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Learning and Discrimination of Individual Cuticular Hydrocarbons by Honeybees (*Apis mellifera*)

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Abstract

In social insect colonies, recognition of nestmates, kinship, caste and reproductive status is crucial both for individuals and for the colony. The recognition cues used are thought to be chemical, with the hydrocarbons found on the cuticle of insects often cited as being particularly important. However, in honeybees (*Apis mellifera*) the role of cuticular hydrocarbons in nestmate recognition is controversial. Here we use the proboscis extension response (PER) conditioning paradigm to determine how well honeybees learn long-chain linear alkanes and (*Z*)-alkenes present on the cuticle of worker bees, and also how well they can discriminate between them. We found large differences both in learning and discrimination abilities with the different cuticular hydrocarbons. Thus, the tested hydrocarbons could be classified into those which the bees learnt and discriminated well (mostly alkenes) and those which they did not (alkanes and some alkenes). These well-learnt alkenes may constitute important compounds used as cues in the social recognition processes.

Key words: chemosensory cues, differential conditioning, guarding, nestmate recognition, proboscis extension response

Introduction

For colonial living organisms, being able to distinguish between colony and non-colony individuals has a number of advantages. For example, in social insects the ability to recognize nestmates helps them to prevent intra- and interspecific parasitism and the theft of colony resources (Breed, 1998). It also enables context specific behavioural modifications by colony members such as reproductive dominance (Heinze *et al.*, 2002; Endler *et al.*, 2004). Nestmate recognition is achieved mainly by chemical communication in social insects (Breed, 1998) and the chemical composition of these cues is starting to be elucidated. One of the groups of compounds thought to play an important role in recognition are the long-chain hydrocarbons on the cuticle, which protect insects against desiccation (Gibbs, 2002).

Many correlation studies have shown a wide variation in the cuticular hydrocarbon profiles between individuals from different colonies (Breed, 1997). Hydrocarbons have been shown to play a part in nestmate recognition in some ant species (Boulay *et al.*, 2000; Lahav *et al.*, 1999) and also in the recognition of reproductive status (Cuvillier-Hot *et al.*, 2002; Endler *et al.*, 2004). They have also been shown to be the cues for nestmate recognition in social wasps (Panek and Gamboa, 2000), with branched alkanes and alkenes likely to be more important recognition cues than linear alkanes (Dani *et al.*, 2001).

However, in honeybees, the evidence for the role of hydrocarbons in nestmate recognition is more controversial. There are colony differences in the composition of cuticular hydrocarbons which have been reported to separate nestmates and even full-sisters from half-sisters (Arnold *et al.*, 2000). Supplementation experiments, i.e. the modification of an individual profile by the addition of specific compounds, have shown an effect on nestmate recognition for some alkanes like hexadecane and octadecane (Breed and Stiller, 1992) but these are absent on the cuticle of worker bees. Alkenes such as *Z*-(9)-tricosene, which are present on the cuticle, have an effect on nestmate recognition by guard bees while other

compounds like dodecane, tricosane and pentacosane do not (Breed, 1998). Recently Dani *et al.* (submitted) showed that supplementation of alkenes rather than alkanes modified the recognition cues of worker honeybees and caused them to be rejected from their own colony. Furthermore, Breed *et al.* (2004) postulated that although hydrocarbons may play a part, fatty acids are more important recognition cues used by honeybees. Thus, the role of hydrocarbons on nestmate recognition is still a controversial issue. Moreover, the learning and discrimination abilities of bees towards individual cuticular hydrocarbons, a prerequisite for their use in chemical communication, have still not been fully investigated.

In the laboratory, honeybees can learn to associate olfactory stimuli with a sucrose reward, according to the proboscis extension response (PER) conditioning paradigm (Kuwabara, 1957; Bitterman *et al.*, 1983). When the antennae of a hungry bee are touched with sucrose solution, the animal reflexively extends its proboscis. Other odours and stimuli presented to the antennae do not usually elicit such a reflex in naive animals. However, if an odour is presented immediately before sucrose solution (forward pairing), an association is formed and the odour will subsequently release the PER in following tests. This effect relies on classical (Pavlovian) conditioning (Bitterman *et al.*, 1983), with the odour as the conditioned stimulus (CS) and the sucrose solution as the reinforcing unconditioned stimulus (US). This paradigm has been used to study the olfactory discrimination abilities of bees and has shown that they can differentiate between many odours (Vareschi, 1971). With this assay, Getz *et al.* (1986, 1988) showed that workers can discriminate adult, larvae and eggs using volatile and contact chemicals, and Getz and Smith (1987) demonstrated that bees can discriminate between different mixtures of tricosane and pentacosane. More recent work by Fröhlich *et al.* (2000, 2001), using different fractions of non-polar and polar compounds, showed that bees could not discriminate the hydrocarbon profiles of different comb waxes, nor drone and worker cuticular waxes, and concluded that compounds other than hydrocarbons were more likely to be involved in recognition.

In the present work, we use PER differential conditioning to determine the discriminatory and learning abilities of bees presented with individually synthesized long-chain alkanes and alkenes present on the cuticle of worker bees. By studying bees' ability to perceive and discriminate cuticular hydrocarbons with differing structures we aimed to determine if bees can use hydrocarbons as recognition cues in general and if specific compounds are more likely to be used in nestmate recognition.

Material and methods

Bees

Workers were collected at random from the top box of a populous colony occupying two Langstroth hive boxes and containing ~20 000 bees. The collected workers were kept for 30 min at 33°C in groups of 10 to starve them. They were then chilled until motionless and placed into plastic straws and held in by means of a pin inserted between the thorax and the abdomen which immobilized them without harming them. This allowed free movement of the head, antennae and forelegs of the workers. Workers were then starved for an additional three h in the restraining device prior to the beginning of the experiments.

Preparation of odours

Aliquots of 320 μg of each compound were made up in crimp top vials. Before each experiment, these aliquots were suspended in 160 μl hexane and then 10 μl was evaporated on a glass rod (heat-sealed Pasteur pipette) so that the tip-most 1 cm of each rod was coated by 20 μg of compound. Sixteen glass rods could be made with each aliquot, which were used within 2 days, eight per day. Rods were kept in an oven at 60°C for at least 15 min prior to testing to ensure that all compounds were liquid when tested. A random sample of glass rods (8) were analysed by injecting a hexane wash in a gas chromatograph-mass spectrometer after use in the conditioning experiments, and all proved to still have at least 98% pure initial compounds, showing that no contamination occurred during the experiments.

PER conditioning

Experiments were performed in a temperature-controlled room kept at 25°C. Each bee, restrained in a straw, was placed in a wooden rack with regularly-spaced slots 4 cm apart and kept there during all experiments. We used differential PER conditioning procedures, in which one hydrocarbon is rewarded (CS+), and another hydrocarbon is unrewarded (CS−). Bees received 6 CS+ presentations and 6 CS− presentations in the following pseudo-randomized order: −++−+−+−++−. The sequence always started with a CS− presentation and then a CS+ presentation, so that possible spontaneous responses to the two odours could be recorded prior to the first presentation of the sucrose US. After the 12 conditioning odour presentations, each bee was subjected to an additional control presentation with a blank rod treated with hexane only, to make sure that the bees were responding to the compound and not to the mechanical stimulation.. Bees responding to the blank rod were discarded from the analysis.

During CS+ presentations, the hydrocarbon was presented for 6 s by touching the antennae of the bee with the hydrocarbon glass rod. Three seconds after onset of the CS, the antennae were contacted with a 30% sucrose solution (w/w). The subsequent proboscis extension was then rewarded by feeding the bee with a drop of the same solution. During CS− presentations, bees were presented with an odour in the same way but without the subsequent presentation of sucrose. The interval between each odour presentation was 15 min.

Individuals showing spontaneous responses at the first presentation of the CS− were recorded, and such spontaneous responses were compared among hydrocarbons (see Results). These individuals were then discarded from later presentations. Individuals showing spontaneous responses to the first CS+ presentation were recorded all through the experiments. However, learning and discrimination abilities were analysed only from individuals showing no spontaneous responses either to the CS− or to the CS+, since later responses of such individuals could not be interpreted as purely associative. Furthermore, only bees that showed a normal proboscis extension when stimulated with the US of sucrose in more than three of the CS+ presentations were kept for the following steps of the experiments.

Hydrocarbons tested

In order to obtain meaningful comparisons between compounds with potential roles in recognition, we chose alkanes and alkenes among the compounds most abundant on the honeybee cuticle (Blomquist *et al.*, 1980; McDaniel *et al.*, 1984; Carlson *et al.*, 1989; Francis *et al.*, 1989; Wakonigg *et al.*, 2000). These were the alkanes heptacosane (C27), nonacosane (C29) and hentriacosane (C31); and the alkenes 9(*Z*)-pentacosene (9-C25:1), 9(*Z*)-heptacosene (9-C27:1), 8(*Z*)-nonacosene (8-C29:1), 9(*Z*)-nonacosene (9-C29:1), 9(*Z*)-hentriacosene (9-C31:1), 10(*Z*)-hentriacosene (10-C31:1) and 10(*Z*)-tritriacosene (10-C33:1), representing respectively 19.5, 14.2, 10.1, 1.8, 1.8, 2.7, 13.0 and 15.8% of foragers cuticular hydrocarbons as reported by McDaniel *et al.* 1984 (alkenes differing by double-bond positions pooled as they were not separated in the original paper), with a pooled total of 78.9% of the total hydrocarbons present on the cuticle.

Linear alkanes were purchased from Fluka (Sigma Aldrich Company, Ltd). Alkenes were synthesized following standard Wittig procedures following methods already described (Dani *et al.*, 2001). As previously reported (Dani *et al.*, 2001), the *Z*-geometrical purity of all alkenes was >98% as assessed by GC-MS.

The rationale behind the choice of odours used for each experiment was to test for differences in discriminatory abilities of compounds differing in chain-length, chemical nature (alkanes versus alkenes) and double-bond position. It was not possible, however, to test every pair of all the compounds of interest, or to test each odour pair on each day. We therefore divided the experiments into five groups of three or four hydrocarbons that could be tested simultaneously in a randomized way. For each pair of hydrocarbons tested, each hydrocarbon was presented both as the rewarded hydrocarbon (CS+) and as the unrewarded hydrocarbon (CS−) because of possible discrimination asymmetries. Different bees were tested with the two combinations simultaneously (on the same days). In this paper, when a pair of hydrocarbons is noted as A+/B−, the first compound is the CS+ and the second the CS−.

Statistical analyses

Since bees were subjected to six presentations of the CS+ (rewarded compound) and six presentations of the CS− (unrewarded compound), and only bees not showing a spontaneous response to the CS+ or the CS− were kept, bees could give between 0 and 5 responses to each odour during the trial. To check whether bees significantly preferred the CS+ over the CS−, we used a Wilcoxon test for matched pairs.

Two indexes were used to get a more detailed analysis of the responses: the first was a learning index, used to characterize the learning abilities of individual bees with the different odours. The index is defined by the number of conditioned responses (CS+) associated with an odour. For graphic purposes, we represented it as a mean proportion of the five possible responses (Figure 3A). The index range is thus between 0 and 1, with 1 representing perfect learning.

We also used a discrimination index, defined as

$$\frac{(CS+)-(CS-)}{(CS+)+(CS-)}$$

where (CS+) is the number of responses to the CS+ and (CS−) is the number of responses to the CS−. This gives an index between −1 and 1, with 0 meaning that the bee responded equally to the CS+ and to the CS−, thus showing no discrimination. A value of 1 would mean that a bee responded only to the CS+, thus showing total discrimination. Numbers below zero mean that the bee showed more responses to the CS− than to the CS+. We used the Kruskal–Wallis test to test for significant differences by comparing indexes for individual bees across odours or odour pairs for each experiment. When significant, it was followed by two-by-two comparisons using the Noether method (Scherrer, 1984) with Dunn–Sidák threshold corrections.

Results

Over a total of five experiments, 20 hydrocarbon pairs were tested both ways (i.e. with each hydrocarbon as the CS+ and the CS−; 40 tested pairs), with one pair (9C29:1 versus 9C31:1) being repeated in two combinations of hydrocarbons (Figure 3). In total, 2012 worker bees were used in the experiments. Of these, 297 (14.7%) responded spontaneously to the CS− at the first trial and were discarded from the experiments. An additional 197 (11.4%) bees responded spontaneously to the CS+ and 130 (7.6%) to the control presentation, and were discarded from the analysis, with some workers responding to both (1.6%). Forty-seven bees (2.5%) did not respond to the unconditioned stimulus more than three times and were also discarded. Overall, 643 bees were rejected (32%), and the results were obtained from 1369 worker bees, with a mean ± SD of 32.6 ± 2.4 workers per hydrocarbon pair.

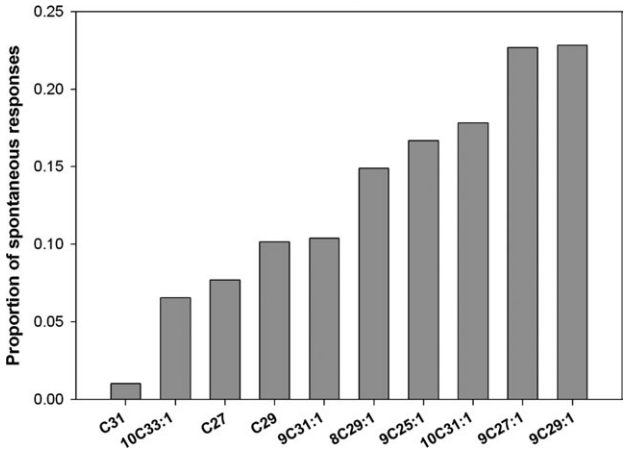

Figure 1 Proportion of spontaneous responses to the tested compound at the first trial of the experiments. Results from all five experiments have been pooled.

Spontaneous responses, learning index and hydrocarbon groups

The proportions of spontaneous responses observed for each hydrocarbon in the first CS− conditioning trial varied between 0.01 for C31 and 0.23 for 9C29:1 (Figure 1). The spontaneous responses were pooled as they are measured during the first odour presentation of the CS−, before any other odour or reward is presented, unlike the measure of the learning index which could vary according to the CS−. There was an overall significant difference in these responses (χ^2 = 19.65, df = 9, P = 0.02). Five compounds, including all the alkanes and two alkenes, 9C31:1 and 10C33:1 gave low levels of spontaneous responses while the other alkenes elicited higher levels of spontaneous responses (Figure 1).

There were also significant differences in the learning success of bees with the different compounds used as CS+ (CS+ curves in Figures 2 and 3A). Alkanes were generally poorly learnt, as shown by the curves for CS+ responses (Figure 2, first three columns), and by the low learning indexes (Figure 3A, 1, 3 and 4), which were always below 0.61. Two alkenes, 9C31:1 and 10C33:1, showed similar low learning performance (Figure 2, respective columns) and low learning indexes, below 0.61 (Figure 3A, 2, 4 and 5). Alkane indexes did not differ significantly from each other or from that of 9C31:1, and although 10C33:1 was not tested together with these compounds, its learning index differed significantly from that of other alkenes such as 9C29:1 and 9C25:1.

All the other alkenes showed high learning curves, reaching 80–100% conditioned responses (Figure 2; see respective columns), and had mean learning indexes between 0.67 and 0.92 (Figure 3A, 2–5). The learning indexes of these alkenes did not differ between each other, but were significantly higher than those of the alkanes or of 9C31:1 and 10C33:1 (Figure 3A, 2–5). Within experiments or overall, there were no significant differences in the learning indexes of individual CS+ odours according to the odour presented as CS− except for 9C31:1 in experiment 2, where the mean learning index when the CS− was 9C29:1 (0.52) was different from when 9C27:1 was the CS− (0.62; Kruskal–Wallis test, P = 0.047; all the other tests gave Ps > 0.3). This indicates that the learning indexes observed for odours when used as CS+ did not vary according to the CS−, therefore validating the use of the learning index.

We observed that the clear differences between odours concerning learning success mirrored the trend observed in the spontaneous responses, since the five least-well-learnt hydrocarbons (the three alkanes and the two alkenes 9C31:1 and 10C33:1) were also those that showed the lowest spontaneous responses (Figure 1). The clear-cut difference observed between hydrocarbons in learning performance suggested that it was meaningful for further analysis to divide the hydrocarbons into two groups according to the learning success: the alkanes and 9C31:1 and 10C33:1 in a low learning index group (LL) and the other alkenes in a high learning index group (HL).

Discrimination success and asymmetries

Out of the 40 tested pairs, 13 pairs of hydrocarbons gave non-significant discrimination results (Wilcoxon matched-pairs test), with bees responding with similar probability to the CS+ and to the CS− (Figure 2). Nine of these involved pairs in which a LL compound was the CS+ (Figure 2). In the four cases where both odours were HL compounds, the pairs could not be discriminated whichever way they were tested, and they involved the compounds with the highest learning indexes, 9C29:1 versus 9C27:1, and two very close compounds with regards to formula, 9C29:1 and 8C29:1 (Figure 2, columns 9C27:1–9C29:1; Figure 3B, experiments 2 and 5).

Although in most cases (27 out of 40 tested HC pairs), bees could discriminate between the CS+ and the CS−, there were important differences in the magnitude of this discrimination. We therefore used the discrimination index, which quantifies such differences, to get a better picture of bees' discrimination ability regarding different cuticular hydrocarbons (Figure 3B).

LL versus LL odours

For pairs of LL hydrocarbons, discrimination was always very low (see e.g. how close together the CS+ and CS− curves are for different alkanes in Figure 2). Discrimination indexes were therefore also low, ranging from 0.11 to 0.55, without any significant differences between them. The biggest difference in a pair was for 9C31:1 versus C31, with bees discriminating better when the alkene was rewarded. Altogether, bees discriminated poorly between alkanes (Figures 2, upper left and 3B, 1), and no clear trend in the discrimination index appeared between them (range 0.11–0.36, Figure 3B, 1).

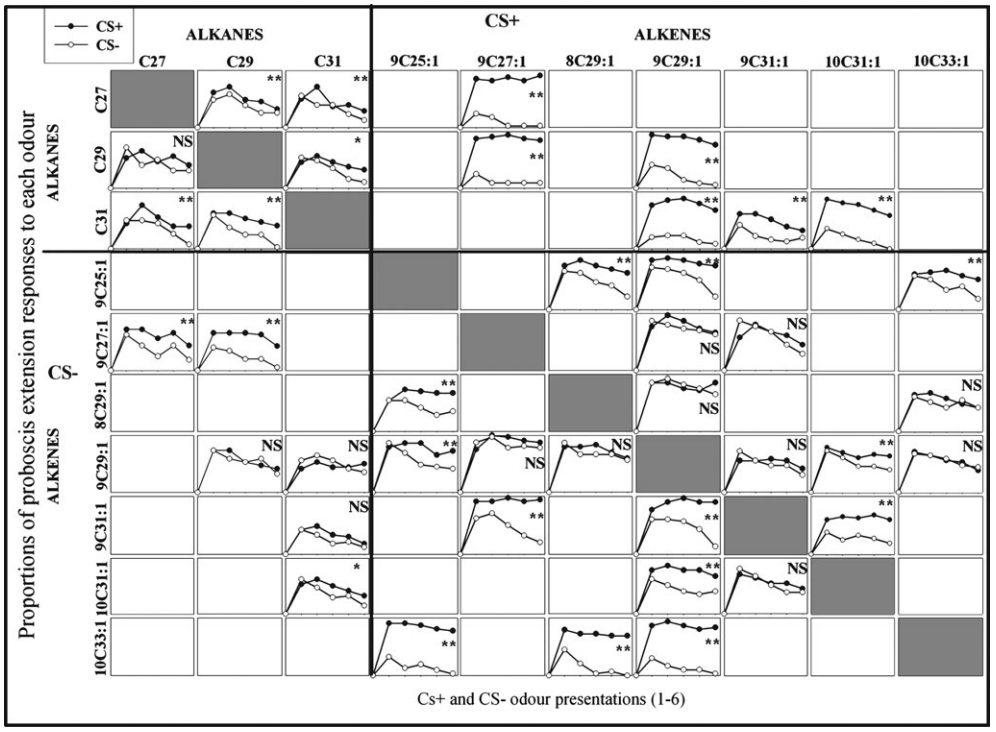

Figure 2 Proportions of proboscis extension responses to the CS+ and the CS− during differential conditioning experiments between each odour pair. Significant differences between the response curves are indicated by $*P < 0.05$; $**P < 0.01$ (Wilcoxon test for matched pairs).

LL versus HL odours

When an HL odour was rewarded (CS+) against an LL hydrocarbon (CS−), the discrimination index was always high, between 0.70 and 0.84 when the LL hydrocarbon was an alkane or 10C33:1 and between 0.38 and 0.53 for 9C31:1. When the LL hydrocarbon was rewarded the discrimination was low, between −0.14 and 0.45. These differences caused a systematic significant asymmetry between the discrimination index for the two hydrocarbons of a pair. In all but one of the 12 pairs tested, the discrimination index for HL+/LL− situations was significantly higher than that for LL+/HL− situations (see respectively the black and white bars in Figure 3B, 2–5). The remaining pair was 9C27:1 versus C29 where C29+/9C27:1− did not differ from the alkene+/alkane- indexes.

HL versus HL odours

When both hydrocarbons were HL, discrimination was generally low. The discrimination index for HL alkenes ranged between 0.03 and 0.35, and never significantly differed between odour pairs.

Discussion

In this work, we used 10 hydrocarbons, representing almost 80% of the honeybee cuticular hydrocarbon profile, and the PER conditioning technique to evaluate how well bees learn and discriminate these compounds. Our results show that bees can discriminate between most of the cuticular hydrocarbons tested, but that there are clear differences in learning and discrimination abilities according to the nature of the compounds. There appears to be a clear divide between alkanes and alkenes, with alkenes being generally much better learnt than alkanes.

Honeybees are known to be able to learn a very wide range of odours in an appetitive context, like in PER or free-flying experiments (Vareschi, 1971; Menzel, 1985; Laska *et al.*, 1999). In particular, they can learn odours with a strong pheromonal value (queen pheromonal compounds, alarm pheromones, social aggregation pheromone) (Vareschi, 1971; Smith and Menzel, 1989; Smith, 1991; Sandoz *et al.*, 2001) or even initially aversive odours (von Frisch, 1965; Kriston, 1971). Thus, the efficiency with which bees learn odours in the PER conditioning context gives us important information about how well such odours are perceived mostly independently of the biological value they might have. We thus think that the odours which were not learned efficiently by bees in our experiments are odours that are not well detected by the bee nervous system, i.e. not very salient odours. This happened mainly with alkanes. This may not be surprising, as alkanes have only one distinguishing feature in the length of their carbon chain, whereas alkenes have at least three distinguishing features—the bend of the double bond, the length of the short chain between one terminus and the double bond, and the length of the long chain between the other terminus

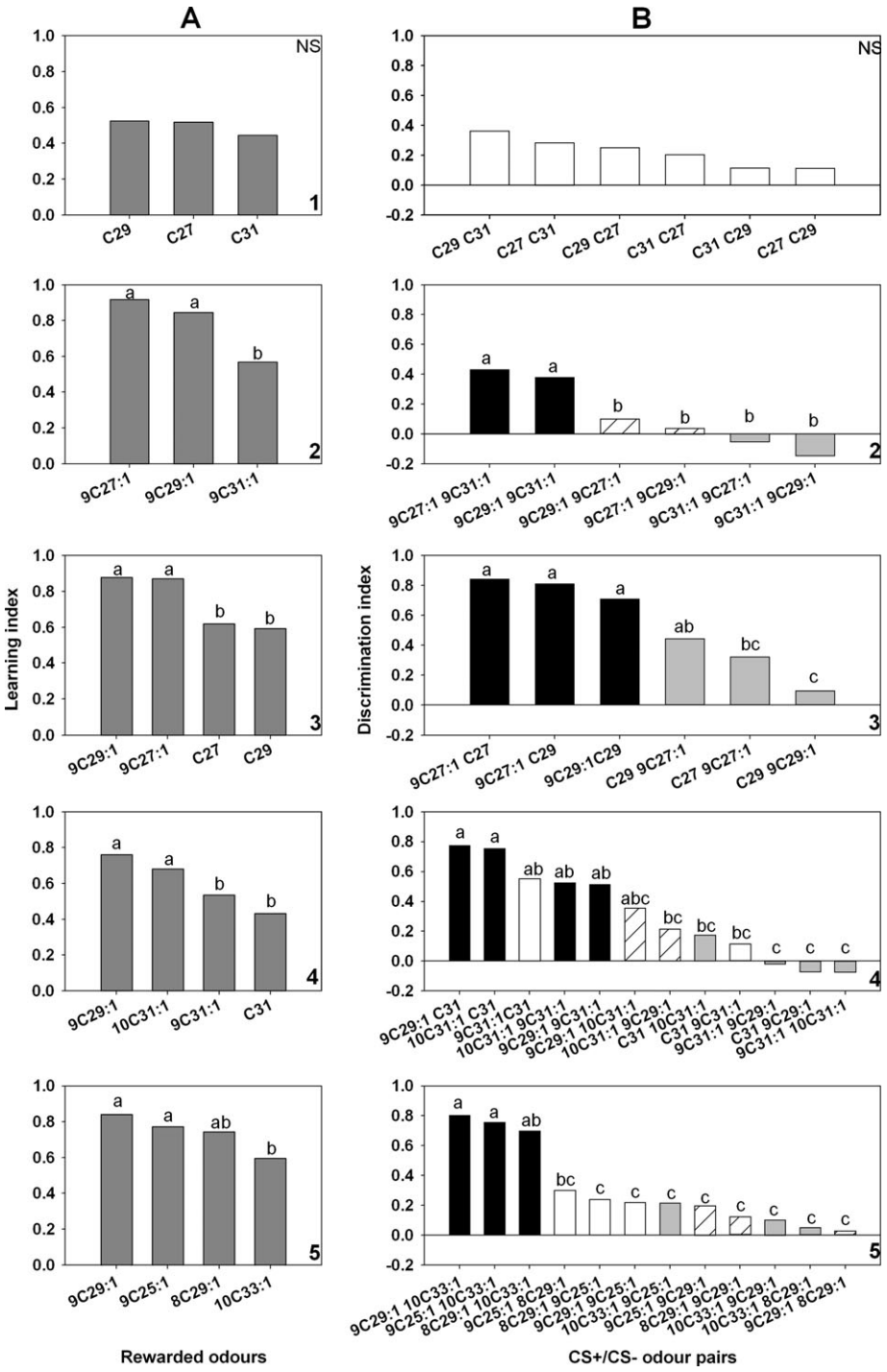

Figure 3 Discrimination and learning ability for the different hydrocarbons and hydrocarbon pairs in the five experiments, numbered 1–5. NS indicates a non-significant overall difference. Different letters indicates significantly different values for experiments where the overall Kruskal–Wallis test was significant. **(A)** Learning index for individual compounds. **(B)** Discrimination index for all the hydrocarbon pairs tested. The first hydrocarbon of each pair is the rewarded hydrocarbon. The colour of the bars indicates the type of comparison between well-learned (HL) and less well-learned odours (LL): black, HL+/LL−; grey, LL+/HL−; white, LL+/LL−; white patterned, HL+/HL−.

and the double bond. What is striking is that the two LL alkenes 9C31:1 and 10C33:1 have long chains of 21 and 22 carbon atoms respectively. This, coupled with the fact that the HL 10C31:1 is distinguishable from the LL 9C31:1, strongly suggests that there is a link between how well the molecules are perceived and the long carbon chain length.

Calcium imaging experiments, as carried out on the honeybee brain, allow odour-evoked activity in olfactory brain

areas to be recorded (Joerges *et al.*, 1997; Faber and Menzel, 2001), giving some insight into how odours are perceived by the brain. In the antennal lobe, the first relay of the olfactory pathway, odours have been shown to elicit glomerular response patterns (Joerges *et al.*, 1997) based on a code which is conserved between individuals (Galizia *et al.*, 1999; Sachse *et al.*, 1999). Since these responses emphasize the activity of sensory neurons (Galizia and Menzel, 2001), and because sensory neurons carrying one type of receptor seem to all project to the same glomerulus (Voshall, 2000), calcium imaging of the antennal lobe gives us an idea of the sensitive range of possible olfactory receptors on the bees' antennae. In one study, Sachse *et al.* (1999) presented bees with C5–C13 hydrocarbons. Results showed that odour-evoked responses were only obtained for the shortest-chained alkanes (C5–C9), in which very few glomeruli responded, which also responded to several other oxygenated compounds with the same chain lengths (alcohols, aldehydes, ketones). No signals appeared for the longer-chained alkanes (C10–C13), and very long-chained alkanes (like our C27, C29 or C31) were not tested. The results obtained by Sachse *et al.* (1999) suggest that, at least on the surface of the antennal lobe, which is accessible to optical imaging studies (about 40 glomeruli out of the 165 present in the lobe), no glomerulus is specifically sensitive to alkanes, and none responds to long-chained alkanes. Because the olfactory code is thought to be highly redundant (Galizia *et al.*, 1999; Galizia and Menzel, 2001), it could be that long-chain alkanes bind only non-specifically onto odour receptors, and do not therefore give rise to very salient or clear neural representations. In this case, they would represent poor substances to act as nestmate recognition cues (see below). However, this remains a hypothesis, since not all regions of the antennal lobe have yet been explored with the imaging technique and long-chain alkanes and alkenes specifically have not been tested.

Our results can allow some hypotheses to be made on the potential use of cuticular hydrocarbons in nestmate recognition. First, compounds which were not well learned appear unlikely to have any role in chemical communication. Interestingly, these compounds include the most abundant compounds on the bee cuticle, namely alkanes and the longer-chain alkenes like 10C33:1 (74–91% of the compounds we tested on the cuticle) (McDaniel *et al.*, 1984). Recent supplementation experiments of cuticular hydrocarbons (Dani *et al.*, submitted) and experiments in other species like *Polistes* (Dani *et al.*, 2001) have also confirmed that these compounds are not likely to be used for nestmate recognition.

On the other hand, the ability of workers to learn shorter-chained alkenes below 29 carbons makes these compounds likely candidates for recognition cues. In our experiments, however, these structurally similar compounds were not always discriminated well, and when they were, the discrimination indices were generally low. This generalization phenomenon is, however, not uncommon for biologically active compounds like pheromones, even when their chemical structure is very different, like the honeybee alarm pheromones 2-heptanone and isoamylacetate (Sandoz *et al.*, 2001). It is possible that in a context other than the appetitive context of conditioning, such as while guarding at the entrance to the hive, the bees could be more motivated to discriminate between these compounds or that the bees would class compounds together in groups. Moreover, we do not have data on how bees would respond to mixtures of the different odours and discrimination could be increased in this case, as was found by Getz and Smith (1987) in an experiment using C23 and C25. Of particular interest would be to test different mixtures of alkenes with the same carbon number but different double-bond position (like 9C31:1 and 10C31:1), the proportions of which have been shown to change according to race but could also vary between colonies (Carlson *et al.*, 1989) and give reliable cues about colony origin. Chemical properties and recognition ability can also change between the compound alone and the compound in a mixture of different compounds, and this can be influenced by the solid or liquid phase of the compounds (Gibbs, 2002). An indication of this comes from the fact that bees examined by guards increase their thorax temperature, possibly to improve chemical communication (Stabentheiner *et al.*, 2002). In contrast to our results, Fröhlich *et al.* (2000, 2001) have found, using PER conditioning, that the hydrocarbon fraction of different comb waxes and cuticular waxes are not discriminated by honeybees, and hence these authors conclude that hydrocarbons cannot be used as cues for nestmate recognition. In their work, however, they have tested the cuticular extracts from two different castes, namely males and workers, coming from the same colony. The colony signature being the same could explain the absence of discrimination in the learning experiments.

In conclusion, our experiments have shown differences in the learning and discrimination ability of cuticular hydrocarbons by honeybees. The most common compounds on the cuticle (alkanes and long-chained alkenes) are learnt least well, which could mean that such compounds are not used for recognition and probably only have a role against desiccation. Less common compounds, like shorter-chained alkenes, were well learnt and easily differentiated. This suggests that bees could have the ability to use such compounds in a recognition context. The PER conditioning approach thus appears a useful method for filtering through the many compounds present on the cuticle. Further tests on cuticular hydrocarbons could include PER conditioning with mixtures of the well-learned compounds. Such tests should be linked with correlational and supplementation studies in order to improve our understanding of nestmate recognition in bees.

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