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**Original article**

# Learning and discrimination of honey odours by the honey bee

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**Abstract** – We used classical conditioning of the proboscis extension response to test whether the natural discrimination ability of honey bees could be used to assess the origin of honeys. Five honeys were used as the conditioning stimuli in the procedure: linden (*Tilia* spp.), oilseed rape (*Brassica napus*), eucalyptus, sunflower (*Helianthus annuus*), and black locust (*Robinia pseudoacacia*). Bees exhibited high levels of conditioned responses to all honey odours. Responses to the conditioned honey were usually the highest, but high levels of generalisation; i.e. behavioural response to other honeys, were recorded. Using a differential conditioning procedure where one honey odour was rewarded and another odour was explicitly unrewarded, we showed that honey bees could not always differentiate between honey types. The potential use of the honey bee as a biological detector to discriminate among honeys is discussed.

**honey bee / olfactory learning / discrimination / honey / melissopalynology**

## 1. INTRODUCTION

Honey is a clearly defined food product, following the European Community law 74/409/EEC. However, official methods for honey analyses are of limited reliability and are difficult to put into practice, which can lead to non-compliance of the regulatory standards for honey trade. Honeys might be adulterated with beet, corn or cane sugar, fraudulently labelled as monofloral, and given an incorrect geographical or industrial origin. Among the existing methods, melissopalynology is the primary method to confirm geographical and botanical origins of honey. However, pollen analyses often cause interpretation problems, and results may differ from one expert to another. Thus, even though this method is essential to establish the floral origin of honeys, in many cases it is necessary to conduct complementary studies. Adulteration of honeys by addition of sugars coming from C4 plants (like corn or sugar cane), for instance, can be detected by microscopic analysis and/or chemical measurements (e.g. $\delta^{13}C$ contents, Kerkvliet and Meijer, 2000). But such methods can be applied only in specific cases where adulteration is suspected. Therefore, it would be highly valuable to introduce novel analytical methods of detecting the origin and quality of honey.

Honey bee, *Apis mellifera* L., foraging behaviour is based on an associative conditioning process, in which bees associate the chemical and visual cues of flowers with the uptake of food. Among floral stimuli, the

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odour-food association is the most efficient for the recognition of floral sources (Kriston, 1973; Menzel et al., 1993). Floral aromas are known to be complex mixtures of tens to hundreds of components, but behavioural and electrophysiological studies have shown that floral volatile extracts are recognised on the basis of a limited range of key-components (Pham-Delègue et al., 1986, 1993; Thiéry et al., 1990; Le Métayer et al. 1997). Honey odours, as flower odours, are characterised by specific compounds, which can be identified using physico-chemical analyses (Bicchi et al., 1982, 1983; Bouseta et al., 1992). Therefore, one can hypothesize that honey bees may be able to discriminate between the odours of honeys of different origins as they do between floral odours. This work is an attempt to test this hypothesis, and to estimate the value of honey bees as biological detectors of honey volatiles and thus, as a tool to better assess the origin and quality of honey.

The ability of honey bees to learn olfactory cues, which is crucial in their foraging behaviour, can be investigated under controlled laboratory conditions. Numerous studies on learning and memory in the honey bee have used a bioassay based on the classical conditioning of the proboscis extension response (PER) (Bitterman et al., 1983; Brandes, 1988; Getz and Smith, 1991; Menzel et al., 1993; Sandoz et al., 1995). In this bioassay, the age, and prior experience of the bees, and the stimulus delivery can be controlled. In the present work, we addressed the question of the possible use of honey bees' olfactory learning abilities to differentiate honey odours, by performing PER conditioning procedures with these odours as conditioned stimuli.

## 2. MATERIALS AND METHODS

### 2.1. Honey sources

Five honeys from various origins were chosen: Linden, *Tilia* spp. (France), oilseed rape *Brassica napus* L. (Cher-Indre, France), eucalyptus *Eucalyptus* spp. (Spain), sunflower *Helianthus annuus* L. (Charentes, France), and black locust *Robinia pseudoacacia* L. honey (Landes, France). To check the botanical origin and quality of these honeys, physico-chemical, human sensory and melissopalynological analyses were carried out using standard methods (colour – Aubert and Gonnet, 1983; Hydroxy-methylfurfural (HMF) dosage – Jeurig and Kuppers, 1980; and organoleptic characteristics – Gonnet and Vache, 1985). The mellissopalynological study included the identification and counting of pollen grains and other particles present in the honey samples. This was done following classical methods (Louveaux et al., 1978). The quantitative analyses were based on a single recording, using the counting method reported by Cour (1974). Contrary to Cour's procedure, no prior acetolysis was done to determine the total pollen content in 10 g of honey.

The results of the physico-chemical and sensory analyses corroborated the monofloral denomination of the five honeys (Tab. I). The total number of pollen grains from the mellissopalynological analysis that corresponded to the reference plant for the monofloral denomination in each honey sample is also reported in Table I. According to Maurizio (1939), the characterisation of a monofloral oilseed rape honey is based upon a percentage of pollen of 88% (between 20 000 and 100 000 grains in 10 g of honey) and that of a monofloral sunflower honey corresponds to a percentage of 15–90% *Helianthus* pollen (the Italian norm indicates 19 000 grains in 10 g of honey). Our data show that the two honeys fit with the denomination. For eucalyptus honey, the sample did not fit with the denomination, since the percentage of *Eucalyptus* pollen found in our sample was 52% (norm: more than 90%). However, this percentage usually varies with the country of origin and can be between 60 and 90% (Ricciardelli d'Albore, 1997). The honey used in our experiment could be considered as a "honey containing eucalyptus" rather than an "eucalyptus honey" stricto sensu. Similarly, a honey is called "linden honey" when it contains 30% of *Tilia* pollen, and called "black locust honey" when it contains 40% of *Robinia* pollen. The two honeys in this study contained respectively 6% of *Tilia* pollen and 6% of *Robinia* pollen, which were far from reaching standard values. Thus, while all honeys fit with the norm on the basis of physico-chemical and sensory analyses, mellissopalynology only confirmed the denomination of two honeys out of five.

### 2.2. Honey bees

Workers of *Apis mellifera* were collected immediately after emergence from combs of outdoor hives. They were caged in groups of 70 individuals and maintained in an incubator (32–34 °C, 55% relative humidity, dark). Bees were supplied sugar and water ad libitum, and with ground pollen of multifloral origin during the first 8 days only. They were used in the conditioning procedures at an age of 14–15 days, when their learning performances are usually the best in this

**Table I.** Organoleptic quality, physical and chemical properties, and melissopalynological characteristics of the five honey samples used in the conditioning experiments.

| Honey type | Organoleptic characteristics | | | Physical and chemical analysis | | | Mellissopalynology | |
|---|---|---|---|---|---|---|---|---|
| | Physical state | Colour | Aroma and flavour | Moisture (%) | Colour scale of pfund (cm) | HMF (mg/kg) | Pollen grains /10 g | % pollen of the reference plant |
| **Linden** (*Tilia* spp.) | Solid, finely crystallized | Amber | Persistent, characteristic, very aromatic | 16.6 | 8.5 | 17.28 | 3 550 | 6 |
| **Oilseed rape** (*Brassica napus* L.) | Solid, very finely crystallized | White | Characteristic, sweet flavour. | 17.6 | 2.0 | 1.73 | 46 810 | 87 |
| **Eucalyptus** (*Eucalyptus* spp.) | Solid, finely crystallized | Amber | Intense, complex, characteristic, strong and persistent | 16.1 | 7.1 | 9.98 | 59 020 | 52 |
| **Sunflower** (*Helianthus annuus* L.) | Solid, crystallized | Yellow amber | Aromatic flavour, characteristic | 17.7 | 5.9 | 3.84 | 4 150 | 16 |
| **Black locust** (*Robinia pseudoacacia* L.) | Liquid | White | Characteristic | 16.4 | 1.7 | 2.69 | 2 230 | 6 |

bioassay (Pham-Delègue et al., 1990a). Bees were mounted individually in glass holders, leaving the antennae and mouth parts free. They were left to starve for 4 hours before the experiments began.

### 2.3. Stimulation apparatus and odour source

The odour stimulation device created a constant flow of 52.5 $cm^3$/s, either scented or unscented, which was delivered to the bees through a 1 cm glass tube. This flow was comprised of a main vector airflow (50 $cm^3$/s), and of a secondary one (2.5 $cm^3$/s) injected into the main airflow used for odour stimulation. The odour source was an $80 \times 3$ mm piece of filter paper soaked with 150 mg of honey or with 10 µL of pure ethyl phenylacetate, which was inserted in a disposable Pasteur pipette. The secondary flow was shifted by the experimenter either through the pipette containing the odour source or through an identical empty pipette. A fan placed opposite the delivery tube extracted the released odours from the experimental room.

### 2.4. Conditioning of the Proboscis Extension Response (PER)

Conditioning trial: At the beginning of each trial, bees were positioned for 15 s in the airflow to become familiarized with the mechanical stimulation. The Conditioned Stimulus (CS), the odour, was then presented for 6 s. Three s after the onset of the CS, the antennae were contacted with a 30% sucrose solution (w/w), the Unconditioned Stimulus (US). The subsequent proboscis extension was then rewarded by feeding the bee with a drop of the same solution. The US was thus a typical compound-US, consisting first in the stimulation of antennae and then in a reward to the proboscis. Individuals showing spontaneous responses at the first presentation of the odour were recorded but then discarded 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 were kept for the following steps of the experiments.

Air-control trial: To check if bees had been conditioned to the odour CS and not to the airflow, a blank trial was performed. After 15 s familiarisation to the airflow, a 6-s stimulation with air (coming from an empty pipette) was given to the bee. Any bee responding to this trial was discarded. Such bees represented 13% of all tested bees.

Test trial: On each trial, bees were placed in the airflow for 15 s and were then exposed to the test stimulus alone for 6 s. Any proboscis extension occurring during the 6-s stimulation period was recorded.

All experiments were carried out with 15-min inter-trial intervals, since such long intervals produce better acquisition and higher retention (Menzel et al., 2001).

### 2.5. Experiment 1 – learning honey odours

We first examined whether bees can learn the odour of the five honeys under investigation. Bees were subjected to 3 conditioning trials with the odour of one honey (acquisition of the conditioned response), followed by an air-control trial, and by five test trials with the same odour (extinction of the conditioned response). Independent groups of bees were used for each honey, and, to avoid confounding the effects of day with a particular group of bees, 6 individuals per group were run daily until about 30 individuals were obtained in each group.

### 2.6. Experiment 2 – generalisation among honey odours

To investigate the specificity of olfactory honey learning, independent groups of bees were conditioned to the odour of one of the five honeys, or to a pure compound, ethyl phenylacetate. This pure chemical was described as a typical descriptor of honey odour for perfume and aroma professionals (Jaubert et al., 1987) and is here used as control for the specificity of responses to honeys. Honey bees were subjected in turn to 3 conditioning trials, an air-control trial, and to a test phase. In the test phase, bees received test trials with all six odours presented in a random order. All groups were run daily and the experiment was repeated until about 30 bees per group were obtained.

### 2.7. Experiment 3 – differential conditioning with honey odours

To test the abilities of bees to differentiate among honeys, we used a differential conditioning procedure where bees were alternately stimulated with two odours. One odour (Positive Conditioned Stimulus – CS+) was always associated with the US, whereas the second odour was never associated with the US (Negative Conditioned Stimulus – CS-). Bees were subjected to a conditioning phase, in which they received five trials with the CS- and

five trials with the CS+ in an alternating order. Five honey odours were tested, so 20 groups had to be run simultaneously (i.e. 10 pairs of honeys, multiplied by 2, each honey of a pair being either CS+ and CS-). Thus, 2 bees per group were run daily, until a total of about 30 individuals per group was obtained.

### 2.8. Statistical analyses

In experiment 1, to follow the performances of bees through the whole procedure, we compared their responses on three given trials: (1) the first conditioning trial (measuring spontaneous responses to honey odours); (2) the last conditioning trial (assessing the efficiency of conditioning); and (3) the last test trial (assessing extinction of the conditioned response). $Chi^2$ tests with n – 1 df (n = 5) were used. In experiment 2, the conditioning procedure was analysed as above following steps 1 and 2. In the test phase, the responses of bees to the different stimuli were compared for each conditioning group using Cochran's Q test (with n – 1 df, n = 6), since this test allows for a within-group comparison of dichotomous variables. For a more comprehensive analysis of the data, we then considered the responses of bees to 3 types of stimuli: the CS, other honey odours and ethyl phenylacetate. Each experimental group was then considered as an observation, and the percentages of response obtained in each case were grouped according to the 3 types of stimuli. A Kruskal-Wallis test was used to compare the responsiveness of bees among them. When significant, it was followed by two-by-two comparisons using the Noether Method (in Scherrer, 1984) with Dunn-Sidák threshold corrections ($\alpha' = 1 - (1 - \alpha)^{1/k}$ where k is the number of two-by-two comparisons in which the same set of data is involved; $\alpha' = 0.025$). In experiment 3, we first evaluated whether by the end of the differential conditioning phase, bees had solved the task presented to them. To do so, we counted for each bee the number of responses it produced to the CS+ and to the CS- in the last 6 trials of the procedure (3 CS+ trials and 3 CS- trials). In each of the 20 groups, these numbers, ranging from 0 to 3, were compared using a Wilcoxon test for matched pairs (1 df). A significant outcome showed that bees significantly differentiated the CS+ and the CS-. In the test phase, the homogeneity of responses to the different honeys was carried out using Cochran's Q test (4 df) as before. A more comprehensive comparison was made on the percentages of responses obtained in all groups to three types of stimuli (CS+, CS- and other honey odours), using a Kruskal-Wallis test and two-by-two comparisons, as in experiment 2.

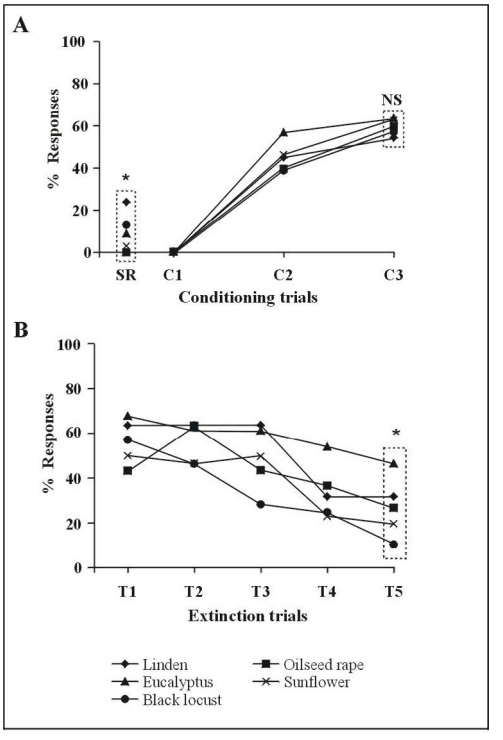


**Figure 1.** (A) Percentages of proboscis extension responses to the five honey odours during conditioning. SR: spontaneous responses; C1-C3: conditioning trials. (B) Responses during the extinction phase. T1-T5: test trials (*: $P < 0.05$; NS: non significant).

## 3. RESULTS

### 3.1. Experiment 1 – learning honey odours

Some bees responded to honey odours before any association with a reward. These spontaneous responses, measured at the first trial of the conditioning procedure (Figs. 1A – SR) showed an heterogeneity between honeys ($\chi^2 = 11.1$, 4 df, $P < 0.05$, sample size between 33 and 35). They ranged between 0 and 13% for oilseed rape, eucalyptus, sunflower and black locust, whereas they reached 24% for linden. To assess the ability of bees to be conditioned to honey odours, individuals responding spontaneously to the CS were not included in further analysis. Conditioned

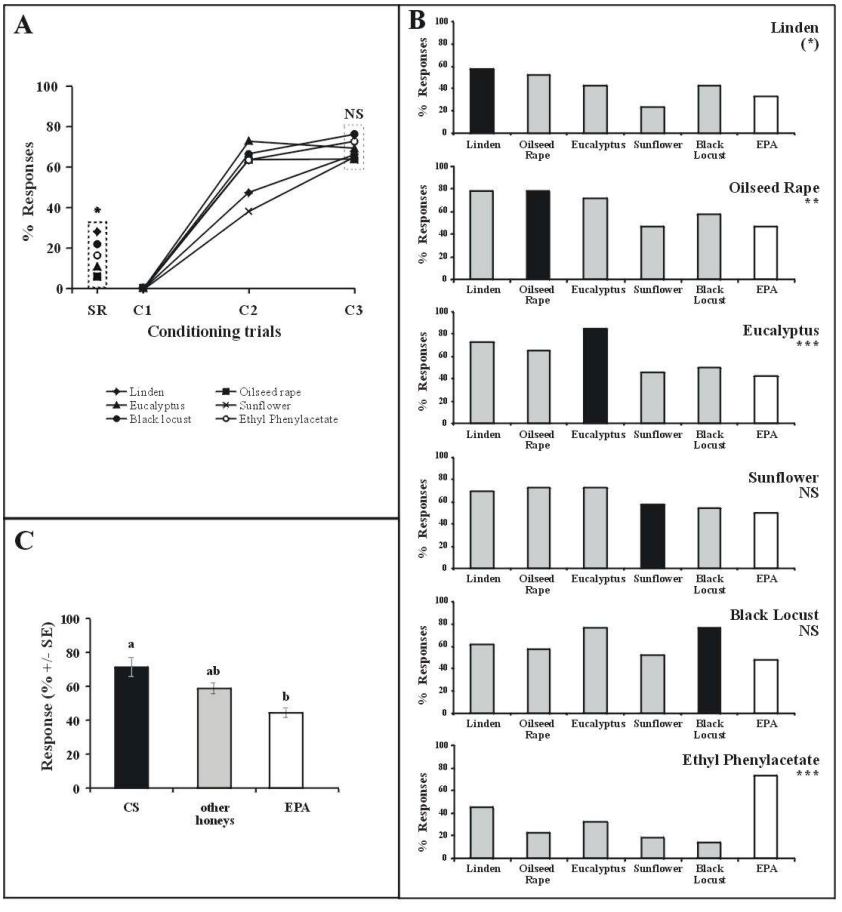


**Figure 2.** (A) Percentages of responses to the five honey odours and to ethyl phenylacetate during conditioning. SR: spontaneous responses; C1-C3: conditioning trials (NS: non significant). (B) Responses of bees tested with all honey odours and with ethyl phenylacetate (EPA), after conditioning to one of these odours (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$; NS: non significant). (C) Responses (mean percentage ± SE) of bees conditioned first to a honey odour, and subsequently tested to stimuli grouped in three categories: the CS, other honeys, and ethyl phenylacetate. Data showing the same letters were not statistically different in two-by-two comparisons (corrected significance threshold $\alpha' = 0.025$).

responses took place very quickly (Fig. 1A), with 39% to 57% of the bees responding after a single CS/US pairing, and 55% to 63% of the bees by the third trial (after two CS/US pairings) without any significant difference between honeys ($\chi^2 = 0.72$, 4 df, NS, sample size between 22 and 30). Bees were thus able to learn all honey odours tested with the same efficiency, despite differences in spontaneous responses. During the extinction phase (Fig. 1B), where bees have to learn not to respond to the honey odour anymore, responses decreased for all honey odours considered. Response levels at the fifth testing trial were heterogeneous ($\chi^2 = 10.17$, 4 df, $P < 0.05$), and ranged between 11% (black locust) and 46% (eucalyptus). The five honey odours were learned equally well by bees, but the decrease of responses during extinction differed between honey types.

### 3.2. Experiment 2 – generalisation among honey odours

Spontaneous responses ranged between 7 and 28% (Fig. 2A – SR), with linden and black

locust honeys eliciting the highest responses. Only near-significant heterogeneity appeared ($\chi^2 = 8.32$, 4 df, $0.05 < P < 0.10$, sample size between 26 and 30). As in experiment 1, the responses increased from the first to the third conditioning trial to reach values ranging between 65% and 76% for honeys and of 73% for ethyl phenylacetate. No statistical difference appeared among odours at this stage ($\chi^2 = 1.1$, 5 df, NS, sample size between 21 and 28). The patterns of responses obtained for the six odours during the test procedure are presented in Figure 2B. After conditioning to a honey odour, the CS induced the highest probability of responses (69%–86%) in all cases except after conditioning to sunflower (61%), but responses to other honeys were also high (24–79%). Responses to ethyl phenylacetate were generally the lowest (33–50%). After conditioning to ethyl phenylacetate, however, the response pattern was more differentiated; bees responded with a much lower probability to honey odours (14–45%) than to ethyl phenylacetate (81%). Significant heterogeneities in response patterns were found after conditioning to oilseed rape and eucalyptus honeys and to ethyl phenylacetate (Cochran's Q test, $Q > 19.8$, 5 df, $P < 0.01$) suggesting that bees do not respond with a similar probability to all presented stimuli. Response heterogeneity after conditioning to linden was nearly significant (Cochran's Q test, $Q = 10.8$, 4 df, $P = 0.055$). A more global representation of the data (Fig. 2C) further showed that the CS generally elicited the highest levels of response. Responses of bees conditioned to a honey odour (n = 5 groups) were grouped according to three categories of stimuli: the CS; all other honeys; and ethyl phenylacetate. A clear heterogeneity appeared among the 3 types of stimuli (Kruskal-Wallis test, $H = 9.8$, $P = 0.007$), the CS elicited 71% of the responses vs. 58% from all other honeys, and 44% from ethyl phenylacetate. Nevertheless, two-by-two comparisons only showed a significant difference between responses to the CS and ethylphenylacetate ($P < 0.025$ corrected threshold). Thus, even if bees generally displayed higher levels of responses to the honey they were conditioned to, they showed high generalisation responses to other honeys. Ethyl phenylacetate, a pure component with honey-like odour, was clearly discriminated from real honey odours.

### 3.3. Experiment 3 – differential conditioning with honey odours

In this experiment, 606 honey bees, divided in 20 groups, were conditioned using a differential conditioning procedure. Spontaneous responses to the CS+ ranged from 3% to 45% in some groups (sample size between 29 and 36 per group). Among honey types, a significant heterogeneity in spontaneous responses appeared ($\chi^2 = 14.5$, 4 df, $P < 0.01$), with linden and black locust honeys eliciting, as in previous experiments, the highest spontaneous response rates, 32% and 29%, respectively. Spontaneous responders (i.e. 123 bees) were removed from further analysis. Differential conditioning between honey types was found to be difficult. Typically, bees generalised strongly between CS+ and CS- in the first trials of the procedure. In numerous cases, even at the end of the procedure, bees showed the same level of responses to both stimuli (Fig. 3, sample sizes between 17 and 31). Only in 7 cases out of 20, did bees respond significantly more to the CS+ than to the CS- (Fig. 3, Wilcoxon matched-pairs test, $P < 0.05$). In particular, in all cases where linden was used as a CS+, bees could differentiate it from other honeys (Fig. 3, linden column). In the test phase, where bees were stimulated with the five honey odours without any reward (Fig. 4), responses to the range of odours showed significant heterogeneity in 15 situations out of 20 (Cochran's Q test, $Q > 10.3$, $P < 0.05$). However, only in 7 cases, the CS+ elicited the highest level of responses, and only in 8 cases the CS-elicited the lowest responses. In 5 cases both conditions were achieved (as CS+/CS-: linden/oilseed rape; linden/black locust; oilseed rape/linden; sunflower/linden; eucalyptus/sunflower). Here again, the linden honey odour showed the highest salience compared to all other honeys odours.

The general responses of bees to three categories of stimuli, CS+, CS-, and other honeys (Fig. 5), showed clear differences in the response rates: responses were elicited preferentially by honey CS+ (62%) and less by honey CS- (32%). Responses to the three other honeys tested reached an intermediate value of

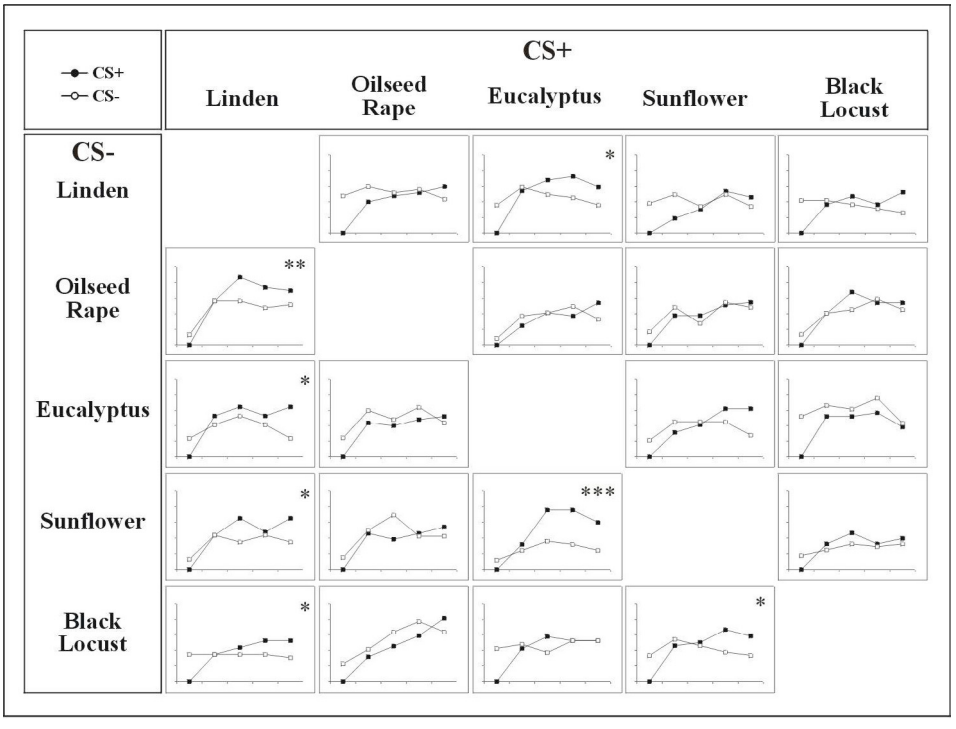


**Figure 3.** Performances of bees in differential conditioning procedures with the five honey odours as CS+ (columns) or CS- (lines). The percentages of responses to the CS+ and to the CS- are shown, for each of the 20 combinations, throughout the 10 trials of the procedure. Asterisks mark pairs of honey which bees significantly differentiated (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$).

48%. The responses among the 3 categories were significantly different (Kruskal-Wallis test, $H = 43.1$, $P < 0.001$), and all two-by-two comparisons were significant ($P < 0.025$ corrected threshold). Thus, although differential conditioning only slightly improved the differentiation between CS+ and other honeys, it clearly decreased responses to the CS- in the test phase.

## 4. DISCUSSION

The reliable identification of honeys from particular monofloral or geographical origins is possible only if reliable methods of confirming their origin are available. At present, mellissopalynological analysis often is considered the only objective method (Maurizio, 1951). From our data, it appears that even though physico-chemical and human sensory analyses tended to confirm the monofloral origin of the five honeys under investigation, the quantity and percentage of pollen from the honey samples did not necessarily fit with standard values, at least for linden and black locust honeys. It was only by combining the three methods that we were able to assign a denomination to the honeys. These results further stress that complementary methods should be designed to confirm honey characterisation. Our purpose was to evaluate whether bees themselves could be used as a new method to check the origin of honeys. In a standard classical conditioning procedure, we showed that honey bees were able to learn honey odours, although they exhibited high spontaneous activity to such odours. Some honeys induced more spontaneous activity than others, but they were all learned equally well. After conditioning, bees responded with

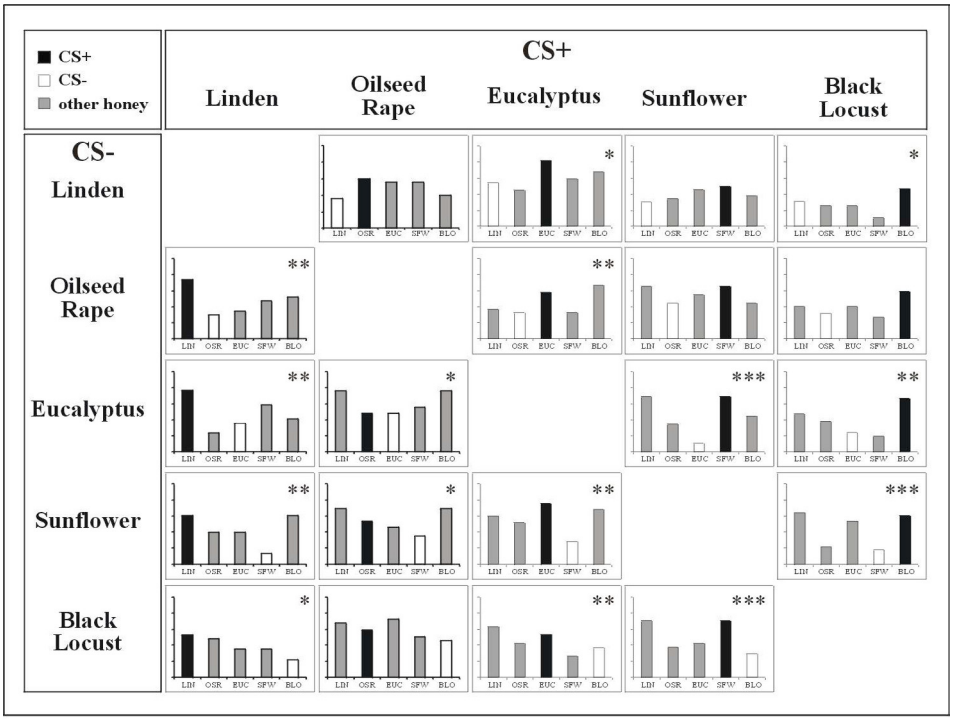


**Figure 4.** Percentages of responses of bees tested with all honey odours after differential conditioning with one of these odours as CS+ and another as CS-. LIN: linden; OSR: oilseed rape; EUC: eucalyptus; SFW: sunflower; BLO: black locust. Asterisks mark significant heterogeneities among honey bee responses (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$).

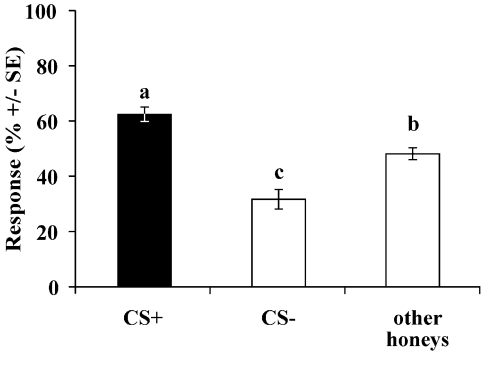


**Figure 5.** Responses (mean percentage ± SE) of bees which were conditioned to one honey as CS+ and another as CS-, to the stimuli grouped in three categories: CS+, CS-, and other honeys. Data showing the different letters were statistically different in two-by-two comparisons (corrected significance threshold $\alpha' = 0.025$).

a high probability to other honeys, showing great generalisation behaviour. Indeed, by subjecting bees to differential conditioning procedures, where one honey was rewarded and another explicitly unrewarded, we showed that bees often cannot discriminate between two honey odours (they significantly discriminated 7 from 20 pairs of honey odours). After such a procedure, the differentiation between honeys was increased in a later test, although bees still showed a high tendency to generalize. Our data thus suggest that only in some cases, honey volatiles have a signature of floral origin characteristic enough to be recognised by bees. Therefore, before PER conditioning can be used as a tool to biologically differentiate honeys, two main problems need to be solved: (i) the high spontaneous activity such that numerous individuals have to be discarded; (ii) the high generalisation behaviour which makes the interpretation of the results difficult.

The spontaneous activity we recorded to honey odours (3 to 45%) was higher than what is usually reported for floral odours in this bioassay (usually less than 20% – Smith, 1991; Sandoz et al., 1995; Laloi et al., 2001). This might be due to a familiarisation at early stages of the bees' development to the honey stored within the hive (although the bees of our study were maintained in an incubator after emergence, they spent both larval and pupal periods within the hive). Indeed the maturation of honey bees' olfactory system takes place from 3 days before emergence to 4–8 days of adult life (Masson and Arnold, 1984) and an olfactory experience during this period affects later behaviours (Pham-Delègue et al., 1990b; Sandoz et al., 2000). However, an exposure to pure volatiles during development rather hindered the acquisition of the PER response and did not change spontaneous activity (Sandoz et al., 2000). Conversely, it cannot be excluded that such a high sensitivity to honey, a signal with a high biological value for bees, is genetically determined. Further experiments will be needed to explore this question and to try and find bees that show lower spontaneous activity to honey.

Although bees did learn honey odours, they did not differentiate well between different honey types. Honey odours, like natural floral odours are complex mixtures that contain a high number of compounds belonging to various chemical classes (Gonnet and Vache, 1985). Differences between honey types are more quantitative than qualitative, although monofloral honeys can produce compounds specific of the plant of origin (Bouseta et al., 1992; Ferreres et al., 1994). In honey bees, the recognition of complex mixtures was shown to depend not on the whole volatile blend, but rather on a smaller fraction or even a limited number of key-components (for floral odours: Pham-Delègue et al., 1986, 1993; Thiéry et al., 1990; Le Métayer et al., 1997; for comb and cuticular waxes: Fröhlich et al., 2000, 2001). Such components could be divided into two categories: (1) those common to the odour of all honeys, and (2) those specific to the plant (or plants) from which the nectar was collected. The first category, probably containing the most components of the blend, would make the odour of different honeys very similar to the bees, inducing the high generalisation responses we observed. The second category could be what the bees rely on to discriminate between honeys in a differential conditioning procedure. It appears from our results that not all pairs of honeys are equally well discriminated. For instance, linden honey presented as CS+ always induced good differentiation (see Fig. 3, column linden), whereas other honeys like oilseed rape or black locust were never well differentiated from other honeys. Also, some particular combinations were very well differentiated (eucalyptus-sunflower), while individual honeys in other combinations were not. Thus, it is probable that some honeys, like linden, contain very specific compounds, whereas other honeys have many common compounds, which can only be differentiated in some cases (e.g., between eucalyptus and sunflower).

We do not know if the 13 pairs of honeys that were not significantly differentiated by bees in our experiments are perceptually so similar to bees that they cannot discriminate between them, or if our experimental procedure was not suitable to record differentiated behaviour between similar but not identical stimuli. Although a 10-trial differential conditioning procedure (as in experiment 3) is a standard procedure that leads to very clear differential responses with pure chemical compounds, the method could be improved. This could be done either by (i) prolonging the whole procedure, subjecting bees for instance to 20 trials with the CS+ and 20 trials with the CS-; or by (ii) increasing the motivation of bees not to respond to the CS- by associating it with an aversive stimulus, such as an electric shock (Smith et al., 1991) or stimulation with a salt solution (Chandra et al., 1998). Another way to increase the motivation of bees is to place them in a situation where they have to produce behavioural responses with a higher energetic cost than a proboscis extension. For instance, after a PER conditioning procedure, bees could have to choose between odour sources, walking in a choice-olfactometer device (Bakchine-Huber et al., 1992) or flying towards artificial food sources (Kriston, 1973; Pham-Delègue et al., 1993). In particular, one could train free-flying bees to visit an odour matrix like the one described by Laska et al.

(1999), with only one rewarded odour among numerous odour sources. In such an apparatus, bees tend to use their discrimination ability fully to limit the energetic costs invested in foraging. By recording all the visits of bees to the different odours, one could thus see how similar a particular honey is to reference honey samples of known origin. The characterisation of new honey samples could thus be standardised.

The present study is an original approach to tentatively characterise honeys by using honey bees as biological detectors. The first results are encouraging since using a rather simple and standardised behavioural procedure, we succeeded in conditioning bees to honey volatiles. The main difficulty encountered relies in the high spontaneous activity to and generalisation between honey odours. By developing methods in which bees are forced to fully use their discrimination abilities, we suggest that the use of honey bees as detectors of the origin and quality of honeys may be obtainable in the future.

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**Résumé – Discrimination olfactive des arômes de miels par les abeilles domestiques.** Les capacités de discrimination olfactive des miels par les abeilles ont été étudiées en utilisant la procédure de conditionnement de l'extension du proboscis sur des individus en contention, l'objectif étant de valider cet essai biologique pour la caractérisation des miels. Les abeilles ont été conditionnées à l'odeur de cinq miels (tilleul, colza, eucalyptus, tournesol, robinier faux-acacia). Les résultats montrent que les abeilles répondent souvent spontanément aux odeurs de miels (3–45 %) mais qu'elles sont capables de les apprendre efficacement (taux de réponses atteignant 60–70 % – Fig. 1A). Bien que les réponses à l'odeur du miel de conditionnement soient les plus élevées (71 % en moyenne), les abeilles généralisent beaucoup leurs réponses aux autres odeurs de miels (58 % en moyenne) (Fig. 2). Au vu de ces résultats, nous avons appliqué une procédure de conditionnement discriminatif, où une odeur est systématiquement renforcée (CS+) et une autre présentée sans renforcement (CS-). Sur 20 paires de miels testés, seulement 7 ont été significativement différenciées par les abeilles (Fig. 3). Néanmoins, cette procédure a permis d´améliorer la spécificité des réponse des abeilles face à d´autres miels. (Figs. 4 et 5). L'utilisation des performances de discrimination olfactive des abeilles pour différencier les miels de diverses origines demandera cependant des améliorations afin de limiter (i) l'importance des réponses spontanées aux miels et (ii) le comportement naturel des abeilles de généralisation entre miels.

***Apis mellifera* / apprentissage olfactif / discrimination / miel / mélissopalynologie**

**Zusammenfassung – Lernen und Unterscheidung von Honigdüften durch Honigbienen.** Die Fähigkeit von Honigbienen, Honige geruchlich zu unterscheiden wurde mit der klassischen Konditionierung des Rüsselstreckreflexes mit fixierten Einzeltieren untersucht. Das Ziel war die Entwicklung eines biologischen Tests zur Charakterisierung von Honigen. Die Bienen wurden auf 5 Honige konditioniert, dies waren Linde (*Tilia* spp.), Raps (*Brassica napus*), Eucalyptus, Sonnenblume (*Helianthus annuus*), Robinien (*Robinia pseudoacacia*). Die Ergebnisse zeigten, dass Bienen häufig spontan auf Honigdüfte reagieren (3–45 %), aber dass sie diese mit großem Erfolg lernen (Prozentsatz der Reaktionen lag bei 60–70 % – Abb. 1A). Die Reaktionen auf den konditionierten Duft waren sehr hoch (71 % im Mittel), aber die Bienen generalisierten häufig ihre Reaktionen auf andere Honigdüfte (58 % im Durchschnitt – Abb. 2). Angesichts dieser Ergebnisse haben wir eine Methode der diskriminierenden Konditionierung angewendet d.h. wir haben einen Duft systematisch belohnt (CS+) und einen anderen deutlich ohne Belohnung angeboten (CS-). Von 20 getesteten Paarungen wurden nur 7 signifikant von den Bienen unterschieden (Abb. 3). Nichtsdestotrotz erlaubte diese Prozedur eine Verfeinerung der Spezifizität der Bienen beim Angebot der anderen Honige (Abb. 4 und 5). Eine Nutzung der geruchlichen Leistungen der Bienen zur Unterscheidung von Honigen verschiedener Herkünfte ist daher von einer Verbesserung der Methoden abhängig wie der Begrenzung des (i) Einflusses von Spontanreaktionen und der (ii) natürlichen Generalisierung zwischen Honigen durch Honigbienen.

**Honigbienen / Lernen von Düften / Diskriminierung / Honig / Melissopalynologie**

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