wax esters (fig. 3D).

Discussion

Insects that live in close association with ants need mechanisms to prevent recognition as non-nestmates and the ensuing ant aggression. One common strategy to achieve this aim is chemical mimicry of the host ants' cuticular hydrocarbon pattern[7]. The cuticular hydrocarbons have been shown to serve for species and nestmate recognition in a number of ant species[33–39]. A number of predators and parasitoids of ants are able to biosynthesize a cuticular hydrocarbon profile nearly identical to that of their prey or host, respectively[7,40,41], while others acquire species-specific odor cues from their host ant[5]. Aphidiid parasitoids which parasitise ant-attended aphid species also need to overcome ant aggression. These species may either mimic the host aphid's cuticular hydrocarbon profile (e.g. *L. cardui*[19,22]) or may actively acquire odor cues of the aphid attending ants, as shown for the root aphid parasitoid *P. eikoae*[31]. In the first group, the parasitoid may coexist with a number of ant species, while the latter strategy restricts the parasitoid to a single ant species.

P. enervis was also treated non-aggressively by different ant species. This pattern was independent of the parasitoid's prior conditioning and was observed even when the ants had intensive antennal contact. The results suggest that this parasitoid species may use odor cues to avoid aggression. The epicuticular lipids of *P. enervis* seem to consist of *n*-alkanes and methyl-branched alkanes which are common components of the insect cuticle[42,43] (fig. 3A). Qualitatively, 50% of the alkanes of the parasitoid cuticle showed an identical RT with those of

its host, *A. corni* (fig. 3B). *P. enervis* also shared a number of peaks with *M. laevinodis* while there were fewer common substances with *L. niger* and *L. flavus* (fig. 3A, C–E). A chemical mimicry of any ant's cuticular hydrocarbon profile by the parasitoid is less likely than a mimicry of the aphid's profile, since *P. enervis* was generally treated non-aggressively by all ant species during simple encounters. For example, if *P. enervis* mimicked the epicuticular lipid pattern of *M. laevinodis* (the ant species which showed most common peaks with the parasitoid), we might expect aggressive responses of *L. niger* workers and vice versa. We suspect that the similarity between the parasitoid's and the aphid's cuticular hydrocarbon patterns may be sufficient to 'fool' the ants and to prevent aggressive ant responses during encounters.

In general, for a chemical mimicry system to work, the mimic only has to copy those cuticular components present in the model that are involved in chemical recognition processes. The mimicking species can also have cuticular components that have no signal function, so the cuticular hydrocarbon pattern of the mimicking organism may differ to some extent from that of its model. We have no evidence that *P. enervis* actively acquires substances from the ants for a camouflage, as shown for *P. eikoae*[31], which gained access to *host* colonies attended by *L. niger* rubbing its legs on the body of an *L. niger* worker. We assume that *P. enervis* may biosynthesize the hydrocarbons which are common with those of its host aphid during immature development, similarly to the aphid parasitoid *L. cardui*[7].

Besides its obvious ability to avoid 'chemical detection' by various ant species, *P. enervis* also mimics tactile communication signals of *L. niger*. This tactile mimicry may serve as an ancillary mechanism to integrate the parasitoid into the host colony[2], as is known, for example, for the myrmecophilous crickets *Myrmecophila acervorum*[8] and *Myrmecophila manni*[44]. Both species live in ants' nests and avoid ant aggression by mimicking tactile signals of their hosts[8]. Other myrmecophiles, like the rove beetle, *Atemeles pubicollis* or the nitidulid beetle, *Amphotis marginata*, use tactile cues to solicit regurgitation[45,46]. For *P. enervis*, the tactile mimicry is also mainly a tool to solicit regurgitation of liquid food from *L. niger*. Both species engaged regularly in trophallaxis after antennal contacts (fig. 1B). By contrast, no such behaviour could be observed after antennal contacts between *P. enervis* and *M. laevinodis*. This difference obviously resulted from the different 'antennal strike frequency' during communication; the rate is much higher in *L. niger* and *P. enervis* than in *M. laevinodis*. Thus, *P. enervis* was not able to communicate with *M. laevinodis*. We suspect that the behaviour of *P. enervis* is genetically fixed, since newly emerged females are already capable of initiating trophallaxis with *L. niger*. On the other hand, the parasitoid seems not to be able to learn to modify its behaviour in order to receive food from other ant species. Such learning was demonstrated for the cricket *M. acervorum*, which adjusts its locomotory behaviour to the movement pattern of the respective host ant species[8]. *P. enervis* responded as first partner with antennating only after contacts with *Lasius* species, but not after contacts with *M. laevinodis* or *T. caespitum* (fig. 1A and B). The 'communication' with *M. laevinodis* may be a reflex behaviour of the parasitoid, which did not occur with *T. caespitum*, since this ant did not respond to encounters with *P. enervis*. Thus, we suspect that *P. enervis* recognizes differences between ant species (e.g. differences in epicuticular lipid patterns). However, we currently have no information about which cues might be involved in such discrimination.

The initial communication obviously succeeded with *L. flavus*, but ended up with aggression by this ant species. A similar interaction pattern was observed for the beetle *A. marginata* and the ant *Lasius fuliginosus*[45]. This beetle waits on the tracks of *L. fuliginosus* and tries to solicit regurgitation from returning ants. It is also heavily attacked after the ant has recognized its 'error', but in contrast to *P. enervis* it survives because of its heavy sclerotization. For *P. enervis*, we have currently no sufficient explanation for the aggressiveness of *L. flavus* workers after they have engaged in trophallaxis. We suspect that a failure of *P. enervis* in mimicking the exact chemical and/or tactile communication signals may be involved in triggering the aggressive ant response.

The consequence of the different outcomes of ant-*P. enervis* interactions is the restriction of *P. enervis* to *L. niger*-attended aphid colonies. A parasitisation of *L. flavus*-attended hosts is prevented by ant aggression, while the lack of *P. enervis* in *M. laevinodis*- or *T. caespitum*-attended resources is the result of the lack of trophallaxis and drastically reduced parasitoid survival. The liquid food provided during trophallaxis is essential for adult self-maintenance (fig. 2). To ensure this feeding by *L. niger*, *P. enervis* may have evolved both behavioural (tactile mimicry) and morphological adaptations (the elongated glossa[47] may facilitate the delivery of liquid food). However, these adaptations seem to be extremely specific: *P. enervis* females were neither able to survive for more than one day when only aphid honeydew (the common adult food of aphid parasitoids[29]) was available as food (expt 10), nor were they able to solicit regurgitation from other ant species like *M. laevinodis*. Parasitoids survived on average only for one day in the absence of *L. niger*. In the field, *P. enervis* may have difficulty in finding new colonies of its very patchily distributed hosts[30,48] within this time. By contrast, newly emerged females which were kept for only 6 h with *L. niger* survived for almost three days. Thus, dispersing females may actively search for *L. niger*-attended colonies to ensure survival of their

progeny. The evolutionary background of this close association may be found in the life history of *P. enervis*. In contrast to other aphidiid wasps, which hibernate as prepupae within the aphid mummy, this species seems to overwinter in the adult stage in ants' nests[29]. During hibernation, parasitoid adults depend on the liquid food provided by their 'host' ants. However, this requires the demonstrated specific adaptations to the host ant's communication system as well as specific morphological adaptations and may therefore be responsible for parasitoid specialization.

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