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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).