

1 **Assessment of pollen rewards by foraging bees**

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8 **Summary**

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- 10 1. The removal of pollen by flower-visiting insects is costly to plants, not only in
11 terms of production, but also via lost reproductive potential. Modern
12 angiosperms have evolved various reward strategies to limit these costs, yet
13 many plant species still offer pollen as a sole or major reward for pollinating
14 insects.
- 15 2. The benefits plants gain by offering pollen as a reward for pollinating are
16 defined by the behaviour of their pollinators, some of which feed on the pollen
17 at the flower, while others collect pollen to provision offspring.
- 18 3. We explore how pollen impacts on the behaviour and foraging decisions of
19 pollen-collecting bees, drawing comparisons with what is known for nectar
20 rewards. This question is of particular interest since foraging bees typically do
21 not ingest pollen during collection, meaning the sensory pathways involved in
22 evaluating this resource are not immediately obvious.
- 23 4. Previous research focussed on whether foraging bees can determine the quality
24 of pollen sources offered by different plant species, and attempted to infer the
25 mechanisms underpinning such evaluations, mainly through observations of
26 collection preferences in the field
- 27 5. More recent experimental research has started to focus on if pollen itself can
28 mediate the detection of, and learning about, pollen sources and associated
29 floral cues.
- 30 6. We review advancements in the understanding of how bees forage for pollen
31 and respond to variation in pollen quality, and discuss future directions for
32 studying how this ancestral floral food reward shapes the behaviour of
33 pollinating insects.

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40 **Introduction**

41

42 Insect pollination is considered the oldest form of pollen transfer (Labandeira &
43 Currano 2013), and the vast majority of modern angiosperms benefit from visitation
44 by insects (Ollerton, Winfree & Tarrant 2011), investing heavily in attractive floral
45 displays and rewards for pollinators. Despite a widespread switch during angiosperm
46 evolution from rewarding with pollen to the provision of nectar for insect visitors,
47 pollen nevertheless remains an important food resource for consumption and
48 collection by flower-visiting insects. While insects wish to maximize the amount of
49 pollen they consume or collect during a flower visit, for plants, pollen removal also
50 comes at a cost, both energetic and in terms of lost reproductive potential
51 (Westerkamp 1997; Hargreaves, Harder & Johnson 2009). Compared to pollen, nectar
52 is considered to be a more convenient pollinator reward for the plant to produce
53 (Simpson & Neff 1983; Heil 2011). From an insects perspective, harvesting nectar
54 requires fewer morphological and behavioural adaptations than pollen collection
55 (Thorp 1979), and is easier to digest (Huber & Mathison 1976). In addition, nectar
56 often contains solutes such as amino acids, meaning pollinators are able to meet a
57 range of nutritional demands with this reward (for reviews see Nicolson 2011; Nepi
58 2014).

59

60 The emergence of nectar-producing organs during the late Cretaceous period, a time
61 characterized by a fast succession of radiation bouts in both plants and the insects that
62 pollinate them (Grimaldi 1999), likely led to the recruitment of novel pollinator
63 clades. However the manner in which pollinator behaviour may have changed in
64 response to this new floral reward is rarely discussed. Most likely behavioural
65 changes exerted new selective pressures that resulted in further co-evolutionary
66 changes in both flowers and insects. One idea that has received little attention is that
67 due to the relative ease with which nutritional quality can be assessed, nectar may be

68 more effective at rewarding learning than pollen, and thus may exert greater control
69 over the behaviour of pollinators. If true, then nectar may also better promote
70 constancy to the flowers visited by insects, enhancing out-crossing potential. In order
71 to compare, we need to know how each reward type affect movement patterns,
72 learning and foraging decisions, and whether this varies between reward types,
73 leading to differential effects on plant-pollinator relationships. The assessment of
74 pollen rewards is yet not fully understood, but with recent advances in research
75 concerning pollen-foraging behaviour, sensory processing and learning in pollinators
76 it is becoming more feasible to evaluate the influence of reward type in shaping plant-
77 pollinator interactions. In this review we will largely focus on bees and include
78 examples and references to work with both social and solitary species that has thus far
79 provided most of the relevant facts and insights.

80

81 For bees and many other flower visitors, pollen is an important source of nutrition for
82 larval development, adult maintenance and sexual maturation. The dietary needs of
83 these insects and their various life stages are diverse, as is the nutritional ‘quality’ of
84 pollen provided by different plant families, species and even individual plants within
85 a population (reviewed by Roulston & Cane 2000). Bee species differ in their ability
86 to digest different pollen types and to cope with the presence of toxins or protective
87 compounds. Pollen type has been shown to dramatically affect both the development
88 and survival of young bees and larvae (e.g. Standifer 1967; Schmidt, Thoenes &
89 Levin 1987; Schmidt *et al.* 1995; Genissel *et al.* 2002; Roulston & Cane 2002; Tasei
90 & Aupinel 2008; Sedivy, Müller & Dorn 2011; Di Pasquale *et al.* 2013), and so it has
91 often been postulated that bees would stand to benefit by being selective in the pollen
92 they choose to collect.

93

94 In the case of nectar foraging it is well established that bees evaluate the nutritional
95 value of this reward instantaneously and over the duration of the foraging trip,
96 accurately assessing the flow rate and sugar content of nectar provided by flowers
97 (Núñez 1970). Pollen is diverse in form and the proportions of key nutrients vary
98 considerably, which is likely to make foraging choices and the assessment of
99 profitability a more complex task. One solution would be to establish foraging
100 selectivity by specialising on pollen of particular plants, and indeed the majority of
101 early bees were oligolectic (Michez *et al.* 2008; Wappler *et al.* 2015). However,

102 among modern bees only a few truly oligolectic species remain, as over evolutionary
103 time increases in the breadth of pollen diets have become more common (Müller
104 1996; Danforth, Conway & Ji 2003). Generalist collection strategies ensure that bees
105 consume a diverse range of nutrients while also diluting plant protection products and
106 toxins (Eckhardt *et al.* 2014). Yet selectivity seems to persist even in highly polylectic
107 species, such as honeybees and bumblebees, which do not collect pollen from all plant
108 species available. Rather, individual foragers concentrate their foraging efforts on a
109 selection of plant species, showing preferences for one pollen type over another (e.g.
110 Schmidt 1982; Müller 1995; Cook *et al.* 2003; Requier *et al.* 2015; Vaudo *et al.* 2016)
111 and a capacity for flower constancy during pollen collection (e.g. Heinrich 1979;
112 Minckley & Roulston 2006). However, whether such preferences are based on
113 individual foragers' assessment of nutritional differences between pollen rewards
114 remains a major outstanding question.

115

116 So far studies attempting to address this issue have yielded mixed results. Many are
117 correlational, relating bees' foraging preferences in the field to the levels of a
118 particular nutrient(s) found in the pollen provided by different plant species
119 (Robertson *et al.* 1999; Hanley *et al.* 2008; Leonhardt & Blüthgen 2012; Somme *et al.*
120 2015). Since pollen is the major source of protein for bees, levels of this
121 macronutrient, and/or the relative abundance of amino acids have frequently been
122 proposed as cues relevant to bees, but results are not consistent, and there appears to
123 be no simple relationship between collection preferences and the nitrogen content of
124 pollen (Levin & Bohart 1955; Schmidt 1982; Schmidt & Johnson 1984; Schmidt
125 1984; van der Moezel *et al.* 1987; Pernal & Currie 2002). For example, when offered
126 a source of protein, in the form of de-fatted soybean flour, diluted to varying degrees
127 with alpha cellulose, a non-nutritional, inert powder, Pernal and Currie (2002)
128 observed no difference in the weight of pollen loads collected by honeybees,
129 suggesting that foragers did not discriminate between pollen samples on the basis of
130 protein content alone. Similarly, Roulston and Cane (2002) reported that sweat bee
131 foragers did not vary how much pollen they provisioned when offered pollen sources
132 enriched in protein content to varying degrees via the addition of soybean meal, even
133 though pollen protein content was shown to affect offspring body size. As such,
134 evidence is lacking for the bees' ability to discriminate between floral pollen on the
135 basis of crude protein content alone, particularly within the range of naturally

136 occurring variation. Further studies have suggested that other macronutrients such as
137 lipids, are equally or more important (Singh, Saini & Jain 1999; Schmidt & Hanna
138 2006; Avni *et al.* 2014; Vaudo *et al.* 2016), or that bees may be guided by the
139 presence of toxins or distasteful compounds (Sedivy, Müller & Dorn 2011).

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141 The lack of consensus among these studies likely arises from the method of
142 investigation. In the first instance, pollen is a complex substance, varying between
143 species and individual plants in a multitude of respects. Though sometimes
144 acknowledged, this is frequently unaccounted for in field studies. However this is
145 perhaps not surprising, given it is impossible to simultaneously control all the
146 dimensions along which pollen varies without the use of artificial pollen surrogates.
147 Furthermore, accurate measurements of the chemical composition of pollen are
148 hampered by methodological limitations arising from the use of fresh plant samples or
149 bee-collected pollen that has been altered through the addition of nectar by foraging
150 corbiculate bees (Roulston & Cane 2000; Campos *et al.* 2008; Nicolson 2011).
151 Finally, such studies often do not consider the sensory experience of an individual
152 forager, as well as their prior experience and other floral cues and environmental
153 factors which may play a role in guiding collection preferences. We argue that in
154 order to determine which component(s) of the pollen reward may be guiding bees'
155 foraging preferences, it is important to consider pollen collection from a behavioural
156 perspective. In this review we examine current evidence regarding what bees can
157 sense during pollen collection, considering which cues are salient and what role
158 learning, prior experience and in the case of social bees, feedback from the nest,
159 might play in determining preferences. We also evaluate to what extent current
160 experimental evidence, and comparisons with nectar foraging behaviour, might
161 explain the factors that guide pollen collection and the formation of associations
162 between floral cues and pollen rewards. We hypothesise that rather than simply
163 detecting and basing foraging decisions on the presence or concentration of particular
164 nutrients, pollen collecting bees are likely to make an overall sensory assessment
165 during foraging, utilising a suite of cues and recalling prior experience.

166 **Do foraging bees taste pollen?**

167 Pollen-collecting bees typically do not ingest pollen at the flower, instead transport it
168 back to the nest for consumption by their offspring, or in the case of social bees, the
169 colony as a whole. Nevertheless, foraging bees may have ample opportunity to sample
170 grains pre-ingestively with their gustatory organs, the mouthparts and antennae, which
171 frequently come into contact with pollen during collection. Bees often probe flowers
172 with the antennae (Ribbands 1949; Lunau 2000) and in some cases, grasp and scrape
173 pollen from the anthers with their mandibles (Thorp 1979). Some species even have
174 specialised hairs on the mouthparts, designed for collecting pollen from flowers with
175 protected anthers. (Parker & Tepedino 1982; Müller 1995). To facilitate adherence of
176 the pollen grains to each other and the pollen baskets, corbiculate bees add
177 regurgitated fluids to the grains, thus potentially providing further opportunities for
178 gustatory sampling through contact between the pollen-covered body and the
179 mouthparts. But what can bees taste?

180 Compared to what is known about both vision and olfaction, the gustatory sense of
181 bees is still poorly understood. Honeybees possess only 10 gustatory receptor genes
182 (Robertson & Wanner 2006; Jung *et al.* 2015); bumblebees have 23 (Sadd *et al.*
183 2015). This is substantially fewer than found in other insects (68 genes in fruit flies,
184 (Liman, Zhang & Montell 2014); 52 genes in mosquitoes (Hill *et al.* 2002)), and has
185 been taken as an indication of bees' limited ability to detect gustatory compounds in
186 their environment. Taste responses are recorded extracellularly at the tip of sensory
187 sensillae and assigned to functional classes of gustatory receptors (GRN). 'Sweet' and
188 'bitter' receptors, genes and pathways (in analogy to the human sense of taste) are
189 well described in *Drosophila*, as well as receptors that respond to salt, water and
190 carbonation (Yarmolinsky, Zuker & Ryba 2009). *Drosophila* is quite insensitive to
191 amino acids and proteins in their food, which occur only at low concentrations in their
192 diet. To date it is the best understood gustatory system amongst insects, and
193 importantly this work shows that taste perception arises from the combined activity of
194 different GRN. GRN sensitive to sugar are found on the antennae, mouthparts and the
195 distal segment (tarsi) of the forelegs in honeybees (Whitehead & Larsen 1976). Some
196 honeybee GRN are sensitive to salts or particular toxins, either when presented alone
197 or in combination with sucrose stimulation (Wright *et al.* 2010; de Brito Sanchez
198 2011; Kessler *et al.* 2015). Honeybees presumably possess an additional receptor type
199 on their mouthparts that mediates responses to either protein (Dethier 1961) or amino

200 acids (Shiraishi & Kuwabara 1970), though this is yet to be tested at the physiological
201 level in bees. In hoverflies (*Eristalis tenax*), a pollinator which consumes pollen at the
202 flower, extracts of pollen diluted in water stimulate the labellar salt receptor cells but
203 not sugar receptors (Wacht, Lunau & Hansen 2000). More studies characterising the
204 response profiles of gustatory receptors and neural pathways in bees and other pollen-
205 collecting insects are certainly needed.

206

207 Behavioural experiments have provided further insights into the gustatory pathways
208 that could be relevant to the assessment of pollen. Bees are sensitive to the presence
209 of amino acids in nectar. When offered the choice, bees preferentially imbibe those
210 containing amino acids over pure sucrose solution, presumably differentiating
211 between the two rewards through pre-ingestive mechanisms (e.g. Inouye & Waller
212 1984; Simcock, Gray & Wright 2014). In restrained bees, when the antennae of
213 unsatiated bees are touched with nectar or artificial sucrose solution, a reflexive
214 extension of the proboscis (PER) is observed, a behaviour characterised as an
215 unconditioned, appetitive response to stimulation with a food reward (Bitterman *et al.*
216 1983). Such a response can be elicited following a single or few repeated pairings
217 with olfactory, visual or tactile stimuli, and is frequently utilised as a paradigm for
218 studying learning with sucrose rewards in harnessed bees (PER conditioning).
219 Reflexive PER responses have also been observed in honeybees stimulated at the
220 antennae with hand-collected almond pollen (Scheiner, Page & Erber 2004) and bee-
221 collected pollen (Grüter, Arenas & Farina 2008; Nicholls & Hempel de Ibarra 2013),
222 supporting the idea that pre-ingestive gustatory pathways are involved in the
223 assessment of pollen rewards. Very few individuals respond with PER to inert alpha-
224 cellulose powder, used to dilute pollen in experiments and as a pollen surrogate,
225 which suggests that bees are able to detect phago-stimulatory compounds in pollen
226 through the antennae. The presence of additional sugars in dry honeybee-collected
227 pollen does not seem to be perceived by honeybees, at least not at the level of the
228 antennae, the most sucrose-sensitive sensory organ. When pollen was delivered to the
229 antennae of honeybees with a small sponge during an attempt to condition the pollen-
230 PER to an odour (Nicholls & Hempel de Ibarra 2013), bees failed to form an
231 association between the odour and reward, responding no differently from a control
232 group that was stimulated with a clean sponge (Fig. 1A). Since bees readily form an
233 association between this odour and sugars presented in solution with water, this

234 suggests that any sugar present in the dry pollen was not detected by bees, as no
235 association was formed.

236

237 More recently, Ruedenauer et al. (2016) trained bumblebees in a different PER
238 conditioning paradigm, in which pollen and a pollen surrogate were paired with a
239 sucrose reward. Pollen and casein were mixed in various concentrations with cellulose
240 and water to form a thick, wet paste that was presented on a small copper plate which
241 bees touched with their antennae. The sucrose reward was delivered to one of the
242 antenna while the other was still in contact with the humid paste. Using chemo-tactile
243 cues, bees learnt to distinguish between pollen and pollen-surrogate stimuli differing
244 in absolute protein concentration, though only when the concentration differences
245 between the two stimuli were sufficiently large. Though it is unclear how these
246 differences might compare to naturally occurring variations in crude protein between
247 pollen species (2-60% protein, Roulston & Cane, 2000), the study offers new methods
248 and insights that are yet another demonstration of the rich sensory capabilities of bees
249 and the multisensory nature of the information extracted from pollen rewards. The
250 numerous controls that were conducted alongside these experiments reflect the
251 difficulties that experimenters face when trying to reliably separate tactile and
252 chemical stimulation (Scheiner, Erber & Page Jr 1999; Giurfa & Malun 2004;
253 Nicholls & Hempel de Ibarra 2013).

254

255 **The importance of olfactory cues**

256

257 Pollen is both fragrant and also often conspicuously coloured, providing additional,
258 potentially highly salient, cues. Indeed it has been suggested that in early
259 angiosperms, prior to the appearance of a well-developed perianth, the androecium
260 itself may have served as the original advertisement for attracting pollinating insects
261 (Faegri & Pijl 1971; Crepet *et al.* 1991). In general, floral odours provide important
262 cues that can guide pollinator foraging decisions (Raguso 2008; Wright & Schiestl
263 2009) and are undoubtedly salient sensory stimuli for pollinators. Bees are renowned
264 for their extraordinary ability to detect, discriminate and learn odours (e.g. Laska *et*
265 *al.* 1999), although they are poor at detecting the odours of amino acids, which as
266 previously discussed, are considered an important nutritional component of the pollen
267 reward (Linander, Hempel de Ibarra & Laska 2012). Most likely insects learn and rely

268 on the overall olfactory signature of pollen-rewarding flowers. For example, bees
269 have been shown to be capable of distinguishing pollen odours from that of the whole
270 flower (von Aufsess 1960; Dobson, Danielson & Wesep 1999; Carr *et al.* 2015),
271 perhaps unsurprising given pollen, particularly the outer pollenkitt layer, emits odour
272 bouquets that differ strikingly in their composition from other floral odours (Dobson
273 & Bergström 2000). Bees in controlled choice experiments have been found to be
274 guided by the presence of previously experienced pollen odours (Hohmann 1970;
275 Pernal & Currie 2002; Konzmann & Lunau 2014; Beekman, Preece & Schaerf 2016),
276 preferring pollen-containing samples that are rich in odour, over odour-poor
277 surrogates, or learning the odour bouquets of different pollen species when rewarded
278 with sucrose (von Aufsess 1960; Cook *et al.* 2005; Ruedenauer, Spaethe & Leonhardt
279 2016).

280

281 In natural settings it is more difficult to measure how pollinators respond to variation
282 in odour concentrations, and to test the significance of pollen odour cues for finding
283 flowers or predicting the amount of pollen available (Galizia *et al.* 2005; Raguso
284 2008; Carr *et al.* 2015), especially when pollen odours are simultaneously presented
285 with other strong sensory cues in the form of floral odour bouquets, colours or
286 patterns. In experimental tests we found that pollen-foraging bumblebees did not
287 utilise a considerable contrast in odour concentration to distinguish between pollen
288 samples, and instead based their choices on differences in visual appearance (Nicholls
289 & Hempel de Ibarra 2014).

290

291 Studies testing olfactory learning where pollen itself serves as the reward can provide
292 further insights. Arenas and Farina (2012) concluded from their experiments with
293 scented pollen feeders that honeybees learn to associate a particular odour with the
294 presence of pollen, although it cannot be fully ruled out that their preferences could
295 have been determined by their earlier olfactory experience (Arenas & Farina 2014). It
296 is more suitable to train less experienced foragers and test under more controlled
297 conditions for demonstrating if and what bees learn when pollen alone serves as the
298 reward.

299

300 The PER conditioning paradigm offers the advantage of simply applying pollen to
301 specific sensory organs to condition bees to an unfamiliar odour under highly

302 controlled conditions. As previously mentioned, the PER paradigm has previously
303 proven extremely valuable for examining the sensory and neural pathways underlying
304 sucrose-rewarded learning in bees and other insects (e.g. Hammer & Menzel 1995;
305 Burke & Waddell 2011). Pollen elicits reflexive proboscis extensions when applied to
306 the antennae, as required for the paradigm, however multiple pairings of odour and
307 pollen presentation do not result in a conditioned response to the odour. This suggests
308 bees are not able to form an association between an odour and a pollen reward under
309 these conditions (Nicholls & Hempel de Ibarra 2013). An earlier study by Grüter,
310 Arenas and Farina (2008) prematurely reported that honeybees could learn to
311 associate a reward mixture of pollen and water (70% pollen w:w) with an odour
312 following three PER training trials. However, without indispensable controls it is not
313 possible to conclude that an observed increase in responsiveness to the odour is the
314 result of bees learning a predictive relationship between the odour and pollen reward.
315 It could be potentially caused by other factors, such as an increase in sensitivity due to
316 repeated antennal stimulation or clogging of the antennae with a sticky substance.

317

318 **Pollen-rewarded learning of visual cues**

319

320 While PER conditioning paradigms permit tight control over the delivery of
321 conditioned and contextual odour stimuli and rewards, it can be challenging to select
322 appropriate stimuli and obtain necessary controls, especially when both the
323 conditioned stimulus and the reward provide cues in the same sensory modality.
324 Furthermore bees are restrained in these experiments, which may negatively impact
325 on the learning process. Visual conditioning of freely-behaving bees thus appears to
326 be a more advantageous method for examining the reward properties of pollen in
327 associative learning.

328

329 Bees and most other pollinating insects have excellent visual capabilities, even though
330 their eyes are small and have low spatial resolution (von Frisch 1967; Kevan & Baker
331 1983; Hempel de Ibarra, Vorobyev & Menzel 2014). When pollen is displayed openly
332 by the flower, it often contributes to flower patterns, though as with pollen odour,
333 visual cues cannot be seen in detail from a distance, but are resolved only once a
334 pollinator has arrived at the flower (reviewed by Hempel de Ibarra, Langridge &
335 Vorobyev 2015). If cues are learnt, it is most likely that foragers are guided by

336 sensory cues displayed by the whole flower or by joint displays of inflorescences and
337 across co-located plants.

338

339 In learning experiments we showed that naïve bumblebees (*Bombus terrestris*) not
340 only learn the colour of pollen samples, but are also able to form an association
341 between the pollen reward and a coloured stimulus surrounding it (Nicholls &
342 Hempel de Ibarra 2014). Bees were offered two colours in combination with two
343 pollen samples that differed in pollen concentration (Fig. 1B). After a short training
344 period, bees shifted their initial preference for the coloured stimulus paired with the
345 low concentration of pollen towards the alternative colour associated with the more
346 concentrated pollen mixture. This was demonstrated for different colour pairings,
347 suggesting that the bees' learning abilities with pollen rewards are not limited to
348 particular colours that might frequently occur in petals of pollen-displaying flowers.
349 Muth, Papaj and Leonard (2016) further observed that bumblebees (*Bombus*
350 *impatiens*) are able to form long-lasting associations of up to seven days between
351 pollen and a coloured stimulus, using artificial flowers with both a coloured 'corolla'
352 and an 'anther', a small chenille brush from which pollen was collected (Fig. 1C).
353 Interestingly, when both flower parts indicated the presence of a reward, bees seemed
354 to attend more closely to the colour of the corolla than the colour of the anther. Again,
355 this can be explained by the poor resolution of bee eyes. Given its larger size, the
356 corolla would be more suited to attracting and guiding the approach of bees to the
357 flower than the smaller anthers.

358

359 **Mechano-sensory feedback during pollen collection**

360

361 The lack of learning with pollen rewards observed in restrained bees in the PER
362 paradigm discussed above may indicate that some component intrinsic to the active
363 collection of pollen is necessary for reinforcing behaviour during pollen foraging,
364 most likely through the activation of specific motor patterns and mechano-sensory
365 feedback during pollen collection. Studies of buzz pollination, where bees use
366 vibrational movements to shake pollen from poricidal anthers, show that both
367 bumblebees and carpenter bees adjust their flower handling time according to the
368 amount of pollen released by a flower (Buchmann & Cane 1989; De Luca *et al.* 2013;
369 Burkart, Schlindwein & Lunau 2014), though this is not necessarily true for all

370 flowers with this type of anthers (Nunes-Silva *et al.* 2013). The vibrational
371 movements can be varied both in duration and amplitude, forming part of a mechano-
372 sensory feedback system that might have the capacity to modulate buzzing behaviour
373 in response to signals about the state and type of flower.

374

375 Mechano-sensory feedback is also likely to be involved in learning during non-
376 buzzing pollen collection. It has been suggested that grain size and shape may
377 influence the manner in which grains pack in to the corbiculae (Vaissiere & Vinson
378 1994; Pernal & Currie 2002; Lunau *et al.* 2015), thus bees may select pollen species
379 in order to maximise packing efficiency. Interestingly, grain size correlates with
380 protein content in a number of species (Baker & Baker 1979; Roulston, Cane &
381 Buchmann 2000). Physical cues could therefore serve as reliable indicators of pollen
382 identity, which in turn could influence the selection of pollen species.

383

384 Recently a hitherto unknown sensory capability of bees was discovered, the detection
385 and discrimination of electric fields that stimulate mechano-sensory hairs located on
386 the bee's body (Clarke *et al.* 2013; Sutton *et al.* 2016). Electrostatic forces can aid
387 pollen transfer (Gan-Mor *et al.* 1995; Vaknin *et al.* 2001), and insect visitation, pollen
388 removal and pollination status all alter the electric potential of a flower. Electric fields
389 may be another important, yet understudied, cue utilised by pollen-collecting bees.

390

391 Efficiency of pollen harvesting behaviour in bees, including handling of the flower to
392 access anthers and grooming of pollen from the body surface, which depend both on
393 the pollen deposition mode and the pollen packing behaviour itself, needs to be
394 studied further to understand under which circumstances the evaluation and learning
395 about pollen rewards is based on the handling requirements for different pollen and
396 flower types. When designing behavioural experiments and field observations it thus
397 seems essential to include more measures and controls that account for the possibility
398 that pollen packing might influence bee foraging decisions.

399

400 **Pollen is a multi-modal stimulus**

401

402 Taking into account their diverse sensory capabilities, from a bees' perspective pollen
403 represents a multi-modal stimulus, simultaneously providing foragers with gustatory,

404 olfactory, visual and mechano-sensory cues, all of which could be used to guide their
405 foraging choices. Different pollen species are likely to provide a widely varying array
406 of sensory signals, making it difficult to address the functions and interactions of
407 sensory modalities or to determine which cues are most salient for bees. Salience may
408 vary depending on context, or bees might rely on multi-modal associations; perceptual
409 information, may vary according to relative saliences, experience and spatio-temporal
410 constraints on their foraging movements.

411

412 There are methodological difficulties to be considered when using pollen or pollen
413 surrogates in experiments that aim to isolate the various dimensions of pollen as a
414 multi-modal stimulus. Different substances vary in both their nutritional and physical
415 properties. Fresh, hand-collected pollen of a single plant species seems to most
416 closely resemble the natural state of pollen encountered by bees at the flower, but it is
417 very difficult to obtain in sufficient quantities and to maintain in a fresh state over the
418 duration of behavioural experiments. Usually experimenters revert to commercially-
419 collected pollen that can be purchased either as single- or mixed-species pollen from
420 different geographic locations. While single-species pollen has the advantage of
421 controlling a particular cue, such as grain size, mixed-species pollen offers a diverse
422 range of nutrients and can be useful for masking potential confounding cues, or
423 diluting the presence of toxins and unpalatable compounds. Bee-collected pollen is
424 easier to obtain in large quantities, and so far there is no evidence to suggest that in
425 the dry form, the sucrose added by foragers is sensed by bees. Pesticide load is likely
426 to be lower than in commercially collected single-species pollen, typically harvested
427 from intensively farmed crops such as fruit orchards, given that honeybee colonies
428 placed in agricultural landscapes typically utilise a range of wild flowers in addition
429 to crops. Hand-collected pollen may also contain anthers and other plant material, and
430 it is often not clear whether experimenters take steps to remove such plant tissue prior
431 to testing. All pollen that is not freshly picked from a plant is usually dried to prolong
432 longevity, and in the case of commercially available hand-collected pollen from crop
433 plants, additionally treated to improve effectiveness in crop plant fertilisation.
434 Sometimes pollen is washed by experimenters to remove surface sugars before being
435 presented to bees as either a dried powder or wet paste. There is a risk that washing
436 may place grains under osmotic pressure, bursting them and expelling their content
437 whilst simultaneously removing other important phago-stimulatory compounds

438 present in the pollenkitt. Details of washing procedures should be reported and
439 assessed, as it can change pollen properties quite substantially (e.g. Ruedenauer,
440 Spaethe & Leonhardt 2015; Nicholls, Chow and Hempel de Ibarra, personal
441 observations). Using surrogates, such as alpha-cellulose and casein, can be very
442 advantageous for manipulating particular chemical and tactile cues in isolation, but
443 are limited in their potential to simulate the diversity and variability of pollen cues
444 present in real flowers. The above mentioned are all challenges that need to be
445 considered when studying the sensory mechanisms underlying pollen foraging and
446 reward assessment.

447

448 **The role of experience in pollen evaluation**

449

450 The act of removing pollen from flowers involves motor patterns that are hard-wired
451 (e.g. Russell *et al.* 2016), though some aspects of this behaviour can be fine-tuned
452 with experience (Raine & Chittka 2007; Morgan *et al.* 2016). Furthermore, individual
453 collection preferences have been shown to be affected by prior foraging experience.
454 Cook *et al.* (2003) found that honeybees preferred pollen species containing a higher
455 concentration of essential amino acids only when they had previous experience of
456 foraging on this pollen type. This suggests that bees undertake an experience-based
457 assessment of pollen quality. Supportive evidences comes from experiments where
458 distinctive responses were recorded in bumblebees offered a choice between pollen
459 mixes diluted to varying degrees with cellulose (Nicholls & Hempel de Ibarra 2014).
460 Some individuals had a preference for the more familiar pollen type, even if it had a
461 lower protein concentration. Preferences changed over time or even disappeared, with
462 bees accepting variable pollen rewards (Konzmann & Lunau 2014; Nicholls &
463 Hempel de Ibarra 2014). It remains open which sensory cues may be involved in this
464 familiarity effect.

465

466 In honeybees, interpretation of the waggle dance offers a unique opportunity to gain
467 insight into individual foraging preferences. When foragers aim to recruit nest mates
468 to a profitable food source, they decide whether to dance and, in the case of nectar
469 sources, how vigorously to perform their dance (Lindauer 1948; von Frisch 1967;
470 Seeley, Mikheyev & Pagano 2000). When pollen stores are low, pollen foragers have
471 been observed to dance not only for flower pollen but also for a range of pollen

472 surrogates such as dry milk, potato powder, wheat or soy flour and for pollen from
473 wind-pollinated plants (e.g. hazel) (Lindauer 1948). Lindauer (1948) also offered
474 potato flower mixed with bitter-tasting wormwood (*Artemisia absinthium*) pollen
475 which contrary to his expectation did not diminish, but increased the dancing activity
476 of bees, with some bees switching to preferentially collect it. Waddington, Nelson and
477 Page (1998) observed that honeybees were less likely to perform a dance to alert their
478 hive-mates to the location of a pollen source diluted with alpha-cellulose, presumably
479 because it was perceived as inferior. However such a conclusion has been disputed by
480 a more recent study. Beekman et al. (2015) found that long-term exposure to a
481 particular pollen type at known locations affects how bees respond to changes of
482 pollen qualities. More research is needed to clearly establish whether individual
483 assessment of pollen resources affects reward evaluation and the propensity to dance.

484

485 **Comparing sucrose- and pollen-rewarded learning**

486

487 Comparisons of nectar and pollen foragers, their behavioural adjustments and
488 similarities or differences of learning processes during nectar and pollen collection
489 can provide interesting insights to understand the assessment of pollen rewards by
490 bees. Recent work has centred on the question of how pollen-rewarded sensory
491 assessment and learning of floral features compare to the associations that are
492 acquired during nectar collection. Studies with sucrose-rewarded bees have found that
493 learning is impaired when individuals are prevented from imbibing the reward
494 (Sandoz, Hammer & Menzel 2002; Wright *et al.* 2007), so it is reasonable to expect
495 that in pollen-collecting insects, pollen may be a less effective behavioural reinforcer
496 than nectar. Another major difference between the two types of learning is the
497 handling time required to collect the reward. The location and extraction of nectar
498 generally takes less time and provides direct pre- and post-ingestive feedback for
499 bees, which might enhance learning and re-learning, speed up decision-making and
500 strengthen flower constancy. On the other hand, longer pollen handling times could
501 influence the perception of reward quality. Once learned, bees might be slower in
502 extinguishing memories, less prone to fully switch to new flower types and therefore
503 possibly show lower levels of flower constancy. Such questions remain unanswered,
504 and only very recently have attempts been made to compare the two types of learning.

505

506 Nicholls, Ehrendreich and Hempel de Ibarra (2015) compared learning and memory
507 recall of naïve pollen and nectar-foraging honeybees trained under similar conditions
508 in the laboratory (pollen odours were present in each condition inside the reward box,
509 Fig. 1D). In simple colour association tasks, pollen and nectar-rewarded bees
510 performed equally well. When bees were required to repeatedly re-learn which colour
511 (Blue or Yellow) was paired with the reward, pollen-rewarded bees initially exhibited
512 longer search times to find the reward following a switch in rewarding colour.
513 Evidence for a difference in the strength of memories formed for the two colours
514 between sucrose- and pollen-rewarded bees comes from their differing responses in a
515 memory test performed one hour after training. Whilst pollen-rewarded bees exhibited
516 an equal preference for both learnt colours, nectar-rewarded bees preferred the colour
517 that was reinforced first, presumably because this association was consolidated
518 rapidly and formed a more robust memory, which could have interfered with the
519 recall of subsequently learnt colour pairings. This is first evidence to suggest that
520 differences might exist in the mechanisms underlying pollen and sucrose-rewarded
521 learning, an idea that needs to be investigated further.

522

523 Muth, Papaj and Leonard (2015) examined how bumblebees' behaviour might be
524 modulated when foraging for both reward types simultaneously. Nectar and pollen
525 were provided in artificial flowers, the colour of which signalled the type of reward
526 provided. Interestingly, while half of the bees tested chose to forage for both types of
527 reward in the same foraging bout and readily learnt both colour associations
528 simultaneously, the rest preferred to collect only one reward both within and across
529 multiple foraging trips. Here also bees learnt each colour-reward association easily,
530 once more supporting the notion that pollen-rewarded learning is fast and establishes
531 robust colour memories (Nicholls & Hempel de Ibarra 2014; Muth, Papaj & Leonard
532 2016). These fast associations are likely to form the basis for individual pollen
533 constancy within and between foraging trips of bees.

534

535 One difficulty that arises when comparing learning in pollen- and nectar-rewarded
536 bees is in controlling the visual and olfactory cues provided by the different types of
537 reward. This can be somewhat overcome by scenting feeders (Arenas & Farina 2012)
538 or constraining bees to collect pollen in the dark (Nicholls, Ehrendreich & Hempel de
539 Ibarra 2015). The visual appearance of pollen may impact on initial colour

540 preferences and/or the acquisition and recall of colour-reward associations (Nicholls
541 & Hempel de Ibarra 2014; Muth, Papaj & Leonard 2015). Furthermore, it is important
542 to establish whether foragers specialising on nectar or pollen may inherently differ in
543 their cognitive abilities.

544

545 It has been proposed that pollen-foraging honeybees form better sucrose-rewarded
546 olfactory associations (Scheiner, Page & Erber 2004) as a result of variation in
547 sucrose sensitivity between forager types leading to differences in individual
548 perception of reward quality (Scheiner *et al.* 2005). It may at first appear paradoxical
549 that bees which forage for pollen are more sensitive to sucrose than those which
550 collect nectar, but Page *et al.* (2006) argue that such specialisation could be adaptive
551 for the colony, since nectar foragers would collect only from flowers producing
552 highly concentrated nectar, thus returning to the hive the best quality resource
553 currently available. Scheiner, Page and Erber (2004) also suggest that sucrose
554 responsiveness is unlikely to be directly responsible for the differences in pollen and
555 nectar forager behaviour, rather that variation in sucrose response thresholds may
556 represent general differences in sensory processing. This view is supported by the fact
557 that sucrose sensitivity is also known to correlate with sensitivity to other modalities
558 such as pollen (Scheiner, Page & Erber 2004) and light (Tsuruda & Page 2009;
559 Scheiner *et al.* 2014). Differences in sensitivity to external stimuli have been
560 demonstrated to have an impact on differences in learning between forager types.
561 Scheiner, Erber and Page Jr (1999) found that pollen foragers learned a tactile PER
562 conditioning task more rapidly, reached a higher asymptote and greater resistance to
563 extinction than nectar foragers. An analogous result was found for olfactory PER
564 conditioning (Scheiner, Barnert & Erber 2003), though differences in the learning
565 performance of foragers reinforced with their respective rewards, has yet to be tested.

566

567 **Social cues and colony feedback**

568

569 While in this review we have advocated a focus on the individual sensory experience
570 of a pollen-collecting bee, the role that social cues may play in guiding pollen
571 foraging behaviour should not be overlooked, especially considering the majority of
572 studies reported here have used social bees as their subjects. For honeybees and
573 bumblebees, levels and quality of collective pollen storage as well as feedback from

574 nest mates may also be important, adding an additional layer of complexity to the
575 process of determining the relative importance of various factors guiding the
576 evaluation of pollen rewards by foraging bees. Pernal and Currie (2001) observed that
577 honeybees altered foraging effort in response to fluctuations in the protein content of
578 stored pollen. There was no difference in the quality or breadth of species collected
579 under either manipulation, which suggests that individual honeybee foragers lack the
580 ability, or at least do not solely attend to the protein content of pollen whilst
581 collecting, and rely on the feedback about colony demand from the nurse bees which
582 unload their pollen sacs. Indeed, young honeybees at the age when they typically
583 engage in nursing, change their feeding behaviour to compensate for protein
584 deprivation (Paoli *et al.* 2014). This mechanism could potentially contribute to the
585 regulation of in-hive interactions between hive bees and pollen foragers.

586

587 Bumblebees are also able to adjust colony collection rates over time to compensate
588 for changes in colony stores and responding to variation in pollen concentrations at
589 artificial feeders (Kitaoka & Nieh 2009). Since bumblebees unload their own pollen
590 baskets, and individually assess brood levels and stored pollen (Dornhaus & Chittka
591 2005), one might predict that it would be more efficient for bumblebees to possess the
592 ability to individually assess some aspect of pollen quality directly at the flower,
593 supplementing information gained inside the colony.

594

595 **Concluding remarks**

596

597 Multiple floral cues have the potential to influence bees' pollen collection behaviour
598 and perception of pollen rewards, in addition to their own experiences and in the case
599 of social bees, the feedback they receive directly or indirectly from their nest mates.
600 Since most foragers can combine pollen and nectar foraging, either on the same trip or
601 throughout their life (Robinson 1992; Weislo & Cane 1996; Hagbery & Nieh 2012;
602 Konzmann & Lunau 2014), this may add to their experience base and further
603 influence navigational and foraging decisions during pollen collection. Nonetheless,
604 what is clearly established is that pollen-foraging bees individually prefer some
605 flowers over others and have the ability to detect differences between pollen(-like)
606 samples of different chemical, colour and/or mechano-sensory qualities.

607

608 Pollen is a complex and diverse food substance and floral reward. Although much
609 effort has been made, we are still lacking answers to fundamental questions necessary
610 to fully understand the adaptive value of floral pollen rewards, regarding their
611 differences in nutritional quality, within the limits set by requirements for plant
612 fertilisation (Roulston, Cane & Buchmann 2000), and sensory cues that mediate
613 pollen foraging and pollen reward evaluation in pollinating insects. How pollen
614 rewards may influence the foraging decisions of pollen-collecting insect pollinators is
615 thus far best studied in bees, since many aspects of their behaviour, the neural
616 pathways and mechanisms are well understood with regards to nectar rewards.

617

618 Still little is known about how nutrients in food rewards, other than sucrose, are
619 encoded and processed pre- and post-ingestively by bees, how and which molecular
620 pathways are shared or diverge, which brain regions are involved in turning reward
621 value into foraging decisions. Preliminary investigations suggest that, as observed in
622 mammals, encoding of reward type in the bee brain may involve a subset of the
623 molecular pathways implicated in a generalized food-based response, though
624 particular brain regions and populations of nerve cells were observed to be uniquely
625 responsive to differences in food type (McNeill *et al.* 2015). Transcriptional changes
626 in the mushroom bodies, the main centres of sensory integration in the insect brain,
627 vitally important for learning and cognitive processes, appear to play an important
628 role in encoding differences in both reward type and value.

629

630 Pollinators will accept a range of pollen rewards of varying nutritional value within a
631 bracket of cost-benefit assessment that considers various aspects – floral and pollen
632 cues, handling requirements, availability of pollen sources and individual experience.
633 To understand the reward functions of pollen, it is important to separate these
634 different factors and describe the varied mechanisms that are involved in the
635 perception of pollen rewards. More studies addressing sensory and learning
636 mechanisms in pollen-foraging bees, and comparisons with nectar-foraging modes in
637 the same individuals or with nectar-foraging conspecifics, are needed for continuing
638 the quest of uncovering the mechanistic basis of pollen foraging. Recent advances in
639 research technologies and genome sequencing provide new avenues for gaining
640 interesting insights in the evolution and functions of flower pollen as a reward for
641 pollinators.

642

643 Data Accessibility

644

645 This manuscript does not use data.

646

647 **Figure 1:** Methods for experimental testing of pollen collection and pollen-rewarded
 648 learning in bees. (A) When stimulated with pollen bees spontaneously respond with a
 649 proboscis extension (PER). In the olfactory PER conditioning paradigm, the typical
 650 sucrose reward was substituted with pollen in an attempt to train bees to associate an
 651 unfamiliar odour with pollen reward (Nicholls & Hempel de Ibarra 2013). Small
 652 cosmetic sponges were dusted in dry pollen and frequently replaced during
 653 conditioning. Bees in the control group were trained to the same unfamiliar odour but
 654 ‘rewarded’ with a clean sponge that was attached to a pollen-coated sponge to provide
 655 pollen scent. (B) Bees accept pollen presented in petri dishes, which can be placed on
 656 a coloured background (Nicholls & Hempel de Ibarra 2014). (C) Sophisticated pollen
 657 feeders, where the pollen is dusted onto small chenille brushes (Muth et al. 2016). The
 658 brushes are placed inside of differently shaped artificial flowers or attached to a
 659 coloured base to form anther-like structures (photos courtesy of A. Russel; from
 660 Russell & Papaj 2016). (D) Bees can be trained to collect sucrose or pollen rewards
 661 inside of dark boxes. One colour marked the entry tube that led to the inside of the
 662 reward box (Nicholls, Ehrendreich & Hempel de Ibarra 2015). The entrance marked
 663 by the alternative colour was blocked at the end by a mesh that would still allow
 664 pollen odour to diffuse.

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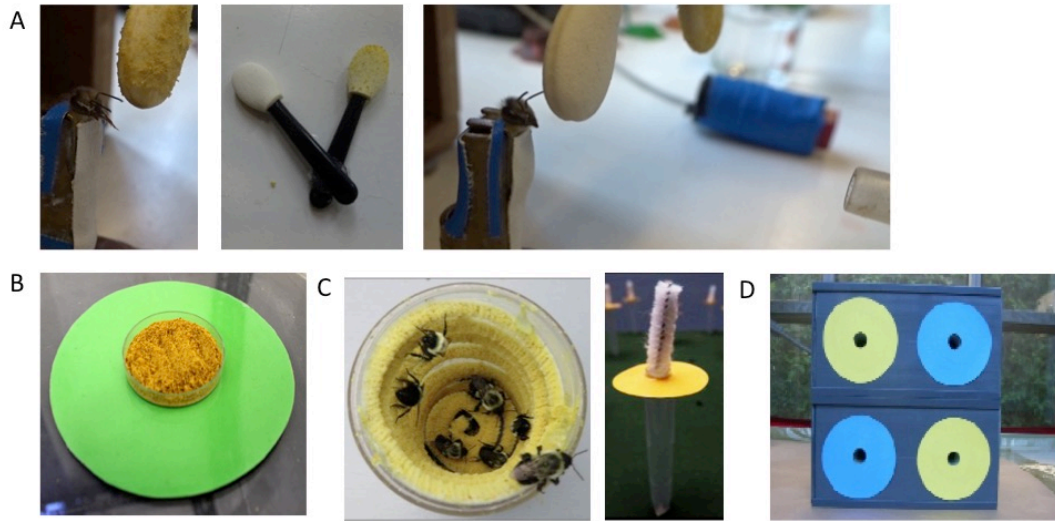
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1034 Figure 1