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Incomplete Homogenization of Chemical Recognition Labels Between *Formica sanguinea* and *Formica rufa* Ants (Hymenoptera: Formicidae) Living in a Mixed Colony

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ABSTRACT. *Formica sanguinea* Latreille (Hymenoptera: Formicidae) is a slave-making species, i.e., it raids colonies of host species and pillages pupae, which are taken to develop into adult workers in a parasite colony. However, it has been unclear if the coexistence of *F. sanguinea* with slave workers requires uniformity of cuticular hydrocarbons (CHCs), among which those other than *n*-alkanes are believed to be the principal nestmate recognition cues utilized by ants. In this study, a mixed colony (MC) of *F. sanguinea* and *Formica rufa* L. as a slave species was used to test the hypothesis that CHCs are exchanged between the species. Chemical analysis of hexane extracts from ants' body surfaces provided evidence for interspecific exchange of alkenes and methyl-branched alkanes. This result was confirmed by behavioral tests during which ants exhibited hostility toward conspecific individuals from the MC but not toward ones from homospecific colonies of their own species. However, it seems that species-specific differences in chemical recognition labels were not eliminated completely because ants from the MC were treated differently depending on whether they were con- or allospecific to the individuals whose behavioral reactions were tested. These findings are discussed in the context of mechanisms of colony's odor formation and effective integration of slaves into parasite colony.

Key Words: nestmate recognition, cuticular hydrocarbon, chemical ecology, slave-making

Social insects are able to recognize colony members and discriminate against intruders. This ability constitutes a means of antiparasite and antipredator defense, which is crucial for the survival of the society. The suggestion that ants recognize nestmates by perceiving their characteristic colony odor dates back to the beginning of the 20th century (Fielde 1903, Forel 1923). More recently, behavioral studies coupled with the use of gas chromatography revealed that in social insects, an individual's recognition label is encoded by hydrocarbons and surface lipids (Bonavita-Cougourdan et al. 1987, Lahav et al. 1999, Dani et al. 2001, 2005). The possible combinations of the three parameters: number of carbon atoms (21–48), the position of methyl branches, and the position of double bonds give rise to a great diversity of cuticular hydrocarbons (CHCs). Nearly 1,000 such compounds have already been detected in different species of ants (Martin and Drijfhout 2009). Colonies may vary depending on both the composition of the hydrocarbon mixture and the relative amounts of particular chemical compounds (Martin et al. 2008a). This variety can be partly accounted for by genetic differences (e.g., Beye et al. 1997, van Zweden et al. 2009). Thus, it is reasonable to postulate that low relatedness between colony members, which could be expected in polygynous societies, may result in differences in the heritable component of the chemical recognition label among nestmates. Nevertheless, highly consistent CHC profiles were observed in polygynous and polydomous colonies of *Formica exsecta* Nylander (Martin et al. 2009). According to the gestalt model, nestmates exchange recognition cues, which lead to the uniformity of the colony odor (Crozier and Dix 1979). It was evidenced that hydrocarbons are transferred between ants by trophallaxis, allogrooming, and physical contact (Soroker et al. 1995, 1998, Dahbi et al. 1999, Boulay et al. 2000, Lenoir et al. 2001a).

One of the possible ways in which animals can recognize members of their own group is phenotype matching (Holmes and Sherman 1983). According to this concept, an individual examines some phenotypic traits of another individual and compares them with the learned neural template. Then, the amount of dissimilarity is assessed and if it passes the rejection threshold, the examined individual is recognized as alien and an appropriate behavioral response follows, e.g., avoidance or aggression. It was suggested that in ants, the internal template is formed at an early stage of adult life on the basis of the perceived characteristic odor of the nestmates (Morel 1983, Errard 1984, Errard and Jaisson 1991). This property is exploited by slave-making ant species which pillage pupae from the nests of host species and bring them to their own nests where adults emerge and successfully integrate into the parasite's colony. However, it was evidenced that colony odor can change over time, which is possibly associated with plasticity of the neural template. Temporal variation might result from changes in the colony composition: assuming that individuals differ in the genetic-based pattern of hydrocarbon biosynthesis, adding new individuals or removing old ones should influence the overall composition of the gestalt odor. In fact, dividing ant colonies into parts which are then reared in standardized conditions was shown to result in divergence of CHC profiles between the separated groups (Dahbi and Lenoir 1998, Lahav et al. 2001). Moreover, in *Formica truncorum* F., temporal changes in colony-specific CHC composition were observed in natural conditions (Nielsen et al. 1999).

The temporal variation in the colony CHC profile could also be partly attributed to environmental factors. Most ant species are general predators, which mean they are able to adjust their diet composition to the temporal and spatial variation in prey availability. Changes in diet composition following seasonal changes in prey abundance were observed in the wood ant *Formica rufa* L. (Skinner 1980). In *Linepithema humile* Mayr and *Acromyrmex subterraneus subterraneus* (Forel), diet was shown to influence nestmate recognition via changes in CHCs (Liang and Silverman 2000, Richard et al. 2004). Moreover, in many other ant species, differences in feeding regimes were demonstrated to

be correlated with hostility between individuals (e.g., Wallis 1962, Le Moli et al. 1992). Thus, food-related temporal changes in the colony gestalt odor are likely to occur in natural conditions, as it was postulated in *Formica aquilonia* Yarrow (Sorvari et al. 2008). Consequently, the neural template used in phenotype matching should be plastic enough to follow these changes. Template plasticity and the ability of adult individuals to learn new odors might enable social-parasite ants to achieve colony integrity even when heterospecific individuals retain their characteristic odor. Interspecific differences in CHC profiles were described in mixed colonies (MCs) of *Polyergus rufescens Latreille*, *Polyergus samurai* Yano, and *Lasius* sp. living with their host species (Bonavita-Cougourdan et al. 1997, 2004; Liu et al. 2000, 2003). This seems to be true also in the facultatively parasitic ant species *F. sanguinea* reared with atypical slaves belonging to the species *Formica polyctena* Foerster. Based on the results of previous behavioral studies, it was proposed that these species only partially adjust their chemical recognition labels to each other (Włodarczyk 2012). However, for this hypothesis to be evidenced directly, it is necessary to conduct chemical studies. Here, we present results of behavioral bioassays coupled with gas chromatography-mass spectrometry analysis of CHCs, using similar experimental set-up as in earlier study. Once again we created somewhat artificial system by the use of *F. rufa,* which in natural conditions extremely rarely coexist with *F. sanguinea* within MCs (see Czechowski 1996, p. 50). This species is closely related to previously used *F. polyctena,* therefore we expected similar processes influencing nestmate recognition in the present system. In particular, we intended to answer the question whether *F. sanguinea* and *F. rufa* ants from MC exchange recognition cues and whether this process, if present, leads inevitably to odor homogenization among heterospecific colony members.

Materials and Methods

Laboratory Colonies. Adult ants and pupae were collected at the beginning of August 2009 from two nests (one per species), which were located by the path in a spruce forest in the vicinity of Ogrodniczki near Białystok, N-E Poland (*F. sanguinea*), and in Knyszyn Forest, 13 km N-E from Białystok (*F. rufa*). Five colonies were established in laboratory conditions between 10 and 22 August: four homospecific (two colonies per each species) and one mixed. The MC was formed by 180 adult *F. sanguinea* workers, which were provided with a total of 360 *F. rufa* pupae and newly emerged callow workers. Using the same total number of *F. rufa* ants with similar proportion of pupae to adults released from cocoons, one homospecific colony was established (control colony [CC]). Analogically, 180 adult *F. sanguinea* workers were used to form a second CC. The two other homospecific colonies (reference colonies [RCs]) consisted initially of roughly 300 adult workers and 2,500 pupae for *F. rufa* and 300 adult workers with a few dozen pupae for *F. sanguinea*. However, by the time the behavioral tests started, *F. rufa* from the RC had suffered high mortality, so that only about 500 individuals survived. Ants were kept in plastic boxes (60 by 45 cm, 30 cm in height), the bottom of which was covered with sandy soil (previously washed twice in deionized water) or with nest-mound material (in the case of *F. rufa* RC), and provided with test tubes fitted at one end with a water container. Upper margins of the walls were coated with fluon to prevent ants from escaping. All colonies were fed with diluted honey and dead crickets in similar proportions and amounts adjusted to the colony size. Colonies were exposed to natural photoperiod and from 19 October were additionally illuminated by lamps fitted with 75-W (935 lumens) bulbs for 8 h a day. This helped to keep the ants active and engaged in social interactions after the cease of their seasonal activity period. The heat produced by lamps increased the temperature in the room to 22–23°C.

Behavioral Studies. A single ant was removed from MC or CC and marked with a white dot. Then it was placed on a Petri dish (8.5 cm in diameter), the bottom of which was covered with a piece of filter paper, along with three or four ants from *F. sanguinea* or *F. rufa* RC, respectively. Two minutes after introducing the last ant, the paper barrier separating the RC ants from the marked ant was removed and the test started. Between trials, the Petri dish was cleaned with 50% ethanol, and the filter paper was replaced with a new one. The behavior of the ants was video recorded for 3 min with a Canon SX110 IS digital camera set on resolution of 640 by 480 pixels and 30 frames per second. Video sequences were then examined at 1-s intervals for the presence and number of behaviors toward the marked ant, classified into three types: amicable (allogrooming, trophallaxis), neutral (antennal contact), and aggressive (antennal contact with open mandibles [threat], attempts to bite [chasing, climbing on, and rapid movements toward the marked ant with open mandibles], biting, attempts to bite with flexed gaster, biting with flexed gaster). Numbers of behaviors of each type as determined by the number of ants engaged in it was summed up across all 180 time intervals in each trial. Behavioral tests were performed in three combinations, separately for *F. sanguinea* and *F. rufa* from RCs. In these combinations, the marked ant was 1) conspecific, coming from the CC, 2) conspecific, from the MC, or 3) allospecific, from the MC. Each combination with *F. sanguinea* RC was performed in 12 replications. Those with the use of *F. rufa* RC members were replicated 13 times. Each ant was used only once. Initially, RC workers to participate in the experiment were collected from outside the nest; after such individuals were no longer available, the workers were taken from inside the nest. This should not bias the outcome of the experiments, as encounter combinations were alternated. Behavioral tests were carried out from 16 November to 5 December 2009. On 21st December, ants in MC were counted with regard to species, giving 117 *F. sanguinea* and 63 *F. rufa* workers. Hence, mortality along with removal of ants for the experiments did not change significantly proportion between heterospecific ants in the MC.

Chemical Analysis. Shortly after behavioral tests had been finished, randomly selected ants from each of the laboratory colonies were killed by freezing and stored in separate glass containers until the time of chemical analysis. During sampling, ants from the MC were classified as either of the two species on the basis of coloration of the thorax (bloody in F.s. and brick red in F.p.) and the occupit (black in F.p. and more or less bloody in F.s.). CHCs were extracted by immersing two dead ants in 300 µl of hexane for 10 min and making several gentle shakes of the vials used. The extract was then evaporated under nitrogen flow and redissolved in 60 µl of hexane. The resulting CHC mixture was analyzed on an Agilent 6890 Gas Chromatograph fitted with Mass Selective Detector 5973 and Autosampler 7683, with an electronic pressure control and a split or splitless injector. Separation was performed on the HP-5ms (I.D.: 30 m by 0.25 mm; film thickness: 0.25 µm) fused silica column. Helium flow rate through the column was 1 ml/min. The injector worked in a splitless mode, and its temperature was set at 250°C. The EIMS spectra were obtained for ionization energy of 70 eV, at the source temperature 230°C and quadrupole temperature 150°C. The MSD was set to scan 41–600 a.m.u. Chromatograms were registered in a programmed regime of linear temperature increase from 50 to 320°C at the rate of 10°C/min. We analyzed 48 extracts divided into 6 equal-sized groups (eight samples per group): four representing each of homospecific colonies and two representing different species from the MC. After the chemical analysis had been finished, ants from the MC were examined for possible misidentification with the use of stereo microscope by a person blinded to previous species affiliation.

Unequivocal identification in GC–MS analyses required both mass spectrum and retention index. Therefore, a hexane solution of C20–C38 *n*-alkanes was chromatographed under the conditions described above. Based on retention time values, linear temperature programmed retention indices I^T were calculated for the ant CHC extracts using the equation proposed by van Den Dool and Kratz (1963). The resulting values of retention indices and mass spectra were compared with those from a database developed by one of the authors (L.S.), as well as from an online library (Linstrom and Mallard 2012)

and literature (Trabalon et al. 1996, Johnson et al. 2001, Saïd et al. 2005, Roux et al. 2009). Mass spectra were interpreted by comparing them with the ones included in the libraries of the GC/MS apparatus software (The NIST Mass Spectral Search Program for the NIST/EPA/NIH Mass Spectral Library Version 2.0 a, built 1 July 2002) and given in literature (Attygalle et al. 1993, Bonavita-Cougourdan et al. 1996, Trabalon et al. 1996, Liu et al. 2001, Nelson et al. 2001, Roux et al. 2009).

For the purposes of data analysis, only those hydrocarbons (or mixtures of hydrocarbons, see Results) were selected, which met two criteria: 1) mean proportion (as measured by peak area) in whole hydrocarbon fraction in at least one of the six groups of samples was not <1% and 2) presence in at least 2 groups. One sample of RC *F. rufa* was decided to be excluded from statistical analysis due to the low number of hydrocarbon peaks, which remained after selection (12; mean for the rest of the group 17.4 ± 2.4). All selected compounds are listed in the Table 1.

Statistical Analyses. The numbers of neutral or aggressive behaviors toward conspecifics from an MC and CC were compared using Mann–Whitney test while amicable behaviors appeared to have been displayed too infrequently to be included in statistical analysis. Similarly, behaviors toward conspecific and allospecific individuals from the MC were compared. Aggressive behaviors were scored from 1 to 5 with increasing levels of hostility (Fig. 2). A similar score had already been applied, e.g., by Errard and Hefetz (1997), Lahav et al. (1998), and Astruc et al. (2001). Next, the aggression index for each test was calculated according to the formulas:

$$A = \frac{1}{n}\sum_{i=1}^{5} n_i s_i \qquad n = \sum_{i=1}^{5} n_i$$

where A is aggression index, n_i number of type i aggressive behaviors in a given test, s_i score for type i aggressive behavior (from 1 to 5).

The values of aggression index were compared using Mann–Whitney test in the same pair combinations as previously. Statistical significance were corrected for multiple comparisons using Bonferroni procedure.

For chemical data, the principal component analysis was performed using the function prcomp() in the R package stats (R Development Core Team 2013) based on the relative abundances of hydrocarbons in each mixture. The original set of variables (% of total ion current of compounds) was reduced to a new set of orthogonal principal components. For each group of samples, 95% confidence ellipses were drawn with the function dataEllipse() in package car. Dendrograms were built with the function hclust() (R Development Core Team 2013) with the following parameters: distance = Euclidean, method = ward for the same data set as in PCA. Both analyses were performed separately for *n*-alkanes and other hydrocarbons.

Results

Aggressiveness Toward Ants From the MC. Individuals from the MC elicited more aggressive behaviors of both *F. rufa* and *F. sanguinea* workers in comparison to conspecific ants from a homospecific colony (Fig. 1). Moreover, in *F. rufa* (but not in *F. sanguinea*) aggression was more pronounced toward allospecific individuals from the MC than toward conspecifics. However, *F. sanguinea* clearly discriminated between individuals of its own and foreign species as indicated by the significant difference in the number of neutral behaviors (antennal contact) toward them. When the mean score of aggressive behaviors is taken into consideration, a very similar pattern is obtained, with *F. rufa* displaying more aggressive attitude toward allospecific ants than toward conspecifics from the MC. This difference in *F. sanguinea* is not significant (Fig. 2).

The behavior of ants from RCs could be assumed to be a reaction to the perceived odor dissimilarity and not a response to the attacks by the

Table 1. Mean relative abundances ± standard deviation calculated separately for *n*-alkanes and other hydrocarbons detected in *F. rufa* and *F. sanguinea* ants from RC, CC, and MC

Peak	Compound	*Formica rufa*			*Formica sanguinea*		
		RC	CC	MC	CC	RC	
n-alkanes							
1	*n*-tricosane	1.85 ± 0.28	3.00 ± 1.29	2.59 ± 0.92	0.84 ± 0.37	0.81 ± 0.15	0.72 ± 0.48
3	*n*-pentacosane	44.74 ± 2.35	42.26 ± 5.63	41.85 ± 5.05	30.91 ± 2.22	27.66 ± 1.78	34.77 ± 2.74
7	*n*-hexacosane	1.80 ± 0.28	2.57 ± 2.28	3.60 ± 0.26	4.86 ± 0.72	5.70 ± 0.32	5.92 ± 0.45
9	*n*-heptacosane	32.68 ± 1.67	31.85 ± 2.56	38.16 ± 3.37	54.54 ± 2.72	55.57 ± 1.15	50.85 ± 1.48
14	*n*-nonacosane	12.23 ± 1.55	12.74 ± 2.77	8.10 ± 3.71	7.96 ± 0.87	8.68 ± 1.03	7.73 ± 1.74
18	*n*-hentriacontane	6.70 ± 1.26	7.59 ± 2.15	5.69 ± 1.69	0.88 ± 0.95	1.58 ± 0.59	0.00 ± 0.00
other hydrocarbons							
2	9-(*Z*)-pentacosene	1.05 ± 0.18	2.40 ± 0.90	3.03 ± 0.66	1.82 ± 0.47	1.62 ± 0.36	1.83 ± 0.36
4	9- + 11-methylpentacosane	0.96 ± 0.26	0.85 ± 0.62	3.63 ± 1.36	3.46 ± 0.58	4.02 ± 0.45	9.68 ± 2.66
5	3-methylpentacosane	0.95 ± 0.15	0.74 ± 0.51	1.91 ± 0.20	1.39 ± 0.85	2.52 ± 0.34	2.23 ± 0.53
6	7(?)-(*Z*)-hexacosene	0.00 ± 0.00	0.00 ± 0.00	0.77 ± 0.64	0.50 ± 0.43	1.83 ± 0.19	4.39 ± 1.34
8	9-(*Z*)-heptacosene	0.27 ± 0.23	0.97 ± 0.56	3.13 ± 1.25	3.08 ± 1.36	7.73 ± 0.83	5.72 ± 0.40
10	9- + 11- + 13-methylpentacosane	0.68 ± 0.42	0.39 ± 0.64	4.17 ± 2.53	5.43 ± 2.42	21.22 ± 2.38	21.16 ± 2.93
11	4-methylheptacosane	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.19 ± 0.82	4.74 ± 2.23	3.02 ± 1.93
12	3-methylheptacosane	1.03 ± 0.18	0.81 ± 0.77	1.60 ± 0.73	3.81 ± 0.96	4.46 ± 1.26	1.57 ± 0.93
13	*x*-octacosene	0.09 ± 0.17	0.00 ± 0.00	2.80 ± 1.37	4.13 ± 0.66	8.12 ± 0.95	11.34 ± 2.24
15	9- + 11- + 13-methylnonacosane	1.63 ± 1.05	1.67 ± 1.07	3.36 ± 1.32	3.48 ± 2.97	6.21 ± 0.64	5.74 ± 2.33
16	4-methylnonacosane	0.00 ± 0.00	0.00 ± 0.00	0.69 ± 0.55	1.01 ± 0.82	3.30 ± 0.33	2.87 ± 1.23
17	2-methyltriacontane	0.00 ± 0.00	0.21 ± 0.52	1.67 ± 0.76	1.52 ± 1.29	2.40 ± 0.42	3.96 ± 1.58
19	9- + 11- + 13-methylhentriacontane	4.57 ± 0.65	6.33 ± 1.40	8.70 ± 2.06	8.97 ± 1.89	7.95 ± 0.60	7.75 ± 0.91
20	5- + 7-methylhentriacontane + *x,y*-dimethylhentriacontane	0.13 ± 0.23	0.34 ± 0.82	2.60 ± 1.60	3.05 ± 1.31	5.30 ± 0.98	8.72 ± 3.00
21	9-(*Z*)-tritriacontene	5.05 ± 1.18	11.68 ± 3.22	5.43 ± 2.13	3.15 ± 1.28	0.00 ± 0.00	0.00 ± 0.00
22	9- + 11- + 13- + 15-methyltritriacontane	11.04 ± 1.36	15.52 ± 2.67	12.15 ± 1.54	13.25 ± 2.50	7.61 ± 1.36	6.79 ± 1.43
23	5-methyltritriacontane + *x,y*-dimethyltritriacontane	18.88 ± 1.82	13.94 ± 3.37	13.77 ± 2.47	14.41 ± 2.58	7.00 ± 2.39	2.52 ± 2.09
24	9- + 11- + 13- + 15-methylpentatriacontane	5.97 ± 2.48	11.40 ± 5.27	6.50 ± 2.87	3.91 ± 3.18	2.76 ± 1.20	0.69 ± 1.21
25	(*x, y*)-dimethylpentriacontane (*x*=11,13,15)	36.36 ± 1.30	27.18 ± 5.15	19.61 ± 1.98	18.97 ± 6.49	0.00 ± 0.00	0.00 ± 0.00
26	(*x,y*)-dimethylheptatriacontane + 4-methylheptatriacontane	11.32 ± 1.54	5.55 ± 5.48	4.47 ± 3.56	3.47 ± 4.46	1.21 ± 1.31	0.00 ± 0.00

Only compounds selected for statistical analysis were included (see Materials and Methods).

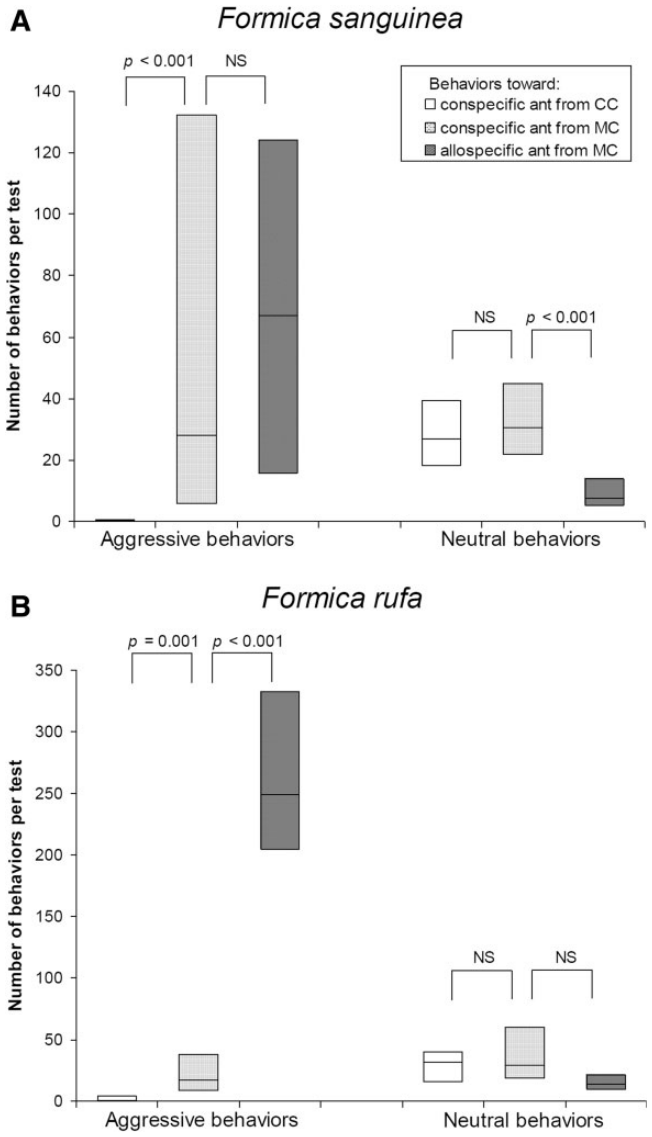

Fig. 1. Number of aggressive and neutral behaviors of *F. sanguinea* (A) and *F. rufa* (B) ants from RCs toward ants of different categories. Bottom margin, inner line, and upper margin of the bars depict 0.25 quartile, median, and 0.75 quartile, respectively. Statistical comparisons were performed by the use of Mann–Whitney test. Significance level after Bonferroni correction for multiple comparisons is $\alpha = 0.00625$.

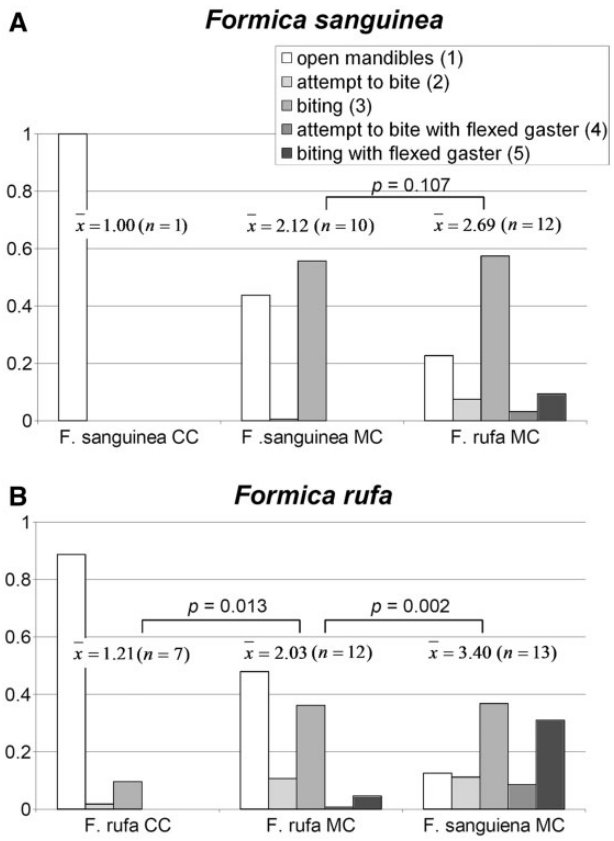

Fig. 2. Mean proportions of different aggressive behaviors displayed by *F. sanguinea* (A) and *F. rufa* (B) toward conspecific individuals from the CC or MC and toward allospecific individuals from the MC. Scores of particular behaviors are given in the legend. Mean aggression score for each encounter combination and the number of tests in which aggressive behaviors occurred are denoted as $\bar{x}$ and n, respectively. Statistical comparisons were performed by the use of Mann–Whitney test. Significance level after Bonferroni correction for multiple comparisons is approximately equal to 0.017.

marked individuals, which exhibited aggressive behaviors only in 4 out of 72 tests. Numerical advantage seemingly gave RC ants behavioral superiority over the single ant from another colony (see Tanner 2008).

Chemical Data. Overall, 26 hydrocarbon peaks were analyzed (Table 1). Some of them were derived from two or more compounds with very similar retention times. The two most abundant compounds in entire hydrocarbon profiles were *n*-pentacosane and *n*-heptacosane. Numerous methyl-branched alkanes were detected, all of which had an odd number of carbon atoms in the main chain. This finding can be explained by the fact that in insects elongation of hydrocarbons takes place by adding two carbon atoms (Lockey 1991). In most of the identified methyl-branched alkanes, methyl groups were located at the 9th, 11th, and 13th carbon atom. We also identified chemicals of others classes: cholesterol, aldehyde (hexacosanal), and esters. Most probably they derived from inside the body and contaminated our samples. Among the analyzed peaks, seven represented species-specific hydrocarbons. Five of them were found in both *F. sanguinea* and *F. rufa* from the MC, which suggests that they had been transferred during heterospecific social interactions (see Vienne et al. 1995). Moreover, hydrocarbons other than *n*-alkanes tend to assume intermediate values in samples from MC when species-specific differences in relative amounts are present (Table 1).

Pattern of samples distribution on PCA plot shows clear divergence between the species when *n*-alkanes are analyzed (Fig. 3A). Analysis based on other hydrocarbons reveals that allospecific nestmates from the MC tend to diverge from their respective kin from the homospecific colonies and shift toward each other along the PC1 axis so that their confidence ellipses overlap (Fig. 3B). A similar picture was provided by cluster analysis. When only *n*-alkanes were taken into consideration, samples diverged at first node according to respective species. Additionally, some samples from MC clustered at close distance with the ones from conspecific pure colonies. In contrast, dataset for other hydrocarbons produced different picture in which *F. sanguinea* from MC clustered together with *F. rufa* samples, and seven out of eight *F. rufa* samples from MC were separated from other conspecific samples as soon as at the second node.

Discussion

In our study, both species clearly differ in respect of relative amounts of *n*-alkanes. However, analysis based on two other classes of hydrocarbons, *n*-alkenes and methyl-branched alkanes, revealed similarity between heterospecific individuals living in the one colony.

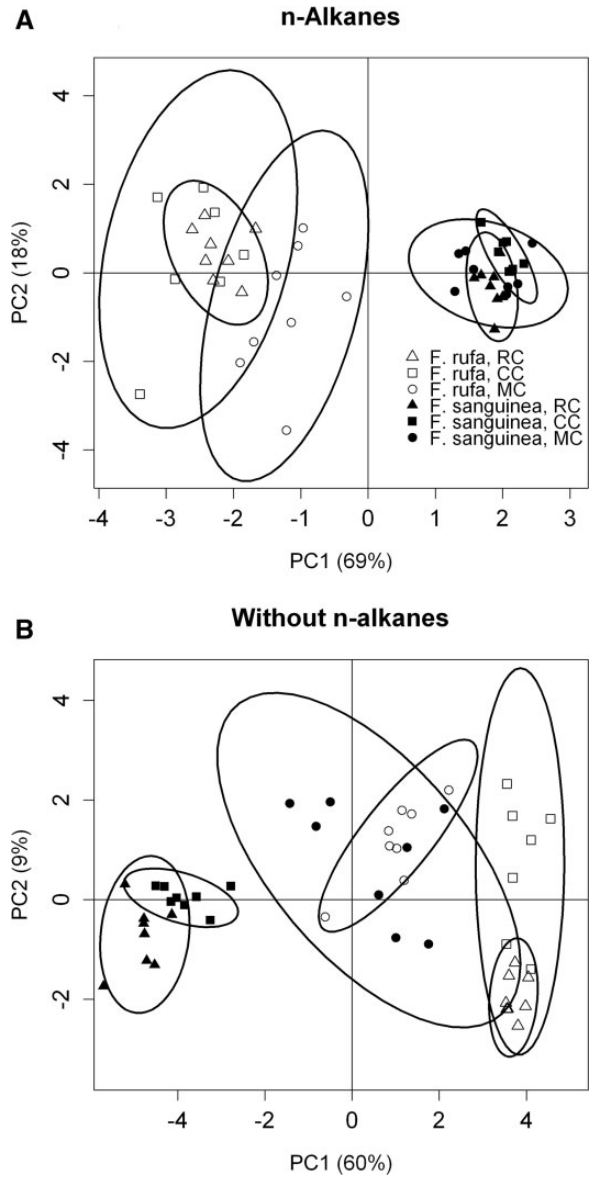

Fig. 3. Results of PCA using relative abundances of *n*-alkanes (A) and other hydrocarbons (B).

Hence, *F. sanguinea* and *F. rufa* have exchanged at least some of their CHCs after a period of cohabitation within the MC. Given the role of CHCs, this could account for the fact that the MC members were treated with hostility by conspecific ants originating from homospecific colonies. However, interspecific exchange of CHC seemed to be asymmetrical, and recognition labels in *F. sanguinea* were more affected by allospecific nestmates than those in *F. rufa*. This pattern could be explained by the fact that *F. sanguinea* workers were half as numerous in the MC as *F. rufa* and thus contributed to a lesser extent to the production and distribution of recognition cues among colony members.

Lack of evidence on transfer of *n*-alkanes in our study could be attributed to physical properties of this hydrocarbon class. Methyl-branched alkanes and alkenes melt at 20–50°C lower temperature than *n*-alkanes with the same carbon backbone length. Thus, they have been hypothesized to occur on insect's cuticle in form of patches of melted phase, which are interspersed by crystals containing *n*-alkanes (Gibbs 2002). Because of weaker affinity between molecules in melted phase, hydrocarbons constituting melted microregions could be expected to be relatively easy transferable via allogrooming and physical contact. This explanation is consistent with results of Martin et al. (2008a) and van Zweden et al. (2010), who established that *n*-alkanes are more variable within colony (hence, less transferable) than alkenes or some of the methyl-branched alkanes. Moreover, these two hydrocarbon classes and not *n*-alkanes are thought to play a role of recognition cues in different social insect taxa (Dani et al. 2001, 2005; Martin et al. 2008c; Guerrieri et al. 2009; van Wilgenburg et al. 2010; van Zweden et al. 2010). However, in the present study, exchange of recognition cues did not result in complete homogenization of the colony odor. Consequently, ants of both species were able to discriminate between representatives of their own species and allospecific individuals. In case of *F. sanguinea* ants, they treated with similar level of hostility both conspecific and allospecific ants from MC. However, conspecific individuals elicited more frequently antennal contact, which might mean that they were needed to be examined longer before aggressive response followed. This, in turn, could be explained by the moderate difference between CHC profiles of individuals involved in the interaction.

Social interactions between nestmates are known to be a very effective way of common gestalt odor formation. Manipulations with the use of radioactive precursors of CHCs have shown that within 1 day, *Camponotus fellah* Dalla Torre workers were able to transfer to other colony members as much as 55% of hydrocarbons stored in their postpharyngeal glands (Lenoir et al. 2001b). On the other hand, in mixed groups of *Formica selysi* Bondroit and *Manica rubida* Latreille, only partial adjustment of species-specific odor traits has been observed (Hefetz et al. 1992, Errard 1994). This could be explained by the difference in genetic patterns of CHC biosynthesis between these species, which can be expected to be much greater than difference resulting from intraspecific genetic variation. Hence, genetic variation could be too large to be effectively counterbalanced by interindividual CHCs exchange. Corbara and Errard (1991) have shown that in mixed groups both *F. selysi* and *M. rubida* workers spend more time on social interactions with conspecific individuals than with heterospecific ones. This might therefore be the main reason why in many studies species within a MC were observed to retain their distinctiveness in terms of recognition cues (see Introduction). Both these reasons, i.e., interspecific genetic differences and the (associated) low degree of heterospecific social interaction, might have contributed to the results of our study. Specifically, lack of reproducing queen in our study system might have contributed to diminished rate of trophalaxis and accompanying CHCs exchange due to lower food demands of colony as a whole.

In our experiment, *F. sanguinea* ants encountered in their nest recently emerged *F. rufa* individuals, which possessed their distinct odor. As both species coexisted peacefully in one colony, *F. sanguinea* had to modify their recognition algorithm, so that to adjust to the new social environment. Similarly, recognition template of *F. rufa* ants had to include odors of both species. Nevertheless, in case of social parasite ant species, it might be crucial in terms of colony performance to reduce within-colony odor variation. Inadequate recognition labels might weaken colony integrity through decreased frequency of heterospecific social interactions (see Lenoir et al. 1982, Corbora and Errard 1991). This might be one of the reasons why slave-making species and their hosts as a rule belong to the same tribe (see Huang and Dornhaus 2008). In a genus *Formica,* phylogenetic distance between species is associated with CHC profile divergence (Martin et al. 2008b). It is possible that in some cases, odor discrepancy might facilitate emancipation of slaves (see Czechowski 2005, 2006). This scenario is supported by the results of Włodarczyk (2012) and our present study, when complemented with observations made by Czechowski (1994), who reported *F. polyctena* slaves to take control over a colony of their parasite *F. sanguinea* in seminatural conditions.

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