Concealed floral rewards and the role of experience in floral sonication by bees

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ABSTRACT

Pollinators frequently use complex motor routines to find and extract floral rewards. Studies of pollinators foraging for nectar rewards indicate these routines are typically learned, and that constraints associated with learning and memory give pollinators incentive to continue foraging on these flowers. However, plants offer rewards besides nectar, including pollen, lipids and essential oils. In particular, bees use a complex motor routine termed floral sonication to extract pollen, their primary source of protein, from the more than 6% of flowering plant species (>22,000 species) that conceal pollen rewards within tube-like poricidal anthers. If floral sonication requires learning, this pollen extraction behaviour could contribute to floral fidelity. However, no studies have quantified the effect of experience on flower handling for bees extracting pollen from poricidal species. We therefore examined the degree to which floral sonication behaviour was modified by experience. We found that the key elements of the sonication motor routine appeared in full-blown form in a flower-naïve bee's first visit to a flower. We additionally found consistent, albeit modest, effects of experience on certain aspects of sonication behaviour. The latency to sonicate slightly decreased with experience. Bees also adjusted the length and amplitude of their sonication buzzes in response to pollen receipt. We conclude that the role of experience in foraging for concealed pollen rewards is different from that reported for nectar rewards. We offer an alternative explanation for its function in sonication. Finally, we discuss alternate hypotheses for the function of poricidal anthers and for how pollen-bearing plants may ensure floral fidelity even in the absence of a significant impact of experience on pollen extraction behaviour.

23 KEY WORDS

24 *Key words: learning, mutualism, floral rewards, bumblebee, Bombus impatiens, pollen*

25 *collection, concealed rewards, experience, buzz pollination, floral sonication*

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INTRODUCTION

Pollinators often use complex motor routines to find and extract floral nectar. For instance, bees may have to enter the flower in the correct way or use their head and legs in a coordinated effort to pry open a flower's corolla to get to the nectar (e.g., Lavery & Plowright 1988; Westerkamp 1999). Such nectar extraction motor routines are frequently learned (cf. Chittka et al. 1999). Accordingly, pollinators may require dozens of trips to become proficient at extracting nectar from the flowers of a given plant species (e.g., Gould 1984; Heinrich 1984; Lewis 1993). Because plant species vary in floral morphology, pollinators such as bees must learn novel nectar extraction routines each time they shift to a new plant species (e.g., Lavery 1994; Gegear & Lavery 1995). Cognitive constraints associated with learning, forgetting, and relearning these motor routines are thought to discourage pollinators from switching back and forth among plant species (Lewis 1993; Gegear & Lavery 1995; Chittka et al. 1999). Pollinators exhibiting floral fidelity provide direct benefits to the plant in terms of reduced pollen wastage and foreign pollen interference (Waser 1986; Gegear & Lavery 1995). In short, floral morphology that requires pollinators to use learned motor routines to access rewards is proposed to be an evolved strategy by which plants promote effective pollination services (Plowright & Lavery 1984; Lewis 1993; Chittka et al. 1999).

Although nectar is a common floral reward, it is not the only one. Bees, which are among our most important pollinators, must also collect pollen, their primary source of protein and a particularly common floral reward (Simpson & Neff 1981; cf. Kevan & Baker 1983; Nicolson & van Wyk 2011). At least 6% of angiosperm species offer only pollen as a reward. Most of these species conceal pollen within specialized tube-like poricidal anthers (>22,000 species across >80

families: Buchmann 1983; Buchmann, Jolles & Kriebel unpubl. data). Pollinators of these so-called poricidal flowers, nearly exclusively bees, must extract the pollen using a complex motor routine termed floral sonication (Pellmyr 1988; Buchmann & Cane 1989). Sonicating bees rapidly contract their indirect flight muscles, thereby generating powerful vibrations (King & Lengoc 1993). These vibrations are transmitted through the bee's clasped mandibles to the poricidal anthers, which causes the pollen to be expelled onto the bee's body where it can be collected (Michener 1962; cf. Switzer et al. 2015). The successful extraction of pollen thus involves coordination of legs (for positioning and grooming), mandibles, and indirect flight muscles.

Floral fidelity could be facilitated if extraction of the pollen reward must be learned and if cognitive constraints like those proposed for nectar extraction result in costs to switch between flower types. In contrast to the role of learning in nectar collection, its role in pollen collection has scarcely been examined. Because floral sonication involves the use and coordination of multiple different motor units, similar in respects to the action of complex learned nectar extraction routines, it is reasonable to ask if sonication is also learned. Previous work addressing this question is limited and has yielded mixed results. Two studies suggested that sonication might be innate (King 1993; Morgan et al. 2016), as bees buzz within their first few visits, while a third (Lavery 1980) reported that bees take time initially to sonicate, as well as to sonicate flowers effectively.

To our knowledge, no studies have quantified the effect of experience on flower handling for bees extracting pollen from poricidal species. In this study we characterized the behaviour of

bees as in their first visits to poricidal flowers and quantified the motor movements involved in floral sonication. Additionally, we examined the possible influence of experience and receipt of a pollen reward on floral sonication behaviour.

METHODS

Subjects

We used 76 workers from six colonies of *Bombus impatiens* in experiments conducted between December 2013 and June 2014. We purchased colonies from Koppert Biological Systems (Howell, MI, USA) or from Biobest USA, Inc (MI, USA). We used equal numbers of bees from at least two colonies for each experiment.

Colonies had access to ad libitum unscented 2M sucrose solution and pulverized honeybee-collected pollen (Koppert Biological Systems, MI, USA) within the foraging arena. Two feeders dispensed sucrose solution via braided cotton wicks (6 inch Braided Cotton Rolls, Richmond Dental) that extended into 40 dram vials through perforations made in the human-white lids (BioQuip Products, Inc.). Pollen was presented using 2 custom-made feeders (Fig. 1a; Russell & Papaj 2016) consisting of human-white chenille fibres, glued to the inside walls of 40 dram vials (BioQuip Products, Inc., USA). Neither type of feeder was scented or coloured in addition to the natural scent or colour of the sucrose solution or pollen. Bumble bees did not sonicate while collecting pollen from chenille fibres: bees always scrabbled for the pollen (additionally, of bees naïve to pollen-foraging that were observed on their first few visits to chenille feeders, none

sonicated). To our knowledge, honeybee-collected pollen is not collected from *Solanum* species (honeybees cannot collect the pollen because they cannot sonicate the poricidal anthers; Buchmann 1983) and could not have been harvested from *Solanum tridynamum*, the focal plant species in our study. This plant species is endemic to Mexico, whereas the honeybee-collected pollen we used was harvested within the Midwestern USA.

We used freshly clipped flowers from eight *Solanum tridynamum* plants in experiments. This species offers only pollen rewards via poricidal anthers (nectar is completely absent from this species). To extract the pollen, bees must vibrate the anthers via sonication. Two *S. tridynamum* were purchased locally (Arizona-Sonora Desert Museum, Tucson, Arizona) and six plants were raised from seeds. Plants were fertilized weekly (Miracle Gro, NPK = 15-30-15) and grown under natural light in a greenhouse.

General Experimental Protocol

All testing took place in a foraging arena (LxWxH 82 x 60 x 60 cm) painted grey on floor and sides to provide a neutral background. To identify naïve bees suitable for testing, we allowed 1-4 flower-naïve workers into the arena simultaneously. When a flower-naïve bee landed on a flower in a test array, we removed the others from the arena immediately with vials. We always tested individual bees that had prior experience with *S. tridynamum* (see Experiment 2) in the absence of other bees, to prevent social influences (cf. Grüter & Leadbeater 2014). Specifically, bees (Experiment 2) on their 2nd and 3rd day of testing were tested individually (in the absence of other bees): thus any changes in behaviour across floral visits would be intrinsic to the bees in

their response to the flower, and not the result of having other bees present or removed from the foraging arena. A bee was allowed to make a predetermined maximum number of visits (varying across sub-experiments), after which we turned off the lights above the foraging arena, causing the bee to stop foraging, and collected the bee after five minutes. For Experiment 1 we also ended a trial if a bee did not approach or land on a flower for five minutes. Upon completion of an assay, we froze and stored the bee at -18 C.

In assays, freshly clipped flowers were horizontally displayed (the usual orientation of the flowers on the plants themselves) on custom built water tubes, mounted on a foam block that matched the foraging arena background (Fig. 1). A single flower was made available to a test bee at any given time and each bee received a fresh, unused flower in each trial.

Figure 1.

Figure 1. (a) Chenille stem feeder loaded with honeybee-collected pollen, reproduced from Russell & Papaj 2016. (b) Grey foam block used to mount water tubes, from which flowers were displayed. Each water tube held only a single flower. Flowers were displayed horizontally and

glued into the water tube, ensuring that floral display was uniform across all treatments and experiments.

Behavioural Assays

Video for all tests was captured at 30fps HD with a digital camcorder (Canon VIXIA HF R400) suspended 2cm from the flower (field of view was 5cm centred on the flower). Audio was input to the camcorder at 3 millisecond sampling intervals using an external microphone (33-3013 Lavalier Microphone, RadioShack) suspended 2 cm from the flower. Video was analysed frame-by-frame using Avidemux software (fixounet@free.fr); audio was analysed using Audition 2.0 (Adobe Systems Incorporated).

We recorded two behaviours: landing and sonication (buzzes) (Table 1). ‘Landings’ were categorized as ‘corolla landings’ and ‘anther landings’. Corolla and anther landings were defined as the bee touching the flower's corolla or anther, respectively, with at least three of the first four legs in the same video frame. Either type of landing marked the beginning of a ‘visit’. The end of a visit was defined as the first video frame in which the bee no longer contacted the flower with its legs. After landing, bees that placed their mandibles or the tarsi of their forelegs on the anther were noted as having located the anthers. We identified digitally recorded ‘sonications’ (a total of 4 186 buzzes; mean per bee $\pm$ SE: 380.5 ± 36.7), which only occurred after landing, by their sound which is distinct from flight buzzes and related sounds (A. Russell pers. obs.). The location of buzzes (‘anther’, ‘corolla’ or ‘off-flower’) was recorded in terms of where the bee's mandibles were clamped at the time of the buzz (5.7% and 0.07% were delivered to the corolla

160 and off-flower, respectively). We also recorded the duration of buzzes. We termed an
 161 ‘acceptance’ to be a visit that involved at least one sonication.

162

163 **Table 1. Behaviours recorded**

Behaviour	Description
approach*	Hovering within 3 cm of the flower.
landing	Corolla and anther landings were defined as the bee touching the flower's corolla or anther, respectively, with at least three of the first four legs in the same video frame.
visit end	The first video frame in which the bee no longer contacted the flower with its legs.
sonication (buzzes)	An attempt at extracting pollen, which only occurred after landing: identified by their distinctive sound.
probing for nectar*	Extending and thrusting tongue against any part of the corolla or anther with a distinctive whole body bobbing action.
antennal contact*	Touching floral tissue for any duration with the terminal antennomer of either antenna.
bites*	The start of a bite was defined as the first frame in which the mandibles were observed clamping onto floral tissue. The end of a bite was defined as the first frame in which the mandibles no longer enclosed floral tissue.

*Only used to construct ethograms

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165 Additionally, we extracted the average amplitude of each buzz using a custom built script
166 (written by Callin Switzer in R v.3.2.0, R Development Core Team). To allow the script to
167 identify buzzes, we manually determined start and end timestamps for the script to read. The
168 script then split each buzz into 512 sampling windows and created an average ‘volume value’ for
169 each buzz. Volume value is a proxy for amplitude, based on bitrate provided by digitally
170 recorded audio, which scales linearly, in contrast to the more commonly used ‘decibel,’ which
171 scales logarithmically. We used these volume values in analyses, hereafter referred to as
172 ‘amplitude’.

173
174 We analysed whether buzz length and amplitude were influenced by experience, because buzzes
175 vary in these two attributes and are the key components of the extraction behaviour that
176 determine whether the bee removes pollen (e.g. De Luca et al. 2013). Some studies (but not all,
177 cf.: Burkart et al. 2011 and Nunes-Silva et al. 2013: the latter being the only study on *Bombus*
178 *impatiens*) that have tested for a relationship between body size and buzz length and amplitude
179 have reported a correlation with buzz length (De Luca et al. 2014) or amplitude (De Luca et al.
180 2013). We therefore tested whether body size differed between treatments and whether buzz
181 length and amplitude were significantly correlated with body size. We found that body size did
182 not differ between treatments and buzz length and amplitude did not correlate significantly with
183 body size (Supplementary Materials).

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Behaviours recorded only for ethograms

To construct ethograms, we recorded four additional behaviours: approaches, antennal contact, probing for nectar, and bites (Table 1). An ‘approach’ was defined as the bee hovering within 3 cm of the flower. ‘Antennal contact’ was defined as the bee touching floral tissue for any duration with the terminal antennomer of either antenna. A ‘nectar probe’ was defined as the bee extending and thrusting its tongue against any part of the corolla or anther with a distinctive whole body bobbing action. The start of a ‘bite’ was defined as the first frame in which the mandibles were observed clamping onto the floral tissue. For each bite on floral tissue where the tips of the bee’s mandibles were visible, we measured the duration of the bite. The end of a bite was defined as the first frame in which the mandibles no longer enclosed floral tissue. The only other occasions where we observed opening or closing of the mandibles were when a bee extended its proboscis during grooming. We did not count these as part of a sonication motor sequence.

We constructed two ethograms. In one ethogram we report the elements up to and including the first buzz or until bees left the flower (Fig. 2). We used 48 bees visiting rewarding flowers for this analysis. The data for these visits were from 18 bees in Experiment 1, 12 bees from Experiment 2, and 18 additional bees that were treated identically to the rewarding treatment in Experiment 1 (but not part of Experiment 1 or 2). In the other ethogram we examined the functional elements of the floral sonication motor routine, including antennal contact, the buzz, the bite, and whether these elements were coincident (Fig. 3). For this analysis we examined the earliest anther buzz or bite that each bee performed on its first visit, for which the mandibles and

antennae were clearly visible. We used 48 bees for this analysis, but discarded 4 of these bees that did not buzz on their first visit. We uncovered additional minor variation in the floral sonication motor routine when we examined the first 85 analysable buzzes of 6 of the undiscarded bees (Table S1).

Experiment 1: Role of experience and receipt of pollen

Here we sought to determine whether foraging behaviour changed with experience and with pollen availability. This experiment used 36 bees from four colonies.

To create unrewarding flowers, we applied drops of glue (Elmer's Glue All, Elmer's Products, Inc) to the tip of each poricidal anther with a clean toothpick and allowed the glue to dry for 5 minutes. This action sealed the anther pore, preventing the release of pollen. We verified with a dissecting microscope that anther pores were closed. If bees broke open anthers during an experiment (usually at the ventral base of the locules) and thus released pollen, we discarded all observations post pollen release (we included observations prior to pollen release in analyses).

To control for the effects of glue scent we applied drops of glue to the distal sides of each anther for all rewarding flowers, without blocking the pores. Glue was allowed to dry for 5 minutes before flowers were used in experiments.

One group of naïve bees was presented with a rewarding flower and another group was presented with an unrewarding flower in this experiment. We allowed bees to make up to ten acceptances

on their particular flower, always in a single foraging bout. However, we report and analyse results only from the first six, because most bees did not complete all ten (bees that dropped out earlier showed the same qualitative pattern as bees that dropped out later). We systematically alternated treatments to control for effects of time and day on behaviour.

A follow-up experiment comparing use of a single flower across all visits for a single bee versus using multiple flowers confirmed that using only a single flower did not affect bee behaviour (see Supplementary Materials).

Experiment 2: Long term retention of behavioural changes

Here we sought to determine whether any changes in pollen collection behaviour persisted for days. We allowed each naïve bee two consecutive acceptances of a fresh flower on the first day, three consecutive acceptances of a fresh flower 24 hours later, and three consecutive acceptances of a fresh flower 48 hours after their first floral experience. Consecutive acceptances were always made within the same foraging bout. Five minutes after completing their first two acceptances bees were labelled with individually numbered plastic coloured tags (The Bee Works, Inc., Oro-Medonte, Canada) attached by superglue to the dorsum of the thorax and returned to the colony box. This experiment used 12 bees from two colonies. We discarded two bees that died before completing the full experiment.

252 *Data Analyses*

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254 All data were analysed using R v.3.2.0 (R Development Core Team).

255

256 For Experiment 1, to determine whether there was an effect of experience and treatment on the
 257 buzz latency across all 6 floral visits we applied a learning curve to each bee's data and analyzed
 258 the estimated parameters. We used a Wright's Cumulative Average Model (Martin Not dated).

259 The model takes the form $Y = aX^b$, where Y = the cumulative buzz latency (measured in
 260 seconds) per floral visit, X = the cumulative number of floral visits, a = estimated buzz latency
 261 for the first floral visit, and b = slope of the function in log-log space. To improve fit we
 262 discarded data from bees that completed fewer than 3 visits (a total of 8 bees; 5 from the
 263 'unrewarding' and 3 from the 'rewarding' treatments). The fit of the model was very good: the
 264 mean coefficient of determination was high (rewarding: 0.79 ± 0.09 ; unrewarding: 0.85 ± 0.07),
 265 and as an additional check, we analyzed whether the estimated parameter 'a' differed
 266 significantly from the actual 'a' for each treatment (it did not: see Supplementary Materials).

267

268 To determine whether the effect of experience (i.e. 'a' and 'b') differed between treatments we
 269 used t -tests, if assumptions of normality and equal variance were met (using Shapiro-Wilk and F
 270 tests, respectively, in the mgcv package: Wood 2015). Otherwise, we used Wilcoxon-signed rank
 271 tests using the wilcox.test() function in R. Likewise, to determine whether there was an effect of
 272 experience at all in each treatment, we used one-sample t -tests or Wilcoxon-signed rank tests to
 273 determine whether estimated parameters differed from zero.

274

We also used a Wright's Cumulative Average Model to determine whether there was an effect of experience on the anther discovery latency across floral visits for the rewarding treatment, following the techniques described above. Data from two bees that had completed fewer than 3 visits was discarded. Because the model could only utilize non-zero numbers and the anther discovery latency was frequently zero (i.e. bees landing on the anthers), we added 0.1 seconds to the anther discovery latency of each visit.

In addition, we examined the difference between the first and the second visit for the rewarding treatment for all 18 bees, as the change in buzz latency was greatest between the first two visits. We used Wilcoxon-signed rank tests to compare latency across the first and second floral visit for the rewarding treatment. We ran Wilcoxon-signed rank tests on the variable 'buzz latency' (measured in seconds) with 'first floral visit' or 'second floral visit' as matched samples.

For Experiment 2 we used repeated measures multivariate analysis of variance (MANOVA) to determine whether the buzz latency drop (difference in the buzz latency from the first to the second floral visit) persisted across days (difference in the buzz latency from the first to the second floral visit on each of 3 consecutive days). We ran this multivariate test (Wilks'-Lambda distribution) using the Anova() function in the car package (Fox & Weisberg 2011).

For Experiment 1 we used linear mixed-effects models (LMM) to determine whether there was an effect of treatment and experience (the 'buzz number', not the visit number) on buzz length or buzz amplitude. We performed two LMMs: one for the response variable 'buzz length', one for

the response variable ‘buzz amplitude’. We log transformed these variables to normalize the residuals. LMMs were specified via the lme() function, in the lme4 package (Bates et al. 2015). For these mixed models we specified ‘buzz length’ or ‘buzz amplitude’ as the response variable, ‘treatment’ (‘rewarding’ or ‘unrewarding’) and ‘buzz number’ (the first 100 buzzes for each initially flower-naïve bee) as fixed factors, with the latter also included as a repeated measures factor within the random factor ‘BeeID’. To examine the possible significance of an interaction between ‘buzz number’ and ‘treatment’, results were first examined using type III sums of squares via the Anova() function. Because the interaction was not significant, we report results with type II Wald chi-square (χ^2) tests via the Anova() function. For each analysis, we performed 2 rounds of backward elimination (as described in Fox 2015).

To determine whether there was a trade-off between buzz length and buzz amplitude we tested for an association between paired samples of buzz amplitude and buzz length for each treatment separately, using the cor.test() function in R.

RESULTS

The basic components of the sonication motor routine are strongly stereotyped

The behaviour of flower-naïve bumblebee workers on their first floral visit to *Solanum* flowers consisted of clearly definable components arranged in a predictable sequence (Figs. 2 and 3). First, the bee approached the flower (Fig. 2a,e). During the approach, bees contacted the flower

with their antennae prior to landing 94% of the time (of the 35 bees where the position of the antennae during landing could be confirmed; Fig. 2b), consistent with the behaviour of bees approaching artificial flowers (Lunau 1992; Evangelista et al. 2010). In the vast majority of cases (92% of 48 bees), a previously flower-naïve bee buzzed on its first floral visit (Fig. 2d,e). Antennal contact with floral tissue preceded buzzing 100% (of 48 bees) of the time (Fig. 3a). Of all bees, 13% (of 48 bees) attempted to probe for nectar (despite nectar not being produced by the flowers of this species) before sonicating thereafter.

The major transitions did not change substantially from the first to second floral visit (Fig. 2e versus 2f). Immediately prior to making their second landing on a flower, bees contacted the flower with their antennae 95% of the time (of 43 bees where the position of the antennae during landing could be confirmed). In all but one case (98% of 47 bees), bees buzzed on their second floral visit (Fig. 2f). Of bees that buzzed, 81% (of 47 bees) buzzed the anthers first, and the remaining bees eventually buzzed the anthers as well (Fig. 2f). No bees attempted to probe for nectar before sonicating on their second visit.

We also observed that components of the sonication routine were coordinated in time and space, even in the very first floral visit. For example, for pollen to be extracted, the bee must buzz while biting the anthers. The location of the buzz is important, because only the anthers hold the

pollen. The sequence is important, because buzzing generates powerful vibrations that eject pollen (King & Lengoc 1993), while biting allows bees to anchor themselves and most effectively transmits the buzz vibrations to the anthers (King & Buchmann 2003). Buzzes not coincident with bites did not result in perceptible amounts of pollen being released; bees that buzzed without biting occasionally ejected themselves from flowers due to the force of the vibrations (A. Russell pers. obs.). However, most bees exhibited a fully functional sonication motor routine even in their very first floral visit (Figs. 2 and 3). Of bees that buzzed on their first floral visit, 89% (of 44 bees) buzzed the anthers first (Fig. 2e). After bees bit the anthers, they almost always buzzed (95% of 44 bees; Fig. 3d-i). Most sonications (80% of 44) were bounded by a single bite and nearly all sonication events (98% of 44) were at least coincident with a bite (Fig. 3d-i).

Figure 2.

Figure 2. (a-d) Key phases in the first floral visit up until the first buzz or until the bee left the flower: (a) approaching the flower (b) antennal contact with the flower indicated with red arrow (c) landing indicated with red arrow (d) buzzing anthers. Anthers indicated by the red letter “A”; corolla indicated by the red letter “C”. (e) Ethogram of the first floral visit. (f) Ethogram of the second floral visit. Arrows indicate the transition from one behavioural component to another. The transition frequency is indicated by both the number and thickness of the arrow. We calculated values by dividing the average number of transitions for a particular component by the total number of transitions derived from a behavioural element. Thus, transition frequencies reflect only the transitions from a given component to any other component (i.e. all transitions from a given component add up to one). We report data from the averaged response of the first and second visit sequence for 48 bees (1 bee discarded for the second sequence, as it made only a single visit).

Figure 3.

Figure 3. (a-c) Key phases in the floral sonication motor routine: (a) antennal contact and clamping the mandibles on floral tissue, (b) sonicating; blur caused by vibration of wings during buzz indicated with red arrow, (c) releasing mandibles. (d-i) The six kinds of floral sonication motor routines observed in the earliest analysable sequence for each bee on its first floral visit: (d) bite begins before and ends after buzz, (e) a bite with no buzz, (f) a buzz with no bite, (g) buzz begins before and ends after bite, (h-i) buzz and bite are offset positively or negatively. Brackets indicate bite duration, stylized sonograms indicate buzz duration, and percentages indicate the relative frequency of each sequence. We analysed the earliest anther buzz or bite that each bee performed on its first visit, for which the mandibles and antennae were clearly visible (mean number of buzzes/bites to find an analysable sequence $\pm$ SE: 3.59 ± 0.52 , $N = 44$ bees).

Amplitude and length of buzzes varies with pollen availability and experience

Bees that encountered and buzzed flowers that could not release pollen had significantly shorter and louder buzzes than bees that encountered and buzzed flowers that released pollen (Fig. 4a; LMM for buzz length: treatment effect, $\chi^2 = 7.4627$, $P < 0.007$; Fig. 4b; LMM for buzz amplitude: treatment effect, $\chi^2 = 16.624$, $P < 0.0001$). These differences correspond to a 26.1% difference in mean duration and a 2.97 decibel difference (a 146.8% difference in mean amplitude and a 198.2% difference in power) in sonications for bees buzzing rewarding versus unrewarding flowers.

In addition, for both treatments the length of buzzes was significantly positively correlated with the amplitude of buzzes: e.g. longer buzzes were also louder (Pearson's correlation $r = 0.278$, $t_{598} = 7.0661$, $P < 0.0001$ and $r = 0.240$, $t_{698} = 6.5347$, $P < 0.0001$, for $N = 6$ and 7 bees in rewarding and unrewarding treatments, respectively). These results indicate that the changes in buzz length and amplitude in response to pollen receipt were not the result of a trade-off between these two characteristics.

For both treatments, bees increased the length and amplitude of their buzzes with experience (Fig. 4a; LMM for buzz length: buzz number effect, $\chi^2 = 4.5921$, $P < 0.033$; Fig. 4b; LMM for buzz amplitude: buzz number effect, $\chi^2 = 4.5608$, $P < 0.033$). There was no significant interaction between experience and treatment for either length or amplitude of buzzes (Fig. 4a; LMM for buzz length: buzz number x treatment effect, $\chi^2 = 0.1126$, $P = 0.737$; LMM for buzz amplitude: buzz number x treatment effect, $\chi^2 = 0.276$, $P = 0.600$).

Figure 4.

Figure 4. (a) Mean duration ($\pm$ S.E.) of sonications and (b) mean amplitude ($\pm$ S.E.) of sonications for the first 100 buzzes. $N = 6$ bees for rewarding and $N = 7$ for unrewarding treatments. Although analyses were performed on log transformed data, the figure shows mean and standard errors for the untransformed data.

Latency to sonicate the flower changes with experience

Naïve bees do not sonicate immediately after landing on a rewarding flower. This initial latency is highly variable across bees, but over subsequent visits the latency to sonicate drops significantly (Fig. 5a; *t*-tests; difference from zero of the learning curve's slope [parameter b]: $t_{14} = -6.4125$, $P < 0.0001$; difference from zero of the learning curve's intercept [parameter a]: $t_{14} = 4.754$, $P < 0.0004$). In fact, from the first to second visit the latency to sonicate drops significantly (latency, 1st visit versus 2nd visit, Wilcoxon rank sum test: $W = 652$, $N = 18$ bees, $P < 0.0008$) and stays low.

However, naïve bees do not significantly improve their ability to find the anthers of a rewarding flower over the first 6 visits (*t*-tests; difference from zero of the learning curve's slope [parameter b]: $t_{14} = 0.7516$, $P = 0.464$, parameter b mean $\pm$ SE = 0.13 ± 0.18 ; difference from zero of the learning curve's intercept [parameter a]: $t_{14} = -1.4682$, $P = 0.163$, parameter a mean $\pm$ SE = -0.23 ± 0.16 ; $N = 16$ bees), despite making a greater proportion of landings on the anthers on the second visit (Fig. 2e versus 2f).

In addition, the pattern of the latency drop is independent of pollen receipt: it did not differ significantly between bees accepting flowers that released or did not release pollen (Fig. 5a; *t*-tests; learning curve's slope [parameter b], treatment effect: $t_{21.752} = -0.5202$, $P = 0.608$; learning curve's intercept [parameter a]: $t_{25.675} = 0.4146$, $P = 0.682$).

Finally, the drop in the latency to sonicate does not persist across days: the magnitude of the latency drop from the first to the second floral visit did not differ across 3 successive days (Fig. 5b; MANOVA: $F_{2,8} = 0.0145$, $P = 0.986$).

Figure 5.

Figure 5. The latency to sonicate (a) on the first six floral visits to a rewarding flower and to an unrewarding flower or (b) on rewarding flowers for the first three floral visits and across days. $N = 15$ and 13 bees for the rewarding and unrewarding treatments, respectively in panel (a). $N = 10$ for bees visiting rewarding flowers in panel (b).

DISCUSSION

Floral fidelity by pollinators is thought to be, at least in part, a consequence of cognitive constraints associated with learning and recalling how to extract concealed floral rewards (Lewis 1993; Chittka et al. 1999; Gegear & Lavery 2005). Concealment of floral rewards has even been proposed as an evolved strategy by which plants maintain floral fidelity by pollinators (Lewis 1993). While pollinators such as bees, many flies, and some butterflies collect both pollen and nectar (Kevan & Baker 1983; Nicolson & van Wyk 2011), the cognitive constraints associated with concealed rewards have only been studied in the context of nectar rewards. However, cognitive constraints – and by extension, floral fidelity – as a result of reward concealment should not depend on whether the reward being concealed is pollen or nectar. Surprisingly, our findings suggest that cognitive constraints do depend on reward, with respect to sonication: bees visiting flowers with poricidal anthers displayed sonication behaviour that was fully expressed and highly effective in their very first floral visit. By contrast, nectar-foraging bees typically fail to find concealed nectar rewards altogether on their first few visits (Lavery 1980, 1994; Lavery & Plowright 1988). The effectiveness of floral sonication appeared to vary little over time, whereas the effectiveness of nectar extraction often changes substantially with experience (cf. Lavery 1994).

While sonication behaviour clearly is spontaneously performed, it nevertheless showed a modest degree of plasticity. For instance, bees decreased the length and increased the amplitude of their sonication buzzes in response to pollen receipt. This result itself is not necessarily learning, but might suggest a capacity for pollen receipt to modify characteristics of sonication buzzes in

response to the particular plant species being foraged from. Pollen receipt is known to modify aspects of behaviour other than sonication. For example, we found previously that bees rapidly adopt landing preferences for the particular buzz-pollinated plant species from which they have successfully collected pollen, and these preferences appear to involve learning of anther cues (Russell et al. 2015).

We additionally observed in the present study that bees modified how quickly they sonicated anthers after landing. Nectar-foraging pollinators show a similar pattern, improving the speed with which they can discover floral nectar with experience ('handling time') (Lewis 1986; Woodward & Lavery 1992; Gegear & Lavery 1998). In fact, changes in handling time are thought to be a major cost of learning to forage for nectar on a novel flower type (cf. Lewis 1993).

Does the change in the latency to sonicate reflect learning how to extract pollen from poricidal anthers? If this change in handling time were chiefly a result of learning to associate floral cues with the acquisition of a pollen reward (that is, associative learning; cf. Giurfa 2007), bees that successfully extracted pollen should sonicate sooner in subsequent floral visits, relative to bees that did not extract pollen. However, in our experiments, bees reduced their handling time whether they were assigned to pollen rewarding or unrewarding treatments. The change in latency might still be associative learning, but might also be a form of non-associative learning. Alternatively, it may not be learning at all, but a priming of general motivation for collecting pollen.

Whether or not the drop in the latency to sonicate constitutes learning, we can still ask if the initial delay in extracting pollen, and any associated cognitive constraints in reducing the delay, will affect foraging efficiency enough to promote floral fidelity. We believe it will not for two reasons. First, the initial delay is short, on the order of 3-6 seconds. This is a much smaller time cost than has been reported in nectar extraction handling time studies (cf. Lavery 1994). Second, the delay is reduced very rapidly, after a single visit. In contrast, bees often take dozens of visits to learn to efficiently locate concealed nectar (Woodward & Lavery 1992; Lavery 1994; Gegear & Lavery 1995). Taking these two factors together, the observed pattern of change with experience seems unlikely to affect foraging efficiency and thus unlikely to mediate floral fidelity directly.

Even if cognitive constraints associated with extracting pollen from poricidal species are unlikely to lead to floral fidelity, poricidal anthers might benefit plants in other ways. For instance, concealment of pollen within poricidal anthers may protect pollen from abiotic factors, such as rain, fluctuations in humidity, and ultraviolet radiation (Gottsberger & Silberbauer-Gottsberger 1988; Johnson & McCormick 2001; Edlund et al. 2004; cf. Zhang et al. 2014). Poricidal morphology may also restrict the amount of pollen a forager can remove for its own use (cf. Castellanos et al. 2006; cf. Hargreaves et al. 2009). Poricidal anthers may pave the way to more efficient pollination in still other ways. Only a limited range of pollinators can collect pollen from poricidal anthers, specifically the 58% of bee species that sonicate (Buchmann 1983; S. Cardinale, A. Russell, & S. Buchmann unpubl. data). Such restriction could facilitate the evolution of floral adaptations tailored to those pollinators that lead to enhanced pollination success (Anderson et al. 2009; cf. Newman et al. 2014). For instance, many poricidal species

have evolved stamens divided into ‘feeding’ and ‘pollinating’ functions (i.e. heteranthery), which further reduces pollen wastage (Vallejo-Marín et al. 2010; cf. Li et al. 2015).

An important question remains: in the absence of cognitive constraints on pollen extraction behaviour, how does a pollen-rewarding plant ensure that the bee shows fidelity and thereby transfers pollen to conspecifics? Possibly, cognitive constraints occur in other components of the foraging sequence. For instance, with experience bees can increase the amount of pollen they transport (Raine & Chittka 2007). Furthermore, as mentioned above, bumblebees form strong, durable landing preferences for pollen-only plant species with which they have experience (Russell et al. 2015); bees are likely learning visual and olfactory cues to identify plant species (Muth et al. 2015, 2016; Russell et al. 2015, but see also Arenas & Farina 2012 and Nicholls & de Ibarra 2014, which use nectar-infused pollen). It is conceivable that the need to learn such cues imposes cognitive constraints that promote floral fidelity. Testing this hypothesis would involve assessing costs associated with switching during pollen collection from one plant species to another, as has been done in the context of nectar collection (Lewis 1986; Gegear & Lavery 1995, 1998). If learning of these floral cues drives floral fidelity, then bees should show significant losses in foraging efficiency when switching back and forth between species. Lastly, while floral fidelity is thought to be a common mechanism resulting in the conspecific transport of pollen, further work will be required to investigate whether bees foraging for pollen exhibit floral fidelity in a manner analogous to bees foraging for nectar (Waser 1986; Gegear & Lavery 1995; Chittka et al. 1999).

We close by suggesting that while sonication behaviour appears not to be learned and thus will not itself drive floral fidelity via cognitive constraints, as proposed for nectar collection behaviour, it may still play an important role in maintaining floral fidelity. We propose that the fact that sonication behaviour is immediately expressed in full-blown form will facilitate the learning of cues that facilitate the finding and recognition of plant species with poricidal anthers (>22,000 species, or more than 6% of angiosperm species). Bees will more immediately receive pollen and receipt of pollen will in turn more immediately reinforce responses to floral signals that identify a rewarding plant species. In this way, the congenital expression of sonication behaviour could make that form of learning a more important driver of floral fidelity than if sonication behaviour itself was learned.

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557 REFERENCES

558 Anderson, B., Alexandersson, R., & Johnson, S. D. (2009). Evolution and coexistence of
 559 pollination ecotypes in an African *Gladiolus* (Iridaceae). *Evolution*, 64(4), 960-972.
 560 <http://dx.doi.org/10.1111/j.1558-5646.2009.00880.x>

561
 562 Anderson, B., Ros, P. Wiese, T. J., & Ellis, A. G. (2014). Intraspecific divergence and
 563 convergence of floral tube length in specialized pollination interactions. *Proceedings of*
 564 *the Royal Society B: Biological Sciences*, 281, 20141420, 1-9.
 565 <http://dx.doi.org/10.1098/rspb.2014.1420>

566
 567 Arenas, A., Farina, W.M. (2014). Bias to pollen odors is affected by early exposure and foraging
 568 experience. *Journal of Insect Physiology*, 66, 28-36.

569
 570 Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models
 571 Using lme4. *Journal of Statistical Software*, 67(1), 1-48. doi:10.18637/jss.v067.i01.

572
 573 Bell, G. The evolution of empty flowers. (1986). *Journal of Theoretical Biology*, 118, 253-258.

574
 575 Buchmann, S. L. (1983). Buzz pollination in angiosperms. In C.E. Jones, & R.J. Little (Eds.),
 576 Handbook of experimental pollination biology (pp. 73–113). New York: Van Nostrand
 577 Reinhold.

578

- 579 Buchmann, S. L., & Buchmann, M. D. (1981). Anthecology of *Mouriri myrtilloides*
 580 (Melastomataceae Memcyleae), an Oil Flower in Panama. *Biotropica*, 13(2), 7-24.
 581
- 582 Buchmann, S. L., & Cane, J. H. (1989). Bees assess pollen returns while sonicating *Solanum*
 583 flowers. *Oecologia*, 81, 289-294.
 584
- 585 Burkart, A., Lunau, K., Schlindwein, C. (2011). Comparative bioacoustical studies on flight and
 586 buzzing of neotropical bees. *Journal of Pollination Ecology*, 6(16), 118-124.
 587
- 588 Castellanos, M. C., Wilson, P., Keller, S. J., & Wolfe, A. D., Thomson, J. D. (2006). Anther
 589 evolution: pollen presentation strategies when pollinators differ. *The American*
 590 *Naturalist*, 167, 2, 288-296.
 591
- 592 Chittka, L., Thomson, J. D. & Waser, N. M. (1999). Flower constancy, insect psychology and
 593 plant evolution. *Naturwissenschaften*, 86, 361-377.
 594
- 595 De Luca, P. A., Bussière, L. F., Souto-Vilaros, D., Goulson, D., Mason, A. C., & Vallejo-Marín,
 596 M. (2013). Variability in bumblebee pollination buzzes affects the quantity of pollen
 597 released from flowers. *Oecologia*. <http://dx.doi.org/10.1007/s00442-012-2535-1>
 598
- 599 De Luca, P.A., Cox, D.A., Vallejo-Marín, M. (2014). Comparison of pollination and defensive
 600 buzzes in bumblebees indicates species-specific and context-dependent vibration.
 601 *Naturwissenschaften*. <http://dx.doi.org/10.1007/s00114-014-1161-7>

602

603 Edlund, A. F., Swanson, R., & Preuss, D. (2004). Pollen and stigma structure and function: the
604 role of diversity in pollination. *The Plant Cell*, 16, S84-S97.
605 www.plantcell.org/cgi/doi/10.1105/tpc.015800

606

607 Evangelista, C., Kraft, P., Dacke, M., Reinhard, J., Srinivasan, M.V. (2010). The moment before
608 touchdown: landing manoeuvres of the honeybee *Apis mellifera*. *The Journal of*
609 *Experimental Biology*, 213, 262-270.

610

611 Fox, J. (2015). *Applied regression analysis and generalized linear models*. 3rd ed. London: Sage
612 Publications, Inc.

613

614 Fox, J., & Weisberg, S. (2011). *An {R} Companion to Applied Regression*, Second Edition.
615 Thousand Oaks CA: Sage. URL:
616 <http://socserv.socsci.mcmaster.ca/jfox/Books/Companion>

617

618 Freitas, L., & Sazima, M. (2003). Floral biology and pollination mechanisms in two viola
619 species—from nectar to pollen flowers?. *Annals of Botany*, 91, 311-317.
620 <http://dx.doi.org/10.1093/aob/mcg025>

621

622 Gegear, R. J., & Lavery, T. M. (1995). Effect of flower complexity on relearning flower-
623 handling skills in bumble bees. *Canadian Journal of Zoology*, 73, 2052-2058.

624

- 625 Gegear, R. J., & Lavery, T. M. (1998) How many flower types can bumble bees work at the
626 same time?. *Canadian Journal of Zoology*, 76, 1358-1365.
627
- 628 Gegear, R. J., & Lavery, T. M. (2005). Flower constancy in bumblebees: a test of the trait
629 variability hypothesis. *Animal Behavior*, 69, 939-949.
630 <http://dx.doi.org/10.1016/j.anbehav.2004.06.029>
631
- 632 Giurfa, M. (2007). Behavioral and neural analysis of associative learning in the honeybee: a taste
633 from the magic well. *Journal of Comparative Physiology*, 1-24.
634 <http://dx.doi.org/10.1007/s00359-007-0235-9>
635
- 636 Gottsberger, G., & Silberbauer-Gottsberger, I. (1988). Evolution of flower structures and
637 pollination in neotropical *Cassiinae* (Caesalpiniaceae) species. *Phyton (Austria)*, 28(2),
638 293-320.
639
- 640 Gould, J. L. (1984). Natural History of Honey Bee Learning. In P. Marler & H. S. Terrace (Eds.)
641 *The Biology of Learning* (pp. 149-180). Berlin, Heidelberg, New York, Tokyo: Springer-
642 Verlag.
643
- 644 Grüter, C. & Leadbeater, L. (2014). Insights from insects about adaptive social information use.
645 *Trends in Ecology & Evolution*, 29(3), 177-184.
646 <http://dx.doi.org/10.1016/j.tree.2014.01.004>
647

- 648 Hargreaves, A. L., Harder, L. D., & Johnson, S. D. (2009). Consumptive emasculation: the
649 ecological and evolutionary consequences of pollen theft. *Biological Reviews*, 84, 259-
650 276. <http://dx.doi.org/10.1111/j.1469-185X.2008.00074.x>
651
- 652 Heinrich, B. (1984). Learning in invertebrates. In P. Marler, & H.S. Terrace (Eds.), *The Biology*
653 *of Learning* (pp. 135-148). Berlin: Springer-Verlag.
654
- 655 Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous Inference in General Parametric
656 Models. *Biometrical Journal* 50(3), 346-363.
657
- 658 Johnson, S. A., & McCormick, S. (2001). Pollen germinates precociously in the anthers of raring-
659 to-go, and *Arabidopsis* gametophytic mutant. *Plant Physiology*, 126, 685-695.
660
- 661 Kevan, P. G. (1983). Insects as flower visitors and pollinators. *Annual Review of Entomology*,
662 28, 407-453.
663
- 664 King, M.J., Buchmann, S.L. (2003). Floral sonication by bees: mesosomal vibration by *Bombus*
665 and *Xylocopa*, but not *Apis* (Hymenoptera: Apidae), ejects pollen from poricidal anthers.
666 *Journal of the Kansas Entomological Society*, 76(2), 295–305.
667
- 668 King, M.J. & Lengoc, L. (1993). Vibratory pollen collection dynamics. *Transactions of the*
669 *American Society of Agricultural Engineers*, 36, 135-140.
670

- 671 Lavery, T. M. (1980). The flower-visiting behavior of bumble bees: floral complexity and
672 learning. *Canadian Journal of Zoology*, 58, 1324-1335.
673
- 674 Lavery, T. M. (1994). Bumble bee learning and flower morphology. *Animal Behavior*, 47, 531-
675 545.
676
- 677 Lavery, T. M., & Plowright, R. C. (1988). Flower handling by bumblebees: a comparison of
678 specialists and generalists. *Animal Behavior*, 36, 733-740.
679
- 680 Leonard, A.S., Dornhaus, & A. Papaj, D.R. (2012). Why are floral signals complex? An outline
681 of functional hypotheses. In S. Patiny. (Ed.), *Evolution of Plant-Pollinator Relationships*
682 (pp. 279-300). Cambridge, UK: Cambridge University Press.
683
- 684 Lewis, A.C. (1986). Memory constraints and flower choice in *Pieris rapae*. *Science*, 232, 863-
685 865.
686
- 687 Lewis, A.C. (1993). Learning and the evolution of resources: pollinators and flower morphology.
688 In: D.R. Papaj, & A.C. Lewis (Eds.), *Insect learning: ecological and evolutionary*
689 *perspectives* (pp. 219–242). New York: Chapman & Hall.
690
- 691 Li, J.K., Song, Y-P., Xu, H., Zhang, Y-W., Zhu, J-Y., & Tang, L-L. (2015). High ratio of
692 illegitimate visitation by small bees severely weakens the potential function of

- 693 heteranthery. *Journal of Plant Ecology*, 8(2), 213-223.
694 <http://dx.doi.org/10.1093/jpe/rtv021>
695
- 696 Lunau, K. (1991). Innate recognition of flowers by bumble bees: orientation of antennae to
697 visual stamen signals. *Canadian Journal of Zoology*, 70, 2139-2144.
698
- 699 Martin, J. R. Not dated. What is a learning curve? Management And Accounting Web. [online]
700 URL: <http://maaw.info/LearningCurveSummary.htm> (accessed May 2016)
701
- 702 Michener, C. D. (1962). An interesting method of pollen collection by bees with tubular flowers.
703 *Revista de Biologica Tropical*, 10(2), 167-175.
704
- 705 Muth, F, Papaj, D. R., & Leonard, A. S. (2015). Colour learning when foraging for nectar and
706 pollen: bees learn two colours at once. *Biology Letters*, 11, 20150628.
707 <http://dx.doi.org/10.1098/rsbl.2015.0628>
708
- 709 Muth, F., Papaj, D. R., & Leonard, A. S. (2016). Bees remember flowers for more than one
710 reason: pollen mediates associative learning. *Animal Behavior*, 111, 93-100.
711 <http://dx.doi.org/10.1016/j.anbehav.2015.09.029>
712
- 713 Newman, E., Manning, J., & Anderson, B. (2014). Matching floral and pollinator traits through
714 guild convergence and pollinator ecotype formation. *Annals of Botany*, 113, 373-384.
715 <http://dx.doi.org/10.1093/aob/mct203>

- 716
717 Nicolson, S. W., & van Wyk, J. H. (2011). Bee food: the chemistry and nutritional value of
718 nectar, pollen and mixtures of the two. *African Zoology*, 46(2), 197-204.
719 <http://dx.doi.org/10.1080/15627020.2011.11407495>
720
- 721 Nicholls, E., Hempel de Ibarra, N. (2014). Bees associate colour cues with differences in pollen
722 rewards. *The Journal of Experimental Biology*, 217, 2783-2788 [http://dx.doi.org](http://dx.doi.org/10.1242/jeb.106120)
723 [/10.1242/jeb.106120](http://dx.doi.org/10.1242/jeb.106120)
724
- 725 Nunes-Silva, P., Hnrcir, M., Shipp, L., Imperatriz-Fonseca, V.L., Kevan, P.G. (2013). The
726 behaviour of *Bombus impatiens* (Apidae, Bombini) on tomato (*Lycopersicon esculentum*
727 mill., Solanaceae) flowers: pollination and reward perception *journal of Pollination*
728 *Ecology*, 11(5), 33-40.
729
- 730 Pélabon, C., Thöne, P., Hansen, T. F., & Armbruster, W. S. (2012). Signal honesty and cost of
731 pollinator rewards in *Dalechampia scandens* (Euphorbiaceae). *Annals of Botany*, 109,
732 1331-1339. <http://dx.doi.org/10.1093/aob/mcs091>
733
- 734 Pellmyr, O. (1988). Bumble bees (Hymenoptera: Aipdae) assess pollen availability in
735 *Anemonopsis macrophylla* (Ranunculaceae) through floral shape. *Annals of the*
736 *Entomological Society of America*, 81, 792-797.
737

- 738 Plowright, R. C., & Lavery, T. M. (1984). The ecology and sociobiology of bumble bees.
739 *Annual Review of Entomology*, 29, 175-199.
740
- 741 R Development Core Team. (2010). R: a language and environment for statistical computing.
742 Vienna (Austria): R Foundation for Statistical Computing.
743
- 744 Raguso, R. A. (2004). Flowers as sensory billboards: progress towards an integrated
745 understanding of floral advertisement. *Current Opinion in Plant Biology*, 7, 434-440.
746 <http://dx.doi.org/10.1016/j.pbi.2004.05.010>
747
- 748 Raine, N.E., Chittka, L. (2007). Pollen foraging: learning a complex motor skill by bumblebees
749 (*Bombus terrestris*). *Naturwissenschaften*, 94, 459-464.
750
- 751 Renner, S. S. (1989). A survey of reproductive biology in neotropical Melastomataceae and
752 Memecylaceae. *Annals of the Missouri Botanical Garden*, 76(2), 496-518.
753
- 754 Russell, A. L., Golden, R. E., Leonard, A. S., & Papaj, D. R. (2015). Bees learn preferences for
755 plant species that offer only pollen as a reward. *Behavioral Ecology*, 00(00), 1-10.
756 <http://dx.doi.org/10.1093/beheco/arv213>
757
- 758 Russell, A.L., Papaj, D.R. (2016). Artificial pollen dispensing flowers and feeders for bee
759 behaviour experiments. *Journal of Pollination Ecology*, 18(3), 13-22.
760

- Simpson, B. B., & Neff, J. T. (1981). Floral rewards: alternatives to pollen and nectar. *Annals of the Missouri Botanical Garden*, 68(2), 301-322.
- Switzer, C. M., Hogendoorn, K., Ravi, S., & Combes, S. A. (2015). Shakers and head bangers: differences in sonication behavior between Australian *Amegilla murrayensis* (blue-banded bees) and North American *Bombus impatiens* (bumblebees). *Arthropod-Plant Interactions*, 1-8. <http://dx.doi.org/10.1007/s11829-015-9407-7>.
- Vallejo-Marín, M., Da Silva, E.M., Sargent, R.D., & Barrett, S.C.H. (2010). Trait correlates and functional significance of heteranthery in flowering plants. *New Phytologist*, 188, 418-425. <http://dx.doi.org/10.1111/j.1469-8137.2010.03430.x>
- Varassin, I. G., Penneys, D. S., & Michelangeli, F. A. (2008). Comparative anatomy and morphology of nectar-producing Melastomataceae. *Annals of Botany*, 102, 899-909. <http://dx.doi.org/10.1093/aob/mcn180>
- Waser, N. M. (1986). Flower constancy: definition, cause and measurement. *The American Naturalist*, 127(5), 593-603.
- Wood, S. (2015). Mixed GAM computation vehicle with GCV/AIC/REML smoothness estimation. R package version 1.9-9. [online] URL: <https://stat.ethz.ch/R-manual/R-devel/library/mgcv/html/mgcv-package.html> (accessed December 2015)

- 784 Woodward, G. L., & Lavery, T. M. (1992). Recall of flower handling skills by bumble bees: a
785 test of Darwin's interference hypothesis. *Animal Behavior*, 44, 1045-1051.
786
- 787 Ye, X.-Q., Meng, J.-L., Zhao, Z.-G., Fan, B.-L., & Du, G.-Z. Optimal pollinator attraction
788 strategies in *Trollius ranunculoides* Hemsl. (Ranunculaceae) at different altitudes:
789 increased floral display or promotion of nectar output?. *Plant Biology*, 13, 551-555.
790
- 791 Zhang, C., Yang, Y.-P., & Duan, Y.-W. (2014). Pollen sensitivity to ultraviolet-B (UVB)
792 suggests floral structure evolution in alpine plants. *Scientific Reports*, 4, 4520.
793 <http://dx.doi.org/10.1038/srep04520>.

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