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Modulation of sucrose response thresholds in honey bees (*Apis mellifera* L.): influence of genotype, feeding, and foraging experience

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Abstract The perception of sugar is important to honey bees for making foraging decisions. We measured bees' perception by determining what concentration of sucrose touched to the antennae elicited the proboscis extension response (response threshold). A low response threshold (extension at low concentration) suggests a high perceptual value of sucrose, and vice versa. Perception of sucrose solutions differed between two artificially selected genotypic strains and was modulated by the bees' recent feeding experiences. Bees offered 10%, 30%, or 50% sucrose solutions in small cages overnight, and in large flight-cages or free-flying in the field for several days, had subsequent response thresholds positively correlated to the concentration offered. Empty bees, whether they were nectar, water or pollen foragers, dancers or non-dancers, had a significantly lower threshold than loaded bees. Crop volume affected response thresholds directly and independently of sucrose concentration. We interpret these findings as multiple mechanisms that operate in different time scales, modulating perception of sucrose. Changes occurred in the time scale of evolutionary processes as demonstrated by genotypic differences. Changes with foraging experience occur in hours or minutes while effects of crop filling are instantaneous.

Keywords Honey bee · Behavior · Neuroethology · Response thresholds

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Abbreviations *PER* proboscis extension response · *RT* response threshold

Introduction

Honey bee foragers make local assessments of the quality of the food they collect which forms the basis for decisions that affect their foraging behavior and the recruitment dynamics of the colony (von Frisch 1967). In order to understand how bees make these decisions, it is necessary to understand the processes of perception and how perception affects the rules that govern the use of the information (Waddington 1982; Page et al. 1995; Waddington et al. 1998).

Bees expend time and energy collecting energy-rich nectar from flowers. They alter their foraging and recruitment behavior on the basis of nectar volume and concentration, and the expenditures of time and energy (von Frisch 1967; Waddington and Gottlieb 1990; Banschbach 1994). Thus, bees must perceive the value of nectar, associate these perceptions with the type of flowers they are visiting (e.g., species of flower), and then use this information to make appropriate foraging decisions.

Sugar has been used repeatedly as a reward stimulus in many studies of foraging behavioral decisions. However, little is known about how individuals differ in their perception of sugar and how these differences affect individual and colony-level foraging decisions.

The recruitment dance of the honey bee (von Frisch 1967) has been used as a quantitative window into the perception of sugar and has suggested functional relationships between sucrose concentration and its perception (Waddington 1982; Waddington and Kirchner 1992; Raveret-Richter and Waddington 1993; Waddington 1997). The bee's evaluation of its own foraging experience can be determined by decoding the dance inside the hive (Waddington 1982; Waddington et al. 1994). For example, concentration of sucrose is encoded in the rate of 180° turns ("vigor") made during the

round dance (Waddington and Kirchner 1992). The dance appears to encode information about the last foraging trip (Waddington and Kirchner 1992), which is modulated by prior foraging experience (Raveret-Richter and Waddington 1993). This suggests that bees are able to perceive the quality of sucrose and adjust their foraging behavior accordingly.

Page et al. (1998) determined a second potential window into honey bees' perception of sugar using the proboscis extension response (PER). PER is routinely used to study classical conditioning in honey bees (Bitterman et al. 1983). Proboscis extension is a reflexive response to a sufficiently concentrated sucrose solution applied to the antennae. By offering increasing concentrations of sucrose and determining the concentration that elicits extension one can determine the response threshold of an individual bee. Using this technique, Page et al. (1998) determined that pollen and nectar foragers perceive the concentration of sucrose differently. Pollen foragers captured at the hive entrance returning from a foraging trip have significantly lower sucrose response thresholds than do similarly captured nectar foragers (Page et al. 1998). Pollen foragers exiting the colony, presumably to initiate a foraging trip, also have significantly lower response thresholds than exiting nectar foragers (Page et al. 1998).

Artificial selection has produced strains of bees that when raised together in the same colony at the same time differ in their probabilities of becoming pollen and nectar foragers. High-strain workers are more likely to forage for pollen and low-strain workers are more likely to forage for nectar (Page and Fondrk 1995). High-strain foragers have low response thresholds to sucrose like pollen foragers and low-strain bees have high response thresholds like nectar foragers (Page et al. 1998; Pankiw and Page 1999). There is a genetic component to response thresholds independent of foraging experience. High strain workers have significantly lower response thresholds than low strain workers in the first week of adult life before foraging begins (Pankiw and Page 1999). Genotype also affects non-pollen foraging decisions. High-strain bees are more likely to collect water and low-strain bees are more likely to return empty. Genotype affects the decision to collect nectar of different concentrations. Low-strain bees return with nectar loads that are significantly more concentrated than high strain nectar foragers (Page et al. 1998; Pankiw and Page 1999). Our interpretation of these results is that high-strain bees have lower response thresholds to sucrose and are more accepting of lower quality nectar (and water) than the low-strain bees.

The study of Pankiw and Page (2000) supports this hypothesis. Response threshold measured in the 1st week of adult life correlates with foraging behavior 2–3 weeks later. Workers with the lowest response thresholds became water foragers, followed with increasing response thresholds by pollen foragers, nectar foragers, bees collecting both pollen and nectar, and finally those returning to the colony empty. Sucrose concentrations of nectar loads were positively correlated with response thresholds measured on 1-week-old pre-foragers (Pankiw and Page 2000). Collectively, these results suggest a common functional link between sucrose perception, foraging roles, and local foraging decisions.

Here we examine how the perception of sucrose concentration varies under a variety of changing conditions, and is constrained by genotype. We show that sucrose perception is constantly changing over different time scales and suggest that the modulation of sucrose perception may be an important enabling mechanism for social foraging.

Materials and methods

Source of bees

The honey bees (*Apis mellifera* L.) used in experiment 1 were derived from separate colonies of the high- and low-pollen hoarding strains of Page and Fondrk (1995). Foragers from the two genotypic strains differ dramatically in their foraging behavior. Individuals of the high-pollen strain show a strong bias for collecting pollen, while those of the low-pollen strain are more likely to specialize on nectar collecting (Page et al. 1995). One queen from each strain was instrumentally inseminated with semen from a single drone from the queen's same strain. Each queen and her daughters were housed in separate hives containing 9–18 combs.

In experiments 4, 5 and part of 6, we used bees from a wild-type commercial colony (bees of unknown history). In experiments 3 and part of 6, free-flying bees, potentially from many colonies in the University of California apiary, were collected for study. All experiments were done during the summer months of 1997–2000 in Davis, California, USA, when bees were actively foraging and rearing brood. Many of the experiments presented compared the effects of specific treatments on bees of different genotypes, derived from the high and low pollen hoarding strains (Page and Fondrk 1995). We controlled for potential effects of the colony environment by co-fostering focal bees in single colonies.

The proboscis extension response

The PER was used to sample a bee's sensitivity to varying concentrations of sucrose (see Page et al. 1998). Extension of the proboscis is reflexive in response to antennal stimulation with solutions of sucrose (Bitterman et al. 1983). The lowest concentration at which an individual bee responds is interpreted as her sucrose response threshold (PER-RT). Bees were captured, immobilized by chilling, and mounted in small brass tubes that restrained body movement but allowed free movement of the antennae and mouthparts (Bitterman et al. 1983). Bees were allowed to recover before testing. During the assay, a droplet of solution was expressed from a syringe and touched to each antenna. The proboscis extension responses of the bees were recorded.

For some experiments bees were tested for PER to a single concentration of sucrose. In others, they were assayed with ascending concentrations to determine their RTs. Concentrations increased in a $\log_{10}$ series of –1.0, –0.5, 0.0, 0.5, 1.0, and 1.5 corresponding to sucrose concentrations of 0.1%, 0.3%, 1%, 3%, 10%, and 30% (wt/wt). A droplet of water was touched to each antenna before the application of 0.1% sucrose and before each subsequent sucrose application (i.e., water, 0.1%; water, 0.3%; etc.). The alternated water trials provided a control for possible effects of sensory sensitization resulting from the antennal stimulation with sucrose (Shepherd 1994). Sucrose solutions were prepared using distilled and Millipore-filtered water as the solvent for Sigma sucrose (99.5% minimum purity).

Experiment 1a: effect of feeding experience and weight of liquid in the crop on PER-RT

The purpose of this experiment was to determine whether manipulating the short-term feeding experience of bees by feeding them different concentrations of sucrose solution modulated sucrose response thresholds. Bees from the high- and low-pollen hoarding strains were used to determine if genotype placed constraints on response threshold modulation.

About 50 exiting foragers were collected from the entrances of two hives, containing bees from the high-pollen strain, the other containing bees from the low-pollen strain. Collections took place between 1000 hours and 1200 hours. Bees were captured in separate cages (15 cm×8 cm×5 cm), and taken immediately indoors. Each cage of high- and low-strain bees was provided with 10%, 30% or 50% (wt/wt) solutions of sucrose ad libitum and held for 20–24 h at room temperature. After this time, we expelled and weighed the crop contents of a sub-sample of bees from each treatment to determine if high- and low-strain workers differentially filled their crops while confined. Two sub-samples of 5 bees were taken per cage. Individuals were anesthetized with CO_2 and their crop contents expressed into pre-weighed capillary tubes (Gary and Lorenzen 1976b). Mass of sucrose solution in the crop was determined for each bee. An additional sub-sample of 12 bees was taken per cage. Bees were chilled for placement in restraining tubes for PER analysis. Bees were tested with the complete sucrose series from 0.1% to 30% as described above. Bee mortality was determined for each cage. Three trials were conducted over 5 days.

Experiment 1b. effect of feeding experience and water satiation on PER-RT

The results of experiment 1a might be a consequence of differential needs for water resulting from feeding different concentrations of sucrose solutions. Therefore, this experiment was conducted to determine whether response thresholds are confounded by differential "thirst" for water.

High- and low-strain bees were captured in separate cages and fed a 10%, 30%, or 50% sucrose solution (wt/wt) as in experiment 1a. Thirty minutes prior to the PER-RT assay all individuals were tested for their responses to water. Individuals that responded to water stimulation were fed water until they no longer responded. We interpreted this as feeding to satiation. The PER-RT assay consisted of the entire sucrose series from 0.1% to 30%, alternating with water, as above. Three trials were conducted over 3 days. The statistical tests were the same as those used in experiment 1a.

Experiment 2: PER-RT of high- and low-strain foragers collecting different concentrations of sucrose

The previous experiments demonstrated that feeding experience modulated sucrose response threshold. The purpose of this experiment was to determine if foraging experience also modulated sucrose response thresholds of high- and low-strain foragers.

Combs containing high- and low-strain pupae were placed into separate screen cages in an incubator for adult emergence. After emerging, adults were paint-marked with colors to distinguish strain, then placed into a hive containing resident bees unrelated to the high and low strains. The hive was then placed inside a screen flight-cage (4 m×4 m×2 m) provided with a sucrose solution foraging station located about 2–3 m from the hive. The foraging station consisted of a plastic dish of sucrose solution with a lid. Bees collected the solution from the ends of cigarette filters that protruded through circular holes in the lid. The stations provided one of the following solutions each for several consecutive days: 10%, 30%, or 50% sucrose. Bees were captured with forceps as they arrived at the station, immediately were taken indoors, and tested in the PER assay. The bees collected almost no solution before capture but they had probably collected the solution over several previous days. We tested each bee about 20 min after she was captured at the feeder. Each bee was tested one time using one sucrose solution (10%, 30%, or 50%) that was either the same as was being offered or was different.

We used saturated (main effects plus interaction terms) categorical analysis of variance to examine the effects of strain, sucrose concentration offered at the feeder, and the concentration offered in the PER assay on proboscis extension response (yes/no) (SAS CATMOD; Stevens 1996). The variable of most interest here was the concentration of the sucrose solution offered at the feeder.

Experiment 3: PER-RT of free-flying wild-type foragers collecting different concentrations of sucrose

Experiment 2 tested the effects of sucrose reward on response threshold of high- and low-strain foragers under strict controls of a flight cage. This experiment tested whether response thresholds to sucrose were likewise modulated in free-flying, unselected, wild-type bees.

We placed a 10%, 30%, or 50% sucrose foraging station in the UC Davis apiary and paint-marked the thoraces of foragers collecting sucrose from the station. A separate color was used for each solution and only one solution was presented on any given day in an ascending concentration order on successive days. Paint marking was done to prevent testing bees that had foraged on all sucrose concentrations. Presenting ascending sucrose concentrations identified foragers willing to collect lower concentrations from those that would accept only higher concentrations. Approximately 200 individuals were paint-marked daily from 0800 hours to1200 hours at which time the solution was removed. Paint-marked bees were recaptured for the PER test at the same time of day. Some were captured as they arrived, others were allowed to collect some sugar solution prior to capture. Bees were transported to the lab individually, immobilized by chilling, mounted, and tested in the PER assay. Immediately after testing, their crop contents were expelled and volume of sugar solution measured. Spearman's correlation coefficients were calculated for PER scores and the concentration of sucrose collected, concentration of sucrose expelled from the crop, and volume expelled from the crop.

Experiment 4: changes in PER-RT of individual bees

Experiment 2 tested for the relationship between the concentration of sucrose collected and PER response in groups of high- and low-strain bees. Because we sampled destructively, differences within the high- and low-strain groups between sugar concentration treatments could have been due to a natural sorting of bees by their thresholds to collect the different concentrations of sugar solution. Thus, perhaps bees with low thresholds foraged at the low concentrations, but bees with high thresholds foraged only at the higher concentrations. We could have been sampling, effectively, different populations of bees at the different concentrations rather than sampling from the same population. The same sorting of populations could have occurred in experiment 3 as well. In experiment 4 we determined whether individual bees changed their response thresholds with changes in the concentration of sucrose solution offered at the foraging station.

A wild-type colony of neither high nor low strain, was placed into a flight cage. A foraging station was provided in the flight cage, as in experiment 2. This experiment was conducted over an 8-day period. From day 1 to day 6 the foraging station contained 10% sucrose solution. From day 3 to day 5 individuals ($n = 28$) arriving at the station were captured, before collecting sucrose solution, tested for their PER-RT, tagged with individual numbered plastic disks (Opalithplättchen) then released back into the cage. At noon on day 6, the 10% sucrose solution was replaced with a 50% solution. Bees collected 50% sucrose solution for the remainder of day 6. On day 7 all tagged bees arriving at the 50% solution were recaptured prior to collecting any solution and tested for PER-RT. We tested whether individual response thresholds were determined by the sucrose concentration collected by performing Paired-Wilcoxon Signed Ranks tests. We tested the PER score when foraging on 10% sucrose against the score when foraging on 50% sucrose.

Experiment 5: forager classes, crop contents, and PER-RT

The previous experiments demonstrated that feeding and foraging experience modulate sucrose response thresholds. This experiment examines the relationship between volume and concentration of crop contents of returning pollen and non-pollen foragers, and PER response thresholds.

A wild-type colony, neither the high- nor low-strain, was established inside a glass-sided observation hive (Gary and Lorenzen 1976a), which was maintained inside a temperature-controlled building. The hive was configured so that free-flying bees entered on the bottom comb on one side of the hive (Waddington et al. 1998). On that side, the bottom piece of glass was replaced with three plates of hinged clear plastic that could easily be lifted to capture selected bees. For several days we paint-marked hundreds of foragers at the hive entrance to facilitate visual observation of returning foragers. We observed individuals on the bottom comb as they returned from foraging and recorded whether the bee was a pollen forager (observed pollen on the corbiculae) or a non-pollen forager (not carrying pollen). Pollen foragers were captured at the moment they began dancing or when they began to deposit pollen into a cell. Non-pollen foragers were captured when they began to dance or when they approached the hive entrance to leave on another foraging trip. Immediately after capture we prepared each bee for the PER assay using the entire sucrose series (see above). Immediately after the PER test we gently squeezed the liquid contents out of the crop into a 25-µl micropipette and determined the volume and sucrose concentration of the nectar collected.

Sampled bees were then classified as (1) pollen, (2) nectar, (3) both (those returning with both nectar and pollen), and (4) empty (those returning without nectar or pollen). No foragers returned with water. We further categorized foragers returning with pollen and/or nectar as either dancers or non-dancers.

Experiment 6: water and pollen foragers

In the previous experiments we examined the relationship of the PER scores to the quantity and quality of the sucrose solution collected by high- and low-strains and wild-type bees. Here we examine the effect of volume in the crop on PER scores independent of the sucrose concentration by testing foragers before and after water collection. We also examined whether modulation of PER scores is independent of the collection of liquids by measuring pollen foragers before and after pollen collection.

Bees were collected at a permanent watering station in the University of California, Davis, apiary before or after they had collected water. These were "wild-type" bees originating from many different colonies. Bees were captured in groups of 10 in order to minimize handling time during the PER assay prior to measuring crop contents. Five bees were captured before collecting and 5 after collecting water for some time. Crop contents were expelled and their volume and sucrose concentration measured. The PER assay was performed on a total of 40 bees before and 40 bees after collection of water.

Wild-type pollen foraging bees from a wild-type colony kept inside a flight-cage (4 m×4 m×2 m) were tested for their PER-RT to sucrose. Over a 3-day period we individually tagged (as in experiment 4) 40 bees that returned to the colony entrance with pollen loads. We measured the PER scores of these individuals as exiting foragers (before collecting pollen) and again as returning pollen foragers (after collecting pollen) on the same day. The order of first and second testing before or after collecting pollen was alternated between individuals to control for any effect of prior testing on subsequent testing.

Statistical Analyses

The results from PER-RT assays with ascending concentrations of sucrose were transformed to mean sucrose and water scores. The mean PER score is directly related to the response threshold of the individual because most bees continue to respond to all increasing concentrations of sucrose following their initial response. For example, a PER score of 6, where an individual responds to all 6 sucrose concentrations beginning with 0.1% sucrose in the PER assay, represents a response threshold 300 times lower than a score of 1 where an individual responds only to 30% sucrose. Saturated categorical model analysis of variance was used to examine the effects of concentration of sucrose solution fed on mean PER scores. We used the "response" statement of the CATMOD procedure in SAS to specify mean scores (Stokes et al. 1997). The sources of variation of most interest here are strain, concentration fed, and their interaction. Spearman's correlation coefficients were calculated for concentration of solution fed, concentration in the crop, PER scores, and volume in crop.

Results

Experiment 1a: effect of feeding experience and weight of liquid in the crop on PER-RT

High-strain bees had significantly higher PER scores than low-strain bees for all sucrose solutions fed (Table 1; Fig. 1 a). The bees' mean PER scores were modulated inversely by the concentration of the sucrose solution fed for both the high and low strains (high rho = –0.588, $P < 0.01$; low rho = –0.503, $P < 0.01$). The relative differences between the strains remained similar between concentrations fed, as indicated by non-significant strain×concentration fed interaction (Table 1).

Crop masses were normally distributed, thus results were analyzed using ANOVA. Although this experiment was composed of three trials over 5 days there was no effect of trial on crop mass ($F = 2.5$, 2 *df*, $P > 0.05$). All trials were pooled for further analyses. There was no main effect of strain on weight of crop contents ($F = 176.01$, 1 *df*, $P > 0.05$; Fig. 2). However, there was a significant effect of the concentration fed on mass of the crop contents ($F = 15.6$, 2 *df*, $P < 0.0001$). Our examination of weight of crop contents between the strains for each solution showed a significant positive relationship between mass and sucrose concentration fed in the high strain (one-way ANOVA $F = 16.4$, 2 *df*, $P < 0.0001$), but not in the low strain ($F = 1.5$, 2 *df*, $P > 0.2$). Low-strain bees fed the 10% sucrose had a mean crop contents mass that was greater than the high strain (*t*-test = –2.0,

Table 1 Effects of variously concentrated sucrose solutions fed to two strains of bees on their proboscis extension response (PER) thresholds to sucrose. Saturated categorical analysis of variance for experiment 1a

Source	*df*	Chi-Square	Probability
Trial	2	8.53	0.0140
Strain[a]	1	117.9	0.0000
Concentration fed[b]	2	141.7	0.0000
Trial×Strain	2	9.7	0.0077
Trial×concentration Fed	4	3.19	0.5268
Strain×concentration fed	2	0.95	0.6233
Trial×strain×concentration fed	4	5.18	0.2692

[a]Strain refers to the high- and low-pollen-hoarding strains of bees
[b]"Concentration fed" refers to the concentrations of sucrose fed to bees (see experiment 1a)
*Non-significant

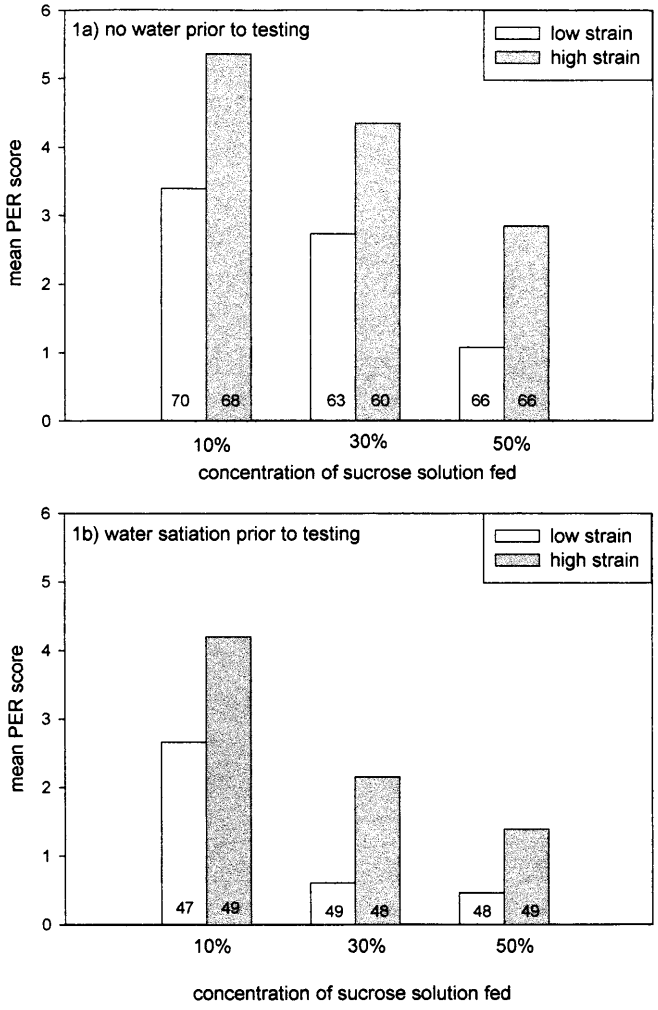

Fig. 1 **a** Experiment 1a. Mean proboscis extension response (PER) scores of high- and low-strain bees fed different concentrations of sucrose while contained in wire mesh cages for 20–24 h. Prior to testing bees were not satiated with water. **b** Experiment 1b. Mean PER scores of high- and low-strain bees fed different concentrations of sucrose. Prior to testing bees were satiated with water. The number of bees tested is indicated in each bar

105 *df*, $P < 0.05$). There was no difference in mass between strains when fed the 30% sucrose solution (*t*-test = 0, 104 *df*, $P > 0.05$); the high-strain bees fed 50% sucrose had a significantly higher crop contents weight than the low-strain bees (*t*-test = 2.8, 104 *df*, $P < 0.006$). PER scores varied inversely with concentration fed but crop filling did not, suggesting that different neurofunctions modulate these responses. Mortality was low: (1) high strain; 10% sucrose – 4 of 124, 30% – 2 of 122, 50% – 2 of 122; (2) low strain; 10% – 3 of 123, 30% – 5 of 130, 50% – 4 of 128.

Experiment 1b: effect of feeding experience and water satiation on PER-RT

There was a significant effect of strain on PER-RT ($X^2 = 144.25$, 1 *df*, $P < 0.0001$; Fig. 1b) as well as a significant effect of the concentration of sucrose fed to bees ($X^2 = 220.57$, 2 *df*, $P < 0.0001$). There was no significant effect of trial on PER-RT ($X^2 = 1.29$, 3 *df*, $P > 0.05$). There was no significant interaction of strain×concentration of sucrose fed ($X^2 = 4.79$, 2 *df*, $P > 0.05$; Fig. 2b), demonstrating that the strains were not differentially modulated by the sucrose concentration fed. There were too few responses to water in the PER-RT assay to permit a saturated model analysis (Stokes et al. 1997), demonstrating that the bees were satiated for water but still responsive to sucrose. No mortality was observed in this experiment.

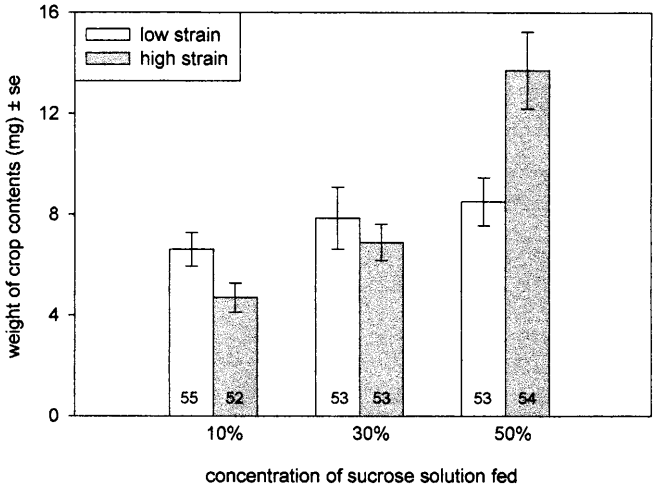

Fig. 2 Experiment 1a. Mean mass of crop contents expelled from high- and low-strain bees fed different concentrations of sucrose for 20–24 h. The number of bees tested is indicated in each bar

Experiment 2: PER-RT of high and low strain foragers collecting different concentrations of sucrose

The concentration of sucrose offered to foragers affected the probability of proboscis extension. There was an inverse relationship between the sucrose concentration offered at the feeders and probability of extending the proboscis to any given concentration used for antennal stimulation (Fig. 3, Table 2). Bees previously foraging on higher concentrations of sucrose were less likely to extend the proboscis than bees foraging on lower concentrations (Fig. 3, Table 2). High-strain bees were more responsive than low-strain bees to the sucrose concentrations in the PER test, independent of the concentration offered at the feeder. As expected, the sucrose concentration used in the PER test also independently affected the probability of response (Fig. 3, Table 2). One interaction term, strain×test concentration, was significant (Table 2). This demonstrates that the strains responded differentially to the changes in concentrations offered in the PER test. In general, high strain bees were more responsive to the increase in test concentration than low strain bees.

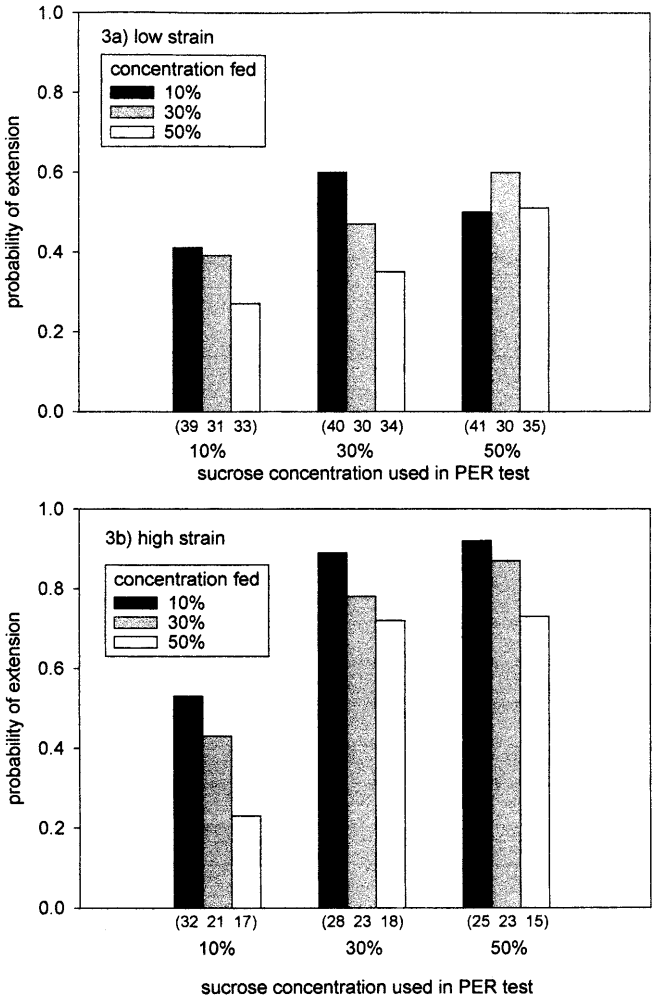

Fig. 3a,b Experiment 2. Probability of proboscis extension during PER tests using three sucrose concentrations in the **a** low-pollen strain and **b** high-pollen strain bees that were foraging on either of the same three sucrose concentrations inside a flight cage (see *inset*). The number of bees tested is indicated in parentheses below each bar of the histogram

Table 2 Effect of foraging experience and PER test concentration on the probability of high- and low-pollen-hoarding strains of bees to extend the proboscis to various concentrations of sucrose. Saturated categorical analysis of variance for experiment 2

Source	*df*	Chi-Square	Probability
Strain	1	30.3	0.0000
Forage Concentration[a]	2	11.8	0.0027
Test Concentration[b]	2	42.1	0.0000
Strain×forage concentration	2	1.2	0.5558
Strain×test concentration	2	9.6	0.0083
Forage concentration×test concentration	4	2.8	0.5868
Strain×forage concentration×test concentration	4	1.9	0.7515

[a]"Forage concentration" refers to the concentration bees foraged on (see experiment 2)
[b]"Test concentration" refers to the sucrose concentration used in the PER assay (see experiment 2)
*Non-significant

Experiment 3: PER-RT of free-flying wild-type foragers collecting different concentrations of sucrose

Free-flying wild-type foragers caught before collecting solution at a foraging station had a mean crop content volume of 1.2 ± 0.4 µl (mean ± SE). The mean crop volume of bees caught after being allowed to collect for some time was 23.3 ± 2.5 µl.

The PER score of bees captured before collecting sucrose solutions in the field was inversely related to the sucrose concentration they had experienced (Spearman's rho = –0.335, $P < 0.05$; Fig. 4). However, a similar comparison of bees after they collected the solutions for some time showed no significant effect of concentration (rho = –0.129, $P > 0.05$) or crop volume (rho = –0.061, $P > 0.05$) on the PER score.

Experiment 4: changes in PER-RT of individual bees

PER scores of bees foraging on 10% sucrose were significantly higher (mean PER score was 2.71 ± 0.4; mean ± SE) than when the same individuals foraged on 50% sucrose (mean PER score was 1.2 ± 0.3; Wilcoxon Signed Ranks Test, $P < 0.01$). This demonstrates that PER modulation observed in Experiments 2 and 3 was not merely a consequence of natural sorting of bees by their thresholds to collect the different concentrations of sugar solution, but rather individuals change their response thresholds with the concentration of sugar solution collected.

Experiment 5: forager classes, crop contents, and PER-RT

Dancing and non-dancing foragers within each of the five forage classes did not differ in their PER scores

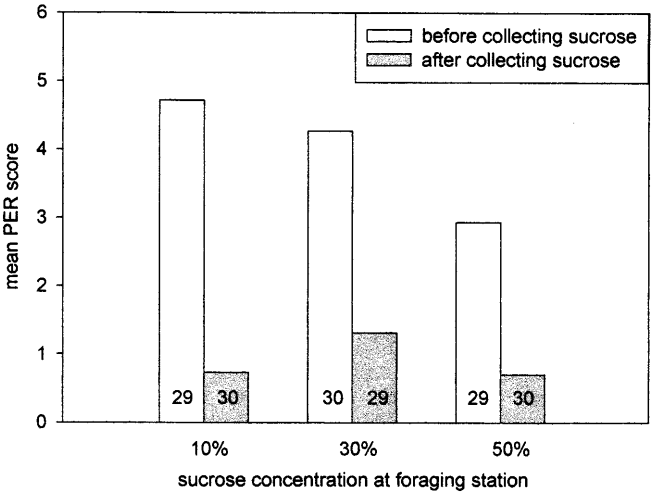

Fig. 4 Experiment 3. Mean PER scores of free-flying wild-type bees before and after collecting sucrose solutions from foraging stations containing different sucrose concentrations. The number of bees tested is indicated in each bar

(nectar foragers: Mann-Whitney $U = 1917.0$; pollen foragers: $U = 425.0$, $P > 0.05$; both foragers: $U = 142.5$, $P > 0.05$; Fig. 5). Consistent with previous studies there were significant differences in PER scores between forage categories (within the non-dancing foragers: Kruskal-Wallis $X^2 = 49.4$, $df = 3$, $P < 0.0001$) and (within the dancing foragers Kruskal-Wallis: $X^2 = 19.5$, $df = 2$, $P < 0.0001$; Fig. 5). Thus the PER scores of bees varied depending on the food they collected.

The PER scores were negatively correlated with crop volume for all classes of foragers that had nectar in their crop (nectar non-dancers, rho = –0.342, $P < 0.01$; nectar dancers, rho = –0.471, $P < 0.01$; both non-dancers, rho = –0.646, $P < 0.01$; both dancers, rho = –0.140, $P > 0.05$). The PER scores for nectar foragers were not correlated with sucrose concentration measured from expelled crop contents (nectar non-dancers, rho = 0.012, $P > 0.05$; nectar dancers, rho = –0.232, $P > 0.05$; both non-dancers, rho = 0.078, $P > 0.05$; both dancers, rho = -0.090, $P > 0.05$). Concentration and volume of crop contents were not significantly correlated (nectar non-dancers, rho = 0.019, $P > 0.05$; nectar dancers, rho = –0.089, $P > 0.05$; both non-dancers, rho = –0.074, $P > 0.05$; both dancers, rho = –0.366, $P > 0.05$).

Note that the volume of nectar we expelled from a crop is not a measurement of how much was collected by a nectar forager during the foraging trip, it is a measure of what the bee was carrying after activity in the hive and during the PER test. A comparison of the bees we sampled that carried nectar in the crop versus those with empty crops showed that empty bees, whether pollen or nectar foragers had significantly higher PER scores (see above) than nectar foragers (Fig. 5). At the time of sampling, the nectar foragers may have already unloaded some of their crop contents to receiver bees. Empty bees, whether they were nectar or pollen foragers, had significantly higher PER scores than bees returning with measurable loads (Fig. 5).

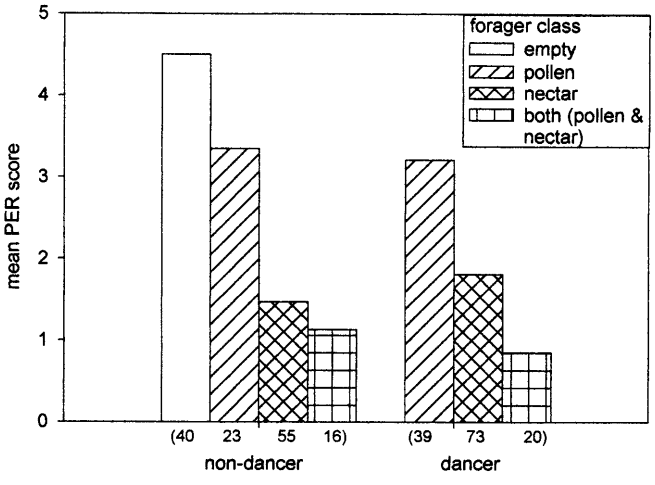

Fig. 5 Experiment 5. Mean PER scores of dancing and non-dancing foragers of different classes. The number of bees tested is indicated in parentheses below each bar of the histogram

Experiment 6: water and pollen foragers

Bees allowed to collect water had significantly lower PER scores than bees that were captured before collecting water (Mann-Whitney $U_{sucrose} = 207.0$, $P < 0.0001$; $U_{water} = 448.5$, $P < 0.0001$; Fig. 6a). PER score was also significantly negatively correlated with volume of water (Fig. 6b) expelled from the crops of bees before water collection began (rho = –0.580, $P < 0.01$). However, there was no such relationship after water collection (rho = –0.020, $P > 0.05$).

Pollen collection did not affect PER scores (Fig. 7). Pollen foragers exiting the hive initiating a foraging trip or returning with a pollen load had similar sucrose and

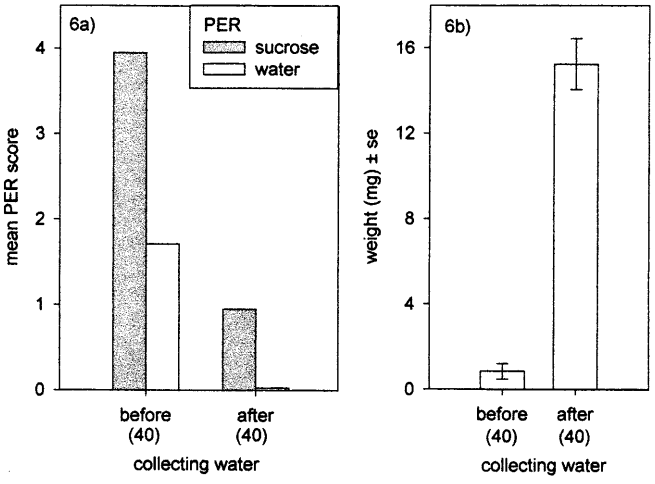

Fig. 6a,b Water foragers of Experiment 6. **a** Sucrose and water PER scores of water foraging bees before and after collecting water for some time. **b** Mean volume of water expelled from the crops of water foragers before and after collecting water. The number of bees tested is indicated in each bar of the histogram

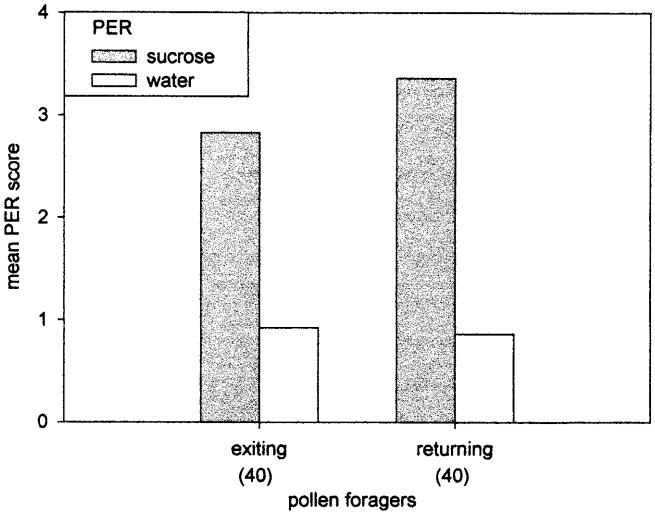

Fig. 7 Experiment 6. Mean PER scores for sucrose and water of pollen foragers before (exiting) and after collecting pollen (returning). Number of bees tested is indicated in parentheses below each bar of the histogram

water PER scores (sign test: $Z_{sucrose} = 1188.5$, $P > 0.05$; $Z_{water} = 1269.5$, $P > 0.05$). The order in which bees were first tested either exiting the colony before collecting pollen or after collecting pollen did not affect the PER score (Mann-Whitney $U_{sucrose} = 586.0$, $P > 0.05$ and $U_{water} = 570.0$, $P > 0.05$).

Discussion

The acquisition of sugar is fundamental to the survival of honey bee colonies. The perception of sugar, as indicated by the sucrose-response threshold, has profound effects on the foraging decisions of bees (Page et al. 1998; Pankiw and Page 1999, 2000) and on learning and memory (Scheiner et al. 1999, 2001a, 2001b). Our results suggest that the perception of sugar is modulated by the feeding and foraging activities of bees, providing the forager with an ever-changing system of sugar evaluation.

Modulation of response thresholds

Response thresholds change with quality of sucrose offered. Bees that consumed 10% sucrose for about 24 h were much more responsive (as measured by proboscis extension response scores) than those offered higher concentrations (experiment 1a). These results cannot be explained on the basis of reduced responsiveness due to starvation because bees fed lower concentrations did not have higher mortality, and were more responsive. An alternative explanation is that bees fed lower concentrations of nectar had greater water deficits and were responding more to the water offered with the test solutions. However, this is unlikely because we controlled for water deficit by satiating bees with water before testing (experiment 1b). The most likely explanation is that the nutritional status of the bee (hunger) affects her perception of sugar.

Response thresholds to sucrose changed with foraging experience, presumably independently of level of hunger. Bees that were confined to flight cages and allowed to forage on lower concentrations of sucrose were more responsive to sucrose (had lower sucrose response thresholds), than those that were foraging on higher concentrations of solution (experiment 2). This result held even though foragers had access to honey and pollen in their hives and should not have been nutritionally deprived. The same result held when free-flying bees from many colonies were tested (experiment 3). Individual foragers showed modulation of sucrose response thresholds based on the concentration of their foraging resource. Foragers arriving at feeders were less responsive to sucrose when they had been collecting higher concentrations of sugar than when they collected lower concentrations.

Volume of crop contents also affected response thresholds to sucrose (experiment 6). Water foragers arriving at a water feeder had lower sucrose response thresholds than those that were allowed to collect water before testing. In this case, immediate nutritional effects as a consequence of feeding while foraging cannot be responsible for the observed changes. However, filling of the crop may provide a feedback system resulting in an increase in responsiveness to sugar, and contribute to the decision of foragers to stop foraging and return to the nest. We also demonstrated that pollen foragers returning from foraging do not differ in their response thresholds to sucrose from those that were initiating a foraging trip (experiment 6). This demonstrates that the act of foraging alone does not modulate sucrose response thresholds.

Response thresholds to sucrose correlated with the roles of returning foragers (experiment 5). Bees returning with pollen had response thresholds lower than those returning with nectar. These results are consistent with those reported by Page et al. (1998) and Pankiw and Page (2000), and represents a robust relationship between the perception of sugar and foraging behavior. Age, gender, caste, and exposure to larval pheromones also have been shown to affect sucrose response thresholds (Pankiw and Page 1999, 2001).

Genotype constrains the modulation of response thresholds. In all cases reported here and elsewhere (Page et al. 1998; Pankiw and Page 1999; Scheiner et al. 1999, 2001a, 2001b) bees from the high pollen strain always had significantly lower response thresholds than bees from the low strain when fostered in the same colonies or cages, and given equal treatments. Response thresholds were not fixed in these strains, they modulated with treatments. The genotypes of the bees, determined by their strain origins, determined the range within which they varied.

Multiple mechanisms modulate sucrose perception and operate in different time scales. The selection of high- and low-pollen hoarding strains by Page and Fondrk (1995) resulted in persistent differences in response thresholds. The changes occurred in a time scale of generations. This is the time scale of evolutionary processes of allelic substitutions within populations. Changes associated with age, as demonstrated by Pankiw and Page (1999) occur in the time scale of the life of an individual. Changes resulting from feeding and foraging experience occur in a time scale of minute or hours (see also Woodring et al. 1993) while those resulting from filling the crop are instantaneous.

Effects of perception of sugar on social foraging

We assume that what we measure as the responsiveness of bees to sugar, the PER response threshold, or PER score, is an indicator of perception. If true, then modulation of sucrose response thresholds may affect the foraging decisions of individuals and the social foraging of colonies. Von Frisch (1967) and Waddington and Kirchner (1992) demonstrated that the probability of performing a wagtail or round recruitment dance is

related to the perceived profitability of the foraging resource. Varying the concentration of sucrose solution offered at artificial feeders modulated dance probabilities and dance rates. Round dance rate in relation to sucrose is further modulated by previous relative foraging experience (Raveret-Richter and Waddington 1993). Seeley (1995) proposed that individual foragers make an assessment of the absolute value of a resource. This assessment is modulated by the current feeding experience of the colony that is determined by the time it takes a forager to unload to receiver bees. A decision is then made to perform or not perform a recruitment dance based on this combined assessment.

Relative evaluations by foragers based on their own genotype, experience, and nutritional status could have profound effects on the foraging dynamics of colonies. This would place constraints on the ability of a colony to allocate its foragers to the most profitable resources unless each bee directly compared the relative value of her own resource against an average for the colony. The colony average may be obtainable through sampling the mixture of incoming nectar represented in the crops of resident bees, resulting from food sharing of all bees in the colony (Lindauer 1961; von Frisch 1967; Núnez 1982). The effects, if any, of modulating sucrose response thresholds on foraging behavior remain to be demonstrated.

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