SHORT COMMUNICATION

Do inexperienced bumblebee foragers use scent marks as social information?

Ellouise Leadbeater · Lars Chittka

Abstract Bumblebees (*Bombus spp.*) foraging in the field typically reject flowers where they detect the olfactory footprints of previous visitors and hence avoid recently emptied inflorescences. A growing number of studies have begun to illustrate that associative learning shapes the development of this process, in both bumblebees and other bee species. This raises the question of what the default response to such marks is, but little is known about how inexperienced foragers use social information. Here, we offered flower-naive bees a choice between scent-marked flowers and unmarked alternatives and found that individuals neither avoided nor preferred marked flowers. Our findings provide no support for 'hard-wired' responses to scent marks in bumblebees and highlight the importance of associative learning in shaping social information use to match local circumstances.

Keywords Scent marks · Bumblebee foraging · Cuticular hydrocarbons · Bumblebee learning · Social information

Introduction

The ability of foraging bees to efficiently find tiny nectar and pollen quantities among vast arrays of blossoming flowers is critically dependent upon associative learning. Although flower-naive bees typically have preferences for flower colours, patterns and scents (Rodríguez et al. 1994; Giurfa et al. 1995; Lunau et al. 1996; Plowright et al. 2006; Seguin and Plowright 2008), such preferences can be quickly magnified or reversed with experience and even entirely new preferences can be learnt (Chittka 1998; Menzel 2001; Raine et al. 2006). In addition to floral features, bees use cues left inadvertently by other flower visitors to predict the rewards offered by flowers in the field (Giurfa and Núñez 1992; Giurfa 1993; Goulson et al. 1998; Leadbeater and Chittka 2005; Worden and Papaj 2005), and social information use can also be modified by learning (Saleh et al. 2006; Leadbeater and Chittka 2009). Is social information use entirely a result of bees' extraordinary ability to learn about how to find nectar and pollen? We know little about whether flower-naïve bees have 'hard-wired' responses to social cues.

Here, we investigate how bumblebees with no foraging experience respond to scent marks left on flowers by previous visitors—a widely documented social cue (Eltz 2006; Giurfa 1993; Goulson et al. 1998; Saleh and Chittka 2007; Stout et al. 1998; Witjes and Eltz 2007). Bumblebees with experience of foraging in the field typically reject scent-marked flowers and thus avoid those that have been recently emptied by conspecific or even heterospecific visitors (Goulson et al. 1998; Stout and Goulson 2001, 2002; Stout et al. 1998). The repellent effect is typically transient, lasting less than an hour in some cases (Stout and Goulson 2001), although the marks themselves can persist for longer (Witjes and Eltz 2009). Although the term 'scent mark' implies active deposition, evidence suggests that such marks are footprints left inadvertently by bees whenever they land upon a substrate, and not only upon flowers (Saleh and Chittka 2007; Wilms and Eltz 2008).

E. Leadbeater (✉)
Institute of Zoology, Zoological Society of London,
Regent's Park, London NW1 4RY, UK
e-mail: ellouise.leadbeater@ioz.ac.uk

L. Chittka
Research Centre for Psychology, School of Biological
and Chemical Sciences, Queen Mary University of London,
Mile End Road, London E1 4NS, UK

Associative learning is clearly important in the interpretation of scent marks. Laboratory studies where previously visited flowers are not empty but highly rewarding have found that the scent marks can become attractive, rather than repellent to foragers (Cameron 1981; Schmitt and Bertsch 1990). Likewise, bees rely on the marks more heavily when visiting a drained flower comes at a relatively high time cost. This suggests that individuals learn about whether the marks are useful in specific foraging contexts (Saleh et al. 2006). Saleh and Chittka (2006) directly compared the behaviour of bees that had experienced the marks in either a rewarding or a nonrewarding context and found that individuals were subsequently attracted or repelled by the marks accordingly.

If associative learning influences interpretation of scent marks so strongly, does the repellent effect of natural scent marks result entirely from repeated exposure to marked flowers that offer low rewards due to recent visitation? What is the 'default' response to scent marks, before associative learning has a chance to take place? Previous studies have never assayed the behaviour of naïve bees, because such individuals cannot be identified in field studies. In the laboratory, shared feeders or artificial flowers are often used to identify potential foragers before experiments begin. Here, we investigate the responses of naive foragers, that have never previously encountered flowers or feeders, to scent marks.

Based on the behaviour of experienced bees, we hypothesise that flower-naïve bees will reject scent-marked flowers and thus choose unmarked flowers over marked alternatives more often in a choice test. However, scent marks left on wild flowers are deposits of cuticular hydrocarbons, which promote tarsal adhesion (for example, to flowers) and reduce desiccation (Eltz 2006; Schmitt et al. 1991; Goulson et al. 2000). These cuticular hydrocarbons also have derived roles in nestmate recognition and communication in social insects (Nieh 2009; van Zweden and D'Ettorre 2010). As such, these scents will be familiar to all foragers from contact with nestmates, and indeed with any substrate associated with the nest. Thus, an alternative hypothesis is that naïve bees will be attracted to the marks because they represent a familiar scent, implying that avoidance of marked flowers by experienced bees is entirely the result of associative learning.

Methods

Subjects came from commercially obtained *Bombus terrestris* colonies (Syngenta Bioline Bees, Weert, the Netherlands), housed in wooden nestboxes comprising a nest chamber and a lined external chamber. Each nestbox was connected to a flight arena (60 cm × 50 cm) by a transparent plastic tube. Access could be controlled with movable shutters, and bees never entered the flight arena before testing. Prior to arrival at the laboratory, colonies had been housed solely in cardboard rearing boxes where sugar solution was available ad libitum inside the nest. Once in the nestbox, sugar solution (50% v/v) was provided directly into the nest compartment. Each colony was supplied with pollen granules twice a week, again directly into the nest compartment. Since bees store food resources in the colony's honey and pollen pots, all individuals experienced food in the nest prior to foraging, as would be the case in wild bee colonies. However, all foragers were flower-naive.

Bees from two colonies were tested in succession (23 subjects from one colony and 27 from the second completed the test). We kept a third colony in a separate nestbox, to provide scent marks. This colony had access to a second flight arena ('marking arena'), where access to ad libitum sugar feeders was permitted.

Bees with no foraging experience typically visit artificial flowers only after very extended periods of exploration (unless they are provided with prior training or conspecific demonstrators). To maximise our sample size, we hence used natural flowers (wild pansy *Viola tricolor*, purple, yellow, orange or bicoloured purple/yellow or purple/white morphs), obtained prior to flowering from a commercial nursery, and kept in an insect-proofed greenhouse to prevent pollinator visitation. All plants contained flowers of one colour morph only. For each test, we selected one plant and chose the two flowers that appeared most alike in size [mean size difference between flowers (widest diameter) = 1.46 ± 1.47 mm (s.d.)]. Other flowers on the plant were removed.

We selected one of the two flowers on the plant at random to be scent-marked by our non-experimental bee colony. This flower was baited with 5 µl of unscented sucrose solution (50% v/v), inserted gently into the deep base of the corolla, while the other was covered using a plastic tub, taking care to avoid touching the petals. The plant was then placed in the marking arena, and bees from the non-experimental colony were released to forage. In every case, a forager visited the flower within 5 min of release. Once the first visitor had departed, the plant was quickly removed from the arena and the plastic covering was removed from the alternative (unmarked) flower. The volume of the nectar bait supplied was not sufficient to fill a bee's nectar crop, so the sucrose in the marked flower had always been entirely drained by this procedure, and we refilled both flowers with 5 µl of sucrose before testing, again inserted into the deep base of the corolla. Previous work has shown that visitation by one forager is sufficient to elicit avoidance of that flower by experienced bees (Goulson et al. 1998).

For testing, we immediately placed the plant, now containing one unmarked and one marked flower, into the test arena approximately 10 cm from the entrance tunnel linked to the experimental colony. One bee was selected from those individuals attempting to leave the colony and allowed to enter the arena. We recorded any approaches (inspections of flowers without landing) and the subject's choice of flower (landing and exploring the flower). Individuals were removed from the arena once their first choice had been recorded; bees that did not make a choice within 10 min ($n = 27$) were also removed. All individuals were marked with a number tag after testing and returned to the colony. Fifty bees completed the test. A fresh plant was used for each bee.

Statistical analyses

We compared the number of bees choosing the scent-marked flower with chance expectations of 50% using a binomial test. To ascertain whether trial outcomes (choice of scent-marked/scent-unmarked flower) varied between colonies, with floral colour morph, or with trial order, we used a generalised linear model, specifying the error distribution as binomial. Nonsignificant terms were dropped sequentially until further removal led to a significant decrease in the explanatory power of the model.

We carried out a simulation-based power analysis to establish the power of our experiment to detect a significant effect, given our sample size of 50 bees. Goulson et al. (1998) found that experienced bees rejected 78% of scent-marked natural flowers offered, compared to 29% of randomly chosen flowers (which may or may not have been scent marked). We thus estimated that, if the behaviour of flower-naïve bees matches those of their experienced counterparts as our primary hypothesis predicts, bees should prefer unmarked flowers according to a ratio of 78:29 (=73% preference for unmarked flowers). We generated random deviates from a binomial distribution with 50 trials in which the probability of choosing a scent-marked flower was 0.73 [R command *rbinom* (Kachitvichyanukul and Schmeiser 1988; R Team 2008)]. We then performed a binomial test on these artificial choices, to establish whether a significant effect was detected. This procedure was repeated 10,000 times, and the number of iterations in which a significant effect was detected was recorded.

Results

In contrast to the experiments with experienced bees (Goulson et al. 2001; Goulson et al. 1998; Stout et al. 1998), rejection rates (approaches without landing) were extremely low for both flower types. Of 50 test bees, only 4 rejected a flower before making their final choice. In two cases, bees rejected both flowers once before making their final choice. One bee rejected the scent-marked flower once but then subsequently probed it, and one bee did the same but with the unmarked flower.

Accordingly, we found neither overall preference nor rejection of the scent-marked flower (Fig. 1). Twenty-three bees landed on and attempted to probe the scent-marked flower, while 27 chose the unmarked alternative (binomial test, $P = 0.67$). We found no influence of floral colour, colony or trial order on preferences for scent-marked flowers (binomial GLM, $P > 0.31$ in each case, Table 1). The power of our experiment to detect a preference for scent-marked flowers equivalent to that described in Goulson et al. (1998) was 89.4% and thus exceeds the standard threshold of 80% (Crawley 2007).

Discussion

We find no evidence that scent marks are meaningful to flower-naïve bees that have had no opportunity to associate them with reward levels. Flower-naïve foragers rarely rejected either scent-marked or scent-unmarked flowers, and as a result showed no preference for either flower type. Thus, scent marks neither acted as a familiar scent that attracts naïve foragers to flowers nor repelled them from potentially unrewarding choices.

Working with naive bees demanded the use of natural flowers, rather than artificial alternatives where colour, size and scent can be closely controlled, and we cannot rule out
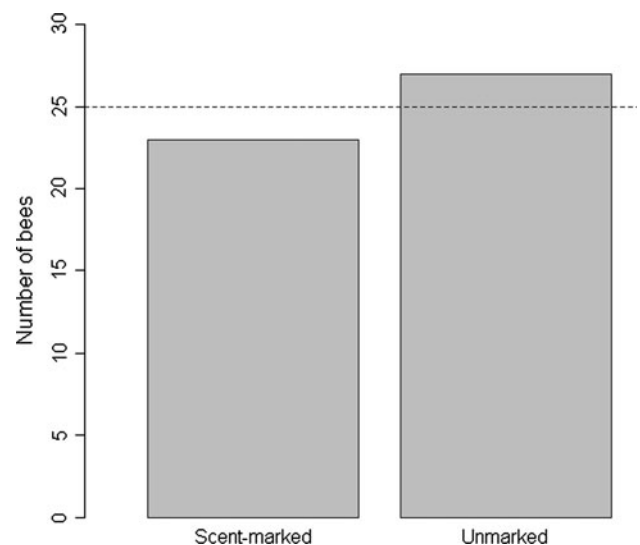

Fig. 1 Number of bees ($n = 50$) choosing the scent-marked flower or the unmarked alternative in a two-choice test. *Dashed line* indicates chance expectations

Table 1 The influence of floral colour, colony and trial order on preferences for scent-marked flowers

	χ^2	Degrees of freedom	P
Floral colour morph	4.78	4	0.31
Colony	0.06	1	0.81
Trial order (day of trial)	<0.00	1	>0.9

χ^2 values and associated P-values were obtained by comparing the null model with the null model plus the variable of interest

that even naive bees would use scent marks when choosing between otherwise identical alternatives. However, a preference that becomes apparent only when bees are faced with identical flowers is unlikely to have ecological relevance, because in the wild, bees are never faced with otherwise identical choices. Our findings suggest that the widely documented avoidance of marked natural flowers by experienced bees is at least magnified by, and may derive entirely from, associative learning through repeated experience with marked, unrewarding flowers.

Scent marks represent a reliable source of social information available to a foraging bee in the field, so why do naïve bees apparently ignore them? Other forms of social information use, such as an attraction to foraging conspecifics [bumblebees (Leadbeater and Chittka 2009)], or preferential visitation of floral scents brought back to the nest by other bees [honeybees (Arenas et al. 2009)], occur in flower-naïve foragers. We suggest two potential explanations as to why flower-naïve bees do not avoid marked flowers.

Firstly, the marks are inadvertent chemical footprints of cuticular hydrocarbons (Saleh and Chittka 2007; Wilms and Eltz 2008; Goulson et al. 2000), which are likely found in abundance within nests and at the nest entrance (Gamboa 1996; van Zweden and D'Ettorre 2010). Since even flower-naive bees also experience nectar and pollen within the nest, a 'hard-wired' response would have to be specific to a foraging context. Nonetheless, social insects are capable of interpreting cuticular hydrocarbons in different ways according to context (Bos et al. 2010), so such specificity may not be unrealistic. Moreover, cuticular hydrocarbons of bees from other colonies (as were used in our study) are more likely to elicit avoidance or aggression than attraction [but note that the response to scent marks is similar irrespective of whether the mark was produced by a conspecific from another colony or the forager itself (Goulson et al. 1998)]. Secondly, scent marks are longer lasting than other forms of social information found in the field, such as conspecific presence on a flower, and bees are likely to encounter them frequently and repeatedly. Thus, there is ample opportunity for rapid, context-specific learning to occur, and it may be that there is little selection for a hard-wired aversion to marked flowers.

Use of hydrocarbon-based deposits at food sources as social information is widespread within social bees (Nieh 2009; Yokoi and Fujisaki 2007), and also occurs in solitary species (Yokoi and Fujisaki 2009). Even in cases where scent marks appear to have a more derived role in communication, such as the odour trails and attractive food-source marks of stingless bee species (Nieh 2004, 2009), evidence that learning is key to their interpretation is growing. For example, stingless bee *Scaptotrigona pectoralis* foragers will follow the trails of foreign colonies only if they experience the scent prior to departure inside their own nests, suggesting that the trail compound is learnt during the recruitment process (Reichle et al. 2011). Odour marks at the food source can be interpreted as attractive or repellent according to experience in the stingless bee *S. mexicana* (Sanchez et al. 2008), just as they are in bumblebees, as we have discussed above. Associative learning allows behaviour to become fine-tuned to the local environment, and as our findings highlight, it may preclude the need for responses to social information in flower-naïve foragers.

References

Arenas A, Fernandez VM, Farina WM (2009) Associative learning during early adulthood enhances later memory retention in honeybees. Plos One 4(12):8. doi:e804610.1371/journal.pone.0008046

Bos N, Guerrieri FJ, D'Ettorre P (2010) Significance of chemical recognition cues is context dependent in ants. Anim Behav 80(5):839–844

Cameron SA (1981) Chemical signals in bumble bee foraging. Behav Ecol Sociobiol 9(4):257–260

Chittka L (1998) Sensorimotor learning in bumblebees: long-term retention and reversal training. J Exp Biol 201(4):515–524

Crawley MJ (2007) The R book. Wiley, Chichester

Eltz T (2006) Tracing pollinator footprints on natural flowers. J Chem Ecol 32(5):907–915

Gamboa GJ (1996) Kin recognition in social wasps. In: Turillazzi S, West-Eberhart MJ (eds) Natural history and evolution of paper-wasps. Oxford Science, Oxford, pp 161–177

Giurfa M (1993) The repellent scent-mark of the honeybee *Apis-Mellifera-Ligustica* and its role as communication cue during foraging. Insect Soc 40(1):59–67

Giurfa M, Núñez JA (1992) Honeybees mark with scent and reject recently visited flowers. Oecologia 89:113–117

Giurfa M, Núñez JA, Chittka L, Menzel R (1995) Colour preferences of flower-naive honeybees. J Comp Physiol A-Sens Neural Behav Physiol 177(3):247–259

Goulson D, Hawson SA, Stout JC (1998) Foraging bumblebees avoid flowers already visited by conspecific or by other bumblebee species. Anim Behav 55:199–206

Goulson D, Stout JC, Langley J, Hughes WOH (2000) Identity and function of scent marks deposited by foraging bumblebees. J Chem Ecol 26(12):2897–2911

Goulson D, Chapman JW, Hughes WOH (2001) Discrimination of unrewarding flowers by bees; direct detection of rewards and use of repellent scent marks. J Insect Behav 14(5):669–678

Kachitvichyanukul V, Schmeiser BW (1988) Binomial random variate generation. Commun ACM 31:216–222

Leadbeater E, Chittka L (2005) A new mode of information transfer in foraging bumblebees? Curr Biol 15(12):R447–R448

Leadbeater E, Chittka L (2009) Bumble-bees learn the value of social cues through experience. Biol Lett 5(3):310–312. doi:10.1098/rsbl.2008.0692

Lunau K, Wacht S, Chittka L (1996) Colour choices of naive bumble bees and their implications for colour perception. J Comp Physiol A-Sens Neural Behav Physiol 178(4):477–489

Menzel R (2001) Behavioural and neural mechanisms of learning and memory as determinants of flower constancy. In: Chittka L, Thomson JD (eds) Cognitive ecology of pollination: animal behaviour and floral evolution. Cambridge University Press, Cambridge, pp 21–40

Nieh JC (2004) Recruitment communication in stingless bees (Hymenoptera, Apidae, Meliponini). Apidologie 35(2):159–182

Nieh JC (2009) Convergent evolution of food recruitment mechanisms in wasps and bees. In: Gadau J, Fewell J (eds) Organization of insect societies: from genome to sociocomplexity. Harvard University Press, Cambridge, pp 264–286

Plowright CMS, Simonds VM, Butler MA (2006) How bumblebees first find flowers: habituation of visual pattern preferences, spontaneous recovery, and dishabituation. Learn Motiv 37(1):66–78. doi:10.1016/j.lmot.2005.03.002

Raine NE, Ings TC, Ramos-Rodriguez O, Chittka L (2006) Intercolony variation in learning performance of a wild British bumblebee population (Hymenoptera : Apidae : Bombus terrestris audax). Entomol Gen 28(4):241–256

Reichle C, Aguilar I, Ayasse M, Jarau S (2011) Stingless bees (Scaptotrigona pectoralis) learn foreign trail pheromones and use them to find food. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 197(3):243–249. doi:10.1007/s00359-010-0605-6

Rodríguez I, Gumbert A, Hempel de Ibarra N, Kunze J, Giurfa M (1994) Symmetry is in the eye of the 'bee holder': innate preference for bilateral symmetry in flower-naïve bumblebees. Naturwissenschaften 91(8):374–377

Saleh N, Chittka L (2006) The importance of experience in the interpretation of conspecific chemical signals. Behav Ecol Sociobiol 61(2):215–220

Saleh N, Chittka L (2007) Bumblebees use incidental footprints to generate adaptive behaviour at flowers and nest. Arthropod-Plant Interact 1:119–127

Saleh N, Ohashi K, Thomson JD, Chittka L (2006) Facultative use of the repellent scent mark in foraging bumblebees: complex versus simple flowers. Anim Behav 71:847–854

Sanchez D, Nieh JC, Vandame R (2008) Experience-based interpretation of visual and chemical information at food sources in the stingless bee Scaptotrigona mexicana. Anim Behav 76:407–414. doi:10.1016/j.anbehav.2008.04.003

Schmitt U, Bertsch A (1990) Do foraging bumblebees scent mark food sources and does it matter? Oecologia 82:137–144

Schmitt U, Lubke G, Francke W (1991) Tarsal secretion marks food sources in bumblebees (Hymenoptera: Apidae). Chemoecology 2:35–40

Seguin FR, Plowright CMS (2008) Assessment of pattern preferences by flower-naive bumblebees. Apidologie 39(2):215–224. doi:10.1051/apido:2007056

Stout JC, Goulson D (2001) The use of conspecific and interspecific scent marks by foraging bumblebees and honeybees. Anim Behav 62:183–189

Stout JC, Goulson D (2002) The influence of nectar secretion rates on the responses of bumblebees (Bombus spp.) to previously visited flowers. Behav Ecol Sociobiol 52(3):239–246

Stout JC, Goulson D, Allen JA (1998) Repellent scent-marking of flowers by a guild of foraging bumblebees (Bombus spp.). Behav Ecol Sociobiol 43(4–5):317–326

R Development Core Team (2008) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org

van Zweden J, D'Ettorre P (2010) Nestmate recognition in social insects and the role of hydrocarbons. In: Blomquist G, Bagneres AG (eds) Insect hydrocarbons. Cambridge University Press, Cambridge

Wilms J, Eltz T (2008) Foraging scent marks of bumblebees: footprint cues rather than pheromone signals. Naturwissenschaften 95(2):149–153. doi:10.1007/s00114-007-0298-z

Witjes S, Eltz T (2007) Influence of scent deposits on flower choice: experiments in an artificial flower array with bumblebees. Apidologie 38(1):12–18. doi:10.1051/apido:2006048

Witjes S, Eltz T (2009) Hydrocarbon footprints as a record of bumblebee flower visitation. J Chem Ecol 35(11):1320–1325. doi:10.1007/s10886-009-9720-7

Worden BD, Papaj DR (2005) Flower choice copying in bumblebees. Biol Lett 1(4):504–507

Yokoi T, Fujisaki K (2007) Repellent scent-marking behaviour of the sweat bee Halictus (Seladonia) aerarius during flower foraging. Apidologie 38(5):474–481. doi:10.1051/apido:2007034

Yokoi T, Fujisaki K (2009) Recognition of scent marks in solitary bees to avoid previously visited flowers. Ecol Res 24(4):803–809. doi:10.1007/s11284-008-0551-8