Jürgen Heinze · Diethe Ortius · Manfred Kaib
Bert Hölldobler

Interspecific aggression in colonies
of the slave-making ant *Harpagoxenus sublaevis*

Abstract Colonies of the slave-making ant, *Harpagoxenus sublaevis*, may simultaneously contain workers of several *Leptothorax* slave species. We observed aggressive interactions among slave-makers, between slave-makers and slaves, and among slaves in 11 mixed colonies. The first two types of aggression appear to be correlated with reproductive competition for the production of males. Aggressive interactions among slaves, however, occurred mainly between slaves belonging to different species. In two colonies, in which one slave species clearly outnumbered the other, the majority attacked and finally expelled all nestmates belonging to the minority species. Our observations thus suggest that in *Harpagoxenus* colonies a homogeneous "colony odor" is not always achieved and that heterospecific slaves may occasionally be mistaken for alien ants. Gas chromatographic analyses of ants from mixed colonies similarly show that cuticular hydrocarbon profiles may differ strongly between heterospecific nestmate slaves.

Key words Slave-making ants
Interspecific aggression · Reproductive competition
Nest odor · Cuticular hydrocarbons

Introduction

The ability of individuals to discriminate between nestmates and non-nestmates is one of the most important factors assuring the integrity of insect societies. Due to the organization of most colonies of ants, bees, wasps, and termites, nestmates are more or less closely related, some polygynous species excepted (Wilson 1971;

J. Heinze (✉) · D. Ortius · B. Hölldobler
LS Verhaltensphysiologie und Soziobiologie,
Theodor-Boveri-Institut, Am Hubland, D-97074 Würzburg,
Germany

M. Kaib
LS Tierphysiologie, Universität Bayreuth, D-95440 Bayreuth,
Germany

Hölldobler and Wilson 1990). Nestmate recognition therefore enables workers to exclude unrelated individuals from the nest and to direct altruistic behavior preferentially towards relatives, thus increasing their own inclusive fitness (Hamilton 1964).

There is evidence for many groups of social insects that nestmate discrimination is based on chemical stimuli on the cuticle. In some sweat bees, individuals learn the non-transferable endogenous labels of all colony members (Greenberg 1979; Buckle and Greenberg 1981). In ants, nestmates are thought to share a common blend of cuticular recognition cues, which may be either acquired from the environment or from the colony's queen (or queens) or be a mixture of the transferable endogenous labels of all nestmates ("gestalt", Crozier and Dix 1979; Hölldobler and Michener 1980; Stuart 1988a; Carlin 1989). The chemical basis of this "colony odor" is still not completely known, though there is some evidence that cuticular hydrocarbons are involved at least in species discrimination and probably also in nestmate recognition (Morel et al. 1988; Bonavita-Cougourdan et al. 1989; Errard et al. 1989; Nowbahari et al. 1990).

Some of the information on recognition was obtained with experimentally mixed groups of ants from different species (Fielde 1903; Carlin and Hölldobler 1983; Errard and Jaisson 1991; Vienne et al. 1992), which, though helpful, expose ants to highly artificial conditions. Mixed colonies of ants, however, regularly occur also in nature: in the nests of some slave-making ants, three or more species may coexist within a single colony (e.g., Buschinger 1966; Buschinger et al. 1988; Heinze et al. 1992). Whether in these cases nestmates are capable of discriminating between nestmates belonging to their own or another species has not yet been examined in detail.

Colonies of the slave-making ant, *Harpagoxenus sublaevis*, are very suitable for the study of interactions among heterospecific ants in naturally occurring mixed colonies. Young queens of *H. sublaevis* found their own colonies by invading nests of any of three *Leptothorax*

(s. str.) species, where they kill or expel the host queens and all of the adult workers and start to reproduce. Workers emerging from the conquered host pupae nurse the slave-maker's brood. To increase the number of slaves and to replenish slave workers which have died, *Harpagoxenus* workers pillage pupae and last-instar larvae from nearby *Leptothorax* colonies. *H. sublaevis* workers indiscriminately raid colonies of the different slave species, hence slave-maker colonies may simultaneously contain slaves from several species (Buschinger 1966, 1974; Buschinger et al. 1980). In the population of the Nürnberger Reichswald in southern Germany, more than half of all *H. sublaevis* nests contain two or even three slave species (Schumann and Buschinger 1991).

We have recently shown that in colonies containing only a single slave species, adult *H. sublaevis* workers somehow acquire or mimic the cuticular hydrocarbon profiles of their respective slaves, *Leptothorax acervorum* or *L. muscorum*. The slave-makers apparently synthesize only minute amounts of their own cuticular hydrocarbons. Slaves and slave-makers from colonies which contained both slave species had hybrid profiles with hydrocarbons from both *Leptothorax* species (Kaib et al. 1993). To examine how heterospecific slaves interact and whether a homogeneous hydrocarbon profile is achieved in mixed societies of *H. sublaevis*, we studied the behavior of slaves and slave-makers in natural and artificially mixed colonies and analysed cuticular hydrocarbons of all three species by gas chromatography.

Methods

Culture and behavioral observations

Complete, queenright colonies of *Harpagoxenus sublaevis* and of two slave species, *Leptothorax acervorum* and *L. muscorum*, were collected in summer 1991 at two sites in the Nürnberger Reichswald, southern Germany. Of the 26 *H. sublaevis* colonies collected 17 contained only slaves of one species, while 9 colonies had slaves from both species. Of the mixed colonies, six were chosen for observations (Table 1a). In addition, we made up experimental colonies by removing all of the adult slaves from natural *H. sublaevis* colonies and adding known numbers of pupae of similar age from single queenright, unparasitized colonies of both slave species (Table 1b). We attempted to control for colony size and to make replicates for fixed slave ratios in this experiment. However, due to high mortality of both the slave-makers and the added pupae in slave-deprived colonies, the composition of colonies was rather heterogeneous by the time they were actually studied.

Colonies were kept in the laboratory following standard procedures (e.g., Buschinger 1974). Ants were fed three times a week with diluted honey and pieces of cockroaches. In an additional colony, which contained *L. acervorum* slaves from two different colonies, all slaves were individually marked with loops of 0.02-mm steel and copper wires.

Dissections were carried out as described by Buschinger and Alloway (1978). During the experimental period, colonies were kept in artificial summer conditions (10 h night, 20°C/14 h day, 30°C). Behavior was observed in individual sessions of 45–90 min for about 6–20 h per colony using a dissecting microscope. The activities of all nestmates were noted at random intervals (scan

Table 1 Composition of mixed colonies in which aggressive interactions among nestmates were studied (only adult individuals)

Colony	*Harpagoxenus sublaevis*	*Leptothorax acervorum*	*L. muscorum*	Total
a. Natural colonies				
Rw 64	40	20	22	82
Rw 7	18	17	18	53
Rd 15	21	40	80	141
Rd 22	34	18	70	122
Rd 29	14	14	35	63
Rd 14	17	40	7	64
b. Artificial colonies				
Rw 58	7	10	12	29
Rw 59	6	16	8	30
R 70	4	3	18	25
R 169	25	9	15	49
Rw 63	3	13	3	19

sampling, Altmann 1974). Data were recorded using D.S. Gladstein's ethogram program (Carlin et al. 1987). In the analysis of data we focused especially on aggressive interactions.

Based on the assumption that individuals interact randomly with homospecific and heterospecific nestmates, we calculated expected frequencies of interactions according to the proportion of individuals of the different species within the nest. The differences between expected and observed frequencies were tested using the χ^2 test and confidence limits of percentages based on the binomial distribution (Sachs 1984; Sokal and Rohlf 1987).

Gas chromatography

Groups of four to ten homospecific ants from the same nest (both from the field and laboratory) were killed by deep-freezing and rinsed for 30 min in n-hexane. Hexane rinses were concentrated to dryness under a stream of nitrogen to eliminate volatile constituents, and reconstituted with n-hexane. The extracts were then applied to a Pasteur pipette silica gel column (70–230 mesh, Fluka) for purification. Hydrocarbons were isolated by eluting the column with n-hexane. The eluate was concentrated under nitrogen. Aliquots of the eluate were assayed by a Siemens Sichromat 3 gas chromatograph equipped with split-splitless injector, flame ionization detector, and a 25m × 0.32 mm i.d. SE 54 CB (0.11 μm film thickness) fused silica capillary (Macherey and Nagel). The carrier gas was helium at a 1.7 ml/min flow rate. The injection mode was splitless for the first 60 s after injection. To separate the hydrocarbons, the oven was programmed from 180°C to 300°C at 2°C/min and then held isothermal. Detector signals were plotted and quantified on a Shimadzu C-R3A computing integrator. Peaks from different chromatograms were classified by comparison of linear retention indices. Retention indices were calculated by the use of an n-alkane series from eicosane to hexatriacontane separated under identical gas chromatographic conditions.

For statistical analysis of the cuticular hydrocarbons we used 33 gas chromatographic peaks. The relative intensity (%) of each peak was calculated on the basis of the total counts of all 33 peaks. To quantify differences of chemical profiles between samples we calculated a matrix of Nei distances for each sample (Kaib et al. 1991). To summarize these matrices we applied a UPGMA algorithm and plotted the results in a dendrogram. Statistical calculations were performed by NTSYS-pc (Rohlf 1990).

Fig. 1 Observed (*left*) and expected number (*right*) of aggressive acts in three colonies (*Rw* 64, *R* 70, *Rd* 14) of the slave-making ant *Harpagoxenus sublaevis* containing the two slave species *Leptothorax acervorum* and *L. muscorum*. The three different species are represented in the diagrams by schematic drawings of their heads: the large-headed *H. sublaevis* is on the *left*, the hairy *L. acervorum* on the *upper right*, and *L. muscorum* on the *lower right*. The *numbers* next to the heads indicate the total number of intraspecific aggressive acts observed (*left*) and expected from the numerical ratio between the different species in the colonies (*right*). *Numbers* near the *arrows* indicate the total number of aggressive interactions between two species. Expected numbers are calculated on the assumption that all nestmates are equally likely to be attacked by an aggressive individual. *Underlined numbers* indicate interactions which were more frequent than expected ($P<0.05$). In Rw 64, as in most other colonies, the slave-makers direct a significantly larger amount of aggressive interactions towards conspecific individuals than expected from their relative frequency in the colony. In Rd 14 and R 70, the majority slave species – *L. acervorum* in Rd 14 and *L. muscorum* in R 70 – attacked the minority slave species more frequently than expected
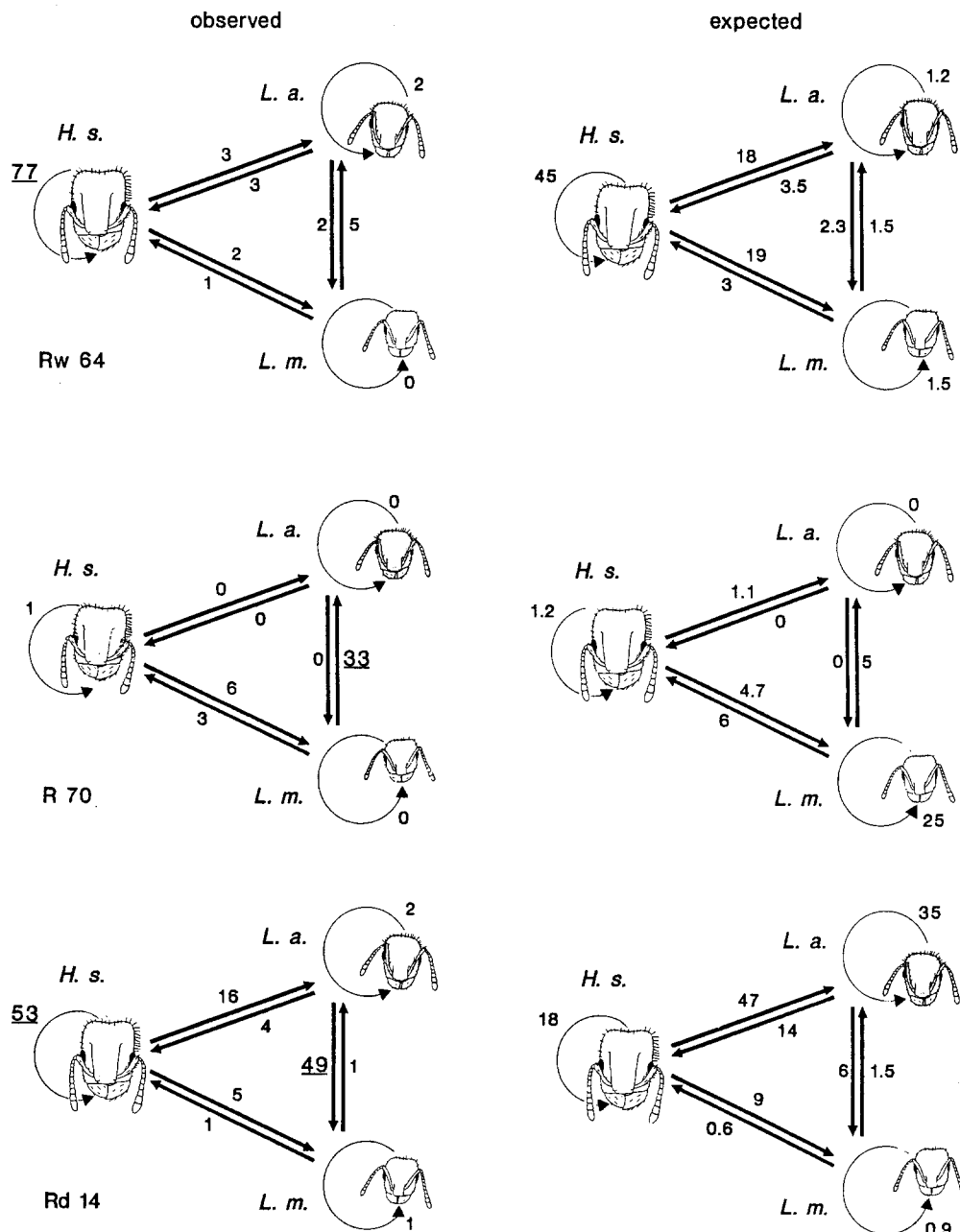
Results

Aggressive interactions among slave-makers

H. sublaevis workers rarely took part in tasks such as colony maintenance, brood-care or foraging. They exhibited, however, a surprisingly high amount of aggressive interaction with other colony members. Especially frequent were "antennal boxing", i.e., one individual heavily antennated the head and thorax of a crouching or receding individual, pulling on another individual's antennae or legs, or grabbing its head and thorax. In some cases, two individuals engaged in mandible fights and violent mutual antennations.

In 8 of 11 observed mixed colonies, *H. sublaevis* workers initiated significantly more aggressive encoun-

ters than expected from their frequency in the colony. Colony Rw 64, for example, consisted of 40 *H. sublaevis*, 20 *L. acervorum* and 22 *L. muscorum*. A total of 95 aggressive acts were observed during 6 h of observation. *H. sublaevis* attacked 82 times, *L. acervorum* 7 times and *L. muscorum* 6 times, which is significantly different from the values expected on the assumption that all individuals were equally aggressive (*H. sublaevis* 46.4, *L. acervorum* 23.1, *L. muscorum* 25.5; χ^2 test, $df = 2$, $\chi^2 = 53.446$; $P<0.001$). Most of the attacks of *H. sublaevis* were directed against other slave-maker workers. Figure 1 gives a representative example: 77 of a total of 82 aggressive acts initiated by *H. sublaevis* in colony Rw 64 were directed against other slave-makers (Fig. 1), which is significantly more than expected on the assumption that attacks are equally likely to be directed

against all nestmates (Fig. 1; χ^2 test, $df = 2$, $\chi^2 = 50.43$; $P < 0.001$).

Since overt aggression and stereotyped dominance, leading to the formation of reproductive hierarchies of *H. sublaevis* from southern Sweden, have already been described and discussed by Bourke (1988) we will not examine this behavior in detail. It is interesting to note, however, that in contrast to Bourke's observations, in one of the colonies from Nürnberger Reichswald, the *Harpagoxenus* queen took part in dominance interactions and violently antennated her workers, which in turn usually moved away from the approaching queen. In the other colonies, no overt aggression between the queen and the workers was observed.

Fig. 2 Highest level of aggression among nestmates in colonies of the slave-making ant *H. sublaevis*: an *H. sublaevis* worker bites the head of an *L. acervorum* slave. By attacking or dominating their slaves, *H. sublaevis* workers probably attempt to minimize the production of slave males in their colonies (drawing by D.O. based on photograph by D.O.)

Aggressive interactions between slave-makers and slaves

As mentioned above, *Harpagoxenus* workers also dominated their slaves (Fig. 1). A typical interaction is shown in Fig. 2. A *Harpagoxenus* slave-maker stands in a stilting posture with the gaster bent forward above a *L. acervorum* slave, grasps the slave's head with the mandibles and beats its antennae over the slave's head and thorax. The slave worker crouches with retracted antennae. With the exception of two colonies (Rd 15, Rw 59), in which *H. sublaevis* attacked the majority slave species more frequently than expected (Rw 59: against *L. acervorum* observed 267, expected 225; against *L. muscorum*, observed 70, expected 112; χ^2 test, $df = 1$, $\chi^2 = 13.27$, $P < 0.001$; Rd 15: against *L. acervorum* observed 1, expected 12; against *L. muscorum* 35, expected 24; $df = 1$, $\chi^2 = 11.36$, $P < 0.001$), the slave-makers did not preferentially direct their aggression towards either of the two *Leptothorax* species. Observations in colonies with *L. acervorum* slaves, which were all individually marked, suggested that slave-makers did not dominate their slaves randomly but were significantly more aggressive towards slaves which had begun to reproduce and whose ovarioles were elongated and contained mature oocytes (Mann-Whitney U-test, 9 slave-workers, excluding L; $z = 2.21$, $P < 0.05$; Table 2). We noted that despite aggression by the slave-makers, slaves deposited eggs in 3 of the 12 experimental colonies. Of five eggs laid during the observation time, two were immediately eaten, one by another slave worker and one by the *Harpagoxenus* queen.

Considerable slave aggression directed against slave-makers occurred only in three colonies, in which *L. muscorum* slaves were more frequent than *L. acervorum* slaves. In colony Rd 15, 84 of a total of 117 attacks initiated by *L. muscorum* were directed against the slave-makers, 23 attacks in Rd 29 (total: 25) and 65 attacks in Rd 22 (total: 78). The observed numbers of attacks against slave-makers were significantly different from the frequencies based on the numeric ratio between the three species in these colonies (χ^2 test, $df = 2$. Rd 15:
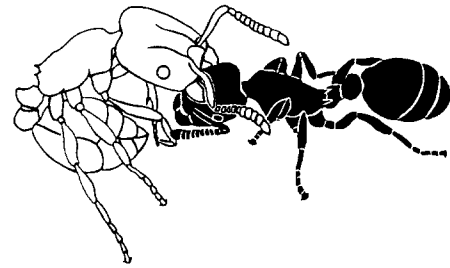
Table 2 Aggression of *H. sublaevis* workers towards *L. acervorum* slaves with different degrees of ovarian development. Slaves with developing oocytes in their ovaries received more attacks than slaves with undeveloped ovaries

L. acervorum worker	A	B	G	J	P	E	F	I	L	R
Number of attacks	9	58	13	17	27	5	6	9	4	7
No. of dev. oocytes	1	2	1	1	2	0	0	0	?	0

$\chi^2 = 318.9$, $P < 0.001$; Rd 29: $\chi^2 = 71.0$, $P < 0.001$; Rd 22: $\chi^2 = 133$, $P < 0.001$).

In other colonies, aggression of slaves towards slave-makers was rare (Fig. 1) but occurred occasionally after a slave had been attacked by a slave-maker. During most attacks, slaves assumed a subordinate posture, i.e., crouched with retracted antennae and legs, or struggled to escape. In some instances, however, they started to pull on the slave-maker's antennae or legs themselves.

Aggressive interactions among slaves

In all colonies, slaves engaged in aggressive interactions with other slaves of both the same species and the other slave species. Artificial and field colonies did not differ in this respect. Interactions consisted of pulling, biting and antennation. In the five colonies in which more than 15 antagonistic interactions among slaves were observed, these occurred almost exclusively among heterospecific pairs (Rd 22:100%; Rd 14:94%; R 70:100%; Rw 58:100%; Rd 15:98%). In these colonies, the frequency of heterospecific fighting was significantly higher than expected from the numeric ratio among the two slave species in these colonies (Tables 3 and 4). In one colony, Rw 7, interactions among slaves belonging to the same species were significantly more frequent than expected. Similarly, the average rate of aggression was significantly higher among heterospecific than homospecific pairs of slaves (heterospecific aggression: 0.037 attacks/worker h; homospecific aggression: 0.012; t-test: $df = 42$, $t = 2.18$, $P < 0.05$; Kolmogorov-Smirnov-test: D $= 0.68$, $P < 0.01$; Table 3). The highest

Table 3 Total number and rate (acts/worker h^{-1}, in parentheses) of aggressive interactions among slaves in *H. sublaevis* colonies. Ac→ac and ac→mu stand for attacks initiated by *L. acervorum* slaves and directed against *L. acervorum* and *L. muscorum* slaves, respectively. Likewise, mu→mu and mu→ac stand for attacks initiated by *L. muscorum* and directed against *L. muscorum* and *L. acervorum*, respectively

Colony (observation time)	Number and rates of attacks					
	Ratio ac:mu	ac→ac	ac→mu	mu→mu	mu→ac	Total
Rw 7 (9.5 h)	17:18	2 (0.012)	0 (0)	9 (0.053)	0 (0)	11 (0.033)
Rw 64 (6 h)	20:22	2 (0.017)	2 (0.017)	0 (0)	5 (0.037)	9 (0.036)
Rw 58 (14 h)	10:12	0 (0)	2 (0.014)	0 (0)	16 (0.095)	18 (0.058)
R 169 (6 h)	9:15	1 (0.018)	0 (0)	4 (0.044)	9 (0.100)	14 (0.097)
Rd 15 (12 h)	40:80	0 (0)	15 (0.031)	1 (0.001)	32 (0.033)	48 (0.033)
Rw 59 (7 h)	16:8	8 (0.071)	3 (0.026)	0 (0)	0 (0)	11 (0.065)
Rd 29 (11.3 h)	14:35	2 (0.013)	0 (0)	0 (0)	2 (0.005)	4 (0.007)
Rd 22 (9 h)	18:70	0 (0)	1 (0.006)	0 (0)	13 (0.021)	14 (0.018)
Rw 63 (7 h)	13:3	2 (0.022)	0 (0)	0 (0)	3 (0.143)	5 (0.045)
Rd 14 (10.5 h)	40:7	2 (0.005)	49 (0.117)	1 (0.014)	1 (0.014)	53 (0.107)
R 70 (12 h)	3:18	0 (0)	0 (0)	0 (0)	33 (0.153)	33 (0.131)

Table 4 Observed percentage, 95% confidence intervals, and expected percentage of the share of heterospecific encounters in all aggressive interations among slaves in colonies of the slave-making ant *H. sublaevis*. The expected percentages were calculated assuming that aggression is randomly directed against other nestmate slaves according to their frequency in the nest. *P* gives the probability that expected and observed % of heterospecific acts are identical

Colony	Total no. of acts	% Heterospecific acts			
		Observed	95% C.I.	Expected	*P*
Rw 7	11	0	0–29	50	>0.05
Rw 64	9	77	46–96	50	>0.05
Rw 58	18	100	85–100	50	<0.05
R 169	14	64	39–85	46	>0.05
Rd 15	48	98	90–100	44	<0.05
Rd 29	4	50	13–90	41	>0.05
Rd 22	15	100	82–100	32	<0.05
Rw 63	5	60	19–92	30	>0.05
Rd 14	5	94	86–98	25	<0.05
R 70	33	100	91–100	24	<0.05

aggression rates were found in two colonies, one natural and one artificial, in which the relative proportions of the two slave species differed most (Rd 14, R 70), and in which the majority species finally expelled the minority (Fig. 1). In these and in other colonies we observed that several or all of the minority slaves were simultaneously attacked by the majority slaves.

There was no evident influence of either colony size or the number of slave-makers present in the colony on the rate of aggression (regression coefficients $b = -0.00035$, SE 0.00032, $df = 9$, t = -1.08, $P = 0.31$ and $b = 0.00071$, SE 0.0011, $df = 9$, $t = -0.655$, $P = 0.53$, respectively).

In most colonies, the expected percentages of heterospecific allogrooming and trophallaxis among all mutual activities between slaves were well within the 95% confidence limits of the observed values. Only in three colonies with extremely uneven ratios between *L. acervorum* and *L. muscorum* (Rd 22, Rd 14, and R 70), the majority groomed the minority more frequently than expected. In Rd 22, for example, *L. muscorum* was observed grooming *L. acervorum* in 57 (expected: 28.4) and *L. muscorum* in 82 (expected 110.6) occasions, respectively (χ^2 test, $df = 1$, $\chi^2 = 13.82$, $P < 0.001$). No fighting among slaves was observed in the colony which contained only *L. acervorum* slaves.
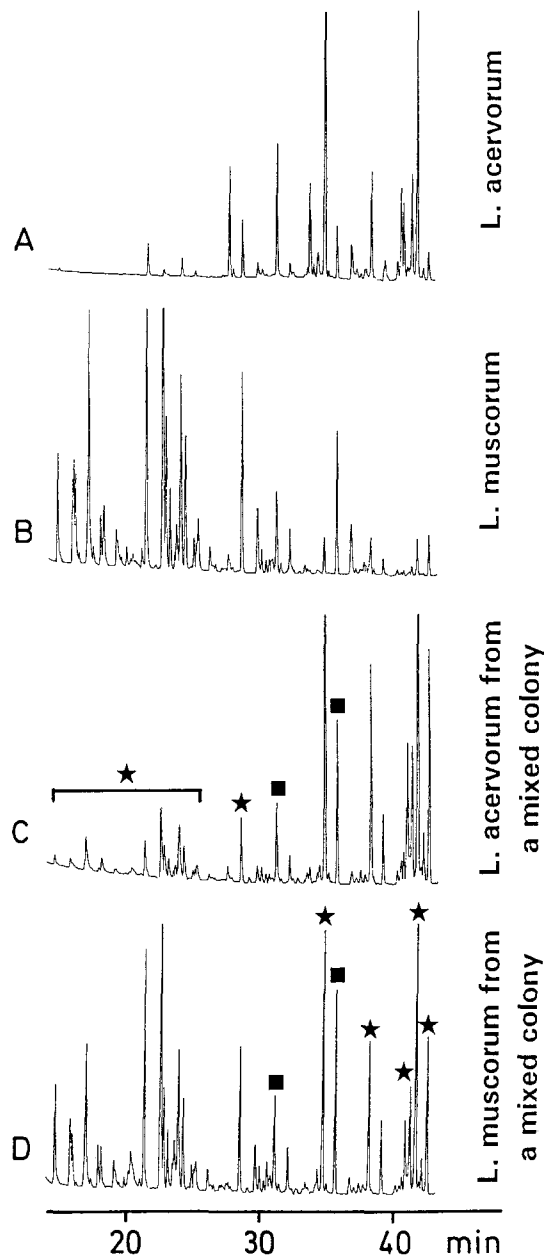
Gas chromatography

Some results on the analyses of cuticular hydrocarbon profiles of *L. acervorum*, *L. muscorum*, and *H. sublaevis* have recently been described and discussed in detail by Kaib et al. (1993). Firstly, adults of both slave species showed species-specific profiles of cuticular hydrocarbons which were not distinct between unenslaved and enslaved *Leptothorax* colonies (Figs 3A, B and Fig. 4). Secondly, slave-makers, which possess insignificant amounts of their own species-specific cuticular hydrocarbons, acquired the profiles of their host species (e.g., 11M and 11H, 14M and 14H, 06A and 06H in Fig. 4).

Workers from colonies with both slave species showed hybrid profiles with substances from both species. The extent of hybridization depended on the ratio between the two slave species. The slave species *L. acervorum*, which produced considerably more hydrocarbons per individual (Kaib et al. 1993), contributed more substances to the hybrid profile than expected from the numbers of *L. acervorum* individuals in the colony.

In colonies with balanced ratios, *L. acervorum* did not change its hydrocarbon profiles (10A in Fig. 4). In contrast, in *L. muscorum* we found profiles which were very similar to those of heterospecific slaves but retained minute amounts of its species-specific hydrocarbons (10M in Fig. 4).

In a colony containing a majority of *L. muscorum* slaves, *L. muscorum* retained their species-specific hydrocarbon profiles (09 M in Fig. 4). Due to the small number of *L. acervorum* in this colony we were not able to obtain hydrocarbon profiles from this species. However, the hydrocarbon profiles of the slave-makers in

Fig. 3 Gas chromatogram of the total cuticular hydrocarbons of **A** *L. acervorum* and **B** *L. muscorum* from two different *H. sublaevis* colonies (profiles of enslaved and unenslaved *Leptothorax* workers do not differ when colonies contain only one slave species, Kaib et al. 1993), of **C** *L. acervorum* which had been experimentally transferred as pupae into a *L. muscorum* colony, and of **D** *L. muscorum* from this colony. *Black asterisks* mark hydrocarbons which are not present in the profiles of unenslaved individuals of this species but are acquired from heterospecific slaves. *Black squares* mark two hydrocarbons which occur in both species, though in differing amounts (for details see text). *A*, *B*, *C* and *D* correspond to 06A/h, 04 M/h, 13A/m(m + a), and 13 M/a(m + a) in Fig. 4, respectively

this colony (09H in Fig. 4) were strongly influenced by hydrocarbons from *L. acervorum*, suggesting that *L. acervorum* had more or less retained its species-specific signature.

In a colony in which *L. acervorum* clearly outnumbered *L. muscorum* both *L. acervorum* and *H. sublaevis*
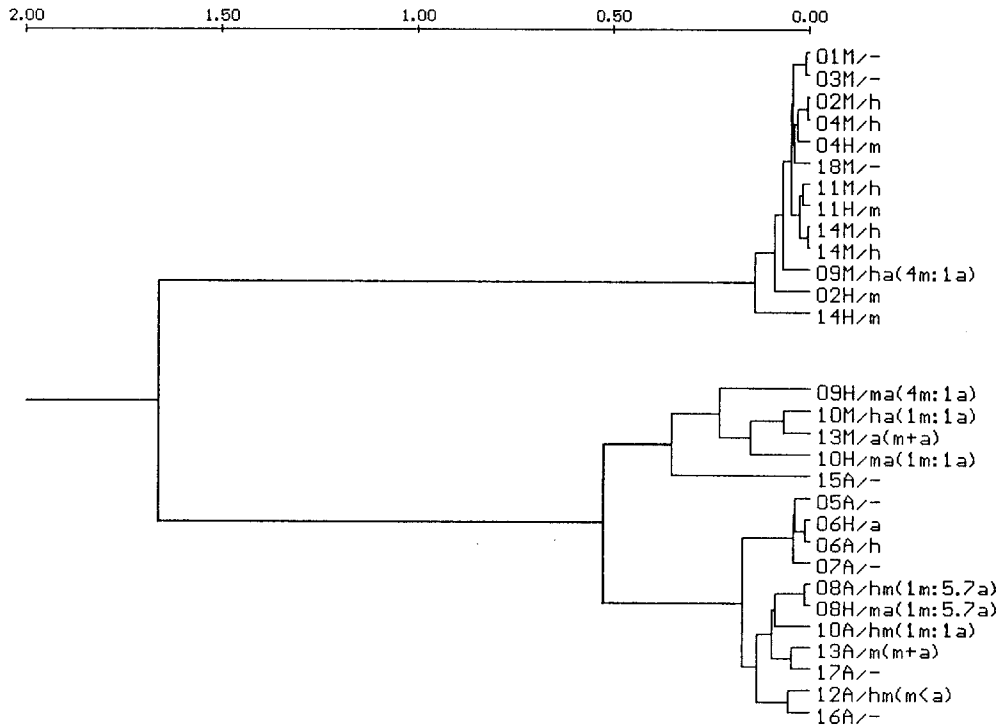
showed congruent profiles (08A and 08H in Fig. 4) more or less similar to unenslaved *L. acervorum*. Again, the minority species could not be analyzed due to low numbers.

To obtain profiles from both majority and minority species in colonies with extreme ratios, ten pupae from an unenslaved *L. acervorum* colony were transferred into a queenright, unenslaved *L. muscorum* colony with approximately 20 workers and 15 larvae. Four *L. acervorum* workers survived in the *L. muscorum* colony for more than four months. Both species had hybrid profiles with homospecific and heterospecific substances, nevertheless the profiles of both species remained distinct (Figs. 3A,C, 13A and 13M in Fig. 4). Significant amounts of substances from both species occurred in *L. muscorum* workers, whereas in the minority species, *L. acervorum*, only traces of *L. muscorum* hydrocarbons were present and its profile was rather similar to the profiles of workers in pure *L. acervorum* colonies.

Discussion

Societies of the palaearctic slave-making ant *Harpago-xenus sublaevis* are characterized by a remarkable amount of aggression among nestmates: agonistic interactions occur among slave-makers, between slave-makers and slaves, and among slaves. *H. sublaevis* workers establish hierarchies, in which the highest-ranking individual engages less in the dangerous tasks outside the nest, receives more food, and lays unfertilized, male-destined eggs in queenright as well as in queenless colonies (Buschinger and Winter 1978; Bourke 1988). Our study suggests that high-ranking *H. sublaevis* workers similarly attack those of their *Leptothorax* slaves with the greatest ovarian development. Aggression of slave-makers against their slaves has previously been reported from *Formica pergandei* and *Polyergus breviceps*, and it was suggested that they thus inhibit the production of slave progeny (Hung 1973). *Harpagoxenus* might similarly attempt to minimize the production of *Leptothorax* males, though the domination of slaves could also be an epiphenomenon of intraspecific dominance among the slave-makers.

Fighting among slaves, however, appears not to be correlated with reproductive competition, but is probably a side-effect of nestmate or species recognition. The coexistence of two or more species within single slave-maker colonies was explained by "imprinting" of ants to the labels of nestmates during the early phase of their adult lifes (Hölldobler 1973, 1977; Jaisson 1975; Le Moli and Mori 1987). Slaves eclosing in the presence of slave-makers will learn the chemical labels of the latter and accept them as nestmates. However, our behavioral data on *Leptothorax* slaves suggest that although all individuals eclosed within the same nest and were exposed to the same chemical environment, intracolonial discrimination may occur among heterospecific nestmates.

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      2.00           1.50            1.00            0.50            0.00
      |───────────────|───────────────|───────────────|───────────────|
                                                                ┌ 01M/-
                                                                └ 03M/-
                                                                ┌ 02M/h
                                                                ├ 04M/h
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                                                                  18M/-
                                                                ┌ 11M/h
                                                                ├ 11H/m
                                                                ├ 14M/h
                                                                └ 14M/h
                                                                  09M/ha(4m:1a)
                                                                  02M/m
                                                                  14H/m

                                                                  09H/ma(4m:1a)
                                                                ┌ 10M/ha(1m:1a)
                                                                ├ 13M/a(m+a)
                                                                └ 10H/ma(1m:1a)
                                                                  15A/-
                                                                ┌ 05A/-
                                                                ├ 06H/a
                                                                ├ 06A/h
                                                                └ 07A/-
                                                                ┌ 08A/hm(1m:5.7a)
                                                                ├ 08H/ma(1m:5.7a)
                                                                ├ 10A/hm(1m:1a)
                                                                ├ 13A/m(m+a)
                                                                ├ 17A/-
                                                                ┌ 12A/hm(m<a)
                                                                └ 16A/-
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Fig. 4 UPGMA cluster diagram of a Nei distance matrix calculated from cuticular hydrocarbon profiles of *H. sublaevis* slave-maker ants and their slaves *L. muscorum* and *L. acervorum*. Specimens from the same colonies bear the same *numbers*. Two homospecific samples of colony 14 were analysed independently. *Capital letters* indicate the species analysed: *M L. muscorum, A L. acervorum, H H. sublaevis. M/-* and *A/-* stand for unenslaved *L. muscorum* and *L. acervorum*, respectively. *M/h* and *A/h* are *L. muscorum* and *L. acervorum* from *H. sublaevis* colonies that contained only a single slave species. *H/m* and *H/a* are the slave-makers from the respective colonies. In colony 13, *L. acervorum* had been experimentally transferred as pupae into an unenslaved *L. muscorum* colony. Only adult workers were analysed [13*A/m(m + a)* and 13 *M/a(m + a)*]. In colonies with two slave species (nos. 8, 9, 10, and 12), the ratio between the two slave species is given in parentheses

Whereas in most colonies slave-makers were rarely attacked by their slaves, in some of the colonies violent fighting among slaves was observed. In most cases, slaves attacked heterospecific nestmate slaves. Aggression apparently was not focused on single individuals, as in the case of attacks initiated by the slave-makers, but was directed against most or all of the heterospecific slaves. It is therefore unlikely that this type of antagonism was related to reproductive hierarchies among slaves. Instead, we suggest that heterospecific colony members were mistaken for non-nestmates, i.e., that a homogeneous "colony odor" was not achieved in some of the mixed colonies.

Our data are probably best explained by assuming that "colony odor" in *Harpagoxenus* nests is a blend of the transferable, endogeneous labels of all colony members. However, rather than producing a uniform "gestalt", individual cues are probably not completely shared among nestmates ("collective hypothesis", Stuart

1988a, b). Though tolerances regarding acceptable mixtures of cues could be fairly broad (Stuart 1988b, 1992), in cases in which a minority bears labels too distinct from the rest of the colony it might well elicit aggression or, as was also observed, intensive grooming. Similarly, Stuart (1992) suggested that aggression towards *Leptothorax* (*Myrafant*) workers that eclosed and aged in alien heterospecific nests upon reintroduction into their parental colonies is correlated with the degree to which introduced workers were contaminated with alien recognition cues.

Gas chromatographic analyses point in a similar direction. Adult workers of *Leptothorax acervorum* and *L. muscorum* have species-specific patterns of cuticular hydrocarbons with only little intraspecific variation. Adult *H. sublaevis* produce only small amounts of their own specific hydrocarbons and apparently acquire or mimic the blend of the slave species present in their colonies (Kaib et al. 1993). Similar results have been documented for the Japanese slave-making ant, *Polyergus samurai* (Yamaoka 1990), whereas its European congener *P. rufescens* seems to produce its own characteristic hydrocarbons (Habersetzer 1993, Habersetzer and Bonavita-Cougourdan 1993). As we show in this study, in *Harpagoxenus* colonies with two slave species, slaves and slave-makers may show hybrid hydrocarbon profiles with substances from both slave species. *Leptothorax* slaves are integrated into slave-maker colonies as pupae, which bear hydrocarbon labels completely different from those of adult workers (Kaib et al. 1993). The occurrence of hybrid profiles in mixed colonies clearly shows that adult slaves produce genetically determined mixtures of hydrocarbons. Hydrocarbons are probably secondarily exchanged between nestmates by allogrooming. In mixed colonies, *L. acervorum* hydrocar-

bons typically dominated the hybrid profiles, even though in some of these colonies *L. acervorum* slaves were clearly less numerous than *L. muscorum*. This may be explained by the observation that individual workers of *L. acervorum* bear twice as much hydrocarbons as individuals of *L. muscorum* (Kaib et al. 1993).

In some mixed colonies, the hydrocarbon patterns differed strongly between heterospecific nestmates. Cuticular hydrocarbons are thus probably not homogeneously transferred throughout the colony but rather as suggested by the "collective hypothesis" (Stuart 1988a, b) for nestmate recognition cues. Though cuticular hydrocarbons are believed to play an important role in species or nestmate recognition (Morel et al. 1988; Bonavita-Cougourdan et al. 1989; Errard et al. 1989; Nowbahari et al. 1990), as yet there is no evidence that they actually are recognition cues at least in leptothoracine ants. In fact, several observations suggest that they are not involved in intercolonial discrimination: the hydrocarbon profiles show little variation between *L. acervorum* from different colonies, but workers nevertheless readily discriminate between nestmates and non-nestmates (Dobrzański 1966; own observations). Furthermore, *H. sublaevis* queens or workers, producing only minute amounts of specific hydrocarbons, are attacked when invading a slave nest for colony founding or during slave raids (Buschinger 1966, 1974). Finally, slave-maker workers differentiate between conspecific nestmates of different rank (Bourke 1988). In agreement with other authors (Obin 1986; Franks et al. 1990; Kaib et al. 1993) our results thus support the idea that cuticular hydrocarbons alone do not provide the chemical basis for all levels of recognition.

As it appears that recognition in ants requires physical contact, recognition cues thus are probably nonvolatile cuticular substances (e.g., Hölldobler and Wilson 1990). It is therefore likely that they are physicochemically linked to cuticular hydrocarbons, i.e., that they are transferred between individuals in a manner similar to hydrocarbons. Heterogeneities in cuticular hydrocarbons might thus be correlated with heterogeneities in recognition cues, which could explain the observed intracolonial discrimination among different slave species. In addition, the predominance of *L. acervorum* labels in the hydrocarbon profiles of the slave-makers, even in those colonies in which only few *L. acervorum* were present, might explain why in these colonies *L. muscorum* repeatedly attacked *H. sublaevis* slave-makers.

Our results complement previous observations in which slaves eliminated heterospecific brood after raids (Schumann and Buschinger 1991). Whereas in our experiments both slave species attacked a heterospecific minority, these authors found that *L. acervorum* killed *L. muscorum* brood but not vice versa. The occurrence of strong aggression between slave species raises the question why the slave-makers do not specialize in raiding slave colonies only of the slave species already present in their nests. In other slave-makers, such as

Polyergus lucidus (Goodloe et al. 1987), or *Chalepoxenus muellerianus* (Buschinger et al. 1988), colonies containing slaves from several slave species are uncommon, though two or more suitable slave species occur in the same area. It might pay, however, to tolerate the eventual loss of a minority of slaves rather than to increase the duration of raiding and scouting activities by specializing on one of two potential slave species (see also Schumann and Buschinger 1991). Furthermore, size differences between *L. acervorum* and *L. muscorum* are large and suggest that the two species occupy different ecological niches. To have slaves from both species might increase the resources available to the *Harpagoxenus* colony. Finally, as was observed in the slave-making *Raptiformica wheeleri* (Wilson 1955), different slave species might specialize on different tasks in the colony. Preliminary results suggest indeed that there may be a division of labor among heterospecific slaves (Ortius 1992).

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