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ORIGINAL ARTICLE

Honey bee foragers balance colony nutritional deficiencies

Harmen P. Hendriksma[1] · Sharoni Shafir[1]

Abstract Honey bee colonies, foraging predominantly on a single pollen source, may encounter nutritional deficits. In the present study, we examined the nutritional resilience of honey bee colonies, testing whether foragers shift their foraging effort towards resources that complement a nutritional deficit. Eight honey bee colonies were kept in screened enclosures and fed for 1 week a pollen substitute diet deficient in a particular essential amino acid. Foragers were subsequently tested for a preference between the same diet previously fed, a different diet that was similarly deficient, or a diet that complemented the deficiency. Foragers preferred the complementary diet over the same or similar diets. Appetitive conditioning tests showed that bees were able to discriminate also between the same and similar diets. Overall, our results support the hypothesis that honey bees prefer dietary diversity, and that they do not just include novel sources but specifically target nutritionally complementary ones. Whereas we specifically focused on deficiencies in essential amino acids, we cannot rule out that bees were also complementing correlated imbalances in other nutrients, most notably essential fatty acids. The ability of honey bees to counter deficient nutrition contributes to the mechanisms which social insects use to sustain homeostasis at the colony level.

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Significance statement Honey bees forage for floral nectar as their main carbohydrate source and for pollen as their main source of amino acids, fatty acids, and micronutrients. It is becoming increasingly appreciated that honey bee colonies require a diet that is nutritionally balanced. Our main finding is that honey bee colonies do not only attempt to diversify their foraging but also that they bias their foraging effort towards a pollen substitute diet that specifically balances colony nutritional deficits. This finding adds a novel ability to honey bee foragers in evaluating collected diet macronutrient composition and in adjusting their foraging effort towards colony nutritional needs. The mechanism by which this is achieved remains to be elucidated. The implication of our study is that honey bee colonies strive to balance their nutrition if appropriate floral resources are available to them.

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✉ Harmen P. Hendriksma
harmenhendriksma@gmail.com

[1] B. Triwaks Bee Research Center, Department of Entomology, Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel

Introduction

The research field of nutritional ecology is gaining momentum with numerous recent studies showing that many animals collect specific proportions of macronutrients in order to maximize growth and reproduction (Simpson and Raubenheimer 2012; Behmer 2014; Lihoreau et al. 2015). A strong theoretical model, the Geometrical Framework for Nutrition, elucidates the behavior of food uptake by animals. To explore how an organism balances and changes nutrient needs in a variable nutritional environment, a multidimensional nutrient space is considered, with as many axes as there are fitness-affecting nutrients. As a result, optimal dietary proportions of carbohydrate (C) to fatty acids (FA) and protein (P) or essential amino acids (EAA) can be identified. The blend of nutrients that form the optimal diet is called the "nutritional target," and organisms evolve behavioral and physiological traits to reach

their target (Behmer 2009; Simpson and Raubenheimer 2012). For instance, for solitary and social insects, the intake of P to C has been in focus. Unbalanced protein nutrition has been shown to induce mortality in ants and honey bees (Dussutour and Simpson 2009, 2012; Pirk et al. 2010; Paoli et al. 2014b). On the contrary, insects may increase vitality by changing their feeding behavior (Povey et al. 2013).

Dietary uptake of essential nutrients is necessary for growth. The underrepresentation of one or more EAA's reduces dietary quality and impairs honey bee development (De Groot 1953). Although recent studies have elucidated on the optimal ratio of EAA:C nutrition for bees (Archer et al. 2014; Paoli et al. 2014a; Stabler et al. 2015), potential behavioral responses to a lack of individual EAA's have not been addressed to date. In the present study, we explore whether honey bees can evade or compensate suboptimal nutrition. In particular, we investigate whether foragers shift their foraging effort among resources in order to complement a deficit in colony nutrition.

Honey bees and bumblebees serve as model organisms for studying the relative uptake of protein and carbohydrate since this ratio is reflected by foragers collecting pollen and nectar, respectively. Honey bee foragers perceive nutritional cues in nectar, e.g., sugars, minerals, and amino acids, and may prefer floral resources accordingly (Von Frisch 1934; Afik et al. 2008; Hendriksma et al. 2014; Simcock et al. 2014). In contrast, pollen choice by foragers seems unlinked to nutritional value, though non-nutritional pollen traits have been reported to induce foraging preference (e.g., color, odor, concentration, pH, or grain size) (Waddington et al. 1998; Pernal 2000; Pernal and Currie 2001, 2002; Hanley et al. 2008; Nicholls and Hempel de Ibarra 2014). Nonetheless, recent EAA:C balance studies with bees in cages show that they uphold specific nutritional intake targets, which suggests the presence of a functional regulatory mechanism to balance the uptake of EAA's in individual bees (Archer et al. 2014; Paoli et al. 2014a, b; Stabler et al. 2015). Accordingly, the aim of this study was to test whether honey bees can actively balance nutritional deficiencies at the colony level. We designed the experiments with specific attention to EAAs; we do not rule out, however, that bees may be also attempting to balance other nutrients that may be correlated with EAAs.

For 1 week, we let honey bee colonies forage on a pollen substitute diet that lacked an EAA. Hereafter, we applied a choice test with three diets to monitor whether forager bees shift diet uptake towards nutritional complementation. The choice options were (i) the same diet as before, (ii) a nutritionally similar diet (also lacking the EAA), and (iii) a complementary diet (with a superfluous level of the previously deficient EAA). Considering the honey bee polylectic feeding behavior, our null hypothesis was that foragers would not nutritionally discriminate among diets (H_0: same = similar = complementary). Alternatively, foragers may

not be able to evaluate the nutritional composition of the diets, but may diversify nutrition by favoring the new diets (H_1: same < similar = complementary) or if they can assess the nutritional composition, they may balance a nutritional deficit by preferring the EAA complementary diet (H_2: same = similar < complementary). To test whether bees could discriminate between diets that were equally chosen (possibly due to nutritional similarity), we conducted additional appetitive conditioning discrimination tests between such diets.

Materials and methods

Diet balancing

Experiments were conducted at the B. Triwaks Bee Research Center in Rehovot, Israel. We first screened 34 pollen substitute diets and performed exploratory data analysis (Online Resource—page 1). We then selected four flours for the nutritional balancing test, based on willingness of foragers to collect the diets, specifically targeting diets with EAA deficiencies. These were yellow peas (YP), red lentils (RL), kamut (KA), and spelt (SP). The minimum levels of methionine and lysine for honey bees are respectively 1.5 and 3.0 % of the total amount dietary protein (De Groot 1953). Considering data of the United States Department of Agriculture's National Nutrient Database for Standard Reference (USDA 2014), legume flours (YP and RL) have a deficient methionine level <1.5 % and sufficient level of lysine >3.0 %. In contrast, cereal flours (KA and SP) have a sufficient methionine level >1.5 % and deficient lysine level <3.0 % (Table 1). In addition, fatty acid percentages of omega 3 (alpha-linolenic acid) are relatively low in the cereal-based diets, and percentages of omega 6 (linoleic acid) are relatively low in the legume flour-based diets, as compared to one another (USDA 2014). We confirmed these values with our own analyses (Table 1).

For the diet balancing test, eight honey bee colonies (*Apis mellifera ligustica*) were standardized to one frame of stored honey, four frames of brood and 5000 bees, approximately. Each colony was placed in a tunnel tent of $250 \times 400 \times 200$ cm ($l \times w \times h$). Colonies were presented a water bucket with cork floats and a feeder with 200 mL sucrose solution at a concentration of 25 % *w/w*, each refilled daily for 15 consecutive days.

After 7 days in the tent, the in-hive pollen stores were depleted as foraging resources for pollen were absent in the tents. In the second week, each colony was offered one of four flour-based diets in powder form. These treatments were provided daily in two 150-mm Ø petri dishes containing 10 g diet each (95 % alimentary flour mixed with 5 % crushed mixed bee-collected pollen pellets as phagostimulant). To monitor diet uptake of the suboptimal diet during the first week of exposure, the remaining diet per colony was weighed daily.

Table 1 Amino acid (AA) and fatty acid (FA) profile analyses of four experimental diets

AA and FA analyses	YP diet	RL diet	KA diet	SP diet
Protein (total AA g/100 g fwt diet)	26.9	28.7	24.1	18.7
MET; LYS (% total AA)	1.11[#]; 6.93	1.11[#]; 6.52	1.77; 2.36[#]	1.72; 2.17[#]
Fat (TFA g/100 g fwt diet)	0.866	0.988	0.942	0.992
$\omega3$; $\omega6$ (% total FA)	14.1; 31.3	15.4; 31.4	12.1; 39.8	10.6; 42.6
Red-Green-Blue (color score)	177-162-122	182-161-131	166-153-114	166-161-140
Hue-Saturation-Intensity (score)	124-204-602	99-171-624	129-214-565	139-100-612

The analyses validate that the 5 % pollen-enriched flour diets for honey bees were nutritionally unbalanced (marked #), either with <1.5 % methionine (MET) or <3.0 % lysine (LYS), following De Groot (1953). In addition, the percentage FA omega 3 is relatively low in the cereal based diets (kamut = KA and spelt = SP) while omega 6 is relatively low in the legume flour based diets (yellow pea = YP and red lentils = RL), as compared to one another. Full AA, FA and color profiles are given in the Online Resource (page 2 and 3)

On the 15th day (June 1, 2013), we performed a choice test between three diets per colony. Synchronously, each of the eight colonies was offered a test arena providing 24 petri dishes of 90 mm Ø, with each dish containing 3-g diet. The dishes were placed on a paper sheet according to a complete block design to balance out potential spatial effects within and among test arenas. Each colony could forage from eight dishes containing the same diet as previously provided, eight dishes with a nutritionally similar diet, and eight with a complementary diet (compensatory on an EAA). The test design was overall balanced for flour types and nutritional roles by eight unique flour treatment combinations: $YP^{YP/RL/KA}$, $YP^{YP/RL/SP}$, $RL^{RL/YP/KA}$, $RL^{RL/YP/SP}$, $KA^{KA/SP/YP}$, $KA^{KA/SP/RL}$, $SP^{SP/KA/YP}$, $SP^{SP/KA/RL}$ (respectively are shown the pre-fed diet, with the "same/similar/complementary" diet choice options in superscript).

A colony test was stopped once one of the dishes in the arena was nearly empty and all petri dishes were weighed to the nearest 0.001 g ($n_{dishes} = 192$). Dietary uptake was calculated from the weight of each dish before and after the experiment. In addition, we photographed the test arenas at 5-min intervals and later counted the number of bees on each dish. On average, each colony was monitored 40 min, with eight photos taken: A total of 3206 bee visits were recorded for analysis. To minimize observer bias, behavioral data were recorded with blinded methods; i.e., weight and count data were collected in the consecutive order of 192 dishes, which alternated in flour type and were unmarked for nutritional role.

Odor discrimination between diets of harnessed bees

We applied the conditioning of the proboscis extension response (PER) paradigm to test whether honey bees could distinguish the four diet odors from air and from one another. Outgoing foragers were collected from healthy colonies ($n_{colonies} = 10$). The bees were anesthetized by cooling and afterwards harnessed and kept under climate-controlled laboratory conditions at 25 °C and 50 % relative humidity. After

1 h, bees were fed 0.8 µl sucrose solution (40 % w/w in water). After 2 h, we checked bee motivation: bees only entered the test if they actively extended their proboscis when touched at the antennae with a 40 % sugar-solution wetted cotton-stick.

The harnessed bees were placed on aluminum rails, sectioned with separating panels. One after another, the bees passed through a cold-light-illuminated test arena where they were individually exposed to 7-s airflow. The flow was regulated with an air pump and a computer-controlled valve. Air was passed through 100-mL Erlenmeyer flasks, either containing 2.0-g diet or nothing (Odor A: conditioned stimulus CS+). At the fourth second, 0.4 µl of 40 % sucrose solution was offered as reward (Sugar: unconditioned stimulus US+). With inter-trial intervals of 6 min, another odor was offered (Odor B: conditioned stimulus CS−), applying at the fourth second a penance by touching an antenna tip with a 2 M NaCl solution with a cotton-stick (Salt: unconditioned stimulus US-). In 12 conditioning trials, the two odor treatments were applied six times each, alternating according to a pseudorandom sequence (ABBABAABABBA).

A total of 290 bees were tested on dual discrimination of diet odors. The test pair possibilities were balanced as conditioned stimulus pairs (CS+ and CS−). The ability to discriminate between air and diet was tested with 80 bees (eight permutations: YP-Air, Air-YP, RL-Air, Air-RL, KA-Air, Air-KA, SP-Air, Air-SP). The discrimination between complementary diets was tested with 120 bees (eight permutations: KA-RL, RL-KA, KA-YP, YP-KA, RL-SP, SP-RL, SP-YP, YP-SP), and similar diet discrimination was tested using 90 bees (four permutations: KA-SP, SP-KA, RL-YP, YP-RL).

Discrimination between similar diets of free-flying bees

Equal foraging on the same and similar diets during the diet balancing experiment could reflect lack of preference between nutritionally similar diets or inability to discriminate between the two diets. We therefore tested the ability of foragers to discriminate between the similar diets in a separate

conditioning experiment (four permutations; KA-SP, SP-KA, RL-YP, YP-RL). For each tested permutation, a different colony was used. In a $400 \times 800 \times 400$-cm flight tent, a 6 frame populated colony was offered a water source and a table with a test arena (50×100-cm white paper). The arena contained 24 petri dishes in a checkerboard pattern: two diets, 12 dishes each, having 5-g diet per dish. At the center of each dish, we placed a 25-mm diameter white cap. For one diet (CS+), the cap was filled with 2.5 ml 30 % w/w sucrose solution (US+), whereas for the other diet (CS−) it was filled with 1 M NaCl salt solution (US−). When foragers had consumed all sucrose solution, feeders were refilled (six times in a day). We then tested the ability of foragers to discriminate between the two diets by placing 24 fresh dishes, half with each of the two diets, now containing feeders with sucrose solution only. We monitored visits to the dishes and collected by vacuum suction every forager that landed on a feeder, counting the number of foragers that visited each diet.

Diet analyses

We obtained amino acid (AA) and fatty acid (FA) profiles of the four test diets. The AA analyses were performed on a Waters Acquity UPLC System. Per diet, 10 mg was hydrolysed (24 h at 110 °C) with 6 N hydrochloric acid (+1 % phenol and norleucine as standard). Aliquots (8×40-mm glass shell vials) of 10 μL supernatant were dried, treated with a redrying solution (methanol: water: triethylamine at 2:2:1), vortex-mixed, vacuum dried (15 min), and derivatized (20 min) with PITC (methanol: water: triethylamine: phenylisothiocyanate at 7:1:1:1). Then, samples were dissolved in a diluent (pH 7.4), and an aliquot was injected into the column (48 °C), running on a modified Pico-Tag gradient for AA detection at 254 nm (Bidlingmeyer et al. 1984). Data were collected and processed using Waters Empower 3 Chromatography software. Generated peak patterns were analyzed on single AA contents per sample to show relative representations of 20 AAs (Online Resource—page 2).

For FA analysis, 200-mg flour diet samples were homogenized in water in a Mini Bead Beater (Biospec Products, Bartlesville, OK, USA). The homogenate was hydrolyzed, and the FAs turned into methyl esters, which were separated by Gas Chromatograph, FID HP5890. Internal standard (Heptanoic acid C17:0, Sigma, IL) was added to each sample in order to quantify FA amounts (Arien et al. 2015) (Online Resource—page 2).

We assessed diet colors by taking photographs (Nikon digital camera D5000) under fixed light conditions (Kaiser 5558 light stand), with subsequent image analyses in MATLAB (2010) to assess RGB and HSI color space, i.e., values for Red, Green, Blue, Hue, Saturation and Intensity (Online Resource—page 3).

Data analyses

Statistics were performed with the JMP Pro software, version 10, SAS Institute Inc. Preceding the choice phase of the diet balancing experiment, diet uptake per day ($n_{colonies} = 8$; $n_{days} = 7$) was compared over time by means of linear regression, in consideration of data dependency (random factor colony identity). Diet choice during the balancing experiment was studied by a two-way analysis of variance. The weights of collected flour diets and the number of bee visits were tested as dependent variables ($n = 192$ petri dishes). Independent variables were the intrinsic preference effect among the YP, RL, KA, and SP flours as nested within legume and cereal diets ("flour-type"; fixed factor; two levels), the potential preference for same, similar, or complementary diets ("nutritional role"; fixed factor; three levels), and the interaction term. The data were grouped in blocks to control for the dependency of the 24 dishes offered per colony (random factor; 8 levels). Model simplification steps involved removing non-significant interaction terms and variables (Crawley 2007), and model residuals were plotted to visually ascertain homogeneity of variance (Zuur et al. 2009).

Olfactory discrimination of diets was assessed by calculating for each bee a discrimination index (ΔPE): the number of proboscis extensions during the last three rewarded odor applications (CS+) minus during the last three penalized odor applications (CS−). The data were analyzed with nonparametric rank sum tests. A Wilcoxon signed rank test was applied to compare the ΔPE to a hypothesized value of zero, and Kruskal-Wallis test was applied to compare ΔPE distributions of the diets relative to one another. In the experiment testing discrimination of free-flying bees between similar diets, we used Chi-square tests to compare the number of foragers that visited the two diets.

Results

Analyses confirmed the deficiencies in essential amino acid levels of test diets (Table 1). In the week of offering the colonies nutritionally unbalanced flour diets, colonies collected a mean total of 88.7-g diet ± 9.15 SE ($n_{colonies} = 8$); the collected amount was stable over the days ($F_{1,54} = 0.25$, $P = 0.62$). After this week, foragers were offered three diets in a choice assay. The models on collected diet and number of bee visits showed neither YP and RL nor KA and SP to differ from one another (post hoc tests $P > 0.05$); hence flour identity, as nested in the factor flour type (legume or cereal), was excluded. The diet amount that foragers collected showed preference between the flour types (Fig. 1a; $F_{1,181.6} = 133.7$, $P < 0.001$), while bees collected relatively more of the nutritionally complementary diets, as compared to the similar and same diets (Fig. 1b; $F_{2,181} = 8.22$, $P < 0.001$) (Interaction term excluded; $P = 0.94$).

Fig. 1 Diet balancing test. Diet type and nutritional role were considered as factors affecting honey bee foraging. *Panels A* and *B* show collected diet amounts per dish and *panels C* and *D* show the number of bee visits. Data are means ± SE. *Different letters above bars* indicate statistically significant differences between groups (Tukey's test; $P < 0.05$)
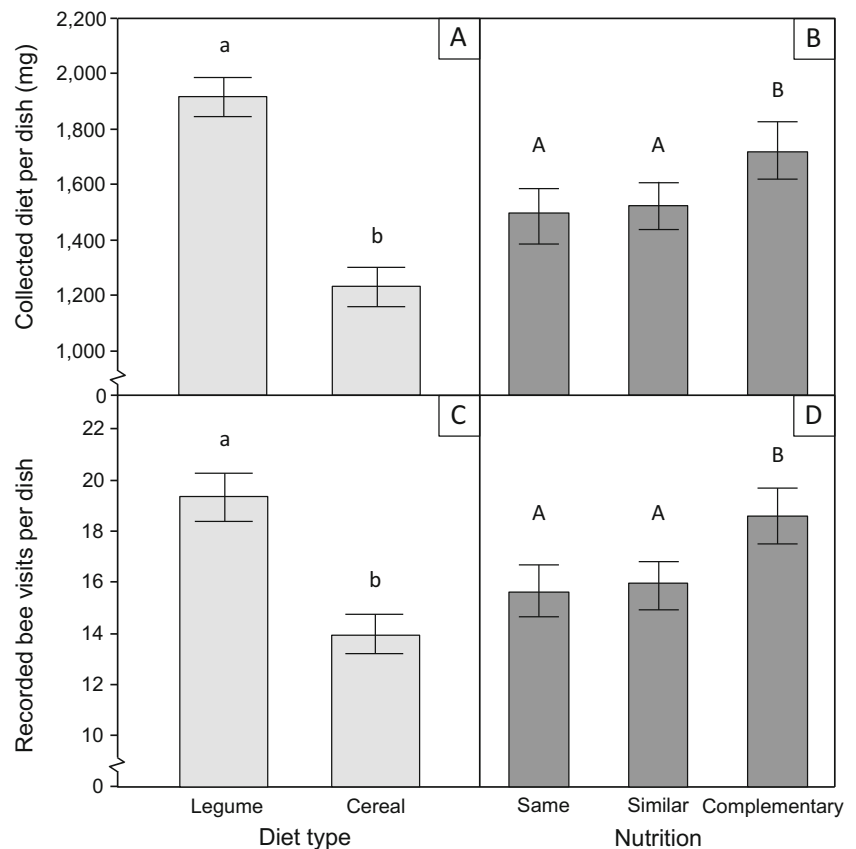
Similarly, forager bee visits differed between the diet types (Fig. 1c; $F_{1,183} = 26.4$, $P < 0.001$), with relatively more visits to the nutritionally complementary diets compared to the similar and same diets (Fig. 1d; $F_{2,181} = 5.10$, $P = 0.007$) (Interaction term excluded; $P = 0.72$). The collected diet weights and the number of forager visits correlated with $R^2 = 48.9$ % ($F_{1,190} = 181.7$, $P < 0.001$).

The PER discrimination experiment showed that bees could distinguish diets by their odor. Bees readily learned to differentiate diet odor from air (Fig. 2a). The number of proboscis extensions differed significantly between the last three rewarded and penalized trials ($\Delta PE > 0$; Wilcoxon signed rank test: $W = 1173$, $Df = 79$, $P < 0.001$). The odors of all four diets were similarly discriminated from air ($\Delta PE_{YP} = \Delta PE_{RL} = \Delta PE_{KA} = \Delta PE_{SP}$, with $n = 20$ bees per diet; Kruskal-Wallis test: $X^2 = 4.01$, $Df = 3$, $P = 0.26$). Similar diets were not significantly distinguished from one another (Fig. 2b; $\Delta PE = 0$, $W = 27.5$, $Df = 84$, $P = 0.36$); yet, the complementary diets were significantly discriminated (Fig. 2c; $\Delta PE > 0$, $W = 266$, $Df = 119$, $P < 0.001$). To ascertain that the difference in statistical significance between the similar ($N = 90$) and complementary ($N = 120$) diets was not an artifact of sample size, we randomly duplicated the results of 30 bees from the first group and randomly deleted results of 30 bees from the second group. Analyses were rerun with these adjusted sample sizes, 20 times. All 20 random permutations of

enlarged datasets still showed no discrimination between odors of similar diets ($\Delta PE_{similar} = 0$; $P > 0.05$), and all 20 reduced datasets still indicated significant discrimination between odors of complementary diets ($\Delta PE_{complementary} \neq 0$; $P < 0.05$).

In the experiment testing free-flying bees, we found that bees could discriminate well between similar diets: bees preferred foraging at the diet that was associated with sugar solutions over that associated with salt solution (Fig. 3) (KA/SP, $X^2 = 164.4$, $Df = 1$, $P < 0.001$; SP/KA, $X^2 = 17.8$, $Df = 1$, $P < 0.001$; YP/RL, $X^2 = 4.15$, $Df = 1$, $P < 0.04$; RL/YP, $X^2 = 14.3$, $Df = 1$, $P < 0.001$).

Discussion

Social bees (Apini, Bombini, Meliponini) form long-living colonies that forage on a range of floral resources. Generalist (polylectic) foraging behavior enables colonies to follow spatial and seasonal opportunities (Hanley et al. 2008; Avni et al. 2009; Requier et al. 2015). Foraging on different resources at the same time is one strategy to passively safeguard nutritional balance. Namely, the probability that essential nutrients are lacking is mitigated when a diet is an average of different resources (McLellan 1978). In our choice experiment, honey bee foragers passively balanced nutrient levels to some extent
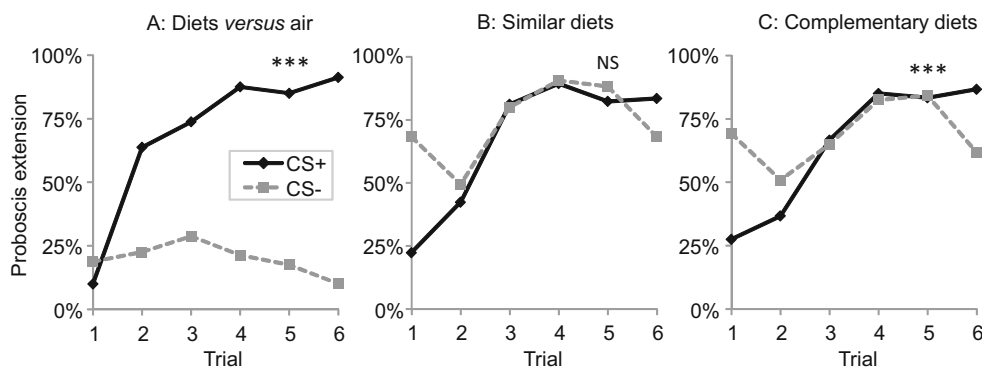
Fig. 2 Olfactory discrimination between diets in proboscis extension response (PER) conditioning tests. In each pair, one odor (CS+) was associated with positive reinforcement (1.5 M sucrose solution; *black*) and the other odor (CS−) with a negative reinforcement (2 M salt; *gray*).

The odors were of diet vs air (**a**), of two similar diets (**b**), or of two complementary diets (**c**). Discrimination during the last three trials was tested statistically (*NS* not significant; ***$P < 0.001$*)

by collecting three diets in parallel. However, we found that bees also actively balanced the colony's diet by biasing foraging towards diets that complemented previous EAA nutrition.

Honey bee foragers may taste or smell EAA's by means of gustatory sensilla on the distal segment of the antennae, on the mouthparts, and on the tarsi of the forelegs (De Brito Sanchez 2011). Honey bees can perceive several essential and non-essential amino acids, either by taste (Inouye and Waller 1984; Kim and Smith 2000; Hendriksma et al. 2014) or by smell (Linander et al. 2012). In addition, amino acids can affect bee learning and memory (Chalisova et al. 2011; Simcock et al. 2014), suggesting a link between foraging behavior and amino acid nutrition. However, empirical studies supporting honey bee amino acid balancing behavior are rare (Cook et al. 2003). We found that foragers preferred a complementary diet to both the same and the similar diet. This preference manifested itself both by the weight of collected

diets and by the number of bees recorded visiting the dishes. We can thus reject our null hypothesis of equal foraging. Bees did not shy away from the same diet in order to diversify (the first alternative hypothesis), but specifically biased their foraging efforts towards the complementary diet. The data thus support our second alternative hypothesis.

It has been described that insects can reject a diet that is deficient in one or several EAA's (Abisgold and Simpson 1987; Simpson et al. 1990; Ribeiro and Dickson 2010; Vargas et al. 2010; Toshima and Tanimura 2012; Bjordal et al. 2014). During the first week of feeding, honey bee foragers in our experiment did not change their efforts for collecting deficient diet. Apparently, honey bees do not exhibit avoidance behavior, evading suboptimal nutrition. An apparent difference between the above studies and ours is these studies relate to solitary insects, whereas the current study is with a eusocial insect. Collected pollen is by enlarged stored in the colony and not consumed by the foragers. In a honey bee colony, the stored reserves may act as a buffer to the specificity of pollen collected by an individual forager during a specific time.

Diet assessment by animals may involve a number of mechanisms, from pre-ingestive cues (e.g., vision, olfaction, or tactile aspects) to ingestive evaluation (e.g., gustation) and post-digestive feedback (Simpson and Raubenheimer 2012). The balancing of nutrients is a complex process in which alimentary cues are integrated with post-ingestive information about food quality to optimize nutrition (Simpson and Raubenheimer 2012). For example, honey bee foragers may use visual cues to discriminate among diets (Fig. 3; Table 1; Online Resource—page 1). Further, perception and learning of odors may play a role in pollen-foraging behavior (Cook et al. 2005). PER conditioning trials showed that honey bees perceived the odor of test diets (Fig. 2a). They quickly learned to discriminate between diet odor and air. Bees were more challenged when having to discriminate between the odors of two diets (Fig. 2b, c). The initial high rate of response to
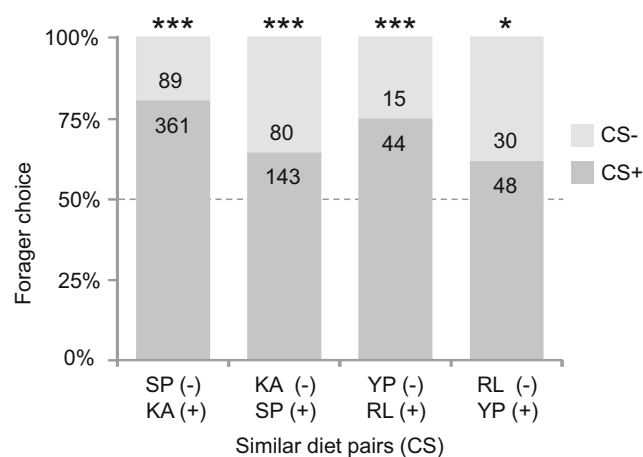
Fig. 3 Discrimination between similar diets in a conditioning experiment. Foragers discriminated between a diet associated with sugar solution (CS+) and a diet associated with salt solution (CS−) in all four diet permutations (Chi-square significance; *$P < 0.05$, ***$P < 0.001$). *Numbers in bars* are the number of foragers that visited each diet. *Dashed line* at 50 % represents indifference

the penalized odor (CS−) reflects generalization between two similar odors. In the pseudorandom sequence that we used, the first trial was always with odor A (CS+) and the second trial with odor B (trial 1 of CS−). Single-trial learning in trial 1 manifested itself in high response rate in the following trial. The later four trials (trials 2–5 of odors A and B) showed lack of discrimination. But by the last trial, bees seemed to have learned to focus on more subtle differences between the odors and to discriminate between them.

Considering the last three trials, olfactory discrimination was statistically significant for the more dissimilar odors (same and complementary) and not statistically significant between the more similar ones (same and similar). Hence, it is possible that the preference for the complementary diet in the free-flight choice experiment was guided by odor discrimination. Bees were indifferent between the same and similar diets in the diet balancing test (Fig. 1b, d). This is consistent with bees actively biasing their foraging towards a nutritionally complementary diet, but could also result from bees trying to diversify their diet (avoiding the same diet) and not being able to discriminate the same from the similar diet. We therefore conducted discrimination tests in which we differentially conditioned bees to the same and similar diets; these tests showed that foragers could discriminate well between the diets (Fig. 3). Our analysis of the reflective properties of the diets suggests that bees may be able to discriminate between them visually (Online Resource—page 3 and 4), though determining the relative importance of different perceptual cues in guiding choice is beyond the scope of the present study.

Intriguingly, in certain insects, an over- or underrepresentation of EAA's in the circulating blood may induce a respective under- and over-sensitive receptor response (e.g., Simpson and Simpson 1992). Whether such receptors are induced in honey bees and accordingly influence forager choice remains to be explored. Further research is needed to corroborate the causality between dietary complementation and EAA deficiency, as compared to other nutritional or non-nutritional cues (Table 1; Online Resource—page 3). In this respect, the essential omega 3 and omega 6 FAs (i.e., alpha-linolenic and linoleic acid, respectively) are of interest as they are potentially limiting nutrients for bees (Avni et al. 2014, Arien et al. 2015). Unlike EAAs, for which an estimate of minimal required amounts exists (De Groot 1953), little is known about bee requirements of essential FAs. The data on dietary contents show that diets contrasted also in FA contents, in addition to AA contents. Hence, bees may have also complemented essential FA needs.

When addressing regulatory balancing behavior for diet uptake in social colonies, the occurrence of age polyethism among honey bees needs to be considered (Seeley 1995; Lihoreau et al. 2014). Nutrient information in social insects has to be communicated across different organizational levels (Dussutour and Simpson 2009; Behmer 2014; Arganda et al. 2014; Lihoreau et al. 2015). Relatively, older worker bees specialize on bringing nectar and pollen to the colony. Thereafter, young nurse bees process the collected foods and distribute it as jelly among larvae, workers, drones, and the queen. A notable quarter of the worker jelly is returned to the forager force (Crailsheim 1991; Camazine et al. 1998). Hence, these trophallactic interactions allow feedback of postdigestive information, from nurses to foragers. Subsequently, honey bee foragers may leave the hive with bias for food sources offering needed nutrients. Projecting this nutrient information pathway onto our experiment, foraging individuals of colonies fed legume or cereal flours, may have been presensitized to collect a methionine-rich or lysine-rich food, respectively.

The screening experiment showed a significant honey bee preference for legume diets over cereal diets (Online Resource—page 1). Consistently, the legumes were generally preferred over the cereals in our main experiment (Fig. 1a, c). Preference among pollen types exists as well (Hanley et al. 2008; Avni et al. 2009). We however could show that, beyond intrinsic preference, forager choices can be affected by unbalanced colony nutrition.

Ongoing global bee declines have been linked to agricultural intensification and decreasing diversity and abundance of floral resources (Ricketts et al. 2008; Potts et al. 2010). Large-scale monocultures may fail to provide polylectic bees sufficient choice, risking monofloral-biased pollen diets for colonies. This can directly restrain development of the colony as a whole, since health, survival, and performance of individual bees are compromised (Schmidt 1984; Alaux et al. 2010; Vanderplanck et al. 2014; Arien et al. 2015). Recent studies in nutritional ecology address the complex question of the influence of dietary nutrients on feeding choice of social bees (Avni et al. 2014; Paoli et al. 2014a; Vanderplanck et al. 2014; Somme et al. 2015). The present study reveals an ability of honey bees to bias their foraging efforts towards nutritional balancing when in an environment that offers such alternatives.

Compliance with ethical standards

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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