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Does sensory deception matter in eusocial obligate food robber systems? A study of *Lestrimelitta* and stingless bee hosts

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Social parasites can break into their host colonies using sensory deception, force, or both. To evaluate the role of sensory deception in eusocial obligate food robbers, we studied the Mesoamerican stingless bee *Lestrimelitta niitkib*—host species system, including preferred and nonpreferred host species. The use of citral as a propaganda substance is documented in *L. niitkib*, but possible mechanisms used by individual scouts to overcome host species recognition have not been studied. We analysed the cuticular profiles of *L. niitkib* and host species, coupled with bioassays of time to aggression (latency) and included data on host species raid frequency. We found that *L. niitkib* has a simple, but not insignificant, cuticular profile. Generally, *L. niitkib* cuticular profiles were similar to (but did not mimic) profiles of its preferred host species and differed from profiles of nonpreferred hosts. As expected, latency generally fitted a recognition system based on the degree of similarity between the cleptobiont's cuticular label and the host species template, with chemically similar species reacting slower and chemically distant species reacting rapidly to *L. niitkib*. There was a positive correlation between raid ratio and latency, indicating that the speed of detection and aggression towards *L. niitkib* scouts could influence host species selection. Cuticular profile similarity of individual *L. niitkib* scouts to host species may help *L. niitkib* scouts evade recognition and attacks from guards. In a further step, unnoticed *L. niitkib* scouts could successfully recruit nestmates to mass-raid host species colonies. The fact that *L. niitkib* can also plunder aggressive species, suggests that obligate cleptobiosis within its narrow biological niche could be characterized by flexibility in invasion strategies to allow exploiting a broad range of host species and be successful over evolutionary times.

The food and nest materials of social insect colonies can attract robbing by conspecifics and allospecifics, a behaviour defined in ecology as cleptobiosis (Breed et al. 2012). Although most social insect species can act as facultative cleptobionts, obligate cleptobiotic species are rare in nature (Breed et al. 2012).

A unique case of obligate cleptobiosis is found in the Neotropical stingless bees in the genus *Lestrimelitta* (Sakagami et al. 1993). The nearly two dozen recognized species of *Lestrimelitta* (Camargo & Pedro 2007) have lost pollen collection structures and have reduced mouthparts, and thus cannot collect nectar or pollen from flowers, and must survive exclusively by robbing food and building material from the nests of other stingless bees (Sakagami et al. 1993; Breed et al. 2012). Interestingly, the different species of *Lestrimelitta* show marked selectivity on host species with species that are frequently raided and others that are rarely or never attacked. Accordingly, the intensiveness of the defensive response varies between both types of host species, ranging from intense fighting to practically no aggression (Wittmann et al. 1990; Sakagami et al. 1993; Radtke 1994). In Brazil and Panama, preferred hosts species of *Lestrimelitta limao* (now known as *Lestrimelitta danuncia*) include *Scaptotrigona pectoralis* (Sakagami et al. 1993), but in Yucatan, Mexico, *L. niitkib* never attacks *S. pectoralis*, and some species, like *Melipona beecheii*, are rarely raided (Quezada-Euán & González-Acereto 2002). In Yucatan, most *L. niitkib* attacks are reported in descending frequency on *Nannotrigona perilampoides*, *Plebeia frontalis* and *Frieseomelitta nigra*, with little aggression from the three species (Quezada-Euán & González-Acereto 2002; J. J. G. Quezada-Euán, unpublished data). Chemical affinities between parasites and hosts may explain reduced aggression by host species (Martin et al. 2008, 2010) and may be the basis for host selectivity in *Lestrimelitta*, but to date no study has been conducted to evaluate this hypothesis.

Nestmate recognition is crucial for the integrity of social insect nests, and parasites use force, chemical deception, or less

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frequently a mixture of both, to overcome their hosts' defenses (Vander Meer & Morel 1998; Dani et al. 2005; van Zweden & D'Ettorre 2010; Cini et al. 2011). The use of force and a general 'propaganda substance' by *Lestrimelitta* during nest raids is well established (Wittmann et al. 1990; Sakagami et al. 1993). However, it has been suggested that *Lestrimelitta* scouts can evade detection, and then recruit massive numbers of additional attackers, which can then use force or propaganda substances (Sakagami et al. 1993; Breed et al. 2012). For evading guard detection, similar to social parasites, *Lestrimelitta* scouts may use strategies based on chemical deception during the initial steps of attacks on hosts. By evading recognition, *Lestrimelitta* scouts could also reduce the possibility of host aggression, but such a relationship has not been studied. Studying the unique association of *Lestrimelitta* and its hosts may help to understand the relative importance of strategies based on force and chemical deception in obligate cleptobiont systems.

In the process of nestmate recognition, cuticular hydrocarbons play an important role in social insects (Vander Meer & Morel 1998; Lahav et al. 1999; Lenoir et al. 2001). In stingless bees, unsaturated cuticular hydrocarbons, alkenes and alkadienes seem to be the main compounds responsible for nestmate recognition (Jungnickel et al. 2004; Buchwald & Breed 2005; Pianaro et al. 2007; Nunes et al. 2008; Nascimento & Nascimento 2012; Septanil et al. 2012). Cerumen (a mixture of bee's wax and plant resins used in nest construction by stingless bees) could also serve as a source of recognition compounds (Nunes et al. 2011), although this view has been recently challenged (Jones et al. 2012). Specificity of unsaturated hydrocarbons has also been shown in honeybees, bumblebees, ants and wasps, and differences in colony and species profiles seem largely responsible for non-nestmate recognition and rejection (Breed 1998; Chaline et al. 2005; Dani et al. 2005; Martin et al. 2007, 2008, 2010; Kather et al. 2011). Individuals guarding the colony's entrance are the first to detect potential invaders (Nash & Boomsma 2008; van Zweden & D'Ettorre 2010). Guard bees can compare the chemical label of an incoming individual with an internal neural template, which if not matched, elicits an aggressive response (Sherman et al. 1997; van Zweden & D'Ettorre 2010). Therefore, measuring aggressive responses seems a good approximation to evaluate chemical recognition abilities in social insects (Martin et al. 2012; Nascimento & Nascimento 2012).

In this study, we analysed the quantitative and qualitative cuticular profiles of *L. niitkib* and some of its potential hosts. We concentrated on the analysis of unsaturated hydrocarbons as these compounds seem the key recognition cues in stingless bees. A significant reduction in the quantity of unsaturated hydrocarbons could be interpreted as chemical insignificance (Uboni et al. 2012). Alternatively, cleptobiont–host cuticular profiles could show similarities in their type of unsaturated hydrocarbons (Martin et al. 2010, 2012). We included bioassays to evaluate whether differences in chemical profiles are related to host aggression that in turn may explain host selectivity by *Lestrimelitta*. We expected that species that were more chemically similar to *L. niitkib* would show lower aggressive responses and vice versa. Finally, we compared data on raid frequency with the aggressive response of the studied host species.

METHODS

Bee Species and Sampling

The study was conducted at the Campus de Ciencias Biológicas y Agropecuarias (CCBA) of the University of Yucatán at Xmatkuil, on the northern part of the Yucatán Peninsula of México. For over 10 years, data on raid frequency have been collected at the stingless bee yard and feral colonies in the CCBA, allowing determination of host preferences of *L. niitkib* in this area. In the study we included colonies of the most preferred species by *L. niitkib*, namely *N. perilampoides*, with about 50% of the total raids recorded on this species, followed by *P. frontalis* and *F. nigra*, both with about 20% of the incursions done by *L. niitkib*. We also included two non-preferred hosts of *L. niitkib*, namely, *S. pectoralis* and *M. beecheii*. Ten worker bees were collected from each of five colonies of each of the six species. All samples of *L. niitkib* were obtained from unmanaged colonies; nests of the other species were obtained either in the stingless bee yard or from feral nests.

Analysis of Cuticular Compounds

Collection and extraction of cuticular compounds took place within 2 weeks during May 2010. Bees were killed by freezing, and all legs were removed prior to extraction to avoid contamination with resins. For the extraction of cuticular compounds, the body of each bee was submerged in 1 ml of hexane for 1 min. After one body was extracted, it was retrieved from the hexane and the body of another bee was submerged in the same hexane for extraction for 1 min. The same procedure was performed successively until the extracts of 10 bees were contained in 1 ml of hexane. In the end there was one extract per colony.

Gas chromatography (HP5890 II GC) using splitless injection and coupled with mass spectrometry (HP5972 MS) served for analyses of extracts. The GC was fitted with a DB-5 MS column (30 m $\times$ 0.25 mm $\times$ 0.25 μm), with an oven programme ranging from 60 °C to 300 °C at 10 °C per minute, and an additional 15 min at the final temperature. Characterization of components was achieved by comparison of mass spectra and retention times with those provided by commercially available mass spectral libraries (Adams 2001; Wiley 275 Mass Spectral Library, J. Wiley, New York, NY, U.S.A.) or reference samples containing *n*-alkanes. Where no characterization above compound class could be accomplished, substances were numbered and added to a custom-made spectral library. Cuticular hydrocarbons were quantified based on peak areas (integrated ion currents) obtained from the chromatograms and, prior to further analyses, integrated ion currents were standardized based on the total number of peak areas for each individual profile.

The relative contribution of each cuticular hydrocarbon (alkanes, alkenes and alkadienes) were calculated per species based on the ion current peak areas obtained for each colony. We focused further analyses on the amounts of unsaturated cuticular hydrocarbons (UCHs).

Chemical Insignificance and Mimicry of L. niitkib

The quantities for UCHs were corrected by dividing the sum of total peak areas (integrated ion currents) by the average fresh body mass (mg) of five specimens of each species (Lenoir et al. 2001; Lorenzi et al. 2011; Uboni et al. 2012). Testing for chemical insignificance in UCHs of *L. niitkib* was done using the total peak areas for each colony and comparing species by means of an ANOVA after Bonferroni correction. Post hoc comparisons were done using Tukey's multiple comparison tests.

To test for chemical similarity between *L. niitkib* and potential hosts, we used two approaches. First, we calculated the chemical distance (CD) between *L. niitkib* and each host species using a 'city block' design as described by Martin et al. (2012). This method uses the sum of the differences in the proportion of each UCH between species and the total is divided by two. Thus, it is a measure of the magnitude of the chemical differences in the proportions of all detected UCHs between species. Proportions of UCHs range from 0, when two UCHs profiles are identical, to 100, when both profiles are completely different (i.e. the species do not share UCHs).

Next, using a principal component analysis (PCA), we combined individual variation in the amount and type of UCHs per sample to derive new variables (components) that concentrated the largest amount of variation in the first axes. We then used these new variables in separate analyses to establish relationships between groups (Tabachnick & Fidell 1996). PCA scores were calculated for the first two components that included the largest amount of variation in the data; this was done for each cohort within each colony sampled. We compared the scores of *L. niitkib* with the other species using ANOVA (with Bonferroni correction). The scores for the first two components were also used to produce plots of the corresponding values for each colony within species onto a bidimensional scale.

Recognition Bioassays and Raid Ratio

Three colonies housed in wooden hives of each of the five host species were used in tests of aggression to cleptobionts. Whole colonies were used in the tests, but observing the behaviour of guard bees when a foreign individual is introduced is difficult under such conditions. To facilitate observations, we attached a small wooden box (10 × 3 × 6 cm) with a glass top to the entrance of each host colony by a plastic tube. Each wooden box was attached to its respective colony 10 days before the tests were started so that guard bees moved to this area and accepted it as part of their colony. Prior to the assays, we individually collected workers at the entrance of a *L. niitkib* colony in Eppendorf tubes. These tubes were placed in a dark room for 15 min to reduce restlessness in the test subjects. We introduced *L. niitkib* scouts into a host colony and recorded the latency to the first aggressive response by host bees. All interactions between the introduced *L. niitkib* scouts and the host bees were observed through the box attached to the colony and in a dark area under a red light to avoid disturbing them. We introduced one test *L. niitkib* into each colony per day to avoid interference of previous tests. One bee was introduced at a time to emulate the behaviour of individual *L. niitkib* scouts (Breed et al. 2012). A total of 10 *L. niitkib* were introduced into each host colony. The data were compared between species using ANOVA. We calculated a raid ratio for the five potential host species using the data recorded over a 10-year period of observations in the stingless bee yard at CCBA. The estimations were done in accordance to Sakagami et al. (1993), dividing the number of recorded raids per species by the number of colonies of that species in the stingless bee yard. We used Spearman rank correlations to compare chemical distances (as calculated above, see Chemical Insignificance and Mimicry of *L. niitkib*) and latency to first aggressive response of host species to *L. niitkib* bees, and latency to first aggressive response and estimates of raid ratios per host species.

All analyses were done using the SAS statistical software (SAS Institute 2008).

RESULTS

A variety of linear alkanes and alkenes were found in the studied species in the range of carbon lengths of C19–C33. Alkadienes were only found in *M. beecheii*, and branched alkanes were not detected in any species. The position and configuration of double bonds in alkenes and alkadienes were not identified. If more than one isomer was present, as indicated by differences in retention time, they were numbered accordingly (Table 1).

Chemical Insignificance of L. niitkib

There was no evidence for chemical reduction in the total quantities of UCHs in *L. niitkib* compared to the other species. Tukey's multiple comparisons showed that *L. niitkib* had similar amounts of UCHs to all species, except *F. nigra*, which had significantly lower amounts ($F_{5,24} = 5.05$, $P < 0.01$; Supplementary Fig. S1).

Table 1
Relative proportions of cuticular hydrocarbons (%) found in *L. niitkib* and stingless bee hosts

Analyte	RT	RI	Species					
			Ln	*Np*	*Pb*	*Mb*	*Sp*	*Fn*
Nonadecane	16.138	1898	–	–	–	0.83	–	–
Heneicosane	18.17	2098	–	–	–	2.10	–	–
Tricosene 1	19.839	2276	–	–	1.45	–	42.38	–
Tricosene 2	19.895	2283	–	–	–	–	2.09	–
Tricosane	20.065	2300	–	–	17.81	–	31.52	–
Pentacosene 1	21.546	2473	–	–	10.71	1.37	3.70	–
Pentacosane	21.781	2500	5.07	23.59	24.41	–	15.66	19.99
Heptacosene 1	23.164	2676	–	9.52	6.33	4.55	0.59	–
Heptacosene 2	23.221	2683	2.25	3.55	5.32	–	–	–
Heptacosene 3	23.25	2687	0.54	0.58	–	–	–	–
Heptacosane	23.362	2700	38.9	19.78	7.77	5.04	2.47	11.24
Nonacosene 1	24.697	2876	36.6	26.52	25.93	7.12	0.58	–
Nonacosene 2	24.727	2880	10.6	9.11	0.78	–	–	–
Nonacosane	24.885	2900	5.07	6.62	–	17.7	0.35	7.87
Hentriacontadiene	26.363	3057	–	–	–	1.33	–	–
Hentriacontene 1	26.56	3076	–	0.71	–	15.24	0.65	3.16
Hentriacontene 2	26.636	3083	–	–	–	10.76	–	3.06
Hentriacontane	26.757	3100	0.98	–	–	20.03	–	8.94
Tritriacontadiene	28.658	nc	–	–	–	1.19	–	–
Tritriacontene1	28.989	nc	–	–	–	–	–	8.58
Tritriacontene 2	29.092	nc	–	–	–	–	–	24.89
Tritriacontene 3	28.958	nc	–	–	–	8.4	–	10.30
Tritriacontane	29.259	nc	–	–	–	4.24	–	1.95

RT: retention time; RI: retention index; *Ln*: *L. niitkib*; *Np*: *N. perilampoides*; *Pb*: *P. frontalis*; *Mb*: *M. beecheii*; *Sp*: *S. pectoralis*; *Fn*: *F. nigra*; nc: not calculated. A dash denotes that the analyte was not identified in the species.

Chemical Similarity of L. niitkib *and Host Species*

Although UCHs isomers can be frequent in bees (Martin et al. 2010), it was not possible to identify them within the scope of this study. Thus, we pooled all alkene isomers of the same chain length under one category. Under this criterion, the type and amount of alkenes in the different species are presented in Fig. 1.

Lestrimelitta niitkib colonies had relatively simple profiles consisting of two main alkene types, C27:1 and C29:1, the latter being highly predominant (95%). Both alkene types were present in different proportions in the potential host species with the exception of *F. nigra*, which completely lacked both. However, in *N. perilampoides* and *P. frontalis*, C27:1 and C29:1 were also the most frequent alkene types. In the case of *N. perilampoides*, both alkenes made up 98% of the total alkene profile.

The values of CD between *L. niitkib* and potential host species showed that the chemical profile of *N. perilampoides* was the most similar to that of the cleptobiont (24%), and *P. frontalis* had a CD value of 42%. In contrast, the other preferred species, *F. nigra*, had the highest CD relative to that of *L. niitkib* (100%), and thus had no shared UHCs types on their cuticles. The two nonpreferred species, *M. beecheii* and *S. pectoralis*, had CD values of 80% and 97% relative to *L. niitkib*, respectively. The results of CD*s* were confirmed by the PCA. The ANOVA of colony scores for each of the two components showed significant differences between species (PC1: $F_{5,24} = 75.33$, $P < 0.001$; PC2: $F_{5,24} = 17.38$, $P < 0.001$; Supplementary Table S1). *Lestrimelitta niitkib* had similar score values along both components with its most preferred species, *N. perilampoides*, which was confirmed by the overlap of both species on the PCA plot (Fig. 2). The values of *P. frontalis* colonies were close to those of the *L. niitkib* cluster, although scores of only two colonies overlapped with those

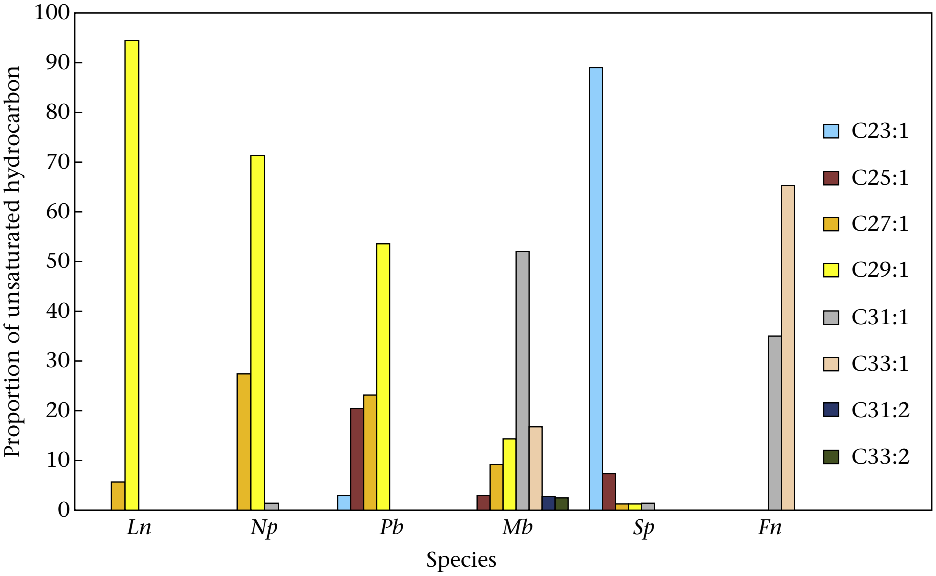

Figure 1. Proportion of unsaturated cuticular hydrocarbons present in *L. niitkib* and host species. *Ln*: *L. niitkib*; *Np*: *N. perilampoides*; *Pb*: *P. frontalis*; *Mb*: *M. beecheii*; *Sp*: *S. pectoralis*; *Fn*: *F. nigra*.

of *L. niitkib*. The score values between *L. niitkib* and *P. frontalis* differed statistically along PC1 but not so for PC2 (Supplementary Table S1). On the other hand, the scores of *L. niitkib* for PC1 and PC2 differed significantly from those of the three most chemically distant species; accordingly, separate clusters of *M. beecheii*, *F. nigra* and *S. pectoralis* with respect to *L. niitkib* were evident on the PCA plot (Fig. 2, Supplementary Table S1).

Recognition Bioassays and Raid Ratio

We introduced a total of 150 individual *L. niitkib* to colonies of the five potential hosts. The numbers of guards at the entrances of experimental boxes differed between host species. On average, there was a single bee at the entrance in *M. beecheii* and *F. nigra*, five bees at the entrance in *P. frontalis*, 10 bees at the entrance in *S. pectoralis* and 15 bees at the entrance in *N. perilampoides*. Latency to first aggression differed significantly between host species ($F_{4,10} = 33.47$, $P < 0.001$). *Scaptotrigona pectoralis* and *M. beecheii* had the shortest latencies to respond, with no significant differences between them (5.01 ± 4.4 s and 8.92 ± 2.3 s, respectively). *Nannotrigona perilampoides* had the longest latency to react to *L. niitkib* (204.9 ± 62.1 s). Latencies to react to *L. niitkib* were similar for *P. frontalis* (38.73 ± 25.5 s) and *F. nigra* (49.4 ± 5.6 s). Both were significantly slower than *N. perilampoides* but longer than *M. beecheii* and *S. pectoralis* (Fig. 3). Spearman rank correlation between latency and CD was −0.47 ($P = 0.09$) when all host species were considered.

The calculated value of raid ratio was highest for *N. perilampoides* with 2.75, followed by *P. frontalis* and *F. nigra*, with ratios of 1.25 and 1.42, respectively. The species with the lowest raid ratios were *M. beecheii* with 0.16 and *S. pectoralis* with 0.05 (Fig. 3).

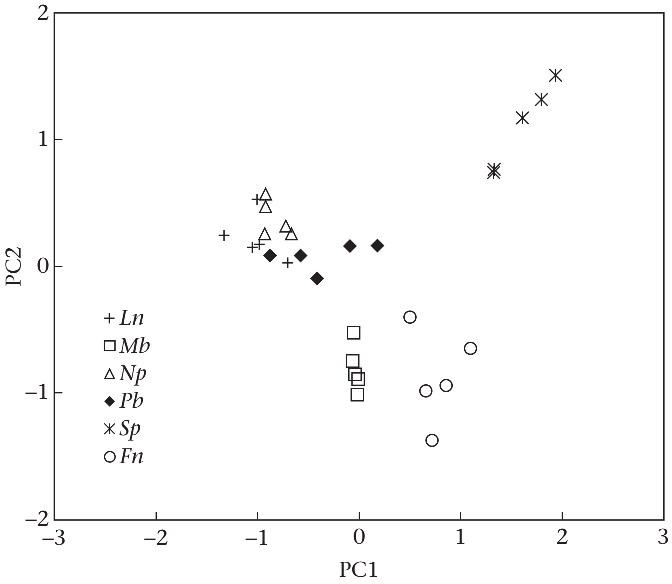

Figure 2. Principal component (PC) scores for stingless bee species plotted against PC1 and PC2 derived from unsaturated cuticular hydrocarbons. *Ln*: *L. niitkib*; *Mb*: *M. beecheii*; *Np*: *N. perilampoides*; *Pb*: *P. frontalis*; *Sp*: *S. pectoralis*; *Fn*: *F. nigra*.

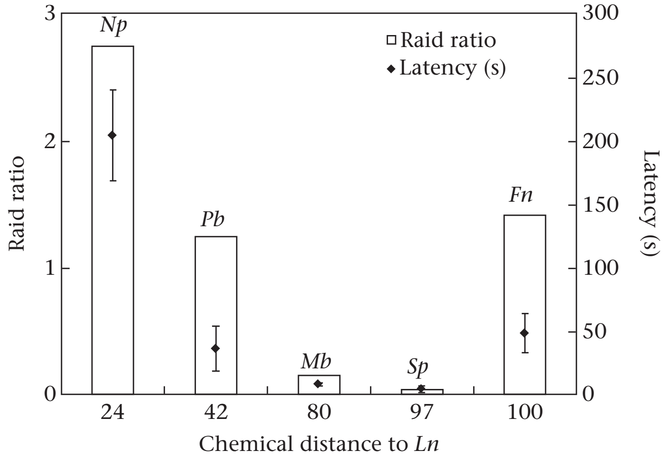

Figure 3. Plot of host species' chemical distances based on unsaturated cuticular hydrocarbons and their average latency time (black diamonds with standard errors) to *L. niitkib* workers, and estimations of raid ratios of *L. niitkib* to each species (bars). *Ln*: *L. niitkib*; *Np*: *N. perilampoides*; *Pb*: *P. frontalis*; *Mb*: *M. beecheii*; *Sp*: *S. pectoralis*; *Fn*: *F. nigra*.

We found significant positive correlation between latency to respond and raid ratio (0.99; $P = 0.001$).

DISCUSSION

Our study represents the first attempt to analyse the dynamics of the chemical interaction between an obligate cleptobiotic species (*L. niitkib*) and its range of possible hosts, in particular to understand the basis for the existence of host selectivity in these systems. We found that *L. niitkib* and preferred host species showed similar UCHs profiles and that the cleptobiont's cuticle showed no reduction in the overall amount of UCHs compared to the studied hosts.

Chemical similarities between *Lestrimelitta* and preferred hosts could derive from common ancestry. *Nannotrigona*, *Plebeia* and *Lestrimelitta* share close phylogenetic relationships, with the latter two genera being possible sister groups (Rasmussen & Cameron 2010). This evidence conforms to Emery's (1909) rule, which predicts that social parasites and their hosts could be close relatives. Sympatric speciation between host and parasite is a possible evolutionary pathway (Choudhary et al. 1994; Sumner et al. 2004; Cini et al. 2011). Alternatively, social parasites may be close relatives but not sister species of their hosts (the 'loose' version of Emery's rule; Sumner et al. 2004) as in the case of *Lestrimelitta* and *Nannotrigona*. Thus, it is possible that chemical similarities found between *L. niitkib* and preferred hosts may reflect such phylogenetic relatedness.

Our bioassays revealed that species chemically closer to *L. niitkib* had a slower aggressive response to the presence of individual cleptobionts. These results are in agreement with the idea that, similar to social parasites (Nash & Boomsma 2008; Martin et al. 2010), individual *L. niitkib* scouts may be chemically unnoticed for some time by host species. Moreover, the latency to aggression towards individual *L. niitkib* workers was positively correlated with the calculated raid ratios of host species. Thus, the speed of detection and further aggression towards individual *L. niitkib* may influence a cleptobiont colony's selection of host species possibly because of the differential risks of physical damage to *L. niitkib* scouts (Radtke 1994; Nash & Boomsma 2008). Similarly, in slavemaking ants, reduced aggression by hosts reduces damage to invaders and increases their success in capturing more brood (Pamminger et al. 2012). From the host's point of view, a nonaggressive response could lower mortality and increase foraging capacity and fitness (Holway et al. 1998). It has been speculated that *Lestrimelitta* may have an innate preference for specific host species or host odours, which that they learned from previous raids, or that they learn to avoid odours of species where they have experienced severe resistance by guards (Jarau 2009). Our results suggest that guard resistance after recognition of individual *L. niitkib* could partially explain the low frequency of raids towards some host species and thus, *L. niitkib* host preferences.

Intraspecific colony recognition in social insects seems based on the relative abundance of individual components rather than on a general model based on the presence or absence of independent recognition cues (Akino et al. 2004). However, a simple presence or absence system may still operate for interspecific recognition when species do not share the same chemical profiles (Couvillon & Ratnieks 2008; Martin et al. 2012), as in our system. An undesirable-absent recognition mode predicts that *L. niitkib* individuals shall be more readily accepted only if their template is similar to that of the invaded species (i.e. that their cues do not differ from those of the host). This seems to be the case, as *L. niitkib* has a relatively simple cuticular profile containing only two alkenes, heptacosene and nonacosene, with one of them, nonacosene, being highly predominant. Both compounds were also predominant in the two most preferred species, *N. perilampoides* and *P. frontalis*, which determined a close CD with *L. niitkib*. Both species showed long latencies to react to *L. niitkib*, presumably because of the similarity of their cuticular profiles to that of *L. niitkib*, which could help *L. niitkib* evade recognition, as predicted by the undesirable-absent model. Two chemically distant nonpreferred hosts, *M. beecheii* and *S. pectoralis*, also fitted this prediction, as revealed by their quick aggressive response to individual *L. niitkib*, presumably resulting from a quick recognition of chemical cues.

However, the undesirable-absent model of recognition does not explain the low aggressive response of *F. nigra* to *L. niitkib*, a chemically distant species, which in theory, should quickly recognize and react to non-nestmate intruders. Studies in ants and wasps have revealed that lack of aggression does not necessarily result from lack of recognition, and thus, that the relationship between cuticular recognition cues and aggression may not be so simple (Martin et al. 2007, 2008, 2009). Parallel mechanisms of reduced aggression could have potentially evolved in some species of stingless bees (Leonhardt et al. 2010). *Trigona collina*, an Asian species, is practically nonaggressive towards homospecific and allospecific intruders. Such a 'peaceful' behaviour presumably derives from the fact that this species extensively collects resins whose terpenes can act as appeasement allomones on other species (Leonhardt et al. 2010). An interesting parallel may be occurring between Paleotropical *T. collina* and Neotropical *F. nigra* regarding interspecific tolerance. The genus *Frieseomelitta* is characteristic for its extensive collection of resins too (Patricio et al. 2002; Quezada-Euán 2005). Notably, the presence of a large fraction of terpenes and other compounds in the cuticular profile of *F. nigra* and in that of other *Frieseomelitta* species may be derived from plants as well (Patricio et al. 2002; this study). Similarly to *T. collina*, *F. nigra* also frequently nests in inter- and intraspecific aggregations (J. J. G. Quezada-Euán, personal observations), which suggests a high intra- and interspecific tolerance too. Our finding that *F. nigra* had the lowest quantities of UCHs of all species studied could be also linked to the importance of other recognition cues in this species. Additionally, the presence of dodecyl acetate in stingless bees has been found for the first time in *P. frontalis* (J. J. G. Quezada-Euán, J. Ramírez, T. Eltz, T. Pokorny, R. Medina & R. Monsreal, unpublished data). Dodecyl acetate has a major role in repelling host attacks by parasite social bumblebees (Martin et al. 2010). These findings point out the need for detailed studies on intra- and interspecific recognition in the highly diverse Meliponini to elucidate the possible role of chemical cues other than UCHs that mediate aggressive responses in this taxon (Martin et al. 2007, 2010; see also Supplementary Material).

Cleptobionts have been divided into two main categories, 'deceptive' and 'forceful' types (Cini et al. 2011; Breed et al. 2012). Invaders whose intrusion is detectable by the host seem to use chemical resemblance to their host's cuticular profiles, while those that use force can break away from this coevolutionary arms race and have different cuticular profiles from those of their hosts (Cini et al. 2011; M. Wurdack, J. Kroiss, O. Niehuis, E. Strohm & T. Schmitt, unpublished data). Our findings of chemical similarity of *L. niitkib* to preferred hosts indicate that this obligate food robber could adopt either strategy (depending on the host), or adopt different strategies at different stages during host raids. The parasitic lifestyle of *Lestrimelitta niitkib*'s temporary incursions for food robbing and the range of species that it attacks fit with the findings of a nonperfect chemical similarity with its hosts (Lohman et al. 2006; Lambardi et al. 2007; Uboni et al. 2012). Perfect mimicry is only expected in social parasites with a long-term interaction with their host as they depend on the latter for care over a long period (Lenoir et al. 2001; Lambardi et al. 2007). However, the degree of chemical similarity of *L. niitkib* to some of its hosts may be sufficient for individual *L. niitkib* scouts to pass by nest guards and not trigger an aggressive response, as occurs in other social insect parasite–host models (Ruano & Tinaut 2004; Errard et al. 2006). This may grant *L. niitkib* scouts sufficient time to enter a host colony and collect information on food reserves and other conditions. In a further step, unnoticed *L. niitkib* scouts could recruit nestmates to engage in the well-recognized mass-raids involving the use of propaganda substances such as citral (Sakagami et al. 1993). Citral produced in *L. niitkib* mandibular glands seems to act as a masking pheromone or a repellent of host bees (Wittmann et al. 1990; Sakagami et al. 1993; Radtke 1994; Pompeu & Silveira 2005). Under this hypothesis, chemical deception could be more important at the initial stages of host invasion when guard bees could more easily repel individual *L. niitkib* scouts attempting to enter their nests.

The most evident cases of invasions by *Lestrimelitta* include the arrival of large groups of bees and their involvement in intense fights, in contrast to the mild raids towards nonaggressive hosts, which frequently go unnoticed (Sakagami & Laroca 1963; Wittmann et al. 1990; Sakagami et al. 1993). Thus, it is evident that workers of *L. niitkib* also possess features that may grant them advantages during fights such as a heavily sclerotized cuticle and sharp mandibles (Breed et al. 2012). By the use of force, *Lestrimelitta* can also successfully attack *Scaptotrigona*, *Melipona* and even *Apis*, which are far more defensive and populous (Sakagami et al. 1993). Thus, fighting may be particularly important in the case of chemically distant species where guards can easily detect and react strongly to the presence of *L. niitkib* invaders (Sakagami et al. 1993; Nogueira-Neto 1997). Note that attacks to nonpreferred species may occur when availability of preferred hosts (or their reserves) decreases (Sakagami et al. 1993).

The system of the obligate food robber *L. niitkib* and its stingless bee hosts seems to fit the hypothesis that both chemical deception and force could evolve simultaneously and play a role in the invasion strategies of social parasites (Brandt et al. 2005; Cini et al. 2011; Green & Field 2011). In this regard, there is a striking parallel between the potential strategies used by *L. niitkib* and *Psithyrus* bumblebee social parasites (Martin et al. 2010). Both, *L. niitkib* and several *Psithyrus* species have simple UCHs profiles dominated by one type of compound, which in both cases is found in a high proportion in their host species. Moreover, some *Psithyrus* can also use substances that repel guard bees of host species with poor chemical similarity (Martin et al. 2010). Thus, it seems that two different groups within Apidae solve a similar problem in a similar way, suggesting possible chemically convergent evolution.

In summary, our findings suggest that cuticular similarities of individual *L. niitkib* scouts to preferred hosts may help them evade recognition and attacks from guarding bees. Unnoticed individual *L. niitkib* scouts could successfully recruit nestmates to mass-raid host colonies. The fact that this cleptobiont is not an exact mimic of its preferred hosts and that other strategies could be used in host invasion (force, pheromones), including eventual attacks on nonpreferred hosts, suggests that obligate cleptobionts may use a relatively flexible range of strategies that may help them to adapt to fluctuations in host availability. So obligate cleptobiosis within its narrow biological niche seems to be characterized by flexibility in invasion strategies to allow exploitation of a broad range of hosts in order to be successful and maintained in time. Continued studies on this and other obligate cleptobiotic systems are needed to corroborate these hypotheses.

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Supplementary Material

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