Insect. Soc. 55 (2008) 425 – 427
0020-1812/08/040425-3
DOI 10.1007/s00040-008-1022-5

Insectes Sociaux

Research article

Does pollen function as a reward for honeybees in associative learning?

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Received 12 February 2008; revised 17 June 2008; accepted 15 July 2008.
Published Online First 11 August 2008

Abstract. The ability to learn an association between floral characteristics such as its odor, color and shape and a reward such as nectar is key to honeybee foraging success. Here, we tested if also pollen could function as a reward for associative learning in honeybees. We found that large proportions of bees with and without field experience showed an unconditioned response, the extension of the proboscis, after touching their antennae with bee-collected pollen. Furthermore, bees readily learn to associate an odor with pollen in a classical conditioning assay. We suggest that pollen might play an important role as a reward for free-flying bees.

Keywords: Apis mellifera, unconditioned stimulus, associative learning, pollen.

Introduction

Associative learning plays an important role in foraging for honeybees (*Apis mellifera*). When a forager discovers a source of nectar, it learns to associate surrounding visual and olfactory cues with the reward (unconditioned stimulus; US) (von Frisch, 1967). This helps bees to return to previously visited food sources or to discover new ones displaying similar characteristics. Bees do not only learn food source characteristics during foraging, but also during social interactions inside the hive (Farina et al., 2005).

However, often bees collect pollen exclusively even if plant species offer both nectar and pollen (Scheiner et al., 2004). Some plant species that are visited by bees, like *Papaver*, *Rosa* and *Solanum,* do not offer nectar at all (Vogel, 1983). Moreover, honeybees can be conditioned to pollen odor either while interacting with dancing pollen foragers (von Frisch 1967) or under laboratory conditions in a proboscis extension response (PER) paradigm (Cook et al., 2005). This raises the question if bees can actually learn to associate relevant colors, shapes and odors in the field or food odors in the hive with pollen. Scheiner et al. (2004) reported that honeybee foragers extend their proboscis after touching their antennae with hand-collected pollen. In honeybees, the PER is an unconditioned response, which is usually elicited after contact with a sucrose solution. Therefore, we tested if pollen itself could function as an unconditioned stimulus for olfactory associative learning. Bees were either captured at the entrance of the hive (with or without pollen loads) or when they were reared under controlled conditions (caged bees) without ever experiencing pollen. Furthermore, we use a classical olfactory conditioning procedure with pollen as reward to test if foragers learn to respond to a previously neutral odor after pairing the odor with the putative US.

Material and methods

Caged bees

Combs with pre-emerging brood were maintained in an incubator (temperature: 36° C, relative humidity 55 %). On the day of emergence, about 50 bees were introduced into a wooden box (10 cm x 10 cm x 10 cm). They were fed exclusively 50 % w/w unscented sucrose solution ad libitum. The boxes were kept in an incubator (25° C, 55 % relative humidity, darkness) for 17 days.

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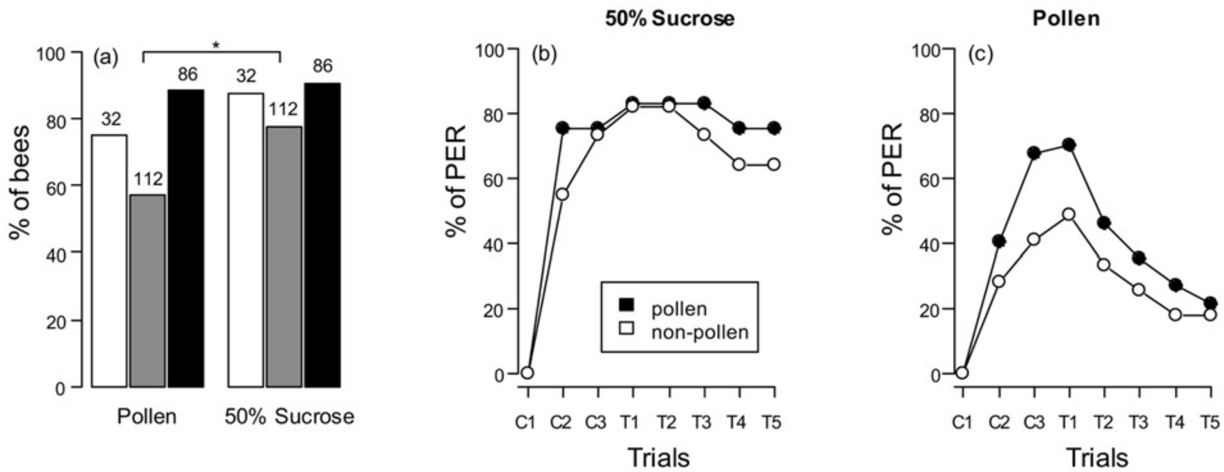

Figure 1. Percentage of bees showing the PER. a) Caged bees (white bars), non-pollen foragers (gray bars) and pollen foragers (black bars) showed the proboscis extension after touching the antennae with pollen or a 50 % sucrose solution. b) Responses in non-pollen foragers (white circles) and pollen foragers (black circles) in a classical conditioning procedure with three conditioning trials (C1-C3) and five test trials (T1-T5) using a 50 % w/w sucrose solution as a reward. c) as in b) but with pollen as reward.

Foragers

Bees were captured with plastic tubes when they tried to enter the colony. We captured similar numbers of bees returning with pollen packages and bees without them, which are likely to be nectar foragers.

PER testing

About 10–20 min after capture and one hour before testing, bees were harnessed in plastic tubes so that they could move freely their mouthparts and antennae (Cook et al., 2005). First, the antennae of bees were touched with water and bees extending the proboscis were fed until satiation. Both antennae were touched with pollen and a 1.8M sucrose solution. The inter-trial interval was about 15 min. One half of the bees was tested first with pollen, the other half with sucrose solution. We checked if bees showed the PER towards the pollen scent immediately before physical contact with the pollen, but observed no such case. Bees were tested with a commercial bee-collected pollen mix (Apícola Calandri). We added 30–50 % w/w water to the dried pollen packages.

Olfactory conditioning

A device that delivered a continuous airflow was used for odorant application (see Cook et al., 2005). Foragers that showed the PER after applying the pollen mix and that did neither respond to the mechanical airflow stimulus nor to the first presentation of the odor were used for the olfactory conditioning. For conditioning we used either a 1.8M sucrose solution or the pollen mix as a reward and Linalool (LIO) as conditioned odor. Test trials lasted for 46 s and consisted of 20 s of airflow, 6 s of odor (CS) and 20 s of airflow (15 min inter-trial interval). Only during the first three trials (conditioning trials; C1-C3), the reward was delivered upon the last 3 s of the CS first to the antennae to release the PER and then to the proboscis. Otherwise; only the CS was presented (test trials; T1-T5).

Results

Figure 1a shows that high proportions of all bees extended their proboscis after contacting their antennae with both pollen and a 50 % sucrose solution (Pollen vs. sugar solution: caged bees, McNemar-Test, N = 32, P = 0.13; pollen foragers, N = 86, P = 0.63, non-pollen foragers, N = 112, P < 0.001). Pollen foragers were more sensitive to both pollen (G-test, $\chi^2 = 24.44$, N = 86/112, P < 0.001, significant after Dunn-Sidak correction for multiple comparison) and sucrose ($\chi^2 = 6.16$, N = 86/112, P = 0.013, significant after correction) than non-pollen foragers.

Figure 1b is an example of a curve for pollen foragers and non-pollen foragers using a 1.8M sucrose solution as US.

Figure 1c shows that 70.3 % of all pollen foragers and 48.7 % of all non-pollen foragers learn to associate LIO with Pollen as a reward after three-conditioning trials (T1, $\chi^2 = 3.62$, N = 37/39, P = 0.057). At T1, more bees respond to LIO if sugar solution was used as US (sugar vs. pollen, $\chi^2 = 4.48$, N = 23/76, P = 0.034, not significant after correction).

Discussion

A high proportion of bees with and without foraging experience show the PER after touching the antennae with pollen (Fig. 1a). While nectar foragers responded more to nectar, pollen foragers responded equally well to nectar and pollen. This suggests that pollen foragers are less demanding regarding the reward, probably because they have lower sucrose response thresholds (Scheiner et al., 2004).

We used bee-collected pollen in our experiment, but Scheiner et al. (2004) showed the same effect when using hand-collected pollen. We also found, that bees learn to respond to an odor using pollen as the US in a classical conditioning PER assay. This strongly suggests that pollen can play a potentially important role as a reward for associative learning in free-flying bees and for recruits inside the hive. The odor-pollen combination can be quickly perceived by foragers when they work pollen with forelegs and mouthparts during collection or when they antennate and lick pollen from the corbiculae of a returning forager (von Frisch, 1967). Additionally, pollen could function as a first-order CS in second-order conditioning if bees previously experienced nectar and pollen simultaneously. However, the PER of caged bees showed that this is not necessary for pollen to function as a US. The main constituents of pollens are proteins, carbohydrates and water. The relative amounts vary greatly between species and bee-collected pollens usually contain more sugars, which are added by the foragers (Solberg and Remedios, 1980). It is likely that it is mainly the sugars the bees respond to, but also amino acids, which are common in pollen, can positively affect learning in honeybees (Kim and Smith, 2000). In other insect species, pollen additionally stimulates salt receptors (Wacht et al., 2000). More studies with types of pollen that contain variable concentrations of the main constituents are needed to determine the importance of the different pollen constituents for learning. Furthermore, the role of pollen as a reward for the learning of ecologically relevant cues such as shapes and colours should be investigated under more natural conditions.

Acknowledgments

This study was supported by funds from ANPCYT, CONICET, University of Buenos Aires and Janggen-Pöhn Stiftung. When we carried out our experiments, we adhered to the legal requirements of Argentina.

References

Cook S.M., Sandoz J.C., Martin A.P., Murray D.A., Poppy G.M. and Williams I.H. 2005. Could learning of pollen odours by honey bees (*Apis mellifera*) play a role in their foraging behaviour? *Physiol. Entomol.* **30:** 164–174

Farina W.M., Grüter C. and Diaz P.C. 2005. Social learning of floral odours within the honeybee hive. *Proc. R. Soc. London Ser. B* **272:** 1923–1928

Kim Y.S. and Smith B.H. 2000. Effect of an amino acid on feeding preferences and learning behavior in the honey bee, *Apis mellifera. J. Insect Physiol.* **46:** 793–801

Scheiner R., Page R.E. and Erber J. 2004. Sucrose responsiveness and behavioral plasticity in honey bees (*Apis mellifera*). *Apidologie* **35:** 133–142

Solberg Y. and Remedios G. 1980. Chemical composition of pure and bee-collected pollen. *Meld. Norg. Landbrukshgskole* **59:** 1–12

Vogel S. 1983. Ecophysiology of zoophilic pollination. In: *Physiological Plant Ecology III* (Lange O.L., Nobel P.S., Osmond C.B. and Ziegier H., Eds). Springer, Berlin Heidelberg New York. pp 559–624.

von Frisch K. 1967. *The Dance Language and Orientation of Bees.* Cambridge, Massachusetts: Harvard University Press. 566 pp

Wacht S., Lunau K. and Hansen K. 2000. Chemosensory control of pollen ingestion in the hoverfly *Eristalis tenax* by labellar taste hairs. *J. Comp. Physiol. A* **186:** 193–203