Behavioral Ecology

The official journal of the
ISBE
International Society for Behavioral Ecology

Behavioral Ecology (2013), 24(5), 1058–1067. doi:10.1093/beheco/art026

Original Article

# Honeybee cognitive ecology in a fluctuating agricultural setting of apple and pear trees

Paula C. Díaz,[a] Andrés Arenas,[a] Vanesa M. Fernández,[a] Cinthia Susic Martin,[a] Alicia M. Basilio,[b] and Walter M. Farina[a]

[a]Grupo de Estudio de Insectos Sociales, Departamento de Biodiversidad y Biología Experimental, IFIBYNE-CONICET, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón II, Ciudad Universitaria (C1428EHA), Buenos Aires, Argentina, and [b]Cátedra de Avicultura, cunicultura y apicultura, Facultad de Agronomía, Universidad de Buenos Aires, Av. San Martín 4453 (C1417DSE), Buenos Aires, Argentina

Received 4 May 2012; revised 14 December 2012; accepted 10 February 2013; Advance Access publication 8 June 2013.

Honeybees' plasticity to adjust responses according to environmental changes has been extensively studied in the laboratory but seldom along temporal variation in natural resources. Apple (*Malus domesticus*) and pear (*Pyrus communis*) trees often coexist in mixed agricultural settings offering different resources, and their blooming periods are slightly shifted. This scenario provides an opportunity to study how changing environments influence the way the honeybees perceive, learn, discriminate, and use odor-resource information along successive flowering events. We found that honeybees preferred to gather pollen on pear flowers and nectar on apple. These individual preferences correlated with variations in the type of resources collected at the colony level according to changes in the floral market. Spontaneous proboscis extension response to pear and apple floral scents of bees captured at the hives located within the crops fluctuated according to changes in floral availability too. The capability of the proboscis extension response-trained honeybees to discriminate between both floral scents at the beginning or at the end of the flowering period was also found, although bees lose this ability when both flowers were fully available. These response patterns suggest that olfactory information experienced on flowers were memorized to predict reward yet susceptible of being reversed when the floral scents were no longer available in the orchard. Combining behavioral assays and field observations, we provide here an example of how honeybees process floral odor information to make decisions about resources in a fluctuating complex environment.

## INTRODUCTION

An approach to study animal behavior is the so-called cognitive ecology—concerned with how animals obtain and process information of their environment, relate it to themselves, and use it to make decisions (Dukas 1998; Healy and Braithwaite 2000; Chittka and Thomson 2001). An important point of this field is integrating ecology (i.e., interactions between organisms and their environment) and cognition (i.e., the neuronal processes through which animals acquire and make use of information) to understand how the surroundings influence the way an animal makes decisions according to their perceptual and learning capabilities.

Abundance and composition of flower species have profound ecological consequences on the insect pollinators because both can change over their short life span (Kearns and Inouye 1993; Willmer and Stone 2004). The ability of nectar and pollen foragers to perceive, learn, discriminate, and thereafter switch their foraging preferences among flower species is critical to adjusting their behavior in an ever-changing environment. Such a statement is supported by numerous studies that assess the relevance of insect behavioral plasticity in response to a wide range of factors that change unpredictably under laboratory conditions (Menzel 1999; Dyer and Chittka 2004; Giurfa 2007). However, few studies have analyzed such plasticity concerning natural floral resource fluctuations in space and time (Gerber et al. 1996; Raine and Chittka 2008).

It is well known that generalist bees, such as honeybees and bumblebees, assess the nectar quality of the flowers offered (von Frisch 1967; Waddington and Gottlieb 1990; Banschbach 1994; Molet et al. 2009), which forms the basis of the decision of whether to forage or not. When a forager bee perceives a food source as suitable (either a nectar or a pollen source), it can associate the surrounding floral cues with the reward and within a few visits

---

Address correspondence to W.M. Farina. E-mail: walter@fbmc.fcen.uba.ar.

A. Arenas is now at the Department of Behavioral Physiology and Sociobiology, Biozentrum, University of Würzburg, Würzburg 97074, Germany.

The authors P.C.D. and A.A. have contributed equally to this work.

establish memories that once retrieved they are helpful in guiding bees toward the learned stimulus (Ribbands 1955; Koltermann 1969; Menzel 1999; Arenas and Farina 2012).

Because at any time certain floral species offering nectar or pollen can become scarce (or depleted), olfactory memories constantly need to be updated on the basis of new information associated with rewarding flowers. At the same time, "former memories" become extinguished (Menzel 1993, 1999) as they are no longer paired to any valuable reward. Then the counterbalance between memorization and extinction of the correct piece of information should be relevant to improving the efficiency over successive feeding bouts, eliminating the time wasted in visiting unrewarding patches.

This issue is particularly challenging for honeybee colonies exposed to forage in environments where mass blooming is a common strategy among plant species (Waser 2001; Dornhaus and Chittka 2004). Under this situation, many individuals of the same colony simultaneously exploit a flower species during narrow periods, and once this resource is no longer available, the collective foraging effort is redirected to an alternative plant species that are also widespread in the surrounding.

Here, we combined ecological and behavioral studies to understand how information associated with natural resources is perceived, learned, and memorized "in real time" by honeybees (*Apis mellifera*) along overlapping flowering events. For this, we chose an agricultural setting dominated by 2 commercial crops with mass-blooming strategies: the pear tree (*Pyrus communis*) and the apple tree (*Malus domesticus*). It is known that these species: 1) exhibit successive and overlapping flowering events; 2) are both commonly visited by honeybees; and 3) diverge substantially in the amount of pollen and nectar they offer (Farkas and Orosz-Kovács 2003; Benedek and Finta 2006).

To provide an ecological framework for the interpretation of honeybee plasticity, we describe some features of the agricultural settings, as well as the foraging behavior of the honeybees in the orchard. We also measured the blooming dynamic of both floral species along a 2-week period and related such outcome to the activity of the honeybee colonies placed in the orchard. Moreover, we registered the type of resources the honeybees preferred to exploit (either pollen or nectar) on each floral species and the moment they switched from pear to apple species. We also performed behavioral controlled assays in situ to test how pear and apple olfactory memories are acquired, retained, and extinguished along these flowering periods by honeybees.

## MATERIALS AND METHODS

### Study site and animals

Field and behavioral studies were performed during the pear–apple blooming seasons in 2007, 2008, and 2011 in a fruit plantation in Ingeniero Huergo (8 ha; located 39°03′ 27.5″S; 67°13′ 53.5″W), province of Río Negro, Argentina. Half of the plantation (4 ha plot) was exclusively dominated by pear trees (*P. communis* sp.; varieties: Packham and D′Anjou), whereas the other half (4 ha plot) consisted of apple trees (*M. domesticus* sp.; varieties: Granny Smith, Gala, and Red Delicious). Pear and apple trees within the plots were planted in 80 rows of about 70 trees.

A total of 35 colonies (5 colonies in 2007, 10 colonies in 2008, and 20 colonies in 2011) of European honeybees (*A. mellifera* L.) containing a mated queen, 3 or 4 frames of capped brood, food reserves, and about 15 000 individuals were located between the pear and the apple plots having equal access to both species. These beehives were the only ones available inside the orchard and within a 3 km radius.

### Blooming period

To describe the flowering period of pear and apple, we studied the phenological stage of branches belonging to different trees of the same variety every morning from 22 September to 2 October 2007, from 15 to 23 October 2008, and from 23 September to 5 October 2011. To this end, 60 new branches were randomly sampled each day. By means of random numbers, we chose 10 rows per plot, 6 trees per row, and finally a branch per tree. This procedure was repeated 4 times: 1 for Packham, 1 for D′Anjou, 1 for Granny Smith, and 1 for Gala and Red Delicious (the last 2 varieties bloomed together). Then we estimated the percentage of opened flowers (hence accessible for the bees) over the total number of flowers per branch.

### Honeybee foraging behavior at the flowers

To sample honeybee foraging behavior in pear and apple flowers, we randomly chose 3 rows in the apple plot and 3 rows in the pear plot. Every day between 25 and 30 September 2007 at 11:00 AM, 3 researchers walked along the rows (1 per row) and scrutinized 1 every 3 trees. Each tree was observed for 1 min, and honeybees observed foraging for nectar, pollen, or both were counted.

Bees protruding their proboscises into the floral nectaries and presumably sucking were considered nectar foragers, and those chewing pollen with their mandibles or packing the grains on their posterior legs were considered pollen foragers. Bees extending their proboscises into the nectaries while carrying pollen on their posterior legs were considered foragers of both resources.

### Colony activity in the orchard

We recorded the number of incoming bees (incoming rate) at the entrance of the hive as an indicator of the general colony activity. We counted the arrivals in 5 colonies for 1 min every morning (9:30–10:00 AM), during the whole experimental period (from 22 September to 1 October 2007). According to the presence or absence of pollen loads on their hind legs, incoming bees were recorded as pollen or nonpollen bees.

To estimate the proportion of nectar foragers among the returning bees without pollen loads (nonpollen bees), we collected a sample of individuals throughout the apple and pear blooming and got them to regurgitate their gut content. This measurement was done in the 2011 season. The bees were captured in plastic tubes (10 mL) at the entrance of 20 hives located within the crop, anesthetized by means of $CO_2$ released inside the tubes and then gently squeezed in the abdomen. Regurgitated contents were emptied onto a refractometer to distinguish nectar foragers from water foragers.

Pollen loads were sampled with conventional pollen traps (frontal entrance trap) fixed at the entrances of the hives for 3 h (11:00 AM–14:00 PM) on 25, 26, 27 and 29 September and 1 October 2007. Afterwards, pollen loads were divided into 4 categories (pear, apple, dandelion, and others pollen) according to their color. When necessary, the analysis was confirmed with palynological practices (using a Labomed microscope CXR III) comparing hand-collected pollens obtained directly from the anthers of the flowers with pollen samples from the bees' loads (Kearns and Inouye 1993).

Using a Mettler Toledo AG 285 balance, we weighed the pollen loads collected from a single colony on 27 and 29 September and 1

October 2007. To reduce errors during weighing, the sample unit was defined as the weight (mg) of 30 pear-pollen loads.

## Spontaneous responses to pear and apple floral odors

We tested whether pear and apple floral olfactory cues were relevant for the bees as food predictors. To achieve this goal, we focused on the proboscis extension response (PER) values obtained during the first presentation of the odors (henceforth, spontaneous response; SR). High percentages of SR would indicate that the olfactory information is relevant to predict food and anticipates the appetitive response: the extension of their proboscis.

This single-PER-trial test was performed every day along the 10-day study period (from 23 September to 2 October 2007). We captured bees at the entrance of the hives and harnessed them in metal tubes so that they could only move their mouthparts and antennae (Takeda 1961). They were fed with 50% w/w sucrose solution for about 3 s and kept in a dark place (30°C, 55% relative humidity) for 2 h until the odor test. During the assay, a constant airflow of 50 mL/s was delivered to the head of the bees through a tube (1 cm diameter) placed 2 cm in front of the bees in which the scents were injected.

Only bees that showed an unconditioned response after applying sugar solution onto the antennae and did not respond to the mechanical stimulus (airflow) were used. The single-PER-trial test lasted 46 s, divided into 3 steps: 20 s of air flow, 6 s of floral odor (CS), and the last 20 s of air flow. The presentation order of the odors was balanced—half of the bees were tested with pear/apple scent in the first presentation and the other half with pear/apple scent in the second presentation. The odors were presented with a 15-min intertrial. The CSs were obtained by an air flow going through a 500 mL Büchner flask (a side-arm glass flask) containing 100 g D´Anjou pear flowers or 100 g Granny Smith apple natural flowers.

## Memory retrieval and discrimination between pear and apple floral odors

To get a better insight into the dynamic of olfactory memories according to currently exploited flora, odor responses in the PER setup were studied again in the following season (Spring 2008). We repeated the measurement of SR levels to pear and apple flowers, and we also performed differential PER conditionings to analyze the learning abilities to discriminate between both floral scents. By conditioning bees to discriminate between apple and pear floral odors (Takeda 1961; Bitterman et al. 1983), we studied how previous experience (i.e., putatively assumed to be exposed to pear and apple olfactory cues) influenced the acquisition of the conditioned response. We expected then that acquisition of CS values (i.e., pear and apple odors) were facilitated or impaired according to experiences acquired in the natural context with the currently exploited flora.

To measure these variables, we focused on 3 different moments during the 2008 season when 1) only pear flowers were available in the surroundings (September 16); 2) pear and apple flowers were both available (September 21); and 3) only apple flowers were available because pear became scarce (September 23, Figure 3D). SR values were obtained exactly as stated in the previous section. Responses to both pear and apple odors were then contrasted with variations in the flowering dynamic during this season.

We used a discrimination assay to train bees, a procedure that involved 2 odors and forced individuals to respond differentially to them (Bitterman et al. 1983). Here, we performed 2 differential PER conditionings using the pear and apple floral odors as CSs. In such procedures, 1 odor was presented as the rewarded conditioned stimulus (CS+) paired to a 50% w/w sucrose solution (i.e., US) and the other as the nonrewarded conditioned stimulus (CS−), which was presented without reward.

Bees were obtained and manipulated as explained in the previous section. Just similar to a single-PER-trial test, conditioning trials lasted 46 s, divided into the same 3 steps: 20 s of air flow, 6 s of floral odor (CS), and last 20 s of air flow. During rewarded trials, the reward (US) was delivered during the last 3 s of CS. The bees were exposed to these stimuli 4 times each in a pseudorandomized order (CS−, CS+, CS+, CS−, CS−, CS+, CS−, CS+). The intertrial interval of the conditioning lasted 15 min. After the conditioning, bees were subjected to a testing phase, during which the bees were exposed to both conditioned odors without reward. The 15-min interval was maintained between the last trial and the first testing event and also between testing events.

To compare the dynamic of learning between both conditionings (CS+: Apple/CS−: Pear; and CS−: Apple/CS+: Pear), we calculated a generalization index (GI; Sandoz et al. 2001; Gramacho and Spivak 2003):

$$GI = 1 - \frac{(R+) - (R-)}{(R+) + (R-)}$$

where $R+$ is the number of positive responses obtained to the CS+ during the 4 trials, and $R-$ is the number of positive response to the CS−. From each bee, we obtained a GI value. The GI value ranges from 0 to 1: 0, when bees respond only to the CS+, and 1, when bees respond equally to the CS+ and the CS−. GI values above 1 could be achieved if bees responded more to the CS− than to the CS+.

The GI and testing response offer different but complementary information: GI indicates how bees generalized between 2 floral scents (i.e., generalization is defined as the tendency of animals to respond to stimuli, which differ from a learnt stimulus; Pearce 1987), whereas testing response shows the outcome of the learning process. Here, we used them together to investigate bee's capacity to learn and discriminate the pear and apple floral scents according to putative information gained from the environment. We used GIs to compare discrimination performance between conditionings, where either apple or pear odor acted as CS+ or CS−, whereas we used PER obtained in the testing phase to describe how the odors involved within a single conditioning (CS+: Apple/CS−: Pear or CS−: Apple/CS+: Pear) were learned in relation to each other.

## Statistics

Behavioral preferences for nectar or pollen at the pear and apple flowers were analyzed using a goodness-of-fit test (Sokal and Rohlf 1995). Because the assumptions of normality and homogeneity of variances were met, the number of incoming foragers per unit time along the blooming period was studied using a 2-way analysis of variance (ANOVA) with repeated measures, with type of forager as a between-group factor (2 levels: pollen or nonpollen foragers) and incoming rate as the repeated measure. When detecting statistical differences in the interaction between factors, we computed simple effects using the corresponding error (Quinn and Keough 2002).

Weights of pollen loads were compared with a Kruskal–Wallis test, followed by a posteriori comparisons between groups (Conover 1971). The proportion of incoming bees that returned carrying nectar was analyzed using the simultaneous-*G*-test procedure of the unplanned test of homogeneity for goodness of fit (Sokal and Rohlf 1995). The proportion of bees that responded toward floral odors before differential PER conditioning (SR) and after the procedure (testing phase) was compared by a Fisher's Exact test. GI values obtained from differential conditioning were analyzed using *t*-tests for independent samples. To reduce the risk of Type I errors due to the multiple use of the same data, the level of significance was corrected using the Bonferroni method ($\alpha' = \alpha/\mathrm{k}$), with $\alpha = 0.05$ and $k = 2$. Thus, our new significance level was $\alpha' = 0.025$.

# RESULTS

## Honeybee foraging behavior at the flowers

Regarding the honeybee behaviors observed at the apple and the pear flowers, we found differences in the type of resource they collected in each plant species. Although the most frequent behavior performed in the apple flowers was collecting nectar ($\chi^2_1 = 82.68$, $P < 0.0001$, $N = 227$), honeybees mainly gathered pollen in the pear flowers ($\chi^2_1 = 99.19$, $P < 0.0001$, $N = 192$; Figure 1). No bees carrying pollen loads were observed licking nectar in either pear or apple flowers.

## Colony activity in the orchard

The rate of incoming bees carrying pollen loads and bees without pollen loads differed along the measuring period (Figure 2A). This was revealed by an ANOVA interaction between foraging type and time (2-way repeated measures ANOVA, $F_{8;\,64} = 8.148$, $P < 0.0001$). Simple effect analysis showed statistical differences ($P < 0.05$) on 24, 26, and 28 September and 1 October (Figure 2A). By the time the orchard was dominated by the pear flowers (beginning of the flowering periods; Figure 2D), a stronger pollen-foraging activity was measured.

On 29 September, when less than the 20% of Granny Smith (apple) flowers were opened (Figure 2D), the proportion of pollen and nonpollen arrivals was reverted. This tendency became statistically significant with almost all incoming nonpollen bees on 1 October ($P < 0.0001$; Figure 2A). By this time, every apple tree was in bloom with most of their flowers opened (Figure 2D).

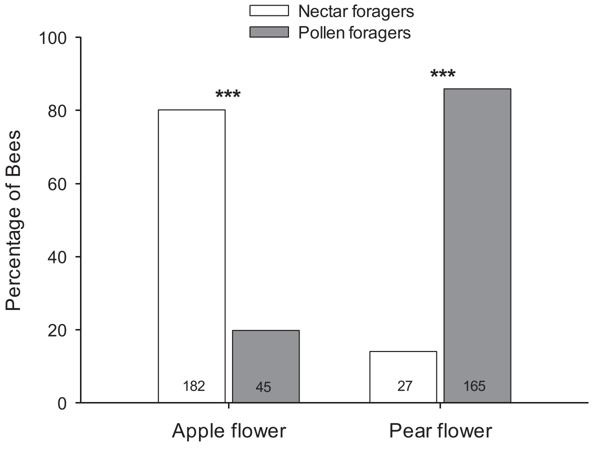

**Figure 1**
Honeybees foraging preferences on apple and pear flowers. Percentage of honeybees showed different foraging behaviors on apple and pear flowers. **, $P < 0.01$, ***, $P < 0.001$.

Accordingly with the intense pollen-foraging activity monitored at the beginning of the season, we measured an increasing amount of pear-pollen loads collected in the traps (Figure 2B). It is important to note that no measurements were done from 22 to 24, 28, and 30 September and 2 October (see Methods). In parallel with the drop in pollen-foraging activity on 1 October, the amount of pear-pollen loads trapped at the hive entrances decreased, whereas the apple-pollen loads slightly increased. Amounts of apple-pollen loads were never as large as pear-pollen loads (Figure 2B,D).

Despite the number of pear-pollen loads decreasing after a peak of collection on 26 September (Figure 2B), the estimated weight of pear-pollen loads collected per foraging bout increased significantly until 1 October ($H = 55.55$, $N = 73$, $P < 0.001$, Kruskal–Wallis test; Table 1).

Table 2 shows that the proportions of returning bees identified as nectar carriers did not differed along the progression of the pear and apple flowering during spring 2011 (G test: $G_H = 6\,283$, $P = 0.18$, $N = 510$, degrees of freedom = 4). From Table 2 it is observed that proportion of nectar foragers remained relatively homogeneous between 26 September and 3 October (when both apple and pear flowers were available). The most extreme values were detected from 45.8% at the very beginning of our measurement (on 23 September) to 30.8% at the end of the recordings. Such fluctuation, by the time new rewarding flowers were discovered and by flower senescence on 5 October, might be related to the adjustment of collective response to changes in the floral market.

## Spontaneous responses to pear and apple floral odors

PER values to the pear and apple floral odors on 23 and 24 of September 2007 (when neither pear nor apple inflorescences were available; Figure 2C) showed low SR levels. Such responses remained relative constant until the earlier variety of pear (D′Anjou) got to 50% flowering on 26 September. By this time, PER values to the pear floral odor increased by 17% and reached to the highest level. It is worth mentioning that SR levels toward pear-odor were not exclusive, and some bees also responded to the apple odor and even to both odors (31.2%). Highest PER values to apple floral odor (53.2%) were detected on 28 September when the first variety of apple, Granny Smith, presented around 5% of opened flowers (Figure 2C). At the end of the measuring period, 100% of apple flowers were already open and started to wither, we found the lowest levels of PER values to this odor.

## Memory retrieval and discrimination between floral odors

On 16 September 2008, when only pear flowers were available (see Figure 3D), SR to pear floral odor was higher than responses to apple floral odor (Fisher's Exact test, $P = 0.0026$; Figure 3A, left panel). This observation suggests that the presence of pear flowers available in the orchard allowed bees to learn pear floral scent in the natural environment and transfer this information to the PER setup. On the other hand, low levels of SR to the apple floral odor would indicate the absence of a specific memory to the apple floral odor and suggest that pear floral-odor memories were not generalized to apple floral scents.

Changes in the floral market also influenced the performance during PER conditioning as revealed in the GI analyses. When bees were trained with pear floral scent as the CS+, we obtained a lower generalization (better discrimination) than those obtained with the pear floral scent as CS− (GI values on 16 September:

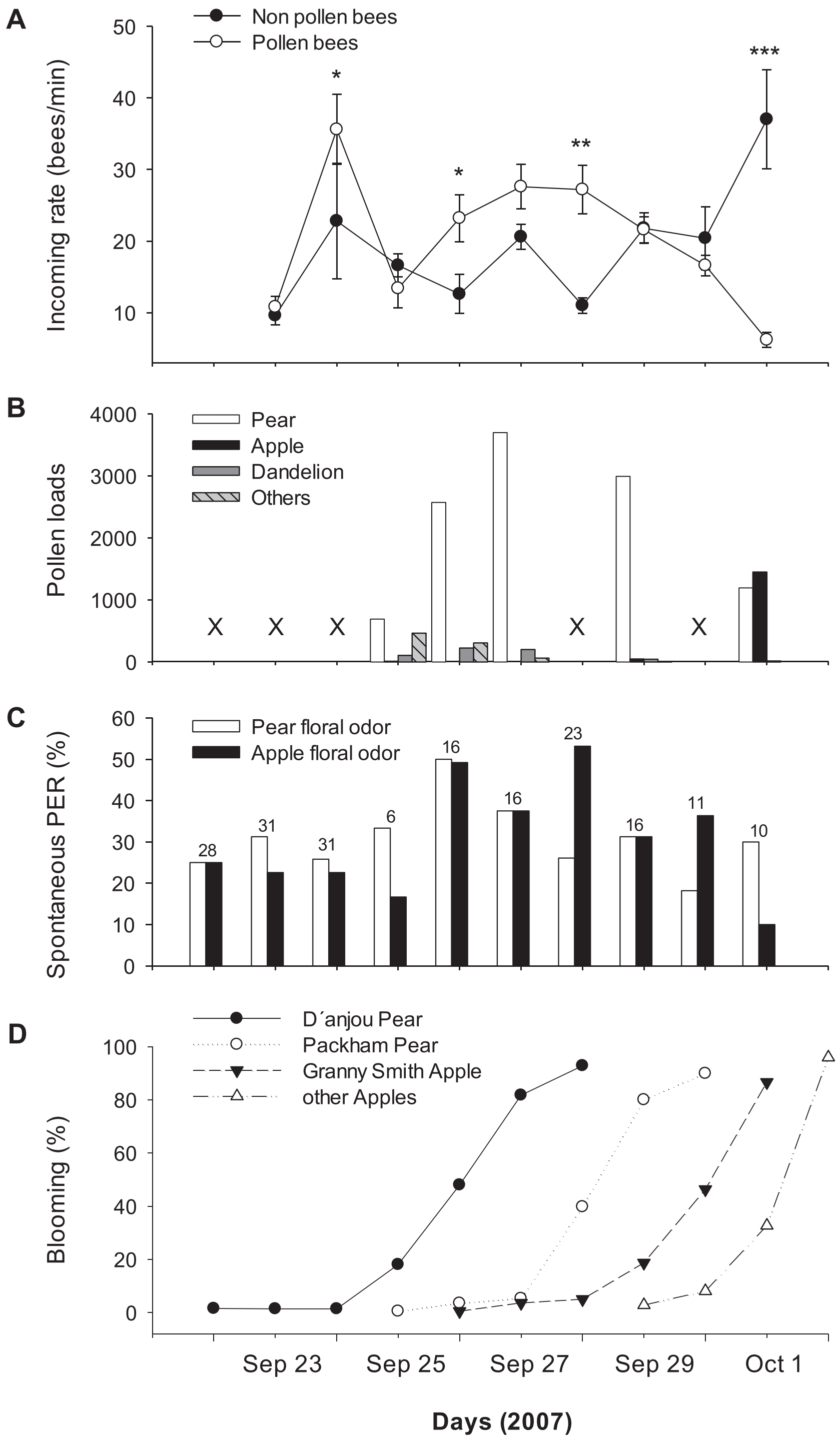

**Figure 2**

Honeybee activity along the apple and pear-blooming periods. (A) Returning bees to their colonies per minute (mean ± standard error). Empty circles represent the number of incoming bees carrying pollen loads, and filled circles represent number of incoming bees without pollen loads in their hind legs. (B) The total incoming pollen loads were collected in traps at the hive entrance during 3 h every morning from 25 September to 1 October. The X indicates that no measurements were done on that date. (C) Percentage of bees that extended the proboscis during the first presentation (spontaneous PER) to the pear floral odor or to the apple floral odor. (D) Percentage of blooming of apple and pear varieties along the experimental period. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$.

Student's *t*-test, $t_{50} = 2.81$, $P = 0.00725$; Figure 3A, center panel). In agreement with putative pear floral olfactory memories, conditioned bees could discriminate successfully between odors when pear floral odor acted as CS+ (Fisher's Exact test, pear CS+: $P = 0.0018$, Figure 3A, right side of the right panel), but they failed to discriminate between scents when pear floral odor acted as CS− (Fisher's Exact test, apple CS+: $P = 0.0708$, Figure 3A, left side of the right panel).

When apple and pear trees were simultaneously in bloom (21 September), honeybees revealed no differences in their

**Table 1**

**Mean weight of the pear pollen collected by 1 beehive on 27 and 29 September 2007 and 1 October 2007**

| | 27 September | 29 September | 1 October |
|---|---|---|---|
| Weight of the pollen loads collected (mean weight, in mg, ± standard error; sample unit: 30 pollen baskets) | $145.9 \pm 2.87^a$ (26) | $213.4 \pm 3.75^b$ (26) | $239.2 \pm 3.43^c$ (21) |
| Weight of each pear-pollen load collected (estimated) | 4.86 | 7.11 | 7.97 |

Values indicate means and standard error 30 pollen loads (sample unit). Sample sizes appear in brackets. The estimated weight of single pollen load is also presented.
Different letters indicate statistical differences ($P < 0.05$).

**Table 2**

**Percentage of blooming of pear and apple varieties along the experimental period during the spring 2011**

| Spring 2011 | Pear blooming (%) | Apple blooming (%) | Incoming nectar carriers (%) | Bees sampled ($N$) |
|---|---|---|---|---|
| 23 September | 30 | 0 | 45.8 | 120 |
| 26 September | 60 | 15 | 34.2 | 120 |
| 28 September | 90 | 30 | 34.2 | 120 |
| 03 October | 97 | 70 | 40 | 30 |
| 05 October | 100 | 85 | 30.8 | 120 |

Percentage of incoming bees carrying nectar was obtained from a sample of $N$ bees captured randomly from 20 hives placed in the experimental orchard.

levels of SR (Fisher's Exact test, $P = 0.0910$, Figure 3B, left panel). Moreover, SR level to a third novel scent not available in the surroundings was tested to control bees' responsiveness. A total of 67 subjects tested to Jasmine showed very low SR levels (16.2%). At this time, GI values obtained from both differential conditionings were high and similar (GI values on 21 September: Student's $t$-test, $t_{59} = -0.606$, $P = 0.547$, Figure 3B, central panel). These results indicate that bees could not discriminate between the CS+ and the CS− probably as both floral scents were currently available in the orchard and associated with reward. The absence of differences during testing (Fisher's Exact $\text{test}_{\text{pear CS+}}$: $P = 0.0518$, Figure 3B, right side of the right panel; Fisher's Exact $\text{test}_{\text{apple CS+}}$: $P = 0.6468$, Figure 3B left side of the right panel) confirmed the presence of stable memories to pear and apple floral scents.

On 23 September, when apple flowers became dominant and pear flowers started withering, SR levels to the apple floral scent remained slightly higher than those obtained to the pear floral odor, although not significant (Fisher's Exact $\text{test}_{\alpha'} = 0.025$, $P = 0.0307$, Figure 3C, left panel). Interestingly, when GI values were analyzed, we detected lower values with apple floral scent than with pear floral scent acting as CS+ (GI values: Student's $t$-test, $t_{57} = -3.2981$, $P = 0.0017$; Figure 3C, central panel). Consistent with the olfactory memories updated according to the flora availability, responses obtained in the testing phase showed higher responses to the apple floral scent when it was rewarded than when it was not (Fisher's Exact $\text{test}_{\text{apple CS+}}$: $P = 0.02$, Figure 3C, left side of the right panel; Fisher's Exact $\text{test}_{\text{pear CS+}}$: $P = 0.07$, Figure 3C, right side of the right panel).

## DISCUSSION

This study addresses honeybees' foraging behavior with an approach that combines ecological aspects (i.e., interactions between honeybees and 2 floral species that provided them with essential resources) with their well-known cognitive abilities. First, we observed that honeybees preferred to gather pollen on pear flowers and nectar on apple (Figure 1) according to profitability of these food sources (Supplementary Figure 1S). Individual preferences observed on these flowers well correlated with the response patterns observed at the entrance of the hive throughout the measuring period (Figure 2).

Second, we observed that the pear-pollen loads became heavier once the pear-blooming period declined (Table 1), suggesting that the honeybees' skills to handle and get pollen from flowers are improved with expertise as it was found in bumblebees (Raine and Chittka 2008). Finally, PER values measured along the flowering periods and at different critical moments of the season suggest that honeybees establish species-specific olfactory memories (Menzel 1993, 1999) while visiting the dominant flowers in the orchard and adjust them according to fluctuations in the resource availability (Figures 2C and 3). These memories would improve the searching for food by reducing the time the bees spend visiting depleted or unrewarded flowers.

### The predictive value of odors is adjusted in response to floral availability

Associative learning processes established under natural conditions (e.g., while visiting natural flowers) are scarce in the literature (Gerber et al. 1996; Raine and Chittka 2007) but important to fit the laboratory data into a complex and realistic scenario. In this study, we describe how honeybees adjust their responses in a changing environment by managing information that relates to it.

In our experiments, as bees were taken from the field and we were not able to controlling their previous experience, we cannot rule out the contribution of innate preferences in the measured response. Because innate responses result from the coevolutionary adaptations between the plant cues and bees' perceptual capabilities (Menzel 1999; Giurfa 2007), we expected their levels to remain constant despite environmental fluctuations. Thereafter, even when initial differences in SR levels between apple and pear odors could be due to differences in innate preferences, changes in the patterns of SR in accordance with changes in the floral market strongly suggest that PERs were adjusted through associative learning.

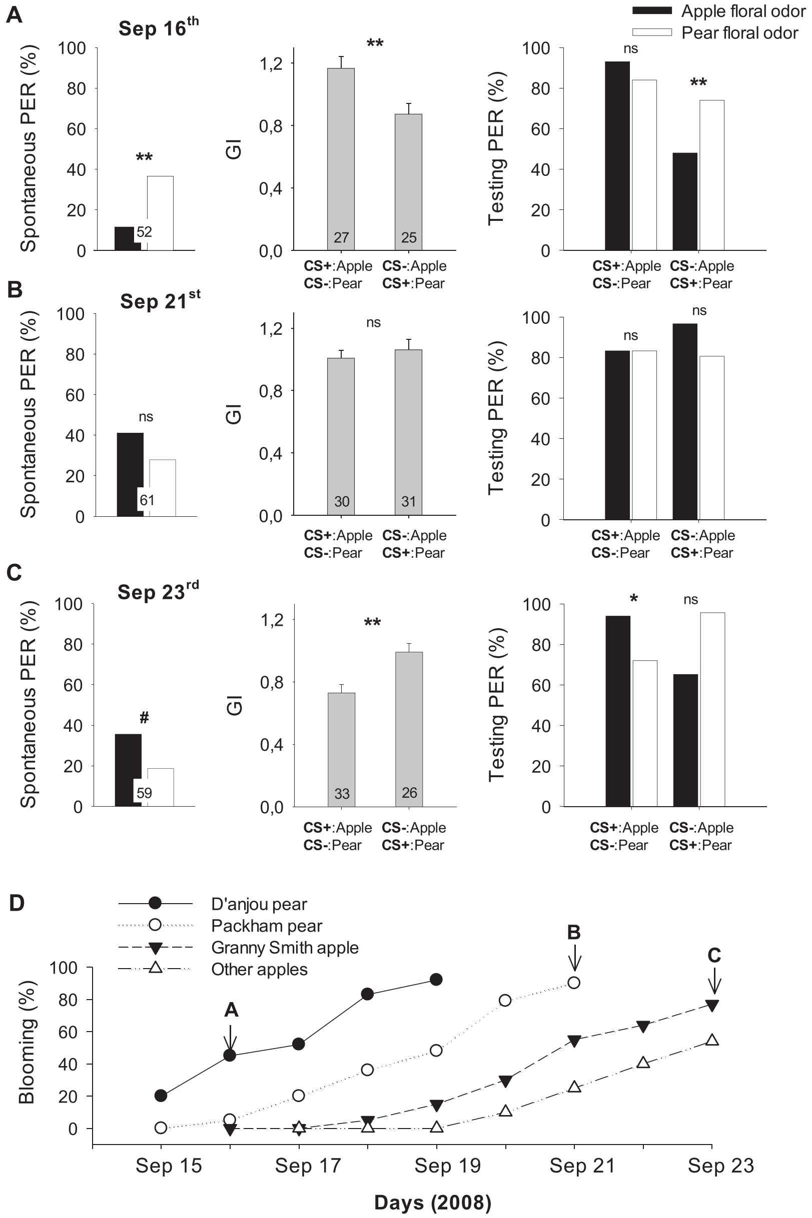

**Figure 3**

Testing olfactory memory and discrimination performance between pear and apple floral odors along the blooming periods. Percentage of bees that extended the proboscis during the first presentation (spontaneous PER) to the pear odor or to the apple odor (left panel). GI values obtained after differential PER conditioning (central panel) and percentage of bees that responded during the testing phase (right panel). PER were recorded when (A) only pear flowers were available in the surroundings, (B) both pear and apple flowers were available, and (C) only apple flowers were available in the field. Samples size is presented at the top of each figure. CS+: conditioning stimulus rewarded, CS−: conditioning stimulus unrewarded. In left and central panels, asterisks indicate statistical differences after Bonferroni correction: **, $P < 0.01$; *, $P < 0.025$; #, $0.025 < P < 0.05$ (not significant after Bonferroni correction); ns: not significant. For testing phase **, $P < 0.01$, *, $P < 0.05$, ns: not significant. (D) Percentage of blooming of apple and pear varieties along the experimental period.

High levels of SR elicited by the pear floral odor at the beginning of the 2008 season (Figure 3A) support the idea that pear-olfactory memories were established when bees exploited pear floral sources. However, the response pattern toward pear odors was different in the previous year. Different trends in the pattern of SR levels measured during 2007 and 2008 might be explained by slight differences in the flowering dynamics. From Figures 2D and 3D, we can see that during the first season, flowerings progressed faster (in about 4–5 days) than those recorded in the following spring (6–7 days). With flowerings that progressed slower, bees might have had more chances to learn pear floral odors without the interference of alternative resources such as the apple flower odor. Pear-odor memories established in the field were also revealed during the PER conditionings, as they impaired the acquisition of information that was in conflict with the information available in the surroundings.

With pear trees in bloom and no apple flowers yet available, we showed that both pear and apple floral odors were clearly discriminated during the PER conditioning in which the pear odor was paired with the reward (Figure 3A, central panel). In contrast, bees were not able to learn the discrimination tasks if the apple odor was paired with the reward, and pear odor remained unrewarded and bees continued responding more to pear odor even when the apple odor was reinforced (GI > 1; Figure 3A, central panel). We conclude that the relevance of pear floral odor as a predictor of reward was high and quite independent of the context in which it was tested (Rescorla et al. 1985; Sandoz et al. 2000). As occurred during a preconditioning procedure, in which animals are exposed to a stimulus in order to prepare them to be more resilient if the same stimulus is tested in the future (Menzel 1999), exposure to pear floral odor in the surroundings would make bees more responsive when this odor was evaluated in the PER protocol.

The increasing number of bees that responded to pear and apple odors by the beginning of the Packham pear and the very beginning of the Granny Smith apple flowering in 2007 might indicate that both olfactory cues were memorized under natural conditions. This idea is consistent with results obtained when flowerings coexisted for a 3-day period in the following spring 2008, when bees were no longer able to discriminate pear and apple floral odors (Figure 3B, central panel). In contrast to odor discrimination attained previously, generalization indexes (GI ≈ 1) indicated that bees responded almost equally to both odors irrespectively of acting as rewarded or unrewarded stimuli. We reason then that olfactory responsiveness during this overlapping period corresponds to the presence of the 2 different species-specific memories rather than a generalized response.

By the end of the measuring periods, when the apple flowering kept on and pear flowers withered, apple-odor memories were more conspicuous than pear-odor memories. This fact was clearly observed through the SR levels at the end of the measuring period (Figure 2C), as well as during the PER conditioning procedures (honeybees could extinguish pear odor but not apple memories when these scents were offered as CS−; Figure 3C, central panel). With a pear floral odor that lost its predictive value, bees were again able to discriminate the rewarded apple odor from the unrewarded pear odor.

Concluding, we reason that odor-rewarded experiences might assist foragers during searching for resources in this heterogeneous agricultural setting. As long as floral odors are species specific, they can provide reliable information for guidance. We observed that previous floral-odor memories continued being retrieved even when a novel odor source starts being intensively exploited. Foraging on both pear and apple resources enables memories to coexist. Interestingly, bees' incapability to discriminate might reflect that olfactory memories established in the field are very stable and that the meaning of these odors could not be easily reversed under laboratory conditions.

## Different behaviors performed at pear and apple flowers

Within the hive, not all the bees perceive rewarded stimuli equally. Pollen foragers, more likely to show high sensitivity to sugar (Page et al. 1998), are able to perceive reward even when it is scarce or offered at low rates or concentrations (Siegel et al. 2012). A recent study showed that nectar foragers learn poorly when conditioned with pollen as the only unconditioned stimulus (Arenas and Farina 2012). In contrast, pollen foragers respond successfully to pollen as reward and also to other gustatory stimuli present in pollen resources such as fatty acids.

We might speculate then that mainly those animals with higher gustatory sensitivity would be prone to visit pear trees, hence able to establish pear-odor memory using pollen as the sole US. It has recently been demonstrated that odor–pollen association leads to memories that are very stable and can guide pollen foragers to the feeding sites (Arenas and Farina 2012). This study showed that memories gained while gathering pollen from scented feeders can be recalled in laboratory assays. These memories were successfully transferred to a dichotomous food-choice device (i.e., a Y-maze); however, they failed to be transferred to the PER paradigm where the bees were harnessed (Arenas and Farina 2012).

Although we cannot exclude the possibility that foragers could acquire pear-odor memories with nectar or even as a combination of nectar and pollen rewards, previous reports showed that nectar offered by pear flowers is very scarce and diluted (Farkas and Orosz-Kovács 2003), and we confirmed that in pear flowers of Packham variety (Supplementary Figure 1S).

On the other hand, it is highly possible that bees learned that apple odor is associated with nectar. Biases toward nectar-foraging behavior in apple trees were consistent with previous findings that stated that apple flowers offered good quality nectars (Free 1993; Benedek and Finta 2006). Previous studies showed that apple flowers offered a high volume secreted from the flowers and high sugar concentration (McGregor 1976; Free 1993; Delaplane and Mayer 2000). More specifically, apple flowers of Granny Smith's variety showed nectar of a higher sucrose concentration and higher volume than pear flowers of Packham's variety (Supplementary Figure 1S). This is due to that olfactory memories in this case were established using nectar as a primary rewarded stimuli. Whether the apple floral odor could be better acquired and/or retained than pear floral odor because of the type of reward remains elusive.

## Increasing efficiency of pollen foragers

Other than the olfactory memories that facilitate searching for food and reduce the time bees would waste visiting unrewarded alternatives, we found evidence that pollen foragers might learn how to handle pear flowers and improve in this way their foraging efficiency. As it has already been reported for bumblebees *Bombus terrestris* (Raine and Chittka 2007), we observed that the amount of pollen collected per foraging bout (the weight of pear-pollen loads) increased along the blooming period of pear flower. The improvement in the efficiency per foraging bout was calculated to be up to 60% by the end of this period (Table 1), and this fact was independent of the total pear-pollen loads collected by the colony, a variable that decreased within the measuring period (Figure 2B). This observation suggests that learning is involved in the motor skills required to extract pollen (Laverty and Plowright 1988; Woodward and Laverty 1992; Laverty 1994; Raine and Chittka 2007). Since the improvement in the efficiency of pollen collection was observed by senescence of pear flowers (Figure 2B,D), we could hypothesize that the low profitability found at the pear flowers was compensated by a higher gathering effort of pollen foragers.

## From individual perception to collective foraging patterns

Individual behavior at the flowers influenced different foraging patterns at a social scale according to fluctuations in nectar and pollen resources. Foraging behavior pattern (Figure 2A) was characterized by 3 phases according to the abundance and composition of the available flora: a first phase with no significant difference between the incoming rates of bees carrying pollen and nonpollen, followed by a phase of intense pollen foraging (mainly bees collecting pollen), followed by a third phase with a reduced activity on pollen, when the number of nonpollen incoming bees dramatically increased.

During the first phase of this foraging pattern, neither pear nor apple trees were in bloom. Collective responses at this time might reflect basal levels of foraging activity on the alternative flora. The second phase was described in parallel with the pear blooming. It is plausible to think then that such a pattern was mainly due to bees with low response gustatory thresholds (Page et al. 1998) motivated to forage on pollen resources. We related the sudden switch from incoming pollen bees to nonpollen bees (Figure 2A) with the appearance of higher profitable sources in terms of nectar such as the apple flowers (Supplementary Figure 1S; Benedek and Finta 2006), which might emerge to optimize the gathering of resources provided by the environment.

The complementary study to estimate the proportion of bees without pollen loads that brought nectar back to the hive showed that the ratio of incoming bees loaded with nectar remained quite constant from 26 September during the apple blooming period (Table 2). The increasing rate of nonpollen incoming bees, together with the constant ratio of bees that returned with nectar, suggests that the number of nectar foragers fluctuated proportionally with the incoming rate of nonpollen bees. We speculate that during the third phase, the activity of nectar foragers increased, whereas the activity of pollen foragers diminished. We relate this fact to the appearance of a profitable nectar source (i.e., apple trees' inflorescences, McGregor 1976; Free 1993; Delaplane and Mayer 2000) that prompted low-sensitivity foragers to increase nectar collection and/or even triggered bees that were initially foraging upon pear-pollen resources to switch to apple nectar gathering. These results provide a good example of how collective tasks are adjusted in response to environmental changes, a process that might have been assisted by information exchange within the hive (Farina et al. 2005, 2007, 2012).

Honeybees and bumblebees have extensively been assessed for plasticity in their response to unpredictable conditions in experiments in which variables change one at a time (Menzel 1999; Dyer and Chittka 2004; Giurfa 2007; Molet et al. 2009). Although necessary to make conclusions, this scenario is not realistic as animals have to extract relevant information from a complex matrix of stimuli in nature to make decisions. Thus, it remains a gap between the processes that were experimentally assayed under highly controlled conditions (i.e., laboratory) and the relevance that the studied stimuli might gain in nature. Here, we attempted to understand the underlying processes that control foraging under natural conditions to have a better insight of insect pollinators' behavior, pollination–plant interactions, and the sharing of food-related information in a highly social insect society.

## SUPPLEMENTARY MATERIAL

Supplementary material can be found at http://www.beheco.oxfordjournals.org/

## FUNDING

Agencia Nacional de Promoción Científica y Tecnológica (PICT 2010 0425); University of Buenos Aires; Consejo Nacional de Investigaciones Científicas y Técnicas (PIP 112-200801-00150); Guggenheim fellowship to W.M.F.

Authors are grateful to M.S. Balbuena, A. Falibene, and G.P. Ramírez for their support during the measuring period. We also thank M. Arenas, J.J. Mange, and Alcázar S.A. for their selfless support at the agricultural setting. Authors also thank the CONICET and the University of Buenos Aires for support.

**Handling editor:** Alexei Maklakov

## REFERENCES

Arenas A, Farina WM. 2012. Learned olfactory cues affect pollen-foraging preferences in honeybees, Apis mellifera. Anim Behav. 83:1023–1033.

Banschbach VS. 1994. Colour association influences honey bee choice between sucrose concentrations. J Comp Physiol A. 175:107–114.

Benedek P, Finta K. 2006.The effect of nectar production to the gathering behavior of honeybees and to the foraging activity of wild bees at apple flowers. Int J Hortic S. 12(2):45–57.

Bitterman ME, Menzel R, Fietz A, Schäfer S. 1983. Classical conditioning of proboscis extension in honeybees (*Apis mellifera*). J Comp Psychol. 97:107–119.

Chittka L, Thomson JD. 2001. Cognitive ecology of pollination—animal behavior and floral evolution. Cambridge, UK: Cambridge University Press.

Conover WJ. 1971. Practical nonparametric statistics. 2nd ed. New York: John Wiley and Son.

Conover WJ. 1971. Practical nonparametric statistics. 2nd ed. New York: John Wiley and Son.

Delaplane KS, Mayer DS. 2000. Crop pollination by bees. Wallingford, NY: CABI Publishing.

Dornhaus A, Chittka L. 2004. Why do honey bees dance? Behav Ecol Sociobiol. 55:395–401.

Dukas R. 1998. Cognitive ecology: the evolutionary ecology of information processing and decision making. Chicago (IL): University of Chicago Press.

Dyer AG, Chittka L. 2004. Fine colour discrimination requires differential conditioning in bumblebees. Naturwissenschaften. 91:224–227.

Farina WM, Grüter C, Acosta L, Mc Cabe S. 2007. Honeybees learn floral odors while receiving nectar from foragers within the hive. Naturwissenschaften. 94:55–60.

Farina WM, Grüter C, Arenas A. 2012. Olfactory information transfer during recruitment in honeybees. In: Galizia G, Eisenhardt D, Giurfa M, editors. Honeybee neurobiology and behavior—a tribute for Randolf Menzel. Heidelberg, Germany: Springer. p. 89–102.

Farina WM, Grüter C, Díaz PC. 2005. Social learning of floral odours inside the honeybee hive. Proc Biol Sci. 272:1923–1928.

Farkas A, Orosz-Kovács Zs. 2003. Nectar secretion dynamics of Hungarian local pear cultivars. Plant Syst Evol. 238:57–67.

Free JB. 1993. Insect pollination of crops. 2nd ed. London: Academic Press.

von Frisch K. 1967. The dance language and orientation of bees. Cambridge, UK: Harvard University Press.

Gerber B, Geberzahn N, Hellstern F, Klein J, Kowalksy O, Wüstenberg D, Menzel R. 1996. Honey bees transfer olfactory memories established during flower visits to a proboscis extension paradigm in the laboratory. Anim Behav. 52:1079–1085.

Giurfa M. 2007. Behavioral and neural analysis of associative learning in the honeybee: a taste from the magic well. J Comp Physiol A Neuroethol Sens Neural Behav Physiol. 193:801–824.

Gramacho KP, Spivak M. 2003. Differences in olfactory sensitivity and behavioral responses among honey bees bred for hygienic behavior. Behav Ecol Sociobiol. 54:472–479.

Healy S, Braithwaite V. 2000. Cognitive ecology: a field of substance? Trends Ecol Evol. 15(1):22–26.

Kearns CA, Inouye DW. 1993. Techniques for pollination biologists. Boulder, CO: University Press of Colorado.

Koltermann R. 1969. Lern- und Vergessensprozesse bei der Honigbiene-aufgezeigt anhand von Duftdressuren. Z vergl Physiologie. 63:310–334.

Laverty TM. 1994. Bumble bee learning and flower morphology. Anim Behav. 47:531–545.

Laverty TM, Plowright RC. 1988. Flower handling by bumblebees: a comparison of specialists and generalists. Anim Behav. 36:733–740.

McGregor SE. 1976. Insect pollination of cultivated crop plants. US Department of Agricultural handbook No. 496. Washington (DC): Agricultural Research Service, U.S. Department of Agriculture.

Menzel R. 1993. Associative learning in honey bees. Apidologie. 24(3):157–168.

Menzel R. 1999. Memory dynamics in the honeybee. J Comp Physiol A. 185:323–340.

Molet M, Chittka L, Raine NE. 2009. How floral odours are learned inside the bumblebee (Bombus terrestris) nest. Naturwissenschaften. 96:213–219.

Page RE Jr, Erber J, Fondrk MK. 1998. The effect of genotype on response thresholds to sucrose and foraging behavior of honey bees (Apis mellifera L.). J Comp Physiol A. 182:489–500.

Pearce JM. 1987. A model for stimulus generalization in Pavlovian conditioning. Psychol Rev. 94:61–73.

Quinn G, Keough M. 2002. Experimental design and data analysis for biologists. Cambridge, UK: Cambridge University Press.

Raine NE, Chittka L. 2007. Pollen foraging: learning a complex motor skill by bumblebees (Bombus terrestris). Naturwissenschaften. 94:459–464.

Raine NE, Chittka L. 2008. The correlation of learning speed and natural foraging success in bumble-bees. Proc Biol Sci. 275:803–808.

Rescorla RA, Durlach PJ, Grau JW. 1985. Context learning in Pavlovian conditioning. In: Balsam PD, Tomie A, editors. Context and learning. Hillsdale (NJ): L. Erlbaum. p. 23–56.

Ribbands CR. 1955. The scent perception of the honeybee. Proc R Entomol Soc Lond B. 143:367–379.

Sandoz JC, Laloi D, Odoux JF, Pham-Delègue MH. 2000. Olfactory information transfer in the honeybee: compared efficiency of classical conditioning and early exposure. Anim Behav. 59:1025–1034.

Sandoz JC, Pham-Delegue MH, Renou M. 2001. Asymmetrical generalization between pheromonal and floral odours in appetitive olfactory conditioning of the honey bee (Apis mellifera L.). J Comp Physiol A. 187:559–568.

Siegel AJ, Freedman C, Page RE Jr. 2012. Ovarian control of nectar collection in the honey bee (*Apis mellifera*). PLoS One. 7:e33465. doi:10.1371/journal.pone.0033465.

Sokal RR, Rohlf FJ. 1995. Biometry: the principles and practice of statistics in biological research. New York: State University of New York. p. 715.

Takeda K. 1961. Classical conditioned response in the honey bee. J Insect Physiol. 6:168–179.

Waddington KD, Gottlieb N. 1990. Actual vs perceived profitability: A study of floral choice of honey bees. J Insect Behav. 3(4):429–441.

Waser NM. 2001. Pollinator behavior and plant speciation: looking beyond the "ethological isolation" paradigm. In: Chittka L, Thomson JD, editors. Cognitive ecology of pollination—animal behavior and floral evolution. Cambridge, UK: Cambridge University Press. p. 318–335.

Willmer PG, Stone GN. 2004. Behavioral, ecological, and physiological determinants of the activity patterns of bees. Adv Stud Behav. 34:347–466.

Woodward GL, Laverty TM. 1992. Recall of flower handling skills by bumble bees: a test of Darwin's interference hypothesis. Anim Behav. 44:1045–1051.