sympatrically in parts of Europe [13]. Although the sex pheromone of the former was estimated to be 100% (+)-dispalure, the gypsy moth possesses cells specialized to (+)- and (–)-dispalure (a pheromone inhibitor), whereas in the nun moth, which was estimated to utilize 90% (–)-, and 10% (+)-dispalure, both cells responded to (+)-dispalure and no (–)-dispalure-sensitive cell was found.

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Cuticular Hydrocarbon Profiles in the Slave-Making Ant *Harpagoxenus sublaevis* and Its Hosts

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The integrity of insect societies (e.g., the colonies of honeybees, ants, or termites) depends on the ability of individuals to recognize nestmates and to deny alien conspecifics access to the colony. Discrimination between nestmates and non-nestmates is thought to be based on colony odors, pheromonal blends which are shared by all colony members but differ between members of different colonies [1]. Several studies suggest that cuticular substances, especially hydrocarbons, provide the chemical basis for intra- and interspecific recognition. In the ant, *Camponotus vagus*, e.g., foreign individuals were treated as nestmates after cuticular hydrocarbons from nestmates had been applied onto their cuticle [2]. Further evidence for the involvement of cuticular hydrocarbons in recognition phenomena derives from studies on parasites, which, despite the discriminatory abilities of ants or termites are well capable for integrating themselves into their colonies. In the cuticles of such myrmecophilous or termitophilous flies [3], beetles [4, 5], wasps [6], and a workerless parasitic ant [7], the same blends of hydrocarbons were found as in the cuticles of their hosts. Parasites either actively synthesize host-specific chemicals (chemical mimicry [3, 4]), or they acquire them passively, e.g., through allogrooming [5–7] („chemical camouflage" [3]).

Parasitic beetles and flies as well as workerless social parasites are present in host colonies only as a small minority and thus probably cannot affect the colony odor of their hosts by adding their own cuticular substances. In contrast, when ants of different species are experimentally kept together in equal proportions, composite hydrocarbon profiles with substances from both species may result [8]. One might, therefore, expect that when parasites and hosts occur in similar numbers (as in the case of slave-making ants) parasites could contribute considerably to the colony odor. It was indeed suggested that slave-makers chemically mark their slaves to prevent them from returning to their own colonies [9].

We here present data on cuticular hydrocarbons of the socially parasitic ant, *Harpagoxenus sublaevis*, and of two of its host species, *Leptothorax acervorum* and *L. muscorum*. After mating, *Harpagoxenus* queens enter *Leptothorax* host colonies and kill or expel all of the adult residents. Newly eclosing *Leptothorax* workers take care of the parasite and its brood, forage, and maintain the nest. Additional host workers are obtained through slave raids, during which *Harpagoxenus* workers pillage pupae and larvae from neighboring *Leptothorax* colonies. Raiding may result in colonies containing both slave species [10].

To study cuticular hydrocarbons queenright ant colonies were collected in the Reichswald forest near Nürnberg, Germany, and – for comparison – from a 150 km distant ant population near Aschaffenburg, Germany. Ants were killed by deep-freezing. Groups of 3 to 12 ants per sample (colony, ant species) were extracted either immediately after collection or after being cultivated for several months in incubators following the cultivating system in [11]. Cuticular constituents for chemical analysis were obtained by soaking the ant samples for up to 30 min in n-hexane. Cuticular rinses were evaporated to dryness to eliminate highly volatile substances, resolved in n-hexane, and then applied to a Pasteur pipet silica gel column (70–230 mesh, Fluka). Hydrocarbons were separated from polar substances by elut-

ing the column with n-hexane. The eluate was concentrated under a nitrogen flow and an aliquot was analyzed. Separated hydrocarbons were quantitatively assayed by gas chromatography as previously described [12]. To quantify the total amount of hydrocarbons per ant, tetracosane and triacontane were added as inner standards during soaking. To calculate the linear retention indices for each peak we used an n-alkane series from eicosane to hexatriacontane. Similarity tests for hydrocarbon profiles were performed by NTSYS-pc [13]. To quantify distances between samples we calculated NEI distances based on the relative abundance of the constituents of the hydrocarbon profiles. The patterns within the NEI-distance matrix have been summarized by principal coordinate analysis. Hydrocarbons were identified by comparison of retention indices and mass spectra with published data [14]. GC/MS analyses were acquired on a VG 70–250 SE mass spectrometer (EI^+, 70 eV, resolution 1 000) coupled to an HP 5890 A gas chromatograph (SE 54, 25 m × 0.32 mm i.d., film thickness 0.25 µm, helium 35 cm/s, 100 °C, 2 min, to 200 °C at 20 °C/min, 200 °C to 300 °C at 2 °C/min, on-column injection). The hydrocarbon profiles of the investigated species showed complex patterns. In the presented study we aimed primarily at the assignment of gross structures. In the investigated species hydrocarbons with odd numbers of carbon atoms clearly dominate. Unsaturated compounds as well as branched hydrocarbons may, however, carry additional information since positions and geometry of double bonds or absolute configurations of chiral hydrocarbons may add to the fine structure of the profiles. Elucidation of these details will be presented separately.

Approximately 1 µg of hydrocarbons could be extracted from a single adult *L. acervorum* worker and approximately 0.5 µg from a single *L. muscorum* worker. Ant body weights were 0.74 and 0.46 mg, respectively (individual average weight from groups of 22 ants each).

The hydrocarbon profiles of unparasitized adult workers of *L. acervorum* and *L. muscorum* are species-specific with very little intraspecific variation due to collecting areas or season and the duration of laboratory cultivation (Figs. 1, 2 a, d). The hydrocarbon patterns of *L. acervorum* are composed of 11 % n-

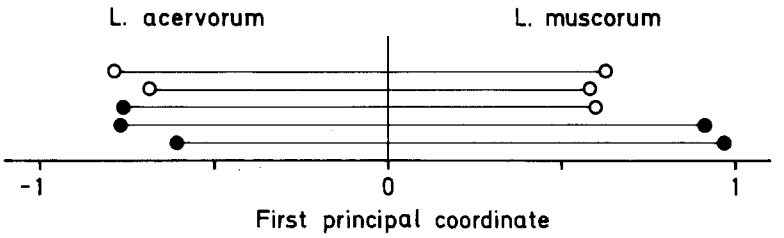

Fig. 1. First principal coordinate of the distance matrix calculated from 20 of the most prominent GC peaks obtained from unenslaved (●) and enslaved (○) *Leptothorax* workers. The first principal coordinate accounts for 99 % of the total variance

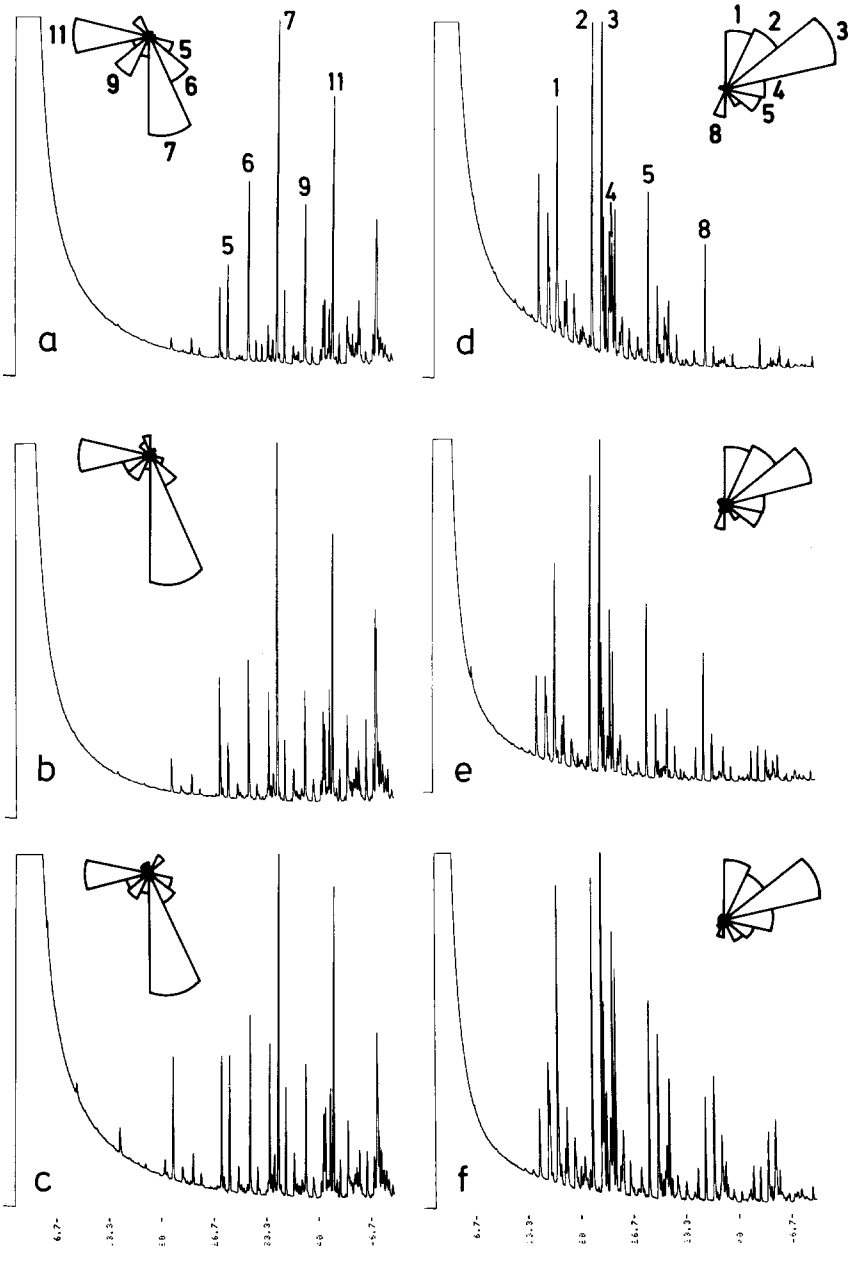

Fig. 2. Gas chromatograph profiles of the total cuticular hydrocarbons of unenslaved (a) and enslaved (b) *Leptothorax acervorum*, of unenslaved (d) and enslaved (e) *L. muscorum*, and of *Harpagoxenus sublaevis* with *L. acervorum* (c) or with *L. muscorum* (f) serving as host species. Peaks are numbered as in Table 1. The star diagrams represent the relative abundance of 14 major cuticular hydrocarbons

alkanes, 59% unsaturated hydrocarbons, and 30% internally branched hydrocarbons. In contrast, the profiles of *L. muscorum* are largely dominated by internally branched saturated hydrocarbons (72%) and by n-alkanes (28%), with traces of unsaturated hydrocarbons (Table 1). The difference in the proportions of alkanes, alkenes, and branched alkanes between the two species strongly suggests distinct biochemical pathways resulting in species-specific hydrocarbon profiles.

In both *Leptothorax* species, cuticular hydrocarbon profiles did not differ qualitatively and quantitatively between unenslaved and enslaved workers (Figs. 1, 2a, b, d, e). Interestingly, the slave-maker ant *H. sublaevis* did not contribute to the colony's cuticular hydrocarbon profile (Fig. 2b, e). Adult slave-maker workers showed the same profiles as their hosts, e.g., that of *L. acervorum* if *L. acervorum* was serving as host (Fig. 2b, c) and that of *L muscorum* if *L. muscorum* was parasitized (Fig. 2e, f).

In colonies, in which ants of both host species were present in approximately equal numbers, all three species showed similar composite profiles with hydrocarbons from both *Leptothorax* species. The influence of *L. acervorum* on the composite patterns was more dominant than that of *L. muscorum*, probably due to their larger amount of hydrocarbons per ant. Young slave-maker workers which had eclosed isolated from their hosts showed very weak hydrocarbon profiles which were fundamentally distinct from both *Leptothorax* species (Fig. 3a, Table 1).

The analysis of the pupal hydrocarbon profiles revealed two surprising results. First, in all three species the pupal hydrocarbon profiles were clearly distinct from that of the respective adults. In contrast to that of adult workers, the profiles of *H. sublaevis* pupae were not influenced by their host species. In pupal hydrocarbon patterns, only trace amounts pointed towards the host's profiles (Fig. 3b, c). Second, and in contrast to the adults' profiles, pupal profiles showed strikingly low variation between species, with a dominance of n-pentacosane, n-heptacosane, and n-nonacosane in all three species (Table 2).

Our study demonstrates some remarkable features of cuticular hydrocarbon patterns which might probably help to understand recognition phenomena in

Table 1. Cuticular hydrocarbons of *Leptothorax acervorum* (L. a.), *L. muscorum* (L. m.), and *Harpagoxenus sublaevis* (H. s.); identification, retention indices (RI), and percent composition. Peaks are numbered as in Fig. 2

No.	Hydrocarbons	RI	L. a.	L. m.	H. s.
	n-C23-an	2300		4.9	4.3
	11-Me-C23-an	2335		4.8	
	7-Me-C23-an	2340		1.9	
1	3-Me-C23-an	2372		9.9	
	n-C24-an	2400		1.3	
	3,11-Dime-C23-an	2407		2.4	
	11-Me-C24-an	2434		2.4	
	C25-en	2470		< 1	< 1
2	n-C25-an	2500	0.5	11.2	16.7
3	11-Me-C25-an, 9-Me-C25-an	2533		19.2	1.3
	7-Me-C25-an	2540		4.2	< 1
	5-Me-C25-an	2549		2.5	< 1
	11,15-Dime-C25-an	2557		6.9	
4	3-Me-C25-an	2572	0.7	6.6	2.1
	5,9-Dime-C25-an	2582		5.6	
	n-C26-an	2600	0.3		
	C27-en	2670	2.8		2.1
	C27-en	2680	0.3		
5	n-C27-an	2700	3.6	5.9	7.8
	11-Me-C27-an, 13-Me-C27-an	2732		4.0	6.0
	7-Me-C27-an	2740		< 1	< 1
	5-Me-C27-an	2749			< 1
	11,15-Dime-C27-an	2757		< 1	
6	3-Me-C27-an	2772	7.3		9.3
	n-C28-an	2800	0.8		
	C29-dien	2843	1.6		
	4-Me-C28-an	2857	1.1		
7	C29-en	2873	14.4		12.0
8	n-C29-an	2900	3.0	4.6	3.5
	11-Me-C29-an	2932	1.1		< 1
	7-Me-C29-an	2940	< 0.3		
	5-Me-C29-an	2950	0.6		
9	3-Me-C29-an	2972	6.4		3.6
	n-C30-an	3000	0.8		
	12-Me-C30-an	3030	< 0.3		
	C31-dien	3039	2.6		
	C31-dien	3044	2.8		
	4-Me-C30-an	3057	< 0.3		
	C31-en	3063			4.2
10	C31-dien	3062	2.5		
11	C31-en	3074	11.2		14.7
12	n-C31-an	3100	1.4	1.1	1.6
	15-Me-C31-an	3130	< 0.3		
	13-Me-C31-an	3130	< 0.3		4.1
	11-Me-C31-an	3130	< 0.3		
	5-Me-C31-an	3156	0.4		
13	3-Me-C31-an	3172	3.2		
	x-Me-C31-an	3172		0.7	
	5,17-Dime-C31-an	3178	< 0.3		
14	n-C32-an	3200	0.7		
	C33-dien	3240	7.1		
	C33-dien	3243	5.2		
	n-C33-an	3300		< 1	
	15-Me-C33-an	3330	< 0.3		
	x-Me-C33-an	3329			3.4
	11,15-Dime-C33-an	3357	3.7		3.4
	13,21-Dime-C33-an	3361	2.2		
	5,17-Dime-C33-an	3378	2.9		
	C35-dien	3441	3.7		

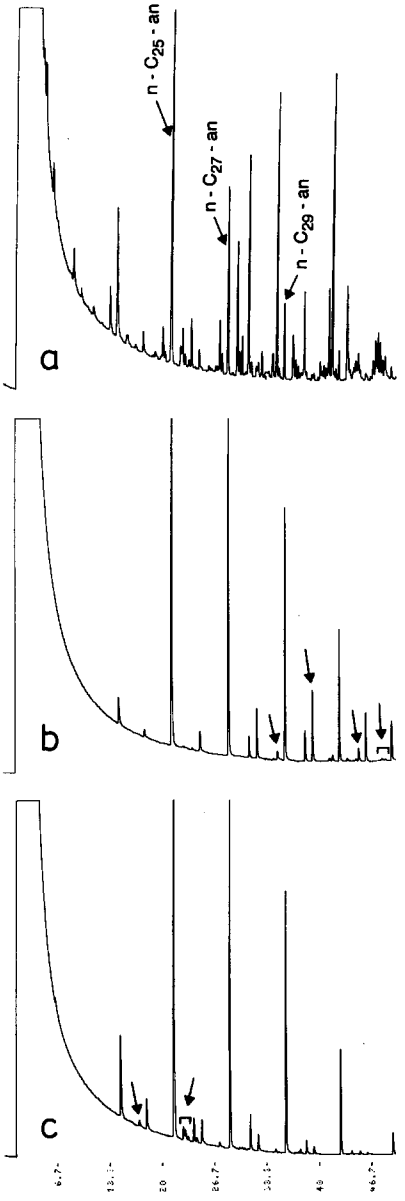

Fig. 3. Gas chromatograph profiles of the total cuticular hydrocarbons of *Harpagoxenus sublaevis* workers (a) which eclosed isolated from their host species and of *H. sublaevis* pupae which have been taken care of by its host species *Leptothorax acervorum* (b) or *L. muscorum* (c). *Arrows* mark trace amounts originating from the respective host species

ants. Hydrocarbon profiles of adult *Leptothorax* workers were significantly different, but those of pupae showed strong similarities. Similar to our findings, cuticular hydrocarbons were almost identical in the larvae of two Japanese carpenter ants, *Camponotus obscuripes* and *C. japonicus*, but differed strongly between adults. Surprisingly, the dominant cuticular hydrocarbons found in

Table 2. Amount (in %) of n-pentacosane, n-heptacosane, and n-nonacosane in the cuticular hydrocarbons of pupae and adults of *Leptothorax acervorum*, *L. muscorum*, and *Harpagoxenus sublaevis* from different origins

	Workers	Pupae
L. acervorum	7.1	58.1
L. muscorum	20.0	69.0
H. sublaevis (from *L. acervorum*)	9.4	72.2
H. sublaevis (from *L. muscorum*)	13.7	81.2
H. sublaevis (eclosed in isolation)	25.5	

Harpagoxenus and *Leptothorax* pupae (Table 2) are identical to the major constituents in *Camponotus* brood [15]. Interspecific similarities between cuticular hydrocarbons in immature stages parallel observations according to which an ant brood can easily by transferred not only between conspecific nests but frequently also between colonies of different species, whereas adult workers are typically rejected when introduced into alien colonies. The strong similarity of profiles among heterospecific ant pupae suggests hypothetic brood substances which may help to explain the transferability of an ant brood [16]. Such substances may facilitate the integration of heterospecific pupae into ant colonies and, thus, may underlie the occurrence of mixed colonies with both host species. Second, these substances may ensure that *H. sublaevis* pupae are taken care of by their slave workers.

When reared to adulthood, heterospecific adopted workers are often attacked or killed by their hosts, probably because they produce genetically determined, species-specific substances which are lacking in their host species and which allow discrimination [16, 17]. According to our results, *Harpagoxenus* workers have the same cuticular hydrocarbon patterns as their hosts, suggesting that the slave-makers do not produce their own specific hydrocarbons. Similar results were recently found in the Japanese slave-maker ant, *Polyergus samurai*, which also shows the same hydrocarbon pattern as its *Formica* hosts [15]. Chemical mimicry or camouflage might help adult slave-makers to be accepted by their hosts and not be recognized as belonging to a different species. In colonies, which contain ants from both *Leptothorax* species in approximately equal numbers, composite hydrocarbon patterns result with components from both slave species, probably because species-specific constituents are transferred through allogrooming. In these colonies, discrimination among heterospecific slaves has not been observed. However, recent results suggest that if one species is clearly more frequent than the other, hydrocarbon profiles may differ and the more common slave species may attack and expel the minority (Heinze, Ortius, Kaib, Hölldobler, unpubl.). If hydrocarbons are indeed correlated with species recognition in these ants, as our data suggest, the lack of specific hydrocarbons in adult *Harpagoxenus* can protect the slave-makers against aggression by their hosts.

In addition to species-specific odors, *Leptothorax* and *Harpagoxenus* have colony-specific cues. When invading a host colony, *Harpagoxenus* workers or queens are frequently attacked by the residents [10, 11], and *L. acervorum* workers are known to discriminate between nestmates and non-nestmates [18], despite similarities in their hydrocarbon profiles. Therefore, we conclude that cuticular hydrocarbons are not the only recognition cues and that other substances which are physicochemically bound to the hydrocarbons, such as cuticular fatty acids and esters, are probably more important for colony-level discrimination (see also [7, 19]).

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Interocular Transfer of Visual Associative Memory in Toads *Bufo bufo spinosus*

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Various studies have shown that anuran amphibians under certain conditions learn to modify their behavior in response to visual stimuli [1]. The question arises whether learning to respond to stimuli present in the visual field of one eye is generalized to the visual field of the other eye. In anurans, this question of "interocular transfer" (IOT) is particularly interesting because – unlike in mammals – the optic nerve fibers cross to the contralateral brain hemisphere, comparable to the situation in birds. Investigations on habituation of prey-catching in toads did not provide evidence of IOT. This kind of nonassociative learning is even specific to that portion of the visual field, in which the same prey stimulus was repetitively offered [1].

The present study investigates IOT in a paradigm of visual associative learning. Common toads, in the course of hand-feeding, associate the experimenter's hand with prey [2]. The prey schema is not expanded just by the conditioned stimulus "hand", rather it is generalized to include other nonprey objects [3], suggesting an associative decrease in configural prey selectivity. During the training of toads in this paradigm, one of their eyes was covered by a black blindfold. When the training criterion was reached, the blindfold was switched to the other eye and the animal's performance using the "naive" eye was tested. The investigation proceeded in the following steps:

(1) Pretraining test. The prey-catching activities of 25 toads were measured in response to three visual objects: a 5×30 mm^2 stripe moving in the horizontal direction of its longer axis ([W]orm configuration), the same stripe whose longer axis was oriented perpendicular to the direction of movement ([A]ntiworm configuration), and a square 30×30 mm^2 in size ([S]quare configuration). A new kind of stimulus presentation was used, in which the computer-generated black stimulus, displayed on a video monitor (Fig. 1), traversed a 120° section of the toad's frontal visual field at $v = 10$ °/s, to and fro. In accordance with previous results, only the W-stimulus was a strong releaser of prey-catching behavior, for both monocular and binocular vision (Fig. 2a).

(2) Training. Of the 25 toads, every day, each individually marked toad – after covering one and the same eye for a period of 30 min – was presented a mealworm with the experimenter's black glove-coated hand. After 48 days of hand-feeding, the training criterion was reached in 17 toads, i.e., they oriented and snapped toward the moving hand (Fig. 3).

(3) Posttraining test. The 17 trained animals, tested according to pretraining, showed a decrease in their prey-selective behavior as expected [3] for the trained eye (Fig. 2b, T) and – unexpected – also

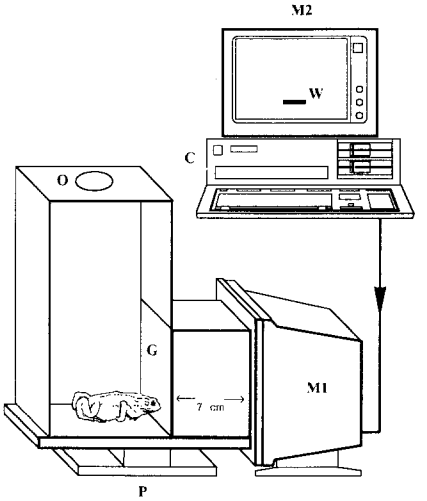

Fig. 1. Experimental procedure; *C* computer, *G* glass window, *M1* monitor, *M2* control monitor, *O* observation window, *P* podest, *W* worm stimulus