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RESEARCH ARTICLE

Hydrocarbons detection levels in ants

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Abstract In social insects the discrimination process of non-nestmates is generally considered to be based on detection of cuticular hydrocarbons. The required quantity of the stimulus is fundamental but unknown. In laboratory conditions, we demonstrated that *Aphaenogaster senilis* ants are able to discriminate the presence of heterocolonial cuticular hydrocarbons on filter papers at concentrations of 0.05 ng/mm^2, the equivalent of 10^{-4} worker. This result does not mean that workers are not able to recognize their own colony odour at lower concentrations, but that the discrimination response appears at very low levels.

Keywords Ants · Hydrocarbons detection · *Aphaenogaster*

Introduction

Social animals discriminate between members of their own colony and conspecific aliens, a process called nestmate recognition. It allows them to maintain colony insularity against competitors and has certainly played an important role in the evolution of eusociality (Wilson and Hölldobler, 2005). Recognition requires the perception of cues emitted by the encountered ant, cues which are compared to the template of the receiver. It is generally thought that cuticular hydrocarbons play an important role in this discrimination (see recent reviews by Hefetz, 2007; d'Ettorre and Lenoir, 2009). A second paradigm involves the role of mandibular gland secretions in nestmate recognition signals (see for example Hernandez et al., 2004), but here we tested the role of non-volatile signals. Nothing is known about the sensibility of the ants to cuticular hydrocarbons. We investigated this question to evaluate the sensibility of workers to a blend of cuticular hydrocarbons.

Materials and methods

Five colonies of *Aphaenogaster senilis* were collected in the Doñana National Park in Southern Spain. They comprised the queen, numerous brood and 400–800 workers and were maintained in the laboratory at 28°C. They were provided with maggots and a sugared solution for bumblebees three times a week and pieces of orange once a week.

Ten workers were randomly picked up from one nest and killed by freezing. Individual thoraces (plus legs) were dipped in 100 µl pentane for 10 min to extract cuticular hydrocarbons (CHCs). We used only thoraces to remove only cuticular hydrocarbons as the head and gaster have different glands. It was verified that this method removes 90% of the hydrocarbons (A.L., unpublished). The identity of the hydrocarbons was previously determined (Lenoir et al., 2001; Boulay et al., 2007). Different solutions were then prepared from the mother solution (1 ant/10 µl): 10^{-1}, 10^{-2}, 10^{-4}, 10^{-6} and 10^{-8} ant equivalent. To determine the discrimination level of an individual ant towards an

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extract, we used rectangular filter papers (5 × 2 mm) previously rinsed several times with acetone and pentane to be “odourless.” We verified that these papers contained no hydrocarbons after treatment. The filter paper was impregnated with 10 μl of one of the extracts or with pure pentane. For the test, a worker was introduced in a Petri dish with edges coated with fluon and left for 10 min. The filter paper was deposited and the ant’s behaviour recorded for 2 min. Ants were tested only once with one filter paper. Behaviours were classified according the following categories: antennal contact, opening mandibles, biting and fighting (see Ichinose et al., 2005). Tests were performed with one worker confronted with extracts of the same colony, or to extracts of alien colonies at the different concentrations, or with pure pentane. 1,153 tests were performed.

Statistics

A principal component analysis was performed on the behavioural data. In a second step, an ANOVA was performed on the first and second components obtained with the PCA analysis using the concentration of extracts and the origin of the extract (pentane, same colony or different colony) as variables.

Results

The first component of PCA explains 35.78% of the variance and it is correlated with the frequency of aggressions (opening mandibles, biting and fight). An ANOVA indicated that there was a significant difference between homocolonial and heterocolonial tests ($F_1 = 21.82$, $P = 0.001$). The extract concentration did not significantly affect aggression when the worker was confronted with a homocolonial extract ($F_5 = 0.157$, $P = 0.978$) (Fig. 1a). On the contrary, the concentration of the heterocolonial extracts did significantly influence aggression (Fig. 1b; $F_5 = 3.38$, $P = 0.0048$). Aggression was absent or low with pentane. The ants were able to discriminate alien extracts at concentrations as low as 10^{-4} ant but not for 10^{-6} and 10^{-8} ant (post hoc Tukey-HSD test). The second axis of the PCA (21.12% of the variance) was correlated with antennal contacts and the ANOVA confirmed the results of component one (not presented here).

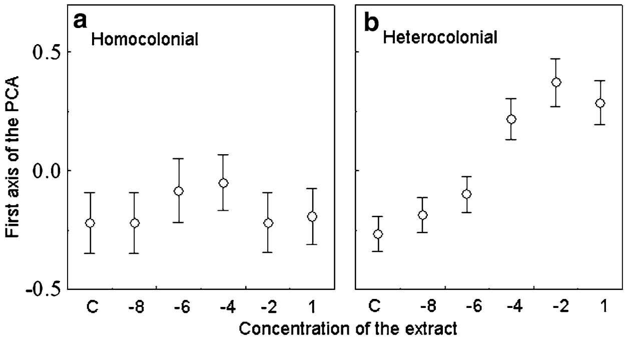

Fig. 1 First component of the PCA (aggression response) ± SE related to the concentration levels of cuticular hydrocarbons (from 1 ant to 10^{-8} ant equivalent). *C* filter paper impregnated with solvent

Discussion

The ant confronted with a worker extract of the same colony did not present the aggressive behaviour which was present when confronted with an alien extract. The detection level for discrimination was very low: at concentrations of 10^{-4} worker equivalent and higher, the workers recognized the alien extract. It has been measured previously that the thorax bears in mean 3,500 ng hydrocarbons (Lenoir et al., 2001), so the worker is sensitive to 0.05 ng/mm^2 on the filter paper, which is very low. The quantity of hydrocarbons on the cuticle related to the surface is not well known and appears to vary considerably with ant species: it is estimated to be from 50 ng/mm^2 in *Cataglyphis iberica* (Dahbi and Lenoir, 1998) to 1,000 ng/mm^2 in *Cataglyphis niger* (Lahav et al., 1998). These strong differences may be due to the calculation methods (see Kroiss et al., 2009), but regardless of the exact value of concentration, our data indicate a discrimination level of 10^{-4} worker equivalent. A similar result has been recently presented on aggressive behaviour of *Camponotus japonicus*: the aggression threshold varying from 10^{-4} to 10^{-1} equivalent of non-nestmate hydrocarbons (Ozaki and Wada-Katsumata, 2010). For other insects only a few data are available: 1,150 ng/mm^2 in the beewolf *Philanthus triangulum* but only 250 ng for the cuckoo wasp parasite *Hedychrum rutilans* which is considered to practice “Chemical insignificance” (Kroiss et al., 2009). For chemical insignificance see Lenoir et al., 1999. The sensitivity of social insects to hydrocarbons is completely unknown. It has been experimentally observed in *Bombus lapidarius* that tricosane (one of the compounds found in the tarsal extracts) at doses $\geq 10^{-12}$ ng/flower resulted in rejection by foraging workers (Goulson et al., 2000). On the contrary, *Polistes dominulus* react to a foreign foundress only when extracts concentrations on a filter paper are higher than two-third wasp equivalent (Cini et al., 2009). The differences are probably due to the experimental designs. One way to estimate the sensitivity of insects to hydrocarbons is to perform electroantennography, but no study has tried to measure it. For example, D’Ettorre et al., 2004 and Ozaki et al., 2005 did not study this question. Batista-Pereira et al., 2004 used a 10^{-2}

worker equivalent extract of the termite *Hodotermes tenuis* which released high EAG activity. More experiments are necessary to progress in the comprehension of the detection level of nestmate recognition cues.

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