

## Chapter I

### ***Apis mellifera* bees modulate their dance according to perceptual properties of a flower patch**

**Abstract** Dance behaviour allows *Apis mellifera* bees to exchange resource-related information. How information about profitability of a nectar source is encoded in the dance has thus far been one of the most studied aspects of dance communication. Correlations between dance parameters and physical measures of reward, such as flow rate or sugar concentration, have been described in depth. However, there are a number of further parameters that influence the subjective expression of dance parameters with respect to the dancer's foraging success. During foraging, bees learn a variety of complex features that allow them to select an appropriate foraging strategy under the current conditions of reward. We addressed the question of how such learning processes interact with the evaluation of reward using the bee dance as a subjective measure for an individual reward estimate. Bees were trained to forage on targets of two colours in an artificial flower patch with constant reward conditions but varying perceptual properties of the patch targets. In the first phase of the experiment, targets of both colours provided similar reward probabilities. In the second phase of the experiment, test bees experienced a similar total reward, but only on targets of one colour, whereas control bees did not encounter a change. Test bees developed a different search pattern while their general income rate and foraging costs stayed constant. Their dance probability before the main unload (the main transfer of nectar to worker bees) increased. Control bees behaved as during the first phase. Since dance probability before the main unload mostly reflects individual experience with the food source, we conclude that the changes in the flower patch's perceptual signals modulated the dance behaviour of the test bees. The results are discussed in the context of energy gain and information flow in the honeybee colony.

## Introduction

*Apis mellifera* bees perform dances inside their hive to communicate with their nestmates about desirable food sources (von Frisch 1927, 1965). The waggle dance is a form of locomotion in which a forager bee, after returning from a food-source, starts walking quickly in figures of eight. An intermediate waggle phase divides the two alternating, opposite circles. During that phase the bee performs a straight waggle run with fast rhythmic, lateral movements of the whole body. For the most part, the whole performance takes place on the vertical comb, while the dancer is being followed by several potential recruits (von Frisch 1965). The dance conveys spatial information related to the food source as well as information about its intrinsic profitability (von Frisch 1965). Several dance parameters, e.g. the number of dancing events and the decision whether to dance or not, are clearly correlated to the profitability of the food source (Lindauer 1948, Núñez 1970, Seeley *et al.* 2000, De Marco and Farina 2001, for reviews see: von Frisch 1965, Seeley 1995, Dyer 2002). Food source profitability, however, as perceived by dancing bees, does not vary linearly. Instead, honeybees appear to derive their final estimate based on a subjective scale (Seeley 1995), and this process depends upon biotic (e.g. the available food stock inside the hive and the income rate of the harvested nectar) as well as abiotic factors (e.g. temperature, rainfall, wind force and season) (Lindauer 1948, Boch 1956, Núñez 1970, Seeley 1986, Seeley and Towne 1992, De Marco 2006, for reviews see: von Frisch 1965, Seeley 1995).

In addition, bees are able to learn and recall specific visual features of an artificial food source (von Frisch 1915, 1919, Menzel 1967, 1968, for reviews see: Opfinger 1931, Daumer 1956) and benefit from these stimuli in order to adapt their foraging strategy to changing reward conditions (Núñez 1971, Greggers and Menzel 1993, Menzel 1999). Furthermore, evidence has been reported indicating that bees compute visual stimuli as well as reward expectations (which appear to depend upon the memories of features that signal the experienced rewards), while deciding where and how to forage within an artificial flower patch (Greggers and Menzel 1993). Although the computation of subjective reward values based on visual features remains unknown. Our study aimed to address the relationship between the reliability of visual stimuli available during foraging (as reward predictors) and the animals' subjective evaluation of the offered reward, as revealed through their subsequent dance behaviour. Indeed, the only evidence of the animals' ability to process complex sets of stimuli is indirect evidence that must be obtained from their ensuing behaviour. In evaluating

whether and how bees perceive variations in the reliability of a visual target, we took advantage of the waggle dance, because it is a self-regulated, finely controlled motion system which appears to be suitable to reveal incentive phenomena (Raveret-Richter and Waddington 1993, De Marco and Farina 2001, De Marco *et al.* 2005). We created signal conditions of different reliabilities by distributing the reward either on targets of two different colours or only on one of two colours. We hypothesised that a food source with a more reliable signal that surpasses the bee's expectation – in this case we offered the reward on only one of the colours – might be rated as a more profitable food source compared to a food source with a more uncertain signal which would correspond to the bee's expectation – in this case we offered the reward on both colours. We assumed that a change in the visual experience during foraging would have an effect on foraging and dance behaviour under conditions of a constant energy balance.

## **Material and Methods**

An observation hive with approximately 5000 honeybees *Apis mellifera ligustica* (supplier: Apiary Mehler, Wallenborn/Vulkaneifel, Germany) was placed in a laboratory at the Institute of Neurobiology of the Freie Universität Berlin (52°31'12'' N, 13°24'36'' E, 35 msn.). The bees had free access to the surrounding gardens to forage for nectar and pollen. The experiment was conducted during late summer, when natural food supply markedly decreases. Bees were daily presented with 50 ml of 20 %-sucrose solution prior to the beginning of the experiments in order to minimise variations of the general nectar influx. The observation hive was kept in darkness except at times of video-recordings.

A group of foragers (5-10 individuals) was trained to forage at the experimental arena (100 m away from the hive entrance) using varying achromatic, grey discs holding drops of sucrose solution of the same concentration as used during the experiment. Single bees were then selected, that learned to feed on the stimuli used in the experiment. Bees were fed *ad libitum* during the pre-training to learn the location and the visual properties of the stimuli. Bees of the test and control groups were trained and tested alternatingly, with a random distribution over the time of day, so that the possible effects of extrinsic conditions would be equally distributed among the different experimental groups.







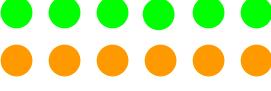


Marked bees foraged individually inside an experimental arena during the single experimental sessions. The arena (1 m x 1 m x 0.3 m) consisted of opaque walls and a plexiglass cover providing its inner space with natural daylight, including ultraviolet light.

An artificial flower patch of 16 coloured stimuli was set inside the arena. The single stimuli (2 cm in height, 8 cm in diameter) were located at equal distances and provided 10  $\mu$ l of 20 %-sucrose solution. Reward was offered through a small hole in the centre of each of the stimuli, so that hovering bees could not see the drops of sugar solution. Rewarded and unrewarded stimuli were always simultaneously offered (see below).

Colours used in the experiment were orange and green (human perception) on a grey, achromatic background. The coloured discs of 8 cm diameter that were placed on the artificial flowers were cut from colourfast papers (HKS 6 N, HKS 53 N, HKS 92 N; K+E Druckfarben, Stuttgart, Germany). Reflectances of the coloured papers were measured with a Spectrometer (SD 2000, Ocean Optics Inc., Florida) and bee-subjective chromatic properties were calculated using the Receptor-Noise-Limited Model of honeybee colour vision (Vorobyev *et al.* 2001). The minimum colour distance between two colours that can still be perceived by honeybees is  $\Delta S = 2.3$ . The  $\Delta S$  value for the two colours used was 8.9, i.e. bees could distinguish them well. Receptor specific contrasts  $q$  for the three different photoreceptor types  $i$  (with  $i = S$  (short), M (medium) and L (long wave receptor)) were  $q_S = 0.74$ ,  $q_M = 0.46$  and  $q_L = 1.97$  for the orange colour and  $q_S = 0.88$ ,  $q_M = 1.29$  and  $q_L = 1.97$  for the green colour. The distances to the background were  $\Delta_{bg} = 10.83$  for orange and  $\Delta_{bg} = 4.60$  for green.

Every bee had to complete 20 foraging bouts (henceforth: trials), divided into two phases each of 10 trials, of which the first phase provided equal conditions for test and control bees (Tab. 1). At random positions, 4 of 16 stimuli were rewarded (2 orange and 2 green stimuli) and 12 were unrewarded (6 orange and 6 green stimuli). During the second phase, test and control groups encountered different conditions. Half of the test bees (henceforth: orange group,  $N = 6$ ) were offered 4 rewarded orange stimuli among the 16 stimuli, of which 4 orange and 8 green stimuli were unrewarded. The other half of the test bees (henceforth: green group,  $N = 6$ ) encountered reversed conditions. Conditions for the control bees ( $N = 6$ ) did not change throughout the experiment. They continued to forage being rewarded on 2 orange and 2 green stimuli among the 16 total stimuli. Each rewarded stimulus contained 10  $\mu$ l of 20 %-sucrose solution, so that the bee could collect up to 40  $\mu$ l of solution in total at each trial.

**Table 1** Experimental procedure. Conditions in the first phase were in each case the same for test and control groups. In the second phase, test bees were only rewarded on one of the colours, but not all of that colour's stimuli were rewarded. Unrewarded stimuli were presented in both colours. The control group encountered the same situation in both phases.

Group	Phase 1 (10 trials) Stimuli	Phase 2 (10 trials)	
		Rewarded stimuli	Unrewarded stimuli
Orange	Rewarded 		
Green	Unrewarded 		
Control			

*Behavioural recordings in the arena*

The following variables were considered for the analysis:

- 1) The time until the first rewarded stimulus was found by the bee (pre search time), the time the bee spent searching from the first until the last rewarded stimulus (main search time) and the time spent inside the arena after the bee had found the last rewarded stimulus (post search time; in seconds).
- 2) The total number of visited stimuli, defined as the number of stimuli on which the bee landed and for the most part actively searched for a reward by means of extending her proboscis and/or sticking her antennae into the centred hole in which the reward might be offered. Landing only was also counted as a visit.
- 3) The total number of rewarded stimuli, defined as the number of stimuli on which the bee landed, searched for a reward as described above and found a reward.
- 4) The number of unrewarded stimuli, defined as landing and/or searching on an unrewarded stimulus. The number of unrewarded stimuli has been separately analysed on the basis of pre, main, and post search time in the arena (see 1)).

*Behavioural recordings in the hive*

The following within-the-hive variables were also analysed:

- 1) The total time spent in the hive (in seconds) between two successive trials (henceforth: hive time).
- 2) The time until the first contact with a nest mate or initial hive search time (in seconds); contact defined as antennating, begging or food unloading.
- 3) The number of trophallactic offering contacts (henceforth: unloads), defined as the number of events in which the experimental bee opened her mandibles during more than one second while one or more food receivers contacted her prementum with their extended proboscises (De Marco and Farina 2001). Additionally the cumulative duration of all food unloads (in seconds) was recorded.
- 4) The time until the beginning of the main unload or search time for main unload, and the duration of the main unload (in seconds), main unload being defined as the longest food unload of each trial.
- 5) The total number of food receivers at the main food unload, defined as the number of receiver nest mates that made contact with the mouthparts of the marked forager for more than one second during the food unload.
- 6) The dance probability, divided into dance probability before and after the main food unload.
- 7) The number of waggle runs.
- 8) The dance strength, defined as the duration of the dance divided by the number of waggle runs (Seeley et al. 2000). Since the distance to the arena was constant, the duration of the dance divided by the number of waggle runs reflects the mean duration of the return runs.

*Statistical analysis*

Six bees per group (two test groups and one control group) were trained and their behaviour recorded. Tests for normality and homogeneity of variances were made with the Kolmogorov-Smirnov Two-Sample Test and the Levene's Test, respectively. For comparisons between the behavioural parameters in the first and second phase we used the Wilcoxon matched pairs test and the T-Test for dependent samples. Proportions of dance probabilities were arcsine transformed before applying the T-Test (Zar 1999).

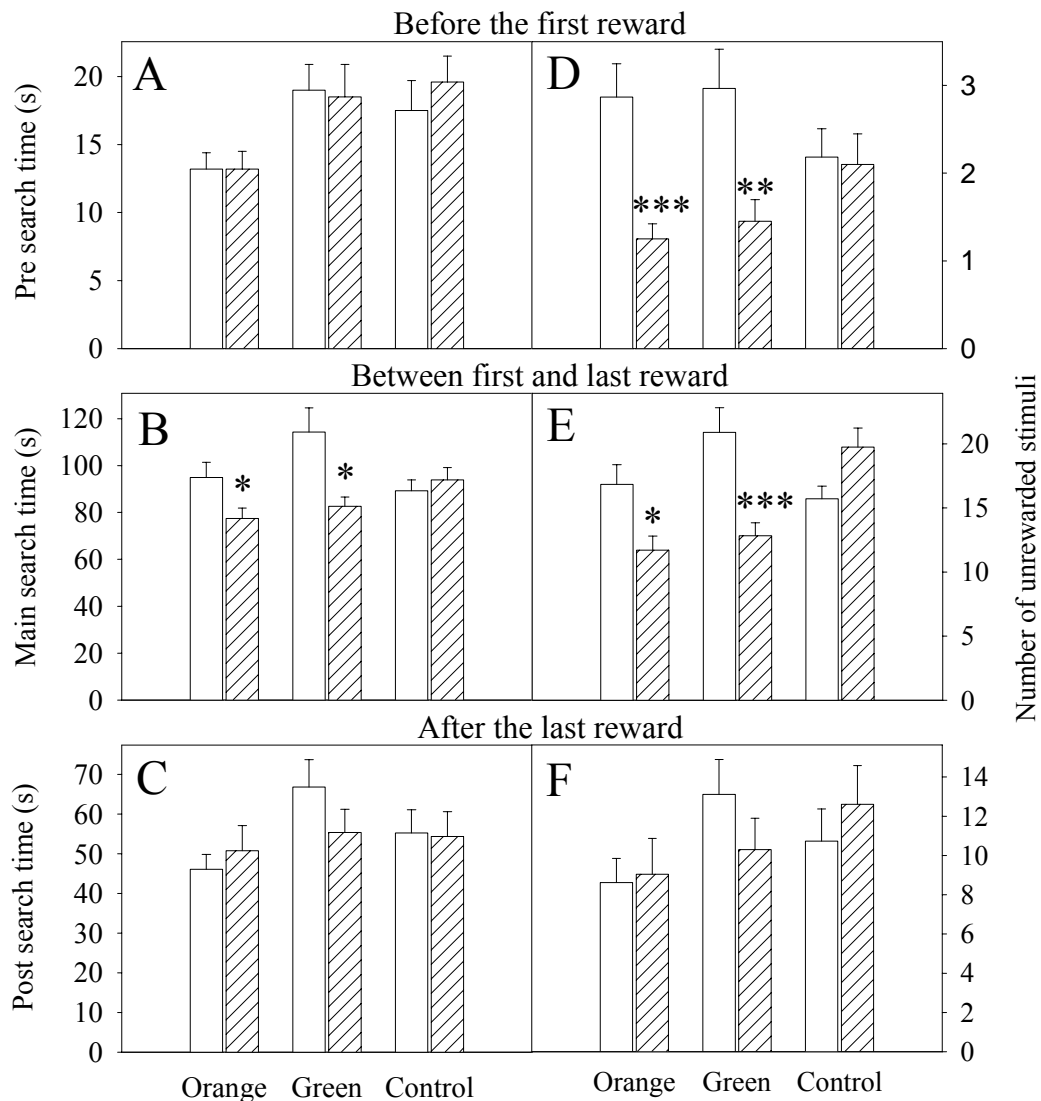
## Results

### *Foraging behaviour in the arena*

We measured the time that bees spent foraging in the arena and divided the total time in pre, main and post search time for each of the two phases of the experiment (Fig. 1). Main search time was assigned to the time between the finding of the first and the last rewarded stimulus. Pre and post search time described the time before finding the first and after finding the last rewarded stimulus, respectively. The test groups and the control group of six bees each showed no variation in pre and post search time when comparing the first and the second phase (Fig. 1A,C; pre search time: orange group:  $T = -0.05$ ,  $P = 0.96$ ; green group:  $T = 0.17$ ,  $P = 0.86$ ; control group:  $T = 0.83$ ,  $P = 0.41$ ; post search time: orange group:  $T = -0.76$ ,  $P = 0.50$ ; green group:  $T = 1.30$ ,  $P = 0.20$ ; control group:  $T = 0.12$ ,  $P = 0.91$ , T-Test). But the main search time decreased in phase 2 for the test groups whereas it did not change in the control group (Fig. 1B; orange group:  $Z = 2.2$ ,  $P < 0.05$ ; green group:  $Z = 2.2$ ,  $P < 0.05$ , Wilcoxon matched pairs test; control group:  $Z = -0.74$ ,  $P = 0.46$ , T-Test). Thus, test bees reduced the time they needed to find the rewarded stimuli during the second phase of the experiment as compared to the first phase, whereas control bees did not. However none of the bee groups varied the time in the arena before finding the first and after finding the last rewarded stimulus during the whole experiment.

Further, we counted the number of rewarded and unrewarded stimuli that were visited by the bees during the pre, main and post search time in the arena. All groups visited the same total number of unrewarded stimuli in both phases (Tab. 2; orange group:  $Z = 1.4$ ,  $P = 0.17$ ; green group:  $Z = 1.6$ ,  $P = 0.12$ ; control group:  $Z = 1.6$ ,  $P = 0.12$ , Wilcoxon matched pairs test) and collected the same amount of reward in both phases (Tab. 2; orange group:  $T = -0.89$ ,  $P = 0.38$ , T-Test; green group:  $Z = 1.6$ ,  $P = 0.12$ ; control group:  $Z = 0.9$ ,  $P = 0.35$ , Wilcoxon matched pairs test). The number of unrewarded stimuli during pre search time decreased for the test groups in the second phase, whereas the control bees did not reduce their visits to unrewarded stimuli (Fig. 1D; orange group:  $Z = 3.51$ ,  $P = 0.0005$ ; green group:  $Z = 2.55$ ,  $P = 0.01$ , Wilcoxon matched pairs test; control group:  $T = 0.16$ ,  $P = 0.87$ , T-Test for independent samples). The same was true for the number of unrewarded stimuli that were visited during main search time (Fig. 1E, orange group:  $Z = 3.04$ ,  $P = 0.002$ ; green group:  $Z = 3.48$ ,  $P = 0.0005$ ; control group:  $Z = 1.85$ ,  $P = 0.06$ , Wilcoxon matched pairs test). The remaining unrewarded stimuli that were visited during post search time did not change in either of the

groups (Fig. 1F; orange group:  $T = -0.21$ ,  $P = 0.84$ ; green group:  $T = 1.15$ ,  $P = 0.25$ , control group:  $T = -0.84$ ,  $P = 0.40$ , T-Test). To summarise, not only was the main search time of the test bees reduced, but also the number of unrewarded stimuli visited during the main search time as well as during pre search time were reduced. The total number of unrewarded stimuli was not significantly different, because all bees continued searching on unrewarded stimuli after finding last rewarded one.



**Fig. 1** Search related arena data: **A** pre search time, **B** main search time and **C** post search time (in seconds), **D** unrewarded stimuli during pre search time, **E** unrewarded stimuli during main search time and **F** unrewarded stimuli during post search time. For a detailed explanation of the variables see Material and Methods. Shown are the mean values and the standard error of the test (orange, green) and control bees in phase 1 (open bars) and 2 (hatched bars). Pre and post search time did not change significantly from the first to the second phase. The main search time of the test groups was reduced in phase 2, whereas the control group's search time did not change during the experiment. The initial number of unrewarded stimuli during pre search time and the main unrewarded stimuli during main search time of the test groups was reduced in phase 2. Corresponding unrewarded stimuli of the control bees was not reduced. Remaining unrewarded stimuli during post search time did not change in neither of the groups. \*  $P < 0.05$  \*\*  $P < 0.005$  \*\*\*  $P < 0.001$ , Wilcoxon matched pairs test.



**Table 2** All recorded variables in the arena and in the hive of the test groups (orange, green) and the control group, separated into phase 1 and 2 (each 10 trials). Shown are the mean values  $\pm$  SE and the p-values of the T-Test for dependent samples and of the Wilcoxon matched pairs Test, respectively. For an explanation of the variables see Material and Methods; for further statistical details see Results.

Variable	Orange			Green			Control		
	Phase 1	Phase 2		Phase 1	Phase 2		Phase 1	Phase 2	
Visited stimuli	32,0 $\pm$ 1,9	25,8 $\pm$ 2,1	<b>p = 0.17<sup>a</sup></b>	40,5 $\pm$ 2,4	28,4 $\pm$ 1,8	<b>p = 0.12<sup>a</sup></b>	32,3 $\pm$ 2,0	38,3 $\pm$ 2,3	<b>p = 0.12<sup>a</sup></b>
Rewarded stimuli	3,7 $\pm$ 0,1	3,8 $\pm$ 0,05	<b>p = 0.38<sup>b</sup></b>	3,7 $\pm$ 0,06	3,9 $\pm$ 0,04	<b>p = 0.12<sup>a</sup></b>	3,7 $\pm$ 0,06	3,9 $\pm$ 0,05	<b>p = 0.35<sup>a</sup></b>
Hive time [s]	121,4 $\pm$ 4,1	150,1 $\pm$ 7,9	<b>p &lt; 0.05<sup>a</sup></b>	126,4 $\pm$ 7,6	119,6 $\pm$ 4,1	<b>p = 0.31<sup>b</sup></b>	139,5 $\pm$ 6,9	191,3 $\pm$ 20,7	<b>p = 0.25<sup>a</sup></b>
Initial hive search time [s]	14,3 $\pm$ 1,4	17,5 $\pm$ 2,3	<b>p = 0.17<sup>b</sup></b>	10,3 $\pm$ 0,7	12,8 $\pm$ 0,9	<b>p &lt; 0.05<sup>b</sup></b>	14,7 $\pm$ 1,3	16,6 $\pm$ 1,4	<b>p = 0.33<sup>b</sup></b>
All unloads	2,9 $\pm$ 0,2	4,1 $\pm$ 0,2	<b>p &lt; 0.001<sup>b</sup></b>	4,0 $\pm$ 0,2	4,1 $\pm$ 0,3	<b>p = 0.63<sup>b</sup></b>	4,0 $\pm$ 0,2	3,9 $\pm$ 0,3	<b>p = 0.95<sup>b</sup></b>
Unloading time [s]	22,9 $\pm$ 0,8	23,4 $\pm$ 1,1	<b>p = 0.71<sup>b</sup></b>	27,1 $\pm$ 0,9	26,4 $\pm$ 1,1	<b>p = 0.55<sup>b</sup></b>	26,9 $\pm$ 0,9	24,6 $\pm$ 0,9	<b>p = 0.08<sup>b</sup></b>
Search time for main unload [s]	23,1 $\pm$ 3,2	40,1 $\pm$ 5,2	<b>p &lt; 0.001<sup>b</sup></b>	19,0 $\pm$ 2,0	21,9 $\pm$ 2,1	<b>p = 0.29<sup>b</sup></b>	23,3 $\pm$ 2,2	28,1 $\pm$ 2,2	<b>p = 0.12<sup>a</sup></b>
Main unloading time [s]	18,4 $\pm$ 0,8	16,5 $\pm$ 0,7	<b>p = 0.25<sup>a</sup></b>	20,0 $\pm$ 0,7	19,9 $\pm$ 0,9	<b>p = 0.92<sup>b</sup></b>	19,8 $\pm$ 0,6	17,6 $\pm$ 0,7	<b>p &lt; 0.05<sup>b</sup></b>
Receivers at main unload	2,6 $\pm$ 0,1	2,6 $\pm$ 0,1	<b>p = 0.78<sup>b</sup></b>	2,4 $\pm$ 0,2	2,4 $\pm$ 0,1	<b>p = 0.86<sup>b</sup></b>	2,5 $\pm$ 0,2	2,3 $\pm$ 0,1	<b>p = 0.27<sup>b</sup></b>
General dance probability	0,5 $\pm$ 0,07	0,8 $\pm$ 0,05	<b>p = 0.08<sup>a</sup></b>	0,5 $\pm$ 0,07	0,8 $\pm$ 0,06	<b>p = 0.28<sup>a</sup></b>	0,6 $\pm$ 0,06	0,7 $\pm$ 0,06	<b>p = 0.94<sup>a</sup></b>
Waggle runs	15,8 $\pm$ 2,7	27,4 $\pm$ 3,1	<b>p = 0.32<sup>b</sup></b>	9,5 $\pm$ 2,0	13,8 $\pm$ 1,9	<b>p = 0.25<sup>b</sup></b>	10,4 $\pm$ 1,9	17,6 $\pm$ 2,4	<b>p = 0.09<sup>b</sup></b>
Dance strength	1,8 $\pm$ 0,1	1,7 $\pm$ 0,07	<b>p = 0.08<sup>a</sup></b>	1,8 $\pm$ 0,1	1,6 $\pm$ 0,04	<b>p = 0.08<sup>a</sup></b>	1,9 $\pm$ 0,1	1,8 $\pm$ 0,05	<b>p = 0.69<sup>a</sup></b>

a = Wilcoxon matched pairs test, b = T-Test for dependent samples

### *Behaviour in the hive*

We measured the time that bees spent in the hive between two consecutive trails separately analysing the total hive time, the initial hive search time, the cumulative unloading time, the search time for the main unload and the main unloading time. Additionally, we counted the number of food-unloading events and the number of receivers at the main unload.

The orange group spent more time in the hive in phase 2 compared to phase 1 whereas the green group and control bees did not change their hive time (Tab. 2; orange group:  $Z = 2.0$ ,  $P < 0.05$ , Wilcoxon matched pairs test; green group:  $T = 1.02$ ,  $P = 0.31$ , T-Test; control group:  $Z = 1.2$ ,  $P = 0.25$ , Wilcoxon matched pairs test). The initial hive search time did not change in the orange and the control groups, but increased in the green group (Tab. 2; orange group:  $T = -1.39$ ,  $P = 0.17$ ; green group:  $T = -2.13$ ,  $P = 0.038$ ; control group:  $T = -0.98$ ,  $P = 0.33$ , T-Test). Since in both cases only one of the test groups differed from the control group, the behaviour most probably did not take place in reaction to the changed environment in the arena.

The number of food-unloading events varied in the orange group. In Phase 2, they divided their unloading time into a higher number of shorter unloads. No differences were found in the green group and the control group (Tab. 2; orange group:  $T = -4.76$ ,  $P = 0.000013$ ; green

group:  $T = -0.48$ ;  $P = 0.63$ ; control group:  $T = 0.06$ ,  $P = 0.95$ , T-Test). Consequently, the cumulative duration of all food-unloading events stayed the same for all three groups throughout the whole experiment (Tab. 2; orange group:  $T = -0.37$ ,  $P = 0.71$ ; green group:  $T = 0.60$ ,  $P = 0.55$ ; control group:  $T = 1.76$ ,  $P = 0.08$ , T-Test).

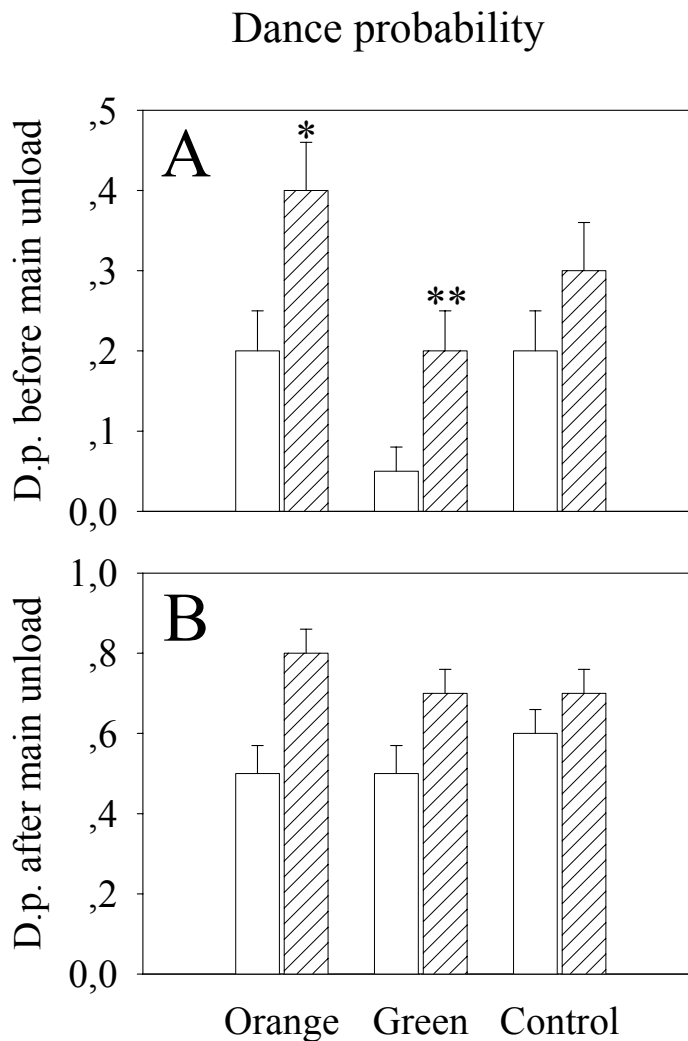
The orange group increased the time until the beginning of the main unload in the second phase. The green group and the control group did not change their behaviour (Tab. 2, orange group:  $T = -3.78$ ,  $P = 0.00037$ ; green group:  $T = -1.07$ ,  $P = 0.29$ , T-Test; control group:  $Z = 1.6$ ,  $P = 0.12$ , Wilcoxon matched pairs test). But the duration of the main unload was the same for both phases in the test groups. The control group decreased the duration in the second phase (Tab. 2; orange group:  $Z = 1.2$ ,  $P = 0.25$ , Wilcoxon matched pairs test; green group:  $T = 0.10$ ,  $P = 0.92$ ; control group:  $T = 2.79$ ,  $P = 0.007$ , T-Test). The latter two cases – search time for main unload and main unloading time – show the same phenomenon as the cases of total hive time and initial hive search time. Only one of the test groups or only the control group showed a different behaviour in the second phase. Therefore we conclude that the changed environment in the arena did not modulate the bees' behaviour.

In all groups, the number of food receivers at the main unload stayed the same throughout the whole experiment (Tab. 2, orange group:  $T = 0.29$ ,  $P = 0.78$ ; green group:  $T = -0.18$ ,  $P = 0.86$ ; control group:  $T = 1.11$ ,  $P = 0.08$ , T-Test).

Finally, we analysed three different dance parameters: dance probability, the number of waggle runs and dance strength (Tab. 2). Dance probability was divided into dance probability before and after the main unload and analysed separately. In all groups, general dance probability did not change (Tab. 2, orange group:  $T = -2.15$ ,  $P = 0.084$ ; green group:  $T = -1.22$ ,  $P = 0.28$ ; control group:  $T = 0.07$ ,  $P = 0.94$ , T-Test of dependent samples). However, the dance probability before the main unload changed in the test groups, where it was higher in the second phase (Fig. 2A, orange group:  $T = -3.42$ ,  $P = 0.019$ ; green group:  $T = -5.69$ ,  $P = 0.0023$ ; control group:  $T = -0.69$ ,  $P = 0.52$ , T-Test of dependent samples). That effect was not noticeable when comparing the dance probabilities after the main unload (Fig. 2B, orange group:  $T = -1.91$ ,  $P = 0.11$ , green group:  $T = -1.22$ ,  $P = 0.28$ ; control group:  $T = -1.07$ ,  $P = 0.33$ , T-Test of dependent samples). The number of waggle runs, which correlates with dance duration (von Frisch 1965), ranged from one to ninety-three. It did not change significantly in either of the groups (Tab. 2, orange group:  $T = -1.02$ ,  $P = 0.32$ ; green group:  $T = -1.18$ ,  $P = 0.25$ , T-Test; control group:  $Z = 1.6$ ,  $P = 0.12$ , Wilcoxon matched pairs test). Nor did the dance strength change from the first to the second phase (Tab. 2; orange group:  $T = 0.93$ ,  $P =$

0.36, T-Test; green group:  $Z = 1.75$ ,  $P = 0.08$ ; control group:  $Z = 0.4$ ,  $P = 0.69$ , Wilcoxon matched pairs test).

To sum up, we found a significant effect with respect to the parameter which reflects the readiness of the animal to dance, i.e. the dance probability, but no changes in the strength of the dance expression once animals did decide to dance. In other words, dance probability before the main unload was enhanced in the test groups during the second phase.



**Fig. 2** Dance probability **A** before the main unload and **B** after the main unload. Shown are the mean values and the standard error of the test (orange, green) and control bees in Phase 1 (open bars) and 2 (hatched bars). The test bees' dance probability before the main unload increased in the second phase, whereas the control bees did not change their behaviour. Dance probability after the main unload did not change from the first to the second phase in either of the groups. \*  $P < 0.05$ , \*\*  $P < 0.005$ , Wilcoxon matched pairs test.

## **Discussion**

### *Behavioural modulation in response to changes in the properties of the patch*

Search behaviour of the test groups in the arena during the second phase changed compared to that of the first phase. They developed a different search pattern, while spending a similar amount of time in the arena (Tab. 2). In that process, they showed a decrease in main search time, whereas the control bees could not reduce their main search time (Fig. 1B). Pre and post search time did not vary significantly (Fig. 1A, C). A second characteristic of the new search pattern was the reduction of visits to unrewarded stimuli. Test bees could reduce their visits to unrewarded stimuli during pre and main search time (Fig. 1E, F), while overall visits to unrewarded stimuli during one trial were not reduced – test bees and control bees visited a similar number of unrewarded stimuli (Tab. 2). Intriguingly enough, although test bees spent a similar amount of time foraging and collected similar amounts of sugar solution inside the arena during both phases, they changed their dance behaviour during the second phase of the experiment (Fig. 2).

Present results allow us to describe how the test bees changed their foraging behaviour inside the arena. Moreover, they clearly point toward a relationship between their experience inside the arena and the changes we observed in their subsequent dances. But how did the test bees evaluate their foraging experience in order to modify their subsequent dances? In other words, how did the changes in the arrangement of the visual stimuli lead to changes in the foragers' dances? Indeed, our results indicate that, based on visual stimuli, bees compute complex features of their goals. Although the regulation of the dance behaviour involves multimodal convergence, the most salient stimuli determining both the occurrence and the strength of the foragers' dances depend upon the amount of energy obtainable at the indicated goal (von Frisch 1965). In the present context, however, we took advantage of the dance behaviour in order to address the dancer's perceptual world, irrespective of the total amount of energy available at the foraging goal. More specifically, we tackled the processing of visual information underlying the evaluation of a complex feature of an artificial, compound flower patch, namely, the reliability of their single coloured targets. Our results indicate that bees are able to compute variations in the reliability of their foraging targets based on visual stimuli.

*The influence of target reliability on foraging strategy and dance communication*

During foraging, the cost of visiting an unrewarded flower is comparatively low (Heinrich 1975). Individual bumblebees, for example, can forage rather quickly, which enhances their number of errors (visits of unrewarded stimuli) or slower by enhancing their accuracy. Both strategies seem to be equally beneficial until unrewarded stimuli in laboratory conditions become penalising. Bees appear to improve their accuracy within the context of these alternative strategies (Chittka *et al.* 2003), although results by Burns (2005) showed that, when performance is measured on the basis of nectar collection rate, fast and inaccurate bees perform better than those being slow and accurate. To achieve accuracy, an efficient detection and discrimination of conditioned stimuli is essential. In the present context, neither the rate of nectar collection nor the overall accuracy and foraging speed changed during the experiments (Tab. 2), but the test bees seem to have identified the more reliable visual stimuli during the second phase of the experiment, and increased both their foraging speed and accuracy during the main-search time (Fig. 1B, E). Menzel and Greggers (1992) have shown that reward expectations associated to specific targets may activate recent memories and control foraging decisions at an artificial flower patch. In our case, the amount of reward associated with only one of the target colours suddenly increased in the second phase, and probably exceeded the bees' expectations that were formed for this colour in the first phase. We conclude that the test bees perceived the higher target reliability of one of the rewarded coloured stimuli during main search time and responded to it via their waggle dances.

Interestingly, bees in our experiment still visited the stimuli with the unrewarded colour during the second phase, once they had found all rewarded stimuli, because they had associated a reward with that colour during the first phase. Such foraging strategies are a consequence of the floral constant foraging behaviour of honeybees (von Frisch 1965, Heinrich 1975, Menzel 1985). Floral constant foragers principally tend to restrict their visits to only a few or one of the available plant species. The intensity of the constancy is dependent on the amount and frequency of available resources (Kevan and Baker 1983, Chittka *et al.* 1999). Oster and Heinrich (1976) developed a model, which indicates that the pure strategy of "majoring" is always better than random foraging if the reward structure remains approximately constant with time. But "minoring", as described above, is a necessary compromise required to track resources changing with time. If two morphologically different species are equally unpredictable in reward or accessibility bees will not develop constancy for one of the species but will switch between flowers, when one

of the species is not profitable enough. When both species are equally rewarding and accessible and profitable enough on their own, bees will specialise to visit only one species (Marden and Waddington 1981, in stingless bees: Slaa et al. 2003). Such behaviour also indicates that target reliability is perceptible to the bee and might influence dance behaviour. Work by Núñez (1970) and De Marco (2006) allows differential interpretation of the probability of the dances that occur before and after the food transfer. De Marco (2006), for instance, showed that the threshold for dancing changes on the basis of stimuli available during the food transfer, and that these stimuli are related to the colony's nectar influx. Indeed, it has long been reported that the probability of the dances that occur after the food transfer depends upon both the properties of the goal being advertised as well as those stimuli arising at the group level within the nest (Lindauer 1948, 1954, Núñez 1970, Seeley 1998, De Marco 2006, for a review see: Seeley 1995). In contrast, dance probability before the food transfer cannot be influenced by information available via the food unloading. If a forager dances immediately after entering the hive, it is reasonable to assume that the triggering of its motor display is influenced by the recent foraging experience at the flower patch. In our experiments, the test bees decreased their thresholds for dancing before the main unload (Fig. 2A), and we conclude that these variations can be explained on the basis of the simultaneous variations in target reliability offered at the flower patch. We found variations neither in the colony nectar influx nor in the dance probability after the food transfer (Tab. 2, Fig. 2B).

### *Energetic balance of foraging bouts*

The profitability of the reward is dependent on various factors: 1) The energy that is being used to find and to take in the reward and finally to bring it home has to be subtracted from 2) the energy that is being gained through foraging depending on amount, sucrose concentration and flow rate of the reward (von Frisch 1965, Heinrich 1975, 1983). And 3) the rate of positive against negative association events with the conditioned stimulus (colour) may also influence the response to the foraging situation (actual and perceived profitability of the reward) through acquisition and extinction (Menzel 1967, 1968, 1969, Bittermann *et al.* 1983, Menzel and Greggers 1992, De Marco and Farina 2003).

Experimental bees of the test and control groups behaved similarly in the arena. They consumed an analogue amount of energy during one trial, as they all had to fly the same distance to the arena and back to the hive, they spent an analogue amount of time flying in

the arena, they landed on a similar number of total stimuli and ingested an analogue amount of reward (Tab. 2, Fig. 1). The reward always had the same viscosity resulting in the same ingestion time (Núñez 1982). Since the individuals of all groups were tested at random, they were on the whole exposed to similar weather conditions. Hence, our conclusion is that the energetic balance did not change from the first to the second phase.

### *Energy gain and information flow in the honeybee colony*

The foraging behaviour of honeybees is organised as a balance between maximising individual efficiency for a short term gain of energy and optimising colony efficiency for long-term gain (Núñez 1982). Núñez described in his work two standpoints of the foraging bee: 1) the energy standpoint; to collect the maximum nectar amount whatever the circumstances are, e.g. in terms of distance to the food source or flow rate of the reward, and 2) the information standpoint; to gain as much information as possible about other potentially better food sources, which can be achieved through a high and short trip frequency. To increase information flow, individual energy gain has to be reduced and *vice versa*. We would like to extend the latter standpoint with the idea that the forager bee also *gives* information. Honeybees, in the consequence of their social behaviour, do not only try to find the best food source by gathering information during their stay in the hive. During the process of optimising the flow of information within the colony, foragers also optimised the distribution of their gathered information in the field to other potential foragers in the hive to increase the colony's income rate. If a bee forages on a low rewarding flower patch, it would not be useful if additional foragers join, because their presence would diminish the first forager's income rate (Heinrich 1983). The same could be true if the flower patch consists of only a few, highly rewarding flowers. The situation would become different, if the reliability of the rewarded flowers increases. If the proportion of rewarded against empty flowers were to increase within one species, while the number of flowers remained constant, there would be a better chance for additional foragers to visit these flowers without disturbing each other and diminishing each other's income rate. As a result, resource uncertainty may have been incorporated into the mechanisms devoted to resource evaluation, i.e. the forager might dance with either a higher probability or with a higher intensity or both when resource uncertainty decreases. We noticed an effect on dance probability, but not on dance strength or number of waggle runs. Interestingly, the information flow (frequency of foraging trips - data not shown) in the second phase of our experiment remained the same, i.e. the forager's

investment in information flow did not increase – certainly not at the cost of its energy gain. There was no trade-off of information flow for energy gain in the second phase of the experiment. There was however an emphasis on the information *given* by the foraging bee to the recruits at the cost of information *gained* by the forager from her nest mates. This phenomenon was also described in reversed conditions by De Marco and Farina (2003). They observed that an increased resource uncertainty enhanced the forager's begging behaviour (short trophallactic contacts with nest mates to gain information about the food quality brought by other foragers).

Overall we conclude that changes in spectral and spatial perceptual properties of a flower patch have led to modulated dance behaviour in honeybees, which reflects their different estimation of resource profitability. These results pose new intriguing questions regarding the cognitive requirements and neural correlates of the complex computation of resource profitability. We see the study of the mechanisms that modulate the dance threshold in relation to reward evaluation as a challenge that should be taken up. How information concerning changes in the environment is transmitted through the bees' dance and received by the recruits and how the recruits, in turn, modulate their subsequent foraging decisions are processes that need to be investigated in order to understand the whole complex system.

### **Acknowledgements**

We want to thank W.M. Farina for thoughtful comments on the experimental design and fruitful discussions. Sincere thanks are given to S. Seefeldt and D. Drenske for motivated help during the experiment. A.M. Wertlen was funded by a doctoral fellowship of the Berlin Program for the Promotion of Equal Opportunities for Women in Science and Higher Education. The present experiments comply with the Principles of Animal Care of the National Institute of Health and the corresponding national current laws.



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