The Journal of Experimental Biology 211, 3392-3400
Published by The Company of Biologists 2008
doi:10.1242/jeb.017624

The response of the honeybee dance to uncertain rewards

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Accepted 1 September 2008

SUMMARY

This work focuses on the responses of dancing bees to uncertain rewards. We varied the distribution of a fixed amount of sugar solution among the several flowers of a patch and recorded the foraging and subsequent dance behaviour of single honeybees collecting such a reward at that patch. Concurrently, we aimed to minimize the well-known modulatory effects of sugar reward on both the probability and the strength of a honeybee's dance. It was under these circumstances that we conceived the honeybee dance as an autonomous information-processing system and asked whether or not such a system is sensitive to uncertainty of reward. Our results suggest that bees can tune their dancing according to the distribution of sugar reward among the several flowers of a patch, and that they seemingly do this based on the number – or the frequency – of their non-rewarding inspections to these flowers: the higher the number of non-rewarding inspections the lower the probability of dancing. As a result, a honeybee's dance appears as 'risk-averse', meaning that dances for uncertain resources are less likely. Presumably, the ultimate result of having 'risk-averse' dances is a colony's ability to diminish delayed rewards and the effects of competition with other flower visitors for limited resources. We conclude that a systems approach to the honeybee dance will help to further analyse the regulation of a honeybee's threshold for dancing, and that theoretical accounts of 'risk-sensitive' dances would prove fruitful in broader studies of honeybee foraging, particularly if one were to examine how recruitment actually translates into fitness.

Key words: *Apis mellifera*, reward uncertainty, recruitment dances, risk-sensitive behaviour, reward learning.

INTRODUCTION

Within a time scale of several hours, pollinators face a seemingly unpredictable scenario because of the high variability in the volumes and concentrations of the nectar found within flowers (Baker and Baker, 1983). Like many other animals, honeybees have evolved strategies to face such variability. Their famous dance communication system (von Frisch, 1967) is an example of that kind of strategies. A honeybee's dance consists of a series of seemingly ritualized movements that foragers perform on the comb surface and use to recruit conspecifics from the nest – or the swarm – to the location of desirable resources. Compelling evidence indicates that it is part and parcel of a series of communication systems enabling a colony to coordinate the activity of its members during foraging and nest-site selection (e.g. Seeley, 1995). This is possible because those colony members that keep close contact with a dancing bee, usually called dance followers, appear to detect a variety of signals emitted by the dancer (e.g. Michelsen, 2003), and process them in such a way that their subsequent behaviours can greatly depend upon the content of these signals.

The key stimulus for dancing is the presence of a sugar reward at a given feeding place. Apparently, a dance is triggered when the amount and sugar concentration of sugar reward exceeds a threshold that has previously been established by a dancer's central nervous system according to several properties of the feeding place (e.g. Seeley, 1986; De Marco and Farina, 2001), the dancer's past experience with such a reward (Raveret-Richter and Waddington, 1993; De Marco et al., 2005) and various stimuli available within the colony (e.g. Núñez, 1970; Seeley, 1986; Seeley, 1989; De Marco, 2006). Thus, the probability and the strength of the dance are presumably graded according to both the dancer's estimate of the overall quality of the feeding place and the current needs of the colony. Yet, what has remained elusive in the study of the honeybee dance is whether and how a dancer responds to variations in the level of uncertainty of reward, as one would reasonably expect from any behaviour that has seemingly evolved to convey information about reward opportunities. This is of considerable interest, given the fact that the dance has long been quoted as an intriguing example of a complex behaviour serving the transfer of such type of information (e.g. von Frisch, 1967; Seeley, 1995; Dyer, 2002; De Marco and Menzel, 2008). Is the honeybee dance sensitive to uncertainty of reward, irrespective of the costs and benefits of a dancer's foraging activities? Information has long been described as an abstract quantity that removes or reduces our current level of uncertainty. Clearly, uncertainty appears as the opposite of information, and depends upon the relative proportion of at least two mutually exclusive inputs (Shannon, 1948). In the present context, reward uncertainty translates into the relative proportion of rewarding and non-rewarding flower inspections.

We present the results of two experimental series addressing the relationship between a honeybee's experience with uncertain rewards and its subsequent dance behaviour within the colony. We varied the distribution of a fixed amount of unscented sucrose solution among the several flowers of a patch, thereby manipulating the arithmetic mean and variance of the volume of sugar reward per flower, and recorded the foraging and dance behaviour of single honeybees. We found evidence supporting the view that a honeybee's dance involves a component that computes not only the energy balance during foraging, but also an estimate of the distribution of food resources among the several flowers recently visited by the dancer. As a consequence, the dance system appears as 'risk averse'. We propose that 'risk-averse' dances help to optimize a colony's energy balance during foraging by diminishing delayed rewards and the effects of competition with other flower visitors for limited resources.

MATERIALS AND METHODS

Overview

In both experimental series, we used a colony of *Apis mellifera* L. bees placed indoors in a two-frame observation hive. We marked forager bees, and trained them to forage on sixteen artificial flowers placed inside a 1 m×1 m×0.3 m arena. Only one bee foraged inside the arena during each experimental session. In both series, the arena was placed ~100 m away from the hive, either outdoors (covered by UV-transmitting Plexiglas) or indoors, in the first and the second series, respectively. Each artificial flower (hereafter referred to as a flower) consisted of a 75 mm wide, 20 mm high plastic cylinder with the upper surface covered by a yellow paper disc (diameter: 75 mm; HKS® 3N; K+E Druckfarben, Germany). We recorded foraging and within-the-hive behaviours of single bees by means of digital video cameras, webcams, and voice recorders. The experiments were conducted during the late summer and the beginning of autumn, when natural resources are scarce. The first experimental series (hereafter, S1) focused on the relationship between the distribution of a fixed amount of unscented sucrose solution among the 16 flowers of the arena and the foraging and subsequent within-the-hive behaviours of the single foragers. The second series (hereafter, S2) was an extension of S1, in that it focused on the same question by means of a similar protocol, although we changed the presentation of the different groups and incorporated additional variables into the analysis.

The first experimental series (S1)

Marked bees were first trained to forage on a close feeder offering unscented 50% (w/w) sucrose solution. Next, the feeder was gradually moved away from the hive towards the vicinity of the arena. After all the marked bees resumed at least five consecutive visits to the arena, a single bee was randomly selected from the group. (The remaining foragers were captured, kept inside small cages, fed with 20% w/w sucrose solution, and released at the end of the experiment.) The selected bee was then allowed to resume six additional visits to the arena. Throughout these visits it was presented with the treatments described below (Table 1), in a semi-random fashion. This enabled us to establish steady-state foraging conditions at the beginning of each session. The floor of the arena provided the bees with a homogeneous grey background. The sixteen flowers were regularly distributed inside the arena, placed at equal distances of 20 cm (centre to centre), and their relative positions were randomly interchanged in-between the single visits of the experimental bee. Each rewarding flower offered unscented 20% w/w sucrose solution by means of a small plastic receptacle (4 mm high) at the centre of the upper surface of the plastic cylinder.

We defined our treatments (Table 1) according to the distribution of a fixed amount of sugar reward among the 16 flowers of the patch. In the first treatment (hereafter, T‘4×8’), each of four flowers offered 8 μl of sucrose solution, while 12 flowers remained empty. In the second treatment (hereafter, T‘8×4’), each of eight flowers offered 4 μl of sucrose solution, while eight flowers remained empty. Finally, in the third treatment (hereafter, T‘16×2’), each of the 16 flowers of the patch offered 2 μl of sucrose solution. Thus, the total amount of sugar reward was 32 μl in all the treatments, and we varied the arithmetic mean and variance of the amount of sugar reward per flower, with reward distributions that included zero rewards in T‘4×8’ and T‘8×4’. Each bee performed a total of 36 successive visits to the arena. Each treatment was assayed three times in ‘blocks’ of four successive visits – a total of 12 visits (three blocks) per treatment per session. Blocks from different treatments were assayed in a semi-random fashion. We tested six bees under these conditions.

The second experimental series (S2)

In S2, we placed the arena indoors, and used 50% w/w sucrose solution as sugar reward. An open window allowed the single bees to access the arena, which had a homogeneous white floor as the background. This helped us to video (using 25 frames per second) the bees’ foraging behaviour by means of a video camera placed above the arena. In addition, we did not use ‘blocks’ of successive, comparable visits in S2. All the treatments were assayed in a semi-random fashion throughout the totality of the successive visits of the bees, meaning that each bee could experience any of the treatments in a given visit, with the exception of that experienced in the previous one. Each animal undertook ten foraging visits per session. We tested ten bees under these conditions.

Measurements

The following measurements were made. (1) The temperature, both inside and outside the arena. (2) The time inside the arena (henceforth, visit time, in seconds), defined as the time interval between when the bee entered the arena and when it left the arena and flew towards the hive. (3) The cumulative flight time and the cumulative non-flight time inside the arena (in seconds). (We made these measurements only in S2.) (4) The cumulative volume of sugar solution collected by the bee at the end of the visit time (henceforth, crop load, in micolitres). This measure allowed us to compute an ‘average solution intake rate’ (henceforth, flow rate), as the ratio between the crop load and the visit time. (5) The number of inspections to flowers offering sugar reward (henceforth, number of rewarding inspections), defined as the number of times a bee landed on a flower that offered sugar reward, searched for and drank the offered sucrose solution. (6) The number of inspections to flowers that did not offer sugar reward (henceforth, number of non-rewarding inspections), defined as the number of times a bee landed on and searched for sucrose solution inside an empty flower. (This last measure also includes those inspections to the flowers that had initially offered sugar solution, but were already empty at the time of landing.) (7) The total number of inspections to the flowers, defined as the number of times a bee landed on any of the 16 flowers of the patch, irrespective of whether or not such flower offered sugar solution. (8) The time outside the hive (in seconds), defined as the time interval between departure from the hive and arrival at the hive after visiting the arena. (9) The time that the bee stayed inside the hive in-between its successive flights toward the arena (henceforth, hive time, in seconds). (10) The probability of dancing (henceforth, dance probability), computed as the proportion of foraging cycles

Table 1. Treatments

Treatment	No. of flowers with sugar reward	No. of flowers without sugar reward	Volume (μl) per flower	Total volume (μl)
T‘4×8’	4	12	8	32
T‘8×4’	8	8	4	32
T‘16×2’	16	0	2	32

in which a marked bee performed either round circuits or waggle-phases (von Frisch, 1967) or both, calculated from the totality of the foraging cycles that the animal made under a similar treatment. Each marked bee was considered as an experimental unit in the analysis, and individual probabilities were averaged for the sake of comparisons across treatments. (Upon entering the hive, bees transfer the content of their crops to food-receivers by means of a common behaviour in social insects called trophallaxis, i.e. the transfer of liquid food by mouth. We separately analysed the dances occurring before and after the animals' food unloading. In doing this, we took into account that bees may transfer the content of their crops through more than one trophallaxis; each time a marked bee performed more than one trophallaxis, we used the longest of such events in order to discriminate between dances occurring before and after the food unloading.) (11) The number of round circuits or waggle-phases recorded throughout the hive time, which allowed us to compute an estimate of the 'dance strength'.

Statistics

Comparisons were made by means of one-way ANOVAs, LSD tests, Lilliefors and Shapiro–Wilk tests, and Pearson correlations (Zar, 1984).

RESULTS

The first experimental series (S1)

In S1, the bees were individually exposed to each of the treatments along 12 foraging visits. These visits were in turn divided into three blocks of four successive visits, assayed in a semi-random fashion throughout each session. We did not find variations in our measurements either within or across the several comparable blocks of four successive visits to the arena (data not shown), and so averaged data from different treatments (groups). Thus, we found group differences in the visit time (Fig. 1A; $F_{(2,15)}$=2.65, P=0.10; one-way ANOVA): it was lower in T‘16×2’ than in T‘4×8’ (Fig. 1A; P=0.04; LSD test). By contrast, we did not find group differences in the crop load (Fig. 1B; $F_{(2,15)}$=1.69, P=0.22; one-way ANOVA) and the flow rate ($F_{(2,15)}$=0.81, P=0.46; one-way ANOVA), with mean (±s.e.m.) flow rates of 0.19 (±0.01), 0.18 (±0.01) and 0.17 (±0.01) μl s^{-1} for T‘16×2’, T‘8×4’ and T‘4×8’, respectively. Similarly, we did not find significant differences among the three groups in the time outside the hive ($F_{(2,15)}$=1.11, P=0.36; one-way ANOVA, after log transformation) and the hive time ($F_{(2,15)}$=0.30, P=0.75; one-way ANOVA). The mean (±s.e.m.) times outside the hive were, 259.1 (±8.0), 310.3 (±35.1) and 313.9 (±32.1) s for T‘16×2’, T‘8×4’ and T‘4×8’, respectively, whereas the mean (±s.e.m.) hive times were of 161.1 (±18.8), 165.3 (±25.8) and 186.5 (±29.1) s for T‘16×2’, T‘8×4’ and T‘4×8’, respectively.

We found significant differences among the three groups in the number of rewarding (Fig. 1C; $F_{(2,15)}$=558.40, P<0.001; one-way ANOVA, after log transformation) and non-rewarding (Fig. 1D; $F_{(2,15)}$=5.20, P=0.02; one-way ANOVA) flower inspections. As expected, the number of rewarding inspections was higher in T‘16×2’ than in T‘4×8’ (Fig. 1C; P<0.001; LSD test), and the number of non-rewarding inspections was higher in T‘4×8’ than in T‘16×2’ (Fig. 1D; P<0.01; LSD test). However, we did not find significant differences among the three groups in the total number of flower inspections ($F_{(2,15)}$=1.54, P=0.23; one-way ANOVA). Next, we calculated a 'relative inspection index' (henceforth, RI), defined as $(a\times b^{-1})\times c$, where a and b are the number of rewarding and non-rewarding inspections to the flowers, respectively, and c is the total number of inspected flowers. This index reflects the proportions of an animal's encounters with rewarding and non-rewarding flowers during the visit time, and clearly changed across groups (Fig. 1E; $F_{(2,15)}$=742.20, P<0.001, one-way ANOVA, after log transformation).

We also found group differences in the probability of dancing before the foragers' food unloading, i.e. immediately after they

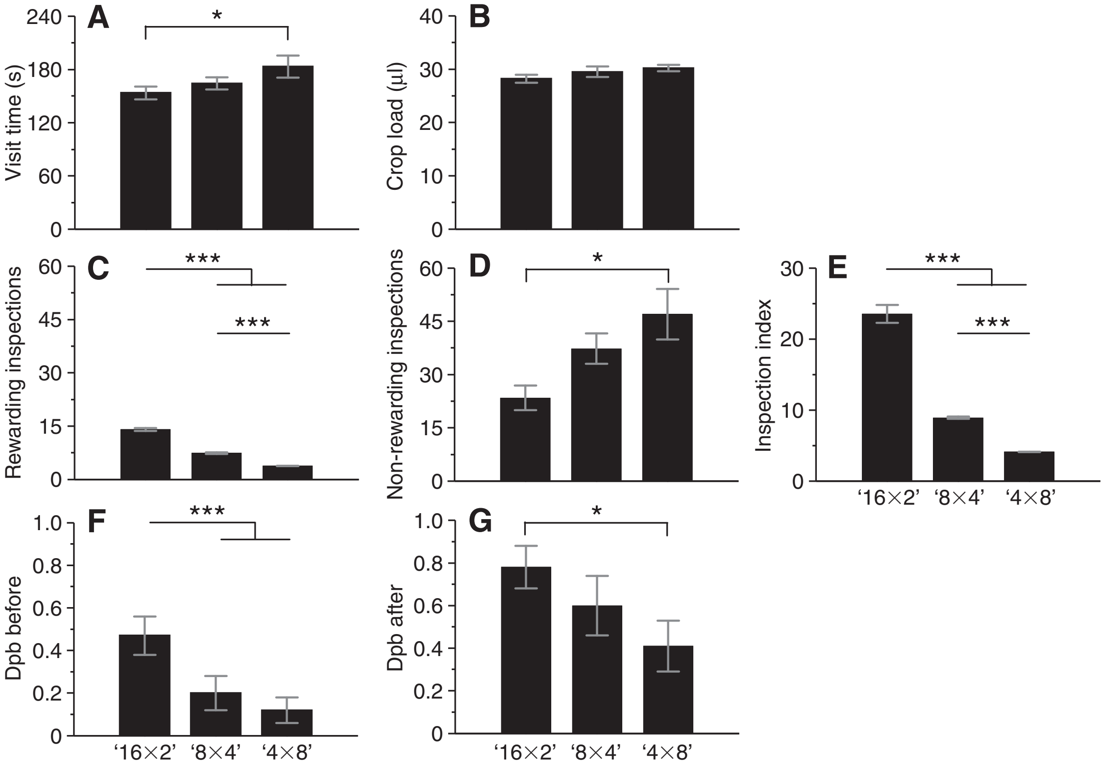

Fig. 1. Comparison of several variables recorded in experimental series S1 as a function of the three different treatments, T‘16×2’, T‘8×4’ and T‘4×8’. (A) Visit time; (B) crop load; (C,D) number of rewarding and non-rewarding flower inspections; (E) inspection index (see Results); (F,G), dance probability (Dpb) before and after the bees' food unloading. Values are means ± s.e.m. N=6. Asterisks indicate statistically significant differences between groups. (See Results for details.)

entered the hive (Fig. 1F; $F_{(2,15)}=4.96$, $P=0.02$; one-way ANOVA): it was higher in T‘16×2’ than in T‘8×4’ and T‘4×8’ (Fig. 1F; $P<0.05$; LSD tests). Similarly, there were group differences in the probability of dancing after the food unloading (Fig. 1G; $F_{(2,15)}=2.45$, $P=0.12$; one-way ANOVA): it was higher in T‘16×2’ than in T‘4×8’ (Fig. 1G; $P=0.04$; LSD test). By contrast, we did not find statistical differences among the three groups in the dance strength, either before ($F_{(2,15)}=0.25$, $P=0.78$; one-way ANOVA) or after ($F_{(2,15)}=0.98$, $P=0.40$; one-way ANOVA) the food unloading.

It is well-known that the regulation of a honeybee’s dance threshold partially depends upon a colony’s nectar intake rate, and that returned foragers sense such rate by computing how quickly (Seeley, 1989) and eagerly (De Marco, 2006) the food-receivers unload their crops. Our treatments were assayed in blocks of four successive visits to the arena, also presented in a semi-random fashion. This means that any possible group difference regarding the bees’ thresholds for dancing could not be accounted for by changes in the colony’s nectar intake rate, simply because such rate cannot vary systematically within a four-visit time window as to promote detectable differences in the likelihood of the foragers’ dances, particularly during the autumn, when natural sources are scarce, and, therefore, variations of a colony’s nectar intake rate are typically small. In spite of this, we measured the speed and eagerness of the foragers’ food unloading by computing all the variables reported by De Marco (De Marco, 2006). As expected, we did not find group differences among these variables (data not shown).

Thus, in spite of having collected the same amount of sugar solution, the bees showed lower thresholds for dancing when all the sixteen flowers of the patch offered a small amount of sugar reward in T‘16×2’ (Fig. 1B,F,G). In principle, this might be accounted for by the slightly diminished visit time found in T‘16×2’ (Fig. 1A), which might have modified the bees’ the energy gain during foraging. That is, a shorter visit time might have changed the bees’ estimate of the net rate of energy intake, $[(G-C)/t]$, or that of the net energy efficiency, $[(G-C)/C]$, where G, C and t are the energy gain, energy cost and the time required to make a round trip to the arena, respectively. Yet, the bees’ thresholds for dancing might also have changed because of the observed variations in the number of rewarding and/or non-rewarding inspections to the flowers (Fig. 1C–E), which might have modified the animals’ estimate of the level of uncertainty associated with the offered reward.

We examined the correlations between the probability of dancing, both before and after the food unloading, and each of the following four measurements: The visit time, the number of rewarding and non-rewarding inspections of the flowers and the relative inspection index. We found no correlation between the visit time and both dance probabilities (before the food unloading: Pearson’s $r=-0.17$, $P=0.50$, $N=18$; after the food unloading: Pearson’s $r=0.11$, $P=0.68$, $N=18$). By contrast, we found a positive correlation between the number of rewarding flower inspections and the dance probability before (Pearson’s $r=0.54$, $P=0.02$, $N=18$), but not after the food unloading (Pearson’s $r=0.42$, $P=0.09$, $N=18$). We also found a negative correlation between the number of non-rewarding flower inspections and the probability of dancing, both before (Pearson’s $r=-0.75$, $P<0.001$, $N=18$) and after the food unloading (Pearson’s $r=-0.72$, $P<0.001$, $N=18$). Similarly, there was a negative correlation between the inspection index and both dance probabilities (before the food unloading: Pearson’s $r=-0.75$, $P<0.001$, $N=18$; after the food unloading: Pearson’s $r=-0.72$, $P<0.001$, $N=18$).

We also examined whether any possible correlation between the probability of dancing and the visit time, as well as between the probability of dancing and the number of non-rewarding flower inspections, changed within a series of four successive, comparable visits to the arena. (We focused on the number of non-rewarding flower inspections because this measure correlated well with the probability of dancing both before and after the food unloading.) After pooling data from all groups and four-visit series, we found that both the visit time (Pearson’s $r=-0.50$, $P=0.04$, $N=18$) and the number of non-rewarding inspections to the flowers (Pearson’s $r=-0.67$, $P=0.003$, $N=18$) correlated with the probability of dancing in the first visit of an average four-visit series. However, only the number of non-rewarding inspections, but not the visit time, correlated with the probability of dancing in the remaining three visits of such sequence (number of non-rewarding inspections *vs* dance probability, visit 2: $r=-0.57$, $P=0.01$, visit 3: $r=-0.72$, $P<0.001$, visit 4: $r=-0.74$, $P<0.001$, $N=18$; Pearson correlations; visit time *vs* dance probability, visit 2: $r=0.11$, $P=0.66$, visit 3: $r=-0.21$, $P=0.40$, visit 4: $r=-0.37$, $P=0.19$, $N=18$; Pearson correlations).

The second experimental series (S2)

In the present context, the visit time and the time outside the hive provide only a rough estimate of a bee’s foraging costs. As a result, it is necessary to quantify a bee’s cumulative flight time inside the arena – a measure that co-varies with the number of flower inspections – in order to estimate more accurately the net rate of energy intake, as well as the net energy efficiency during foraging. This is why we conducted a new series of experiments in which we measured the cumulative flight time inside the arena. In this new series, in addition, we presented the animals with the different treatments in a semi-random fashion throughout the totality of their successive visits to the arena.

In S2, we did not find group differences in the visit time ($F_{(2,27)}=1.49$, $P=0.24$; one-way ANOVA), which gave means (±s.e.m.) of 225.51 (±18.14), 227.93 (±25.52) and 271.86 (±19.79) s for T‘16×2’, T‘8×4’ and T‘4×8’, respectively. Likewise, we did not find significant differences among the different groups in the cumulative flight time (Fig. 2A; $F_{(2,27)}=2.22$, $P=0.13$; one-way ANOVA) and the cumulative non-flight time ($F_{(2,27)}=0.51$, $P=0.61$; one-way ANOVA). For the latter the means (±s.e.m.) were 152.6 (±9.2), 144.8 (±8.6) and 157.6 (±9.1) s for T‘16×2’, T‘8×4’ and T‘4×8’, respectively. Similarly, we did not find group differences in either the crop load attained by the foraging bees (Fig. 2B; $F_{(2,27)}=0.87$, $P=0.43$; one-way ANOVA) or the flow rate that they experienced during the visit time ($F_{(2,27)}=1.34$, $P=0.28$; one-way ANOVA). For the flow rates the means (±s.e.m.) were 0.12 (±0.01), 0.14 (±0.01) and 0.12 (±0.01) $\mu l\,s^{-1}$ for T‘16×2’, T‘8×4’ and T‘4×8’, respectively. Moreover, we did not find significant differences among the different groups in the time outside the hive ($F_{(2,27)}=1.64$, $P=0.21$; one-way ANOVA) and in the hive time ($F_{(2,27)}=0.29$, $P=0.75$; one-way ANOVA). The time outside the hive gave means (±s.e.m.) of 300.8 (±20.5), 325.9 (±32.2) and 367.5 (±24.9) s for T‘16×2’, T‘8×4’ and T‘4×8’, respectively, whereas the hive time gave means (±s.e.m.) of 155.6 (±23.9), 188.1 (±40.4) and 183.5 (±32.4) s for T‘16×2’, T‘8×4’ and T‘4×8’, respectively. The temperature, both outside and inside the arena, did not vary across the different groups (outside, $F_{(2,27)}=0.002$, $P=0.998$; inside, $F_{(2,27)}=0.01$, $P=0.997$; one way ANOVAs).

By contrast, we found group differences in the number of rewarding and non-rewarding flower inspections (Fig. 2C; $F_{(2,27)}=171.03$, $P<0.001$; one way ANOVA, after log transformation), (Fig. 2D; $F_{(2,27)}=3.88$, $P=0.03$; one way ANOVA). The number of rewarding inspections were maximum in the T‘16×2’group, intermediate in T‘8×4’ and minimum in T‘4×8’ (Fig. 2C; $P<0.001$; LSD tests).

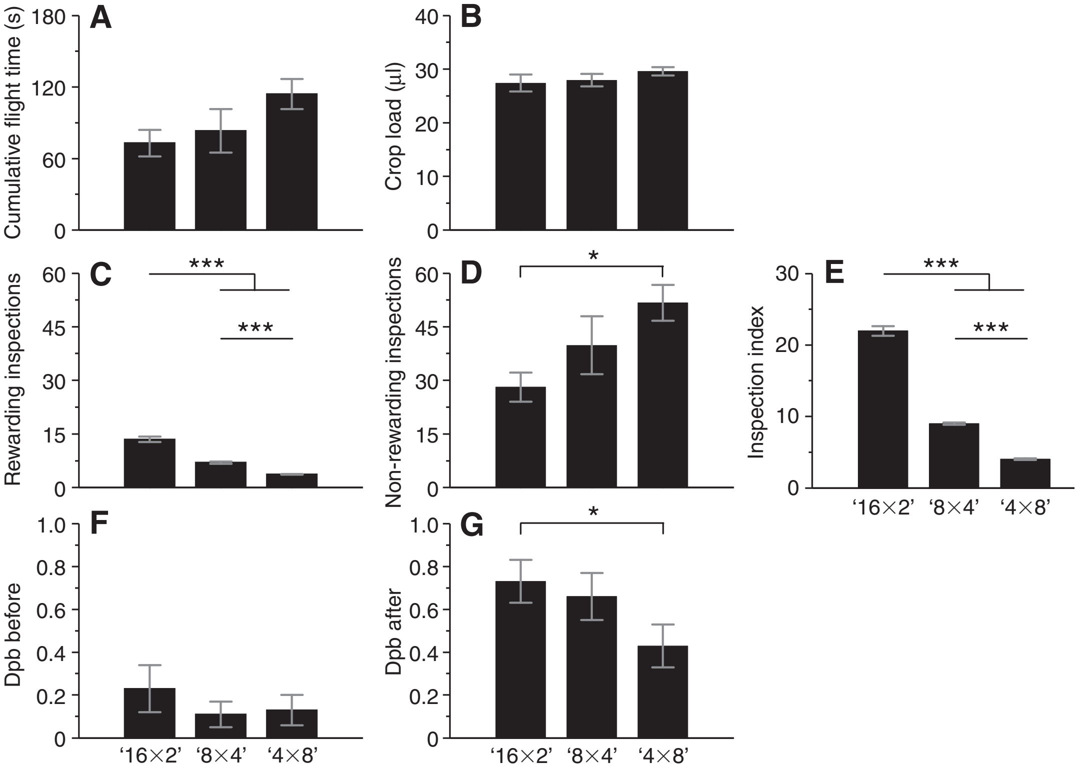

Fig. 2. Comparison of several variables recorded in S2 as a function of the three different treatments, T‘16×2’, T‘8×4’, and T‘4×8’. (A) Cumulative flight time inside the arena; (B) crop load; (C,D) number of rewarding and non-rewarding flower inspections; (E) inspection index (see Results); (F,G) dance probability (Dpb) before and after the bees’ food unloading. Values are means ± s.e.m. N=10. Asterisks indicate statistically significant differences between groups. (See Results for details.)

Moreover, the number of non-rewarding inspections in T‘16×2’ was lower than in T‘4×8’ (Fig. 2D; P=0.01; LSD test). As in S1, we did not find significant differences among the three groups in the total number of inspections ($F_{(2,27)}$=1.24, P=0.30; one-way ANOVA). As expected, we found group differences in RI (Fig. 2E; $F_{(2,27)}$=965.20, P<0.001; one way ANOVA): maximum, intermediate and minimum values in T‘16×2’, T‘8×4’ and T‘4×8’, respectively (Fig. 2E; P<0.001; LSD tests).

Multiple comparisons among all three groups gave no statistical differences for the probability of dancing. It did not change either before (Fig. 2F; $F_{(2,27)}$=0.57, P=0.57; one-way ANOVA) or after (Fig. 2G; $F_{(2,27)}$=2.29, P=0.12; one-way ANOVA) the foragers’ food unloading. However, the probability of dancing after the food unloading was higher in T‘16×2’ than in T‘4×8’ (Fig. 2G; P=0.04; LSD test). Thus, the bees of S2 also showed lower thresholds for dancing when all the sixteen flowers of the patch offered a small amount of sugar reward in T‘16×2’ (Fig. 2B,G). This happened when the several variables (see above) defining the speed and eagerness of the foragers’ food unloading did not change across groups (data not shown). As in S1, we did not find group differences in the dance strength either before (insufficient data prevents statistical analyses) or after ($F_{(2,27)}$=0.30, P=0.74; one-way ANOVA) the food unloading.

Detecting the influence of the energy gain on a honeybee’s threshold for dancing

The bees of S1 and S2 attained similar crop loads and experienced similar flow rates. In S1, they showed lower dance thresholds in T‘16×2’, and such thresholds were positively and negatively correlated with the number of rewarding and non-rewarding flower inspections, respectively. The bees of S2 also showed lower thresholds for dancing in T‘16×2’, and such thresholds were negatively correlated to the number of non-rewarding inspections. In S2, in addition, neither the visit time nor the cumulative flight time varied across groups (see above), suggesting that the observed variations in the bees’ dance thresholds may be accounted for, at least partially, by variations in the animals’ estimates of the level of reward uncertainty, as derived from the computation of their rewarding and non-rewarding flower inspections. However, both the visit time (Pearson’s r=0.85, P<0.0001, N=30) and the cumulative flight time (Pearson’s r=0.88, P<0.0001, N=30) co-vary with the number of non-rewarding flower inspections. This is interesting because we know that these time-based measures co-vary with a honeybee’s estimate of its energy balance during foraging [as derived from the net rate of energy intake or the net energy efficiency, $(G–C)/t$ or $(G–C)/C$, respectively], and that such balance influences, in turn, the animal’s threshold for dancing: the higher the energy gain the lower the threshold for dancing (Seeley, 1986). Detection of the latter relationship in the present context may prove fruitful to check upon the robustness of the correlations reported above. To this end, we followed an approach already described by Seeley (Seeley, 1986), thereby bringing together our own database and the work of other authors.

To estimate the energy gain G per trip first requires quantification of the animals’ crop loads, the mean values of which were found to be 27.4, 27.9 and 29.6 µl in T‘16×2’, T‘8×4’ and T‘4×8’, respectively (Fig. 2B). This value, together with the standard value for the energetic equivalence of sucrose, 5.8 J µlmol (Kleiber, 1961), gave us an estimate of G, the gross energy gain per trip, using the equation:

$$G = cl \times c \times 5.8\,, \tag{1}$$

where cl is the mean crop load and c is the concentration (in $\text{mol}\,\text{l}^{-1}$) of the offered sucrose solution, which for T‘16×2’ was $(158.9\times c)$ J, for T‘8×4’ was $(161.8\times c)$ J and for T‘4×8’ was $(171.7\times c)$ J.

We directly measured the time outside the hive during the experiments (t), i.e. the time required to fly toward the arena, collect the offered solution and fly back to the hive. Since we also measured the time that the bees spent inside the arena, we also calculated the difference between the time outside the hive and the visit time, and divided this difference by 2, in order to approximate the average duration of the outbound and the inbound components of the foraging excursion, and to assign each of these two flight components with different costs based on the mass of the load: 13.7, 14.0 and 14.8 mg in T‘16×2’, T‘8×4’ and T‘4×8’, respectively.

To estimate the energy expended per trip, *C*, we used 484 W Kg^{-1} for the metabolic rate of flying bees (Heinrich, 1980; Withers, 1981; Louw and Hadley, 1986), and 80 W Kg^{-1} for the metabolic rate of walking or feeding bees (Cahill and Lustick, 1976; Withers, 1981; Louw and Hadley, 1986; Seeley, 1986). For the mass of the bees during their outbound flight, we used an average mass of 80 mg (Núñez, 1970). For the mass of a bee flying back to the hive, we used the initial mass of 80 mg plus the mass of the load obtained for each treatment, i.e. 93.7, 94.0 and 94.8 mg in T‘16×2’, T‘8×4’, and T‘4×8’, respectively. Next, we used the average between these two masses, 86.9, 87.0 and 87.4 in T‘16×2’, T‘8×4’ and T‘4×8’, respectively, to estimate the mass of a bee foraging inside the arena. We then calculated the cost per trip, as the sum of the energy expended during the three phases of the foraging trip, i.e. the outbound flight, the time inside the arena (including the cumulative flight time) and the inbound flight:

$$C = mrf \times m_1 \times (t - t_V \times 2^{-1}) + mrf \times m_2 \times t_F + mrw \times m_2 \times t_{NF} + mrf \times m_3 \times (t - t_V \times 2^{-1}), \qquad (2)$$

where *mrf* is the metabolic rate of flying bees (in J mg^{-1} s^{-1}), *mrw* is the metabolic rate of walking or feeding bees (in J mg^{-1} s^{-1}), m_1, m_2 and m_3 are the masses (in mg) of bees flying towards the arena, foraging inside the arena and flying towards the hive, respectively, t, t_V, t_F and t_{NF} are the time outside the hive (s), the visit time (s), the cumulative flight time (s) and the cumulative non-flight time (s) inside the arena, respectively. Using Eqns 1 and 2, as well as the time outside the hive, one can easily compute estimates of $(G–C)/t$ and $(G–C)/C$, and test the relationship between these variables and the probability of dancing.

Both $(G–C)/t$ (Pearson’s r=0.43, P=0.02, N=29) and $(G–C)/C$ (Pearson’s r=0.47, P=0.01, N=29) positively correlated with the probability of dancing after the foragers’ food unloading, and their corresponding coefficients of determination (r_s) were 0.18 and 0.22, respectively. Thus, the influence of the energy gain during foraging on a honeybee’s threshold for dancing can also be detected in the context of our experiments, where both the total amount of sugar reward and the foraging time remained stable. This suggests that any possible correlation between the probability of dancing and the number of flower inspections, being either rewarding or non-rewarding, will be at least as reliable as the correlation between the energy gain and the probability of dancing. We then focused on the relationship between the probability of dancing and the number of rewarding and non-rewarding inspections to the flowers. We found no correlation between the probability of dancing and the number of the rewarding flower inspections (Pearson’s r=0.28, P=0.18, N=29). By contrast, we found a negative correlation between the probability of dancing and the number of non-rewarding flower inspections (Pearson’s r=–0.61, P<0.001, N=29), whose coefficient of determination (r_s) was 0.37.

DISCUSSION

We varied the distribution of a fixed amount of unscented sucrose solution among the several flowers of a patch and recorded the foraging and dance behaviour of single honeybees collecting such a reward at that patch. Concurrently, we aimed to minimize the well-known modulatory effects of sugar reward on both the probability and the strength of a honeybee’s dance. Our bees thus attained similar crop loads and experienced similar flow rates during foraging. It was under these circumstances that we conceived the honeybee dance as an autonomous information-processing system and asked whether or not such system is sensitive to uncertainty of reward. By definition, uncertainty depends upon the relative proportion of two mutually exclusive inputs (Shannon, 1948). In our context, this translates into the relative proportion of rewarding and non-rewarding flower inspections. Intriguingly, we found group differences in the bees’ thresholds for dancing when the total amount of sugar reward, the cumulative flight time, and the total time invested during foraging remained stable (see Results), meaning that the bees tuned their dancing according to the distribution of sugar reward among the several flowers of the patch.

Additionally, the correlation between the number of non-rewarding flower inspections and the probability of dancing, as compared to the correlation between the bees’ energy balance during foraging and the probability of dancing, appeared to be more salient in the first experimental series. It was in fact in the first series that the bees were repeatedly exposed to each of the different treatments throughout blocks of four successive visits to the arena. We found a correlation between the number of non-rewarding flower inspections and the probability of dancing in all four visits of such blocks in such a situation, but it was only in the first of those four successive visits that we found a correlation between the bees’ energy balance during foraging and the probability of dancing (see Results). This is interesting because it is the level of reward uncertainty that, under comparable circumstances, presumably diminishes with increasing experience. If one assumes that a honeybee’s dance actually conveys information, then it is a bee’s prediction of such a level of uncertainty that is expected to be manifested through variations of the animal’s threshold for dancing.

The question of whether the honeybee dance is sensitive to uncertainty of reward is important because such sensitivity is a prerequisite for the dance to be communicative. In addressing such question, however, a very important obstacle arises from the fact that sugar reward has modulatory effects on both the probability and the strength of a dance (von Frisch, 1967). Moreover, a dancer’s behaviour is also influenced by past experience with an offered reward (Raveret-Richter and Waddington, 1993; De Marco et al., 2005). This is why it is virtually impossible to isolate completely any possible effect of uncertainty of reward on a dancer’s behaviour. In searching for such an effect, therefore, one needs to set experimental circumstances in which the dance system, the outcome of which depends upon various, mostly unknown parameters, appears to be highly regulated, thereby exhibiting only slight oscillations in the probability and the strength of a dance. For obvious reasons, minimizing the modulatory effects of sugar reward on a dancer’s performance also means maximizing the effects of the energy expenditure during foraging on a honeybee’s dance behaviour: the higher the energy expenditure the lower the probability of dancing. This happens because a honeybee’s dance is finely tuned to the energy gain during foraging (e.g. Seeley, 1986). In such context, in fact, one would also need to focus on dances occurring before and after a dancer’s food unloading, simply because the process of food unloading itself conveys to dancers stimuli that partially modify the probability and the strength of the dance (e.g. Núñez, 1970; Seeley, 1986; Seeley, 1989; De Marco, 2006). (Such stimuli can only increase the variability associated with the dancer's performance, thereby overshadowing any possible influence of uncertainty of reward on the honeybee dance.) It becomes clear that asking whether the dance is sensitive to uncertainty of reward is more of an entropy problem, rather than a problem of energy gradients. In order to answer such a question, one needs to rely on experimental conditions leading to virtually insignificant variations in a dancer's performance. Eventually, one would benefit from simple patterns of simultaneous variations to examine whether or not the energy balance during foraging co-varies with a suitable, robust estimate of reward uncertainty. The above comments are relevant to understanding the limitations and specificities of our experiments. In particular because, due to our specific experimental conditions and the ensuing complexity of our analysis, our interpretations arise from a relatively small sample size and are based on assumptions and statistical differences that reveal tendencies, rather than unambiguous evidence for acceptance or rejection of null hypotheses. However, our focus is on the well-known relationship between energy gain and dance performance, on the one hand, and a, hitherto, 'presumed' relationship between reward uncertainty and dance performance, on the other. The question here is whether these relationships show similar or dissimilar patterns of variation. The former arises from inherent, scale-invariant properties of the dance system (already supported by empirical evidence), meaning that the relationship between energy gain and dance probability transcend unambiguously variations across species. The later presumably arises from analogous properties based on the system's information processing capacities. (Eventually, the question of what is the actual equivalence between energy gain and uncertainty of reward will still remain elusive.)

There has long been controversy regarding the fundamental measure underlying a honeybee's estimate of the quality of a patch, particularly with respect to the adaptive significance of energy efficiency at the individual level (Schmid-Hempel et al., 1985; Schmid-Hempel, 1993), as opposed to energy intake rate and information flow rate at the individual and group level, respectively (Varjú and Núñez, 1991; Varjú and Núñez, 1993). We found that both the net energy efficiency and the net rate of energy intake accounted for ~20% of the variance of a forager's dance threshold when the total amount of sugar reward and the time invested during foraging remained stable. Under such circumstances, we also found that the number, or the frequency, of the foragers' non-rewarding flower inspections accounted for ~40% of such variance, suggesting that the honeybee dance system computes not only the energy balance that a dancer has recently achieved during foraging, but also an estimate of the level of uncertainty of reward derived from the distribution of food resources across the several flowers that the animal inspected prior to dancing. The latter computation, which unambiguously relates to the level of reward uncertainty, might well be the subject of adjustments depending on the overall flow rate of reward that the dancer experiences during foraging.

In line with these findings, recent studies of choice behaviour in harnessed honeybees (Shafir et al., 1999; Drezner-Levy and Shafir, 2007) support the view that subjects on a positive energy budget invariably prefer less variable reward magnitudes if the variable reward distribution includes zero rewards, as it does in our experiments. The same pattern has been reported by analyses of human studies (Weber et al., 2004), where the subjects strongly avoided reward variability if the variable distribution included zero rewards and had a large coefficient of variation. Response to reward variability has frequently been referred to as risk-sensitive behaviour (Caraco, 1980; Stephens, 1981; Pyke, 1984; Real, 1992; McNamara and Houston, 1992; Kacelnik and Bateson, 1996; Kacelnik and Abreu, 1998). Experimental studies of risk-sensitive behaviour typically focus on how foraging choices depend upon reward variability (e.g. Real and Caraco, 1986; Stephens and Krebs, 1986). For example, while foraging on two types of artificial flowers offering different amounts of nectar, bumblebees rapidly switched their preference for a flower type when reward contingencies were switched between the flowers (Real, 1981). Bees also showed a strong preference for landing on constant rewarding flowers, as opposed to variably rewarding flowers offering the same mean reward (Real, 1981; Waddington, 1981). This type of response, also found in other animals (Kacelnik and Bateson, 1996), is frequently referred to as risk-averse behaviour, in contrast to risk-prone or risk-indifferent behaviour, and has traditionally been accounted for by hypothesizing a nonlinear subjective 'utility function' for reward (von Neumann and Morganstern, 1944; Real, 1992; Smallwood, 1996).

Analyses of other bee studies have shown no preferences for either nectar constancy or variability (Banschbach and Waddington, 1994; Waddington, 1995; Fülöp and Menzel, 2000). For example, in studies with several bee species, variance of nectar concentration had no influence on the animals' choice behaviour (Banschbach and Waddington, 1994; Waddington, 1995; Perez and Waddington, 1996), although bumblebees showed preferences for constancy when the variance of concentration was large and the arithmetic mean of reward was low (Waddington, 2001). The discrepancies among these and other studies (e.g. Shapiro, 2000; Shapiro et al., 2001), as it has previously been stated by Perez and Waddington (Perez and Waddington, 1996), are most probably based on the identities of the targeted species, differences in the size of the colonies and social conditions of the subjects, the foraging arenas employed in the experiments, the manipulation of reward during the experiments, and the presence or absence of non-rewarding flowers.

We support the view that testing risk-sensitive behaviours demands the consideration of alternative hypotheses and predictions from additional mechanisms (Cartar and Smallwood, 1996). One should also ask whether such sensitivity would be relevant for an organism's survival and fitness. Intriguingly, a honeybee's dance has long been described as a behavioural response evolved to convey information on desirable resources (e.g. Dyer, 2002; De Marco and Menzel, 2008). If it conveys information on desirable nectar, for example, then it should be sensitive to the level of uncertainty of sugar reward, which depends, of course, upon how variable such a reward actually is. It follows, therefore, that a honeybee's dance for nectar should appear as 'risk averse'. Our results indeed suggest that honeybees foraging on several flowers are able to compute an estimate of the variance of the volume of sugar reward per flower if the variable volume distribution includes zero values, and subsequently adjust their dance thresholds according to the ensuing level of uncertainty of reward.

This is interesting because the foraging strategy of a honeybee colony strongly relies on collective foraging, which is unambiguously enhanced by dances (Seeley, 1995). In addition, foraging is a form of reinforcement learning (Sutton and Barto, 1998), i.e. learning how to map situations to actions, so as to maximize a reward signal. Thus, a forager's overall response to uncertain rewards might result from two parallel maximization processes (Varjú and Núñez, 1993). The first one, working at the individual level, would tend to maximize a honeybee's energy gain during foraging (Greggers and Menzel, 1993), whereas the second, working at the social level, would tend to maximize a colony's energy gain by mapping the probability of recruitment dances to the distribution of food resources among the several flowers inspected by the dancers. We hypothesize that the ultimate result of having ‘risk-averse’ dances is a colony’s ability to diminish delayed rewards and the effects of competition with other flower visitors for limited resources.

We suggest that theoretical accounts of ‘risk-sensitive’ dances would prove fruitful in further analyses of the honeybee dance system, particularly if one were to examine how the overlap region of recruitment and risk-sensitive behaviour eventually translates into fitness. Further experiments will be aimed at elucidating the responses of dancing bees to variance of sugar concentration, as well as the relationship between a forager’s dance performance and the coefficient of reward variance in relation to both volume and concentration. This will allow further evaluations of the influence of non-rewarding flower inspections on a honeybee’s perception of reward uncertainty. Experiments manipulating the contingency between different floral signals and nectar concentrations might also provide an evaluation of the influence of visual and olfactory signals on the foragers’ perception of reward uncertainty. It would also be interesting to compare the responses of dancing bees of different lines to uncertain rewards. It has been documented that the threshold for recruiting conspecifics is lower in African than in European bees (Núñez, 1979). It follows that any possible relationship between the probability of dancing and the level of reward uncertainty might well appear to be shifted in African bees, as compared to that of European bees, in that the same level of reward uncertainty may lead to a larger increase in the animals’ threshold for dancing. Eventually, our approach would also prove fruitful in further analyses of the mechanisms underlying the regulation of a honeybee’s threshold for dancing. For example, octopamine (OA) release in the honeybee brain is sucrose-responsive, and capable of modulating downstream behaviours (Hammer, 1997). It has recently been shown that a honeybee’s dance threshold can be decreased – thereby increasing dance likelihood – by applying controlled doses of OA on the dancer’s thorax prior to dancing (Barron et al., 2007). It would be interesting to re-examine the correlation between the probability of dancing and the number of non-rewarding flower inspections in OA-treated bees. This would help to further evaluate to what extent such correlation depends upon an increased overall sensitivity to sugar reward, or, instead, a more integrative variation of a honeybee’s perception of uncertainty of reward.

We are indebted to Josué A. Núñez, Diego Vázquez, Randolf Menzel, and two anonymous reviewers for fruitful suggestions and helpful comments on an early version of this manuscript. We are also grateful to Natalie Hempel de Ibarra, Doreen Drenske, Johannes Felsenberg, and Antje Richlitzki for helpful assistance during the experiments. The present experiment complies with the *Principles of Animal Care* (publication No. 86-23, revised 1985) of the National Institutes of Health and the corresponding current national laws. Funding for this work was provided by the Deutscher Akademischer Austauschdienst.

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