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Animal behaviour

Bees use the taste of pollen to determine which flowers to visit

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Pollen plays a dual role as both a gametophyte and a nutritional reward for pollinators. Although pollen chemistry varies across plant species, its functional significance in pollination has remained obscure, in part because little is known about how floral visitors assess it. Bees rely on pollen for protein, but whether foragers evaluate its chemistry is unclear, as it is primarily consumed by larvae. We asked whether the chemical composition of pollen influences bumblebees' foraging behaviour. Using putatively sweet and bitter pollen blends, we found that chemical composition influenced two aspects of bee behaviour relevant to plant fitness: the amount of pollen collected and the likelihood of subsequently visiting a visually similar flower. These findings offer a new perspective on the nutritional ecology of plant–pollinator interactions, as they show that pollen's taste may mediate its collection and transfer.

1. Introduction

Plants offer nutritional rewards to pollinators comprised of both attractive and deterrent components [1]. For example, nectar can contain secondary compounds that limit its consumption, with consequences for both plant and pollinator fitness [1,2]. Pollinators also collect pollen, yet how pollen might influence pollinator preferences has received considerably less attention. For the plant, pollen is a somewhat paradoxical reward, in that its collection is necessary for reproduction, but represents a cost if gametes do not reach conspecific stigmas [3]. Despite this, many angiosperms reward pollinators with both nectar and pollen, and 8–10% offer exclusively pollen [4]. Thus, many plants face a trade-off between encouraging pollen collection (e.g. via visual and olfactory cues [5,6]) while controlling its removal (e.g. through dosing, mechanical and toxic components [3]). However, how pollinators discriminate between pollen types and whether plants might use pollen chemistry to limit removal has been little explored. This is surprising, because the pollen of bee-pollinated species varies widely in its composition: pollen protein content ranges from approximately 2% to 60% [7], and the 'pollen-kitt' surrounding pollen grains consisting of lipids, pigments, secondary compounds and carbohydrates also varies across species [8].

Because bees use olfactory [5] and visual [6] cues to detect pollen, they may also use these cues to discriminate between different pollens. Indeed, training bees to associate scent or chemotactile cues (via antennal contact) with a sucrose reward shows that bees are at least physiologically capable of discriminating between pollens [9], but whether bees actually use these cues when foraging is unclear. Wild-foraging bees seem to preferentially collect higher-quality pollen (based on protein content) [10], but these results contrast with some of those from laboratory-reared experimentally tested bees, which only discriminate at the most extreme comparisons [11]. However, recent work has also found that bumblebees will preferentially collect higher-quality pollen without using odour cues to distinguish between pollens [12]. Bees also attend to tactile cues, discriminating against mechanically defended pollen [13] and favouring fine grains over
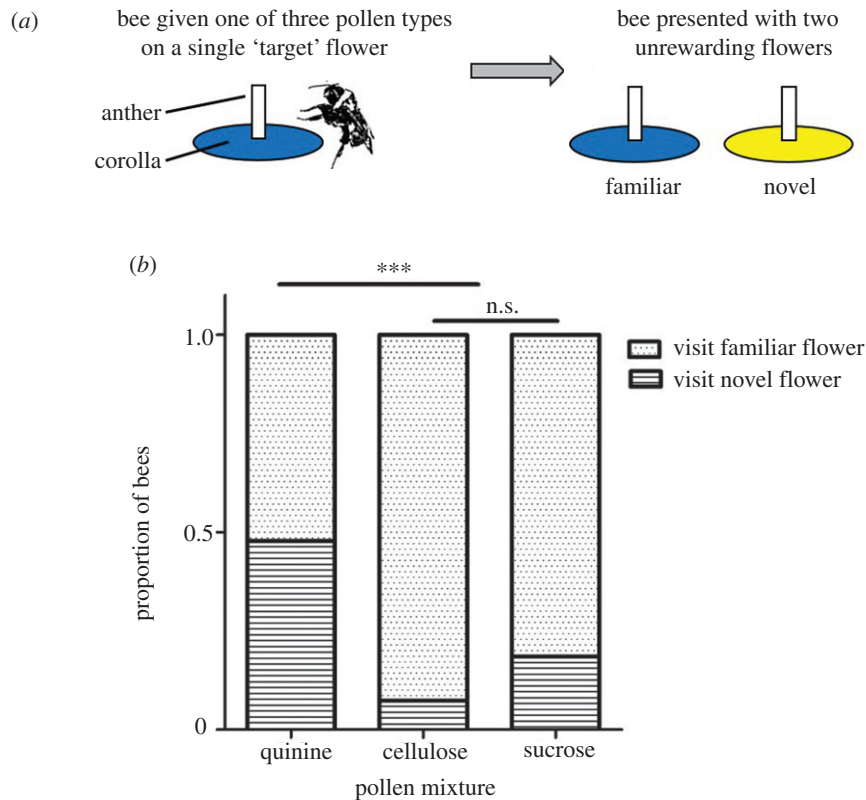
Figure 1. After (a) collecting pollen from the target flower (blue or yellow), (b) bees' tendency to visit either a familiar or novel flower colour across the three treatments. Bees were more likely to switch to a novel flower colour if they had experienced quinine (compared with the other two treatments), whereas bees that collected sucrose- or cellulose-laden pollen were equally likely to switch. (Online version in colour.)

larger ones [14]. However, the possibility that foraging bees assess pollen through taste has not been directly tested.

We addressed whether bumblebees prefer pollens based on taste, and therefore, whether such preferences might allow plants to adjust the trade-off between pollen collection and transfer. We asked whether bees responded differently to one of three experimental blends of pollen in terms of the duration and amount of pollen collection, thoracic temperature (previously shown to correlate with assessment of reward quality [15]), and subsequent landing decisions.

2. Material and methods

(a) Subjects

We connected six sequential colonies of *Bombus impatiens* (Koppert Biological Systems, MI, USA) to a foraging arena (L × W × H: 122 × 59 × 59 cm) by a gated passageway. We selected subjects by allowing a colony access to a training feeder (for 1–2 days) with a white artificial anther (chenille stem) loaded with approximately 50 mg *Prunus avium* cherry pollen (Tieton variety; Antles Pollen Supplies Inc., WA; see electronic supplementary material). We marked foragers visiting this feeder using numbered tags (E.H. Thorne Ltd, Wragby, Lincolnshire, UK) and provided sucrose (30% w/w) via a feeder connected to the colony. We tested bees for their tendency to collect a given pollen type by presenting them with one of three types of pollen, either pollen adulterated (10% by mass) with powdered quinine (a US− in nectar), cellulose (control) or sucrose (a US+ in nectar) from a white artificial anther. These three compounds are all odourless at room temperature (granule sizes in electronic supplementary material, figures S1 and S2). All analyses were carried out in R v. 3.2.3. (R Development Core Team 2010), for models used, see the electronic supplementary material.

(b) Experiment 1: behavioural response to pollen treatments

We presented individual bees ($n = 90$) with a single floral target (blue or yellow, for details, see electronic supplementary material, Methods), which offered 6.5 ± 0.70 mg (mean ± s.d.) of one of the three adulterated pollens ($n = 30$ bees per treatment, represented across three colonies). When the bee contacted the anther, we immediately placed two test flowers (blue and yellow) approximately 10 cm from the target flower. This meant that the bee was presented with one flower of the same 'familiar' colour as the flower she had just collected pollen from, and one novel colour (figure 1a). Test flowers were empty except for pollen-scented anthers [16,17], requiring bees to decide between flowers based only on their previous experience with pollen. We filmed the bee's interaction with the flowers from above (Sony camcorder, 30 fps) for 5 min before euthanizing the forager.

From the recorded videos, we determined (i) the number and duration of anther visits and (ii) the colour (electronic supplementary material, figure S3) of the first test flower chosen. We defined a visit as antennal or leg contact with the anther, including 'scrabbling' pollen collection [16]. We also weighed dried pollen loads (48 h at 40°C).

(c) Experiment 2: temperature response to pollen treatments

To determine whether bees' maximum temperatures while foraging differed across pollen blends, we filmed a visit of an individual bee to an artificial anther (electronic supplementary material) bearing approximately 50 mg of one of the three pollen types (15 bees per treatment, represented across three colonies), using a FLIR T420 thermal imaging camera (FLIR systems, Inc., USA) 30 cm from the flower, which gives the maximum temperature of the object being filmed (see electronic supplementary material, video S1).
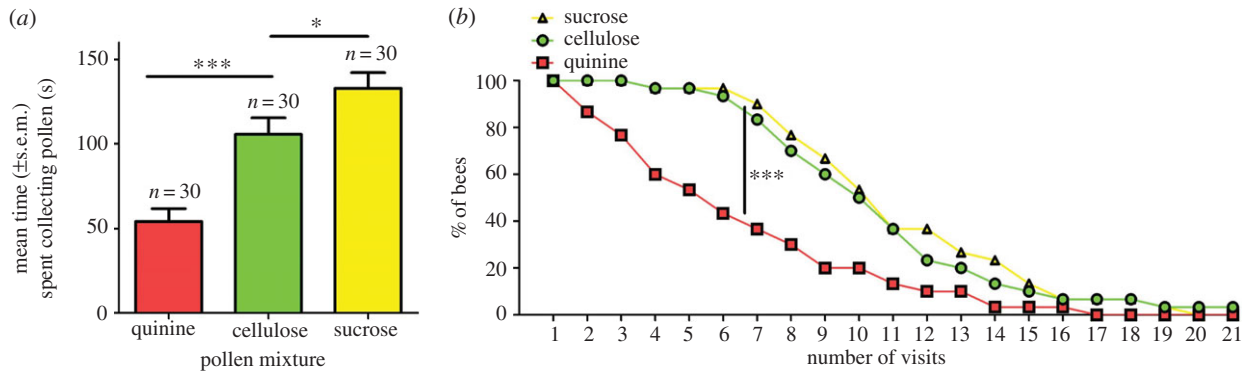
Figure 2. (a) The average amount of time bees spent collecting pollen over the 5 min test period: bees spent the most time collecting sucrose-laden pollen and the least time collecting quinine-laden pollen (all three groups significantly differ to each other). (b) The percentage of bees that visited flowers with a particular frequency across the three treatments. Bees presented with quinine-laden pollen made the fewest visits to flowers, whereas bees visiting sucrose- and cellulose-laden pollen did not differ from each other. Statistical significance denoted: $*p < 0.05$, $**p < 0.01$, $***p < 0.005$. (Online version in colour.)

3. Results

In experiment 1, the chemical composition of the pollen affected how long a bee spent collecting it (model 1: $F_{2,84} = 22.670$; $p < 0.0001$; figure 2a). Over the course of the 5 min trial, bees spent the most time collecting sucrose-laden pollen, and the least time collecting quinine-laden pollen (Tukey *post hoc* tests show all treatments significantly differ at $p < 0.05$). This difference in the overall duration collecting pollen was not explained by differences in individual visit durations: treatments did not differ from each other in either the duration of the first visit (model 2: $F_{2,84} = 1.393$; $p = 0.254$) or the mean duration across their first 10 visits (model 3: $F_{2,86} = 0.001$; $p = 0.100$). In experiment 2, bees also did not show thoracic temperature differences across treatments (model 4: $F_{2,30} = 0.412$; $p = 0.666$).

Instead, bees in the sucrose- and cellulose-laden pollen treatments (in experiment 1) made significantly more visits to target flowers than did bees in the quinine treatment (model 5: AICs with and without 'treatment' included: 507.75, 550.69; $\chi_4^2 = 50.935$; $p < 0.0001$; figure 2b). This resulted in bees that collected quinine-pollen having smaller loads than the other two treatments (model 6: $F_{2,61} = 30.203$; $p < 0.0001$, differences confirmed with Tukey *post hoc* test; electronic supplementary material, figure S4).

In experiment 1, pollen composition also influenced bees' probability of making a colour-constant transfer after leaving the target flower: bees that collected quinine-laced pollen were more likely to switch from the target flower to the novel test flower colour than bees in the other two treatments (χ^2 test: $\chi_2^2 = 11.88$; $p < 0.005$; figure 1b).

4. Discussion

Pollen is a critical resource for bees, providing protein and lipids that fuel development and survival. As interest grows in understanding how bees respond to human-driven perturbations to their nutritional resources [18], several basic questions regarding bees' pollen foraging routines remain open. We found that bumblebees taste the pollen they collect, spending more time collecting sugar- and less time collecting quinine-adulterated pollen, differences not explained by granule size (electronic supplementary materials, Results) or scent (the three diluents were odourless). Therefore, just as

for nectar [19], plants may use gustatory features of pollen to manipulate pollinator behaviour.

The timing of apparent pollen discrimination indicates that bees may not assess pollen upon initial collection as when collecting nectar, but instead after grooming in flight. On their first floral visit bees across treatments did not differ in either the time they spent collecting pollen or in their thoracic temperatures (previously reported as an indicator of quality assessment [15]). Indeed, because gustatory sensilla on the tarsi and antennae are not known to respond to bitter substances (at least in honeybees; [20,21]), bumblebees in the quinine treatment may not have tasted the pollen until they regurgitated nectar using their probosces (electronic supplementary material, video S1).

These findings thus raise new questions about the timing of reinforcement involved in pollen-based learning of floral features [16]. Interestingly, even after a bee tasted the quinine-laced pollen, she often did not cease collection immediately as found with equivalent concentrations of this chemical in nectar [22]. This raises the possibility that bees may be more tolerant of secondary compounds in pollen than in nectar. This tolerance would be in line with the prediction that plants are more likely to add secondary compounds to pollen than nectar, because pollen is directly tied to plant fitness [23].

From the plant perspective, our results show that taste shapes the trade-off between two determinants of plant fitness: pollen removal versus transfer [24]. First, we found that pollen chemistry mediated how much time bees spend collecting pollen and how much they collected into corbicular loads. While quinine has not been reported as a component of pollen, it is commonly used as an aversive compound in nectar-based behavioural assays [22] and alkaloids are present in the pollen of many bee-pollinated plants [25–27]. Sugars, likewise, are components of pollen [8], although how they might influence pollinator behaviour has not previously been assessed. Here, we found that bees spent more time collecting sugar-rich pollen. While bees collected the same amount of sugar- and cellulose-laced pollen overall (as measured by corbicular load), this was likely due to bees collecting all of the pollen available during the test period in those treatments. We also found that bees exposed to quinine-laced pollen were more likely to switch to a novel colour, i.e. make a colour-inconstant transition. Floral constancy is a key determinant of plant reproductive success, as heterospecific pollen transfer represents lost male fitness and can interfere with fertilization.

Taken as a whole, these findings suggest plants may balance offering pollen that is distasteful enough to limit excessive removal [3] but not so distasteful that the bee switches to a new plant species. Determining how bees' behavioural response depends on the dose of aversive compounds, as well as in combination with associated floral nectar is an obvious avenue for future study.

References

1. Adler L. 2000 The ecological significance of toxic nectar. *Oikos* **91**, 409–420. (doi:10.1034/j.1600-0706.2000.910301.x)

2. Richardson LL, Adler LS, Leonard AS, Andicoechea J, Regan KH, Anthony WE, Manson JS, Irwin RE. 2015 Secondary metabolites in floral nectar reduce parasite infections in bumblebees. *Proc. R. Soc. B* **282**, 20142471. (doi:10.1098/rspb.2014.2471)

3. Hargreaves A, Harder L, Johnson S. 2009 Consumptive emasculation: the ecological and evolutionary consequences of pollen theft. *Biol. Rev.* **84**, 259–276. (doi:10.1111/j.1469-185X.2008.00074.x)

4. Buchmann SL. 1983 Buzz pollination in angiosperms. In *Handbook of experimental pollination biology* (eds CE Jones, RJ Little), pp. 73–113. New York, NY: Van Nostrand Reinhold Company.

5. Dobson HEM, Bergström G. 2000 The ecology and evolution of pollen odors. *Plant Syst. Evol.* **222**, 63–87. (doi:10.1007/BF00984096)

6. Lunau K. 2000 The ecology and evolution of visual pollen signals. *Plant Syst. Evol.* **222**, 89–111. (doi:10.1007/BF00984097)

7. Roulston T, Cane J, Buchmann S. 2000 What governs protein content of pollen: pollinator preferences, pollen–pistil interactions, or phylogeny? *Ecol. Monogr.* **70**, 617–643. (doi:10.1890/0012-9615(2000)070[0617:WGPCOP]2.0.CO;2)

8. Pacini E, Hesse M. 2005 Pollenkitt — its composition, forms and functions. *Flora - Morphol. Distrib. Funct. Ecol. Plants* **200**, 399–415. (doi:10.1016/j.flora.2005.02.006)

9. Ruedenauer FA, Spaethe J, Leonhardt SD. 2015 How to know which food is good for you: bumblebees use taste to discriminate between different concentrations of food differing in nutrient content. *J. Exp. Biol.* **218**, 2233–2240. (doi:10.1242/jeb.118554)

10. Leonhardt SD, Blüthgen N. 2012 The same, but different: pollen foraging in honeybee and bumblebee colonies. *Apidologie* **43**, 449–464. (doi:10.1007/s13592-011-0112-y)

11. Konzmann S, Lunau K. 2014 Divergent rules for pollen and nectar foraging bumblebees—a laboratory study with artificial flowers offering diluted nectar substitute and pollen surrogate. *PLoS ONE* **9**, e91900. (doi:10.1371/journal.pone.0091900)

12. Ruedenauer FA, Spaethe J, Leonhardt SD. 2016 Hungry for quality—individual bumblebees forage flexibly to collect high-quality pollen. *Behav. Ecol. Sociobiol.* (doi:10.1007/s00265-016-2129-8)

13. Lunau K, Piorek V, Krohn O, Pacini E. 2015 Just spines—mechanical defense of malvaceous pollen against collection by corbiculate bees. *Apidologie* **46**, 144–149. (doi:10.1007/s13592-014-0310-5)

14. Pernal S, Currie R. 2002 Discrimination and preferences for pollen-based cues by foraging honeybees, *Apis mellifera* L. *Anim. Behav.* **63**, 369–390. (doi:10.1006/anbe.2001.1904)

15. Mapalad KS, Leu D, Nieh JC. 2008 Bumble bees heat up for high quality pollen. *J. Exp. Biol.* **211**, 2239–2242. (doi:10.1242/jeb.016642)

16. Muth F, Papaj DR, Leonard AS. 2016 Bees remember flowers for more than one reason: pollen mediates associative learning. *Anim. Behav.* **111**, 93–100. (doi:10.1016/j.anbehav.2015.09.029)

17. Muth F, Papaj DR, Leonard AS. 2015 Colour learning when foraging for nectar and pollen: bees learn two colours at once. *Biol. Lett.* **11**, 20150628. (doi:10.1098/rsbl.2015.0628)

18. Bommarco R, Biesmeijer JC, Meyer B, Potts SG, Pöyry J, Roberts SPM, Steffan-Dewenter I, Ockinger E. 2010 Dispersal capacity and diet breadth modify the response of wild bees to habitat loss. *Proc. R. Soc. B* **277**, 2075–2082. (doi:10.1098/rspb.2009.2221)

19. Nicolson SW, Thornburg R. 2007 Nectar chemistry. In *Nectaries and nectar* (eds S Nicolson, M Nepi, E Pacini), pp. 215–264. Amsterdam, The Netherlands: Springer. (cited 17 December 2014).

20. de Brito Sanchez MG, Giurfa M, de Paula Mota TR, Gauthier M. 2005 Electrophysiological and behavioural characterization of gustatory responses to antennal 'bitter' taste in honeybees. *Eur. J. Neurosci.* **22**, 3161–3170. (doi:10.1111/j.1460-9568.2005.04516.x)

21. de Brito Sanchez MG. 2007 Taste perception in honeybees: just a taste of honey? *Arthropod. Plant. Interact.* **1**, 69–76. (doi:10.1093/chemse/bjr040)

22. Chittka L, Dyer AG, Bock F, Dornhaus A. 2003 Psychophysics: bees trade off foraging speed for accuracy. *Nature* **424**, 388. (doi:10.1038/424388a)

23. Cook D, Manson JS, Gardner DR, Welch KD, Irwin RE. 2013 Norditerpene alkaloid concentrations in tissues and floral rewards of larkspurs and impacts on pollinators. *Biochem. Syst. Ecol.* **48**, 123–131. (doi:10.1016/j.bse.2012.11.015)

24. Thomson JD. 1986 Pollen transport and deposition by bumble bees in *Erythronium*: influences of floral nectar and bee grooming. *J. Ecol.* **74**, 329. (doi:10.2307/2260258)

25. Kempf M, Heil S, Haßlauer I, Schmidt L, von der Ohe K, Theuring C, Reinhard A, Schreier P, Beuerle T. 2010 Pyrrolizidine alkaloids in pollen and pollen products. *Mol. Nutr. Food Res.* **54**, 292–300. (doi:10.1002/mnfr.200900289)

26. Praz CJ, Müller A, Dorn S. 2008 Specialized bees fail to develop on non-host pollen: do plants chemically protect their pollen? *Ecology* **89**, 795–804. (doi:10.1890/07-0751.1)

27. Roulston T, Cane J. 2000 Pollen nutritional content and digestibility for animals. *Plant Syst. Evol.* **222**, 187–209. (doi:10.1007/BF00984102)