Multiple Rewards Have Asymmetric Effects on Learning in Bumblebees

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1 In their natural environments, most animals must learn about multiple kinds of rewards, both
2 within and across contexts. Despite this, the majority of research on animal learning involves
3 a single reward type. For example, bees are an important model system for the study of
4 cognition and its ecological consequences, but nearly all research to date on their learning
5 concerns a single reward, nectar (carbohydrates), even though foragers often simultaneously
6 collect pollen (protein). Features of learning under more ecologically realistic conditions
7 involving multiple reward types are thus largely unexplored. To address this gap, we
8 compared performance on a colour learning task when floral surrogates offered bumblebees
9 *Bombus impatiens* a single type of floral reward vs. multiple, nutritionally distinct, rewards.
10 In one experiment, bees learned a floral association with nectar either alone or while
11 simultaneously collecting pollen. In a reciprocal experiment, bees learned a floral association
12 with pollen either alone or while simultaneously collecting nectar. Bees that collected pollen
13 while learning about nectar did not suffer any detriment to learning which flower colour
14 offered nectar. However, this was not the case for the reciprocal task: collecting nectar
15 impaired bees' ability to learn and remember associations between floral colour and pollen.
16 Our findings offer new insight into how bees learn in relation to ecologically realistic
17 rewards, and how cognitive constraints may shape their behaviour under ecologically realistic
18 foraging scenarios.

20 **Key words**

21 *Bombus impatiens, Bumblebee, learning, memory, multitasking, nectar, pollen, pollination*

Although research on animal learning often involves a single reward or task type, animals in the wild must often perform multiple tasks, learn multiple associations, and correctly remember stimuli across different contexts. These multi-faceted demands are generally expected to tax attention, slow acquisition, and impair recall. In our own species, cognitive psychology supports the common wisdom that learning proceeds more slowly when subjects are asked to simultaneously perform a second task (Foerde, Poldrack, & Knowlton, 2007; Pashler, 1994; Waldron & Ashby, 2001); more broadly, efficiency and accuracy are often lowered when attention is divided between different activities (Dukas, 2002). For example, blue jays *Cyanocitta cristata* detect multiple prey types more slowly compared to a single type (Dukas, 2001) and when bees learn multiple conflicting nectar-foraging tasks, their performance is impaired (Cheng & Wignall, 2006; Chittka & Thomson, 1997). Animals may also suffer impairments to learning when learning about multiple, conflicting, stimuli in different contexts (e.g. foraging, nest location and oviposition sites; Weiss & Papaj, 2003; Worden, Skemp, & Papaj, 2005, but see Colborn, Ahmad-Annuar, Fauria, & Collett, 1999). However, it is not clear whether these impairments were due to learning a second association generally, or learning a second association that conflicted with the first (i.e. that a given stimulus was rewarding in one context but not in the other).

One scenario routinely faced by generalist foragers but rarely explored in research on animal learning concerns learning associations while concurrently collecting multiple resource types. Within this single context (foraging), foragers may encounter prey or diet items that differ in handling techniques and nutritional composition (Simpson & Raubenheimer, 2012). Animals can clearly discriminate between different resources when foraging (Mayntz, 2005; Simpson, Sibly, Lee, Behmer, & Raubenheimer, 2004), and employ different strategies accordingly (Sulikowski & Burke, 2007). Foragers can also learn to associate different stimuli with multiple types of food reward (bees: Muth, Papaj, & Leonard,

2015; locusts: Raubenheimer & Tucker, 1997). However, whether animals incur costs to performance in terms of acquisition or recall when foraging for multiple items is not clear. Understanding the relative costs of learning about a single vs. multiple resources is broadly relevant to understanding the factors that govern the degree of short- or long-term specialization in foraging-related tasks.

Generalist bees offer an ideal opportunity to explore how foragers ‘cope’ with learning about multiple reward types. Honeybees (*Apis mellifera*) and bumblebees (*Bombus* spp.) visit many different plant species, and in doing so they rapidly form associations between the multimodal “sensory billboard” of the floral display (Leonard & Masek, 2014; Raguso, 2004) and a reward (Leonard, Dornhaus, & Papaj, 2011b). As such, bees have served as important models for our understanding of cognition (Giurfa, 2007; Menzel, 2012; Menzel & Giurfa, 2001). We define cognition as the mechanisms by which animals acquire, process, store and act on information (Healy & Rowe, 2010; Shettleworth, 2010). However, despite the wide range of resources plants offer pollinators (e.g. resins, oils, oviposition sites etc.; Renner 2006; Armbruster 2011), study of how learning mediates pollination mutualisms has focused almost exclusively on learning in relation to a single type of floral reward, usually nectar (top row in Table 1). In reality, bee-pollinated plants often offer two major, nutritionally complementary resources: nectar and pollen (Nicolson, 2011) in diverse combinations (Table 1). In natural settings, therefore, bees encounter learning scenarios considerably more complex than those typical of lab-based studies.

Recent work has established that bees can learn associations between floral stimuli and pollen rewards (Grüter, Arenas & Farina 2008; Nicholls & Hempel de Ibarra 2014; Russell *et al.* 2015; Muth, Papaj & Leonard 2016), advancing our understanding of how they learn in relation to non-nectar rewards. Further, bees can learn simultaneously that one floral colour offers only nectar and a second colour only pollen (Muth et al. 2015). Yet, despite the

fact that individuals of many bee species (including bumblebees) collect both resources on a foraging bout (Goulson, 2003; Hagbery & Nieh, 2012; Hofstede & Sommeijer, 2006; O'Donnell, Reichardt, & Foster, 2000) we know nearly nothing about how learning performance is affected by simultaneously collecting both rewards. Given that bees' learning of floral stimuli has important consequences from both bee and plant perspectives, our current understanding of learning performance under realistic reward scenarios leaves our picture of the cognitive ecology of pollination surprisingly incomplete.

Table 1: Examples of plant strategies for offering nectar and pollen as pollinator rewards. The majority of research on bee cognition approximates the top row reward scenario.

Reward (Nectar ● Pollen ●)	Examples	Reference
Nectar only	<i>Asclepias</i> Orchidaceae	Pleasants & Chaplin 1983 Sun et al. 2011
Pollen only	<i>Pyrola</i> <i>Solanum</i> <i>Dodecatheon</i> <i>Papaver</i>	Knudsen & Olesen 1993 Buchmann 1983 Harder & Barclay 1994 Raine & Chittka 2007
Various combinations of Nectar and Pollen	<i>Aster</i> Apiaceae Cucurbitaceae <i>Salix</i>	Niesenbaum et al. 1999 Langenberger & Davis 2002 Nepi et al. 2001 Mosquin 1971
Transition from Nectar and Pollen to Pollen only (Protogyny)	<i>Campanula rotundiflora</i> <i>Erythronium grandiflorum</i>	Cresswell & Robertson 1994 Thomson 1986
Transition from Nectar and Pollen to Nectar only (Protandry)	<i>Lavandula stoechas</i> <i>Phacelia linearis</i> <i>Alstroemeria aurea</i> <i>Trachymene incisa</i>	Gonzalez et al. 1995 Eckhart 1991 Aizen & Basilio 1998 Davila et al. 2007
One reward type becomes temporally available AM PM	<i>Rhus hirta</i> Sudworth <i>Aralia hispida</i> <i>Lavandula latifolia</i>	Greco et al. 1996 Thomson et al. 1989 Herrera et al. 1990

We addressed how learning of a floral feature (colour) was affected when bees foraged for multiple rewards. In a series of free-flying behavioural assays using bumblebees *Bombus impatiens* as subjects, we compared the learning of a reward-colour association

when bees learned an association with nectar, with or without simultaneously collecting pollen (Exp. 1) and when bees learned an association with pollen, with or without simultaneously collecting nectar (Exp. 2a).

If collecting multiple types of reward impairs learning, then we expected that bees collecting two reward types would find it more difficult to learn an association between a given reward type and floral feature than bees learning this association in isolation. Alternatively, since individual bumblebees forage for both rewards under natural conditions, and both are critical for colony survival, they may be well-equipped to learn associations while concurrently performing these two activities without incurring a cost to task performance. After Experiment 2a showed that nectar impaired learning of pollen-colour associations, we explored the mechanism behind this performance decrement in a follow-up experiment (Exp. 2b). This experiment tested whether the learning impairment was simply due to carrying out a second task (i.e. collecting nectar) or specifically due to experiencing different rewards on different, conflicting stimuli (Fig. 1).

General Methods

Subjects and maintenance

We used a total of 208 bumblebees from eleven colonies of *Bombus impatiens* (Koppert Biological Systems MI, USA) represented equally across treatments within each experiment, with at least 11 subjects per treatment included in the final analysis (for sample sizes see Table 1 in Supplementary Material). Colonies were connected to a central foraging arena (L x W x H: 122 × 59 × 59 cm) where all training and testing took place. We selected individuals as subjects by allowing a colony access to the test arena containing both a feeder with a cotton wick that offered 50% (w/w) sucrose (hereafter, “nectar”) and a chenille stem

feeder loaded with ~50mg of flower-collected cherry pollen (*Prunus avium* “Bing” variety, Firman Pollen, Yakima WA, hereafter “*Prunus*”). Bumblebees are major pollinators of cherry crops (Velthuis & van Doorn, 2006) and readily collect this pollen under lab conditions (Muth et al., 2016; Muth et al., 2015). We numbered foragers individually using thorax tags (E. H. Thorne Ltd, Wragby, Lincolnshire, UK).

We maintained colonies on honeybee-collected pollen (Koppert Biological Systems) prior to experiments, but only used *Prunus* pollen in experiments. During experiments, bees only had access to the pollen that they collected during training and testing. We maintained colonies on 50% (w/w) scented sucrose (500ml water, 500g sugar and 2µl linalool). By giving bees controlled amounts of nectar and pollen (~4 honeypots filled with 50% sucrose and ~100mg of pollen at the start of the test day and half their honeypots filled with 50% sucrose and ~700mg of pollen at the end of the test day) we kept the majority of foragers motivated to collect both nectar and pollen.

Floral arrays and rewards

In all experiments we used arrays of 20 artificial flowers (10 human-blue, 10 human-yellow; for positions in bee colour space see Supp. Fig. 1), each consisting of a coloured corolla and artificial anther, arranged in a 5 × 4 grid. Flowers (Supp. Fig. 2) were spaced 7 cm apart at the base (5 cm apart at the top), and consisted of 3D-printed 5 cm diameter circular discs (Makerbot, NY, USA) placed on inverted plastic tubes (8 cm in height), with a coloured circle (the ‘corolla’, printed on laminated waterproof paper: National Geographic Co., USA). The centre of the corolla contained two holes: one fitted with a nectar well (diameter: 4mm) and one (diameter: 1mm) from which the ‘anther’ (a 25 mm beige chenille stem: Creatology, China) protruded vertically. Bees readily collect pollen from these artificial anthers (for a

description and video see Muth et al. 2015; 2016). The design of our artificial flowers made nectar- and pollen-collection behaviour (i.e. probing the well vs. contacting the chenille stem) easily distinguishable. Flowers were always oriented such that the nectar wells faced bees as they entered the arena. Between each trial we replaced all nectar wells and anthers. The foraging arena was lit directly from above (59cm) by both an LED light (2100 Lumens, Lithonia Lighting, USA) and a human-white LED strip around the top of the inside of the arena (36 watt, LED Wholesalers, China), as well as indirectly from fluorescent and natural room light.

During training, flowers contained either 12 μ l of 50% scented sucrose (for nectar-rewarding flowers) or 12 μ l of scented water (for nectar-unrewarding flowers), both scented with 2 μ l of linalool per 1000ml solution to encourage their discovery. Flowers also either contained ~3-5mg of *Prunus* pollen on their anther (for pollen-rewarding flowers) or an empty, scented anther (for pollen-unrewarding flowers). We scented unrewarding anthers with pollen (as in Muth *et al.* 2015, 2016) to increase the chance that bees would land on these flowers and to make it more difficult for bees to distinguish flowers based on scent alone. We did this by storing the artificial anthers overnight in mesh bag within a sealed container of the *Prunus* pollen, with the bag above the pollen such that they did not come into direct contact. The quantities of nectar and pollen used per flower in the current study have been shown in previous studies to be sufficient to fill the crops and pollen baskets of an average-sized forager after collecting the reward from 10 flowers (e.g. nectar: Muth, Keasar & Dornhaus 2015; pollen: Muth *et al.* 2016).

General protocol

In all treatments bees had to learn that one of two flower colours predicted a reward, either in isolation or with a second reward also present on flowers (termed the ‘secondary reward’; Fig. 1). In Experiment 1 and 2a the secondary reward was present on all the flowers. Bees in all experiments progressed through the following stages: pre-training 1; pre-training 2; training and testing. We carried out both pre-trainings on the same day, and training and testing one to three days later, also on the same day. The number of days between pre-training and training was represented equally across treatments (all values are mean number of days $\pm$ standard deviation: Exp. 1: nectar and pollen: 1.4 ± 0.7 ; nectar only: 1.3 ± 1.0 ; Exp. 2: nectar and pollen: 1.1 ± 0.3 ; pollen only: 1.3 ± 0.5). Within each experiment, the colour (blue vs. yellow) offering the reward was counterbalanced within treatments, across subjects.

Pre-training

The pre-training stages allowed us to first select foragers that collected both nectar and pollen (~ 80% of foragers within a colony in the current experiment) and then train them to visit the array used in training and testing phases. Thus, foragers randomly assigned to either single reward or dual reward treatments had all previously demonstrated that they collected both nectar and pollen. We did this to ensure that differences between foragers’ behaviour were the result of experimental treatments rather than any pre-existing differences among foragers.

For pre-training 1 we gave all previously-marked foragers access to an array of 20 flowers, identical to those that would be used in training and testing, except that their corollas were grey rather than blue or yellow (Supp. Fig. 1). Each flower contained 36 μ l of 50% sucrose and ~10mg of pollen. Bees that collected both nectar and pollen from these flowers progressed to pre-training 2. In pre-training 2 bees were given access to an array containing 10 blue and 10 yellow flowers. In Exp. 1, we gave individual bees (n=94) flowers that were

all rewarding for both nectar and pollen. The pre-training 2 for Exp. 2a was conducted as described above, with the exception the flowers for the pollen-only treatment only offered pollen rewards (n=35) while the nectar and pollen treatment offered both rewards (n=71). We carried out pre-training in Exp. 2a in this way because pilot trials showed that bees would not readily transition from foraging on flowers with both nectar and pollen to pollen-only flowers (often abandoning the array after not finding nectar; an effect not observed when transitioning from dual-reward flowers to nectar-only flowers). However, to rule out the possibility that this difference in pre-training generated the difference in our findings in Experiments 1 and 2a, in Exp. 2b we carried out pre-training in the same way as Exp. 1 (with both nectar and pollen present). The main difference between the results of Exp. 1 and 2a was replicated within this single experiment (see Exp. 2b Results).

Training and testing

In all experiments and for each training trial we let an individual bee into the foraging arena with free access to the floral array. During training and testing, a bee could make two kinds of errors (attempting to collect pollen from an unrewarding anther or probing an unrewarding nectar well, for more details see Supplementary Material) and two kinds of correct decisions (collecting pollen from a rewarding anther or drinking from a rewarding nectar well). A bee was freely allowed to collect nectar and pollen in a trial before returning to the colony. If a bee stopped foraging and did not return to the array she was returned to the colony after 10 minutes had elapsed. In Exp. 1 we gave all bees a minimum of two and maximum of six training trials; when a bee reached a learning criterion of 8 out of 10 sequential correct visits to the flower colour rewarding for nectar (A. Leonard, Dornhaus, & Papaj, 2011a) we proceeded to the test trial. In Exp. 2a, training and testing were identical to Exp. 1, with the

exception that all bees were given a fixed number of training trials (two). This was because visits to anthers occurred more quickly than visits for nectar, so floral choice for pollen could not be accurately observed live. In previous work (Muth et al., 2016; Muth, Papaj, et al., 2015) we found that under these conditions, the vast majority of bees reached the 8 of 10 visits learning criterion within two training trials; review of recordings from Exp. 2a established that all bees but one reached this learning criterion. Additionally, while it took bees in Experiment 2a fewer trials to learn than in Exp. 1, bees made many more visits to flowers per trial (compare Figs 2a and 2b): unlike when nectar foraging, bees foraging for pollen returned multiple times to a single flower and successfully extracted pollen from it.

After training, we gave all bees a test (probe) trial, where we presented an individual with an array identical to the training array with the exception that all the flowers were unrewarding for nectar and pollen (i.e. containing only scented anthers and scented water).

Behaviour recorded

We filmed training and testing using an HD Sony camcorder (30 fps) mounted on a tripod placed on top of the test arena facing downwards. From the videos we coded each flower visit made by a bee. This included: 1) the colour of the flower (blue or yellow); 2) whether the bee attempted to collect pollen or nectar (i.e. from the anther or nectar well respectively); and 3) whether she was successful at gaining the reward or not ('rewarded' or 'unrewarded'). Visits to collect nectar were only coded if the bee probed the nectar well with her proboscis and visits to collect pollen were only coded if the bee made contact with the anther with her antennae or legs. Pollen-collecting behaviours are described in more detail in the supplementary material as well as in Muth *et al.* (2015, 2016).

Data analysis

To compare learning across treatments (single reward versus two rewards, separately for all three experiments), we used the number of correct visits within a block of 10 consecutive visits as the response variable. Because different bees within treatments made a different total number of visits to flowers, we set the upper value of visits at the block where there were fewer than seven bees still visiting flowers (i.e. Exp. 1: 4 blocks, Exp. 2a, 8 blocks). We excluded bees that did not make at least 15 visits to the trained reward type (Exp. 1: $n = 3$; Exp. 2a $n = 7$) and bees that did not collect at least two rewards of the secondary reward type during the time they took to visit 20 flowers for the trained reward type (Exp. 1: $n = 0$; Exp. 2a $n = 3$). Bees that did not reach the learning criterion within the maximum number of training trials (Exp. 1: nectar-only: $n=1$, nectar and pollen: $n=3$; Exp. 2a: nectar and pollen: $n=1$) were still included in the learning analysis as they all showed a statistically significant improvement in their proportion of visits to the rewarding flower type (i.e. learning).

For each learning analysis we fitted a generalized linear mixed model (GLMER) with a Poisson error distribution using the response variable [number of correct visits within a block of 10 consecutive visits], with the explanatory factors treatment (single reward or two rewards), colour of reward being trained to (blue or yellow), the continuous variable visit block number (blocked in groups of 10) and the random factors bee nested within colony ('Model 1'). We measured test performance as the number of correct visits in the first 10 visits a bee made and compared treatments with unpaired t -tests.

We carried out all analyses in R version 2.15.1 (R Development Core Team 2010). GLMMs were carried out using the `glmer()` function in `lme4` package (Bates, Maechler, Bolker & Walker 2015) and LMMs were carried out using the `lme()` function in `nlme` package, specifying type three sum of squares and sum contrasts in cases where there were

interactions (Pinheiro, Bates, DebRoy, Sarkar & R Core Team 2015). For all models, maximal models were run initially, and then non-significant interactions were removed in a step-wise fashion. In all cases we verified that model assumptions were met.

Ethical Note:

We maintained ethical standards throughout testing and euthanized bees via freezing.

Experiment 1 and 2a: Results

Experiment 1: Learning nectar associations while concurrently collecting pollen

Bees' ability to learn a nectar-colour association was not impaired by concurrently collecting pollen. Bees in both treatments learned that one colour predicted a nectar reward, making fewer visits to the alternative colour to probe for nectar across visits (Poisson GLMM: $z = 4.814$, $p < 0.0001$; Fig. 2a) and the number of errors during learning did not differ between treatments ($z = -2.654$, $p = 0.107$). Blue-trained bees made more correct choices than yellow-trained bees across both reward treatments ($z = 1.613$, $p < 0.01$). There was no difference between treatments in the number of correct choices bees made in the unrewarded test phase (unpaired t -test: $t_{48} = 0.407$; $p = 0.686$; Fig. 2a).

Experiment 2a: Learning pollen associations while concurrently collecting nectar

In contrast to results of Exp. 1, bees' ability to learn a pollen-colour association was impaired when they concurrently collected nectar. In a pollen-colour learning task, bees in each of two training conditions learned the flower colour associated with a pollen reward, making fewer

errors (i.e. searching for pollen on unrewarding anthers) across visits (Poisson GLMM: $z = 5.22$, $p < 0.0001$; Fig. 2b). However, bees that collected nectar while learning this colour association with pollen made more errors while learning than bees that foraged on flowers with no nectar ($z = -4.66$, $p < 0.0001$). As in Exp. 1, blue-trained bees made more correct choices than yellow-trained bees across both reward treatments ($z = -3.42$, $p < 0.0001$). Bees that collected both nectar and pollen performed worse during the unrewarded test phase than bees that foraged for nectar alone (unpaired t -test: $t_{43} = 3.36$; $p < 0.005$; Fig. 2b).

Motivation to collect rewards across treatments

Because we did not control the amount of each reward bees collected, we were interested in whether any differences in learning performance might reflect treatment-related differences in bees' motivation to collect particular rewards. When we compared bees' attempts to collect nectar and pollen in Experiments 1 and 2a, we found that the presence of pollen on flowers did not lessen bees' motivation to collect nectar, whereas the presence of nectar on flowers appeared to decrease bees' motivation to collect pollen. In Exp. 1, bees made the same number of attempts to collect nectar, regardless of whether they foraged for nectar alone or simultaneously foraged for pollen (unpaired t -test: $t_{59} = 0.023$, $p = 0.982$; Fig. 3). In contrast, in Exp. 2a, bees made fewer attempts to forage for pollen when they simultaneously foraged for nectar compared to the bees that foraged for pollen alone (unpaired t -test: $t_{62} = 4.411$, $p < 0.0001$; Fig. 3). A related analysis regarding how bees' collection of nectar or pollen changed over successive visits differentially based on treatment returned a similar finding (Supplemental material).

Experiment 2b

Because we were interested in why bees that collected nectar were impaired in their ability to learn about pollen, we conducted a follow-up experiment to determine whether this impairment was caused by motivational changes associated with the mere act of collecting nectar, or whether it was driven more specifically by collecting nectar on the colour that was unrewarding for pollen (i.e. via interference; Weiss & Papaj 2003; Worden *et al.* 2005). We trained bees that yellow flowers contained pollen either: 1) when no flowers offered nectar; 2) when yellow flowers also offered nectar or; 3) when blue flowers offered nectar (Fig. 1).

If collecting nectar makes bees less motivated to collect pollen and more motivated to collect nectar, we expected a detriment to learning in either treatment where bees collected nectar (regardless of which colour of flower they collected it from) relative to bees that foraged for pollen alone. Alternately, it is possible that collecting nectar on the flower that was unrewarding for pollen may have interfered with a bee's ability to learn about pollen (as in Worden *et al.* 2005): a bee remembered a blue flower as rewarding but misremembered which reward she found there, so mistakenly searched for pollen instead of nectar on blue flowers. If so, we expected that only the bees in the treatment where nectar was offered on blue flowers would show impaired learning relative to the two other treatment groups.

Experiment 2b: Methods

All bees (n=71) were pre-trained as described above, on arrays containing both nectar and pollen. Training and testing was carried out in the same way as Exp. 2a, with all bees given two training trials and one test trial. We trained bees that yellow flowers contained pollen either: 1) when no flowers offered nectar (n=18); 2) when yellow flowers also offered nectar (n=22) or; 3) when blue flowers offered nectar (n=23) (Fig. 1). We only trained bees that yellow flowers were rewarding for pollen (and not blue) because the largest effect we found

in Exp. 2a was for yellow-trained bees (the less-preferred colour). To determine whether bees made more visits to the rewarding flower type across successive visits and whether this differed between treatments, we fitted a GLMM using the same variables as ‘Model 1’ (see data analysis section above), except this time the factor ‘treatment’ had three levels and there was no factor of ‘colour of reward being trained to’ since all bees were trained to pollen on yellow flowers. To determine whether the three treatments differed from each other in their test performance we fitted a linear mixed model (LMM) with the response ‘number of correct choices’, the explanatory factor ‘treatment’ (3 levels) and the random factor ‘colony’. Tukey post-hoc tests were carried out using the lsmeans package.

Exp. 2b Results

Why did nectar impair learning of pollen-colour associations?

We found that the mere act of collecting nectar, regardless of whether it was on the same or different flower as pollen, impaired bees’ ability to learn the pollen-colour association (Fig. 4). This finding is consistent with the hypothesis that nectar collection impairs performance in pollen learning by affecting motivation. Across all treatments, bees chose more ‘correct’ flowers rewarding for pollen across successive visits than incorrect flowers (Poisson GLMM: $z = 2.287, p < 0.05$). However, their performance differed between treatments, with bees in the pollen-only treatment performing better than bees in the other two pollen-nectar treatments (no difference between the two nectar treatments: $z = 0.436, p = 0.663$; significant difference between pollen-only and other two treatments: $z = 2.227, p < 0.05$). The test phase showed the same pattern of results: bees in the pollen-only group performed better than the other two treatments (LMM: $F_{2, 33} = 3.467; p < 0.05$; Fig. 4).

348

349 **Discussion**

350 Learning is an essential feature of the interaction between plants and their pollinators, yet the
351 study of its mechanisms and consequences usually involves a single reward type scenario
352 (Giurfa, 2007; Leonard & Masek, 2014). This setup fails to capture the complexity of bees'
353 real-life foraging situations, which often also involves collection of pollen (Table 1). If
354 studies of pollinator cognition are to be relevant for research into the ecology and evolution
355 of plant-pollinator relationships, it is critical to understand how bees cope with learning
356 associations in relation to multiple rewards. Beyond providing insights into how nutritionally
357 complex rewards mediate interactions between plants and their pollinators, these questions
358 are also relevant to any foraging scenario in which an animal must learn multiple associations
359 for nutritionally distinct resources.

360 Here we present the first evidence that foraging simultaneously for multiple floral
361 rewards has asymmetric effects on bee learning. Bees that collected pollen while also
362 foraging for nectar did not suffer any detriment to their ability to learn which flowers offered
363 nectar. This finding supports the assumption that using nectar-only floral surrogates (the
364 approach used in the majority of bee cognition research) produces reasonable estimates of
365 nectar-based learning ability under more realistic conditions. However, this was not the case
366 for the reciprocal task: bees learning a pollen-colour association made more errors (searching
367 for pollen on unrewarding anthers) when they simultaneously collected nectar. The
368 unexpected asymmetry in cognitive costs associated with foraging for two rewards at once
369 raises new questions about how bees handle learning multiple associations, with
370 consequences for understanding the evolution of floral reward complexity.

From the pollinator perspective, our results uncover a constraint on bees' ability to learn when foraging for multiple rewards, although only in a single direction. In other systems, carrying out multiple tasks or task-switching can lead to lower performance (Cheng & Wignall, 2006; Courage, Bakhtiar, Fitzpatrick, Kenny, & Brandeau, 2015; Monsell, 2003). A detriment to learning is also found when insects learn associations with the same conditioned stimuli in two different contexts (bees: Worden *et al.* 2005; butterflies: Weiss & Papaj 2003) or learn two tasks with conflicting response requirements (bees: Cheng & Wignall, 2006; Chittka & Thomson, 1997). In these cases, it seems that bees suffer from interference between the two tasks. However, in our experiment, collecting (and potentially learning about) two rewards simultaneously was not sufficient to cause any detriment to learning, since bees were only impaired when learning about pollen while simultaneously collecting nectar. Additionally, the detriment to learning was not simply caused by bees' confusion over which reward a given conditioned stimulus contained (as in Weiss & Papaj, 2003). For example, it was not the case that bees only suffered an impairment to learning that pollen was found on yellow flowers when nectar was found on blue, because bees suffered the same impairment to learning even when they simultaneously collected nectar from the same colour that was rewarding for pollen (Exp. 2b). This implies that the learning impairment was caused by collecting nectar *per se*, regardless of the colour of flower bees collected it from.

Combined with our finding that collection of pollen did not impair nectar learning (Exp. 1), it seems that, rather than a general cost to learning about multiple reward types, there is something specific regarding nectar impairing bees' learning about pollen. This asymmetry in nectar and pollen's effects on learning about the other imply that nectar is a more salient reward to bees than pollen, being more immediately motivating. This is further supported by our finding that bees collected less pollen when also foraging for nectar (Exp.

2a), but did not change their effort to collect nectar when also foraging for pollen (Exp. 1). Furthermore, motivation to collect pollen waned over time in Exp. 1 (supplemental results) whereas bees remained motivated to collect nectar in Exp. 2a. When collecting both rewards at the same time, a higher inherent motivation to collect nectar could be driven by bees' immediate need for carbohydrates to fuel costly flight and foraging (Goulson, 2003; Heinrich, 2004), in contrast to pollen which infers relatively long-term benefits to offspring survival and colony growth. While the mechanism by which nectar acts as a reward to bees has been established (Hammer & Menzel, 1995), precisely how pollen rewards bees is not clear, although both the taste (Muth, Francis, & Leonard, 2016, Ruedenauer, Spaethe, & Leonhardt 2015) and nutritional content (Vaudo, Patch, Mortensen, Tooker, & Grozinger, 2016) are important factors in determining bumblebee floral preferences and may influence learning. Elucidating the mechanism by which pollen reinforces bee behaviour will be critical in determining precisely how bees integrate information about nectar and pollen rewards when foraging for both.

An obvious next step will be to determine whether these results hold under a range of different nectar and pollen qualities, and whether our findings change with colony nutritional state. However, with respect to the current experiment, we kept colonies maintained on diets restricted for both reward types in an attempt to offer rewards of equal value to bees. This manipulation kept the majority of bees motivated to collect both rewards. Further, the nectar and pollen used seemed to reinforce learning to the same extent when they were collected in isolation, supported by our finding that nectar-only bees in Exp. 1 and pollen-only bees in Exp. 2a had similar rates of learning (compare single-reward learning curves in Figs 2a and 2b).

The asymmetry we found is perhaps analogous to a previous finding in honeybees *Apis mellifera* that either learned two associations (one appetitive, one aversive) or a single

association (Vergoz, Roussel, Sandoz, & Giurfa, 2007). Bees that learned both associations did not suffer any impairment to learning relative to bees that learned a single aversive association (odour + shock), but were impaired relative to bees that learned a single appetitive association (odour + sucrose). The impairment to appetitive learning was specifically due to *learning* the aversive association rather than simply being shocked: bees that were shocked in a protocol where the odour stimulus was unpaired from the shock did not suffer the impairment to appetitive learning. Therefore, just as the bee brain may prioritise learning aversive shock associations over appetitive sucrose associations (Vergoz et al., 2007), so too the current experiment suggests that nectar-learning may be prioritised over pollen-learning.

Our finding that when flowers offered nectar, bees found it more difficult to learn about floral pollen (Exp. 2b), also has implications for understanding the functional ecology of floral rewards from the plant perspective. While a pollinator's aptitude for learning floral associations is often considered from the individual's or colony's perspective (Raine & Chittka, 2008), bees' learning performance is often equally as significant from the plant's perspective, since pollinators that make more errors while learning an association or show poorer recall of an association are more likely to transfer pollen to heterospecific plants (Chittka & Thomson 2001). While floral reward diversity has been the subject of a number of overviews (Armbruster, 2011; Renner, 2006), functional explanations for why any one plant might benefit from offering more than one reward type have been relatively unexplored. Any reward strategy is likely to be shaped by multiple agents, and previous explanations have highlighted the potential benefits of increased pollinator diversity (Ghazoul, 2006) or meeting the multi-nutrient target of a given pollinator (Francis, Muth, Papaj, & Leonard, 2016). Our findings complement and extend these ideas, showing that nectar reduces bees' ability to learn effectively about collecting pollen and makes bees less motivated to collect pollen

overall. Since nectar is thought to be a relatively ‘cheap’ reward for the plant compared to reproductively-costly pollen (Hargreaves, Harder & Johnson 2009), it would presumably benefit the flower to encourage learning of its features by inducing the bee to collect this reward rather than pollen. While it is necessary for plants to encourage some pollen collection for successful reproduction, restriction of pollen collection by a single individual is evident from other mechanisms such as dosing via anther morphology (e.g. Harder and Thomson 1989), pollen architecture (Lunau, Piorek, Krohn, & Pacini 2015) and pollen chemistry (Muth et al. 2016b).

The idea that plants benefit from the cognitive limitations of their pollinators is not a novel one: floral constancy, the tendency of individuals to sequentially visit one flower type while bypassing others, may also be explained by cognitive constraints on a pollinator’s ability to recognize or handle multiple types (Chittka, Thomson, & Waser, 1999; Dukas, 1998; Goulson, 2000; Lewis, 1993; Waser, 1983, 1986). Likewise, sexually or food deceptive species may benefit not only from pollinators’ innate attractions to elements of their display, but also from difficulty in learning to avoid them (Jersáková, Johnson, & Kindlmann, 2006; Pohl, Watolla, & Lunau, 2008). Such a constraint on bees’ ability to learn effectively about pollen associations when collecting nectar could in turn feed back onto the evolution of bee foraging strategies: if bees are constrained in their ability to learn about multiple rewards, this could help explain the evolution of nectar- and pollen-foraging specialists in large social colonies such as honeybees (Page, Scheiner, Erber, & Amdam, 2006). Similarly, it may explain why individuals of some species (e.g. bumblebee species) often specialise on one reward in the short term (Francis et al., 2016), only generalising when necessary (Hagbery & Nieh, 2012). This is supported by findings from three wild *Bombus* species that were less efficient when switching from pollen to nectar collection (Cartar, 1992) than when collecting a single nutrient. Thus there may be a trade-off between the cognitive cost of collecting both

rewards and the energetic advantage of doing so, especially when limited by the number of foragers in a colony.

The complex interplay between plants and their pollinators offer a useful system in which to address further more general questions of how multiple, different rewards that differ in quality can mediate learning. In this system, reward types are not only tractable to separate experimentally, but the pattern of natural diversity in plant reward strategies (Table 1) offers reward scenarios in which each type is or is not offered alongside the other. Analogous scenarios might play out in other contexts, for example a generalist predator sampling prey items that vary in nutritional content (Mayntz, 2005), and learning aspects of their anti-predator displays, or a seed-disperser learning information about the location and quality of fruits while interspersing collection of insect prey. In each of these cases, the speed and accuracy of learning will have ecological effects: in understanding these effects, our results highlight the need to consider the possibility that multiple “rewards” can have different and interactive effects on cognitive performance. Animals’ cognitive abilities are expected to evolve in response to selection (Dukas, 2008), but by experimentally investigating learning with a single reward, we may not be gaining a realistic picture of the ecological relevance of learning.

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Figure Headings

Figure 1: Summary of the experiments and treatments. In each case, bees were trained to a colour-reward association with a single reward (Exp. 1: nectar; Exp. 2: pollen) either in the presence or absence of a ‘secondary’ reward (Exp. 1: pollen; Exp. 2: nectar). Red plus signs (next to circular ‘nectar wells’) indicate nectar present; light grey plus signs (next to rectangular ‘anthers’) indicate pollen present.

Figure 2: Results from the test phase: a) Exp. 1: The number of correct visits when bees landed and probed for nectar, averaged across blocks of 10 visits when bees either foraged for nectar alone (n=28) or concurrently collected pollen from both flowers (n=29); b) Exp. 2a: The number of correct visits when bees searched for pollen on anthers, averaged across blocks of 10 visits when bees either foraged for pollen alone (n=30) or concurrently collected nectar from both flowers (n=24).

Figure 3: The mean number of attempts bees made during training to collect a) nectar (Exp. 1) or b) pollen (Exp. 2a) either when collecting that reward alone or when collecting both rewards.

Figure 4: Exp. 2b: The mean number of correct visits to anthers (of yellow flowers) to search for pollen during the test phase, when bees either foraged for pollen alone on yellow flowers (n=16) or concurrently collected nectar from either blue flowers (n=11) or yellow (n=13).

References

- Aizen, M. & Basilio, A. (1998) Sex differential nectar secretion in protandrous *Alstroemeria aurea* (Alstroemeriaceae): is production altered by pollen removal and receipt? *American Journal of Botany*, **85**, 245–252.
- Armbruster, W. (2011). Evolution and ecological implications of “specialized” pollinator rewards. In S. Patiny (Ed.), *Evolution of Plant-Pollinator Relationships* (pp. 44–67). Cambridge University Press, UK.
- Cartar, R. V. (1992). Adjustment of foraging effort and task switching in energy-manipulated wild bumblebee colonies. *Animal Behaviour*, *44*(1), 75–87.
[http://doi.org/10.1016/S0003-3472\(05\)80757-2](http://doi.org/10.1016/S0003-3472(05)80757-2)
- Cheng, K., & Wignall, A. E. (2006). Honeybees (*Apis mellifera*) holding on to memories: response competition causes retroactive interference effects. *Animal Cognition*, *9*(2), 141–150. <http://doi.org/10.1007/s10071-005-0012-5>
- Chittka, L., & Thomson, J. D. (1997). Sensori-motor learning and its relevance for task specialization in bumble bees. *Behavioral Ecology and Sociobiology*, *41*(6), 385–398.
<http://doi.org/10.1007/s002650050400>
- Chittka, L., Thomson, J. D., & Waser, N. M. (1999). Flower constancy, insect psychology, and plant evolution. *Naturwissenschaften*, *86*(8), 361–377.
<http://doi.org/10.1007/s001140050636>
- Colborn, M., Ahmad-Annuar, A., Fauria, K., & Collett, T. S. (1999). Contextual modulation of visuomotor associations in bumble-bees (*Bombus terrestris*). *Proceedings of the Royal Society B: Biological Sciences*, *266*(1436), 2413.
<http://doi.org/10.1098/rspb.1999.0940>

537 Courage, M. L., Bakhtiar, A., Fitzpatrick, C., Kenny, S., & Brandeau, K. (2015). Growing up
538 multitasking: The costs and benefits for cognitive development. *Developmental Review*,
539 35, 5–41. <http://doi.org/10.1016/j.dr.2014.12.002>

540 Dukas, R. (1998). *Cognitive ecology: The Evolutionary Ecology of Information Processing*
541 *and Decision Making*. (R. Dukas, Ed.). University of Chicago Press, Chicago.

542 Dukas, R. (2001). Limited attention: the constraint underlying search image. *Behavioral*
543 *Ecology*, 12(2), 192–199. <http://doi.org/10.1093/beheco/12.2.192>

544 Dukas, R. (2002). Behavioural and ecological consequences of limited attention.
545 *Philosophical Transactions of the Royal Society B: Biological Sciences*, 357(1427),
546 1539–1547. <http://doi.org/10.1098/rstb.2002.1063>

547 Dukas, R. (2008). Evolutionary biology of insect learning. *Annual Review of Entomology*,
548 53(1), 145–160. <http://doi.org/10.1146/annurev.ento.53.103106.093343>

549 Foerde, K., Poldrack, R., & Knowlton, B. (2007). Secondary-task effects on classification
550 learning. *Memory & Cognition*, 35, 864–874.

551 Francis, J. S., Muth, F., Papaj, D. R., & Leonard, A. S. (2016). Nutritional complexity and
552 the structure of bee foraging bouts. *Behavioral Ecology*, 27(3), 903–911
553 <http://doi.org/10.1093/beheco/arv229>

554 Ghazoul, J. (2006). Floral diversity and the facilitation of pollination. *Journal of Ecology*,
555 94(2), 295–304. <http://doi.org/10.1111/j.1365-2745.2006.01098.x>

556 Giurfa, M. (2007). Behavioral and neural analysis of associative learning in the honeybee: A
557 taste from the magic well. *Journal of Comparative Physiology. A, Neuroethology*,
558 *Sensory, Neural, and Behavioral Physiology*, 193(8), 801–24.
559 <http://doi.org/10.1007/s00359-007-0235-9>

560 Goulson, D. (2000). Are insects flower constant because they use search images to find
 561 flowers? *Oikos*, 88(3), 547–552. <http://doi.org/10.1034/j.1600-0706.2000.880311.x>

562 Goulson, D. (2003). *Bumblebees: Their Behaviour and Ecology*. Oxford, UK: OUP.

563 Grüter, C., Arenas, A., & Farina, W. M. (2008). Does pollen function as a reward for
 564 honeybees in associative learning? *Insectes Sociaux*, 55(4), 425–427.
 565 <http://doi.org/10.1007/s00040-008-1022-5>

566 Hagbery, J., & Nieh, J. (2012). Individual lifetime pollen and nectar foraging preferences in
 567 bumble bees. *Naturwissenschaften*, 99, 821–832.

568 Hargreaves, A., Harder, L., & Johnson, S. (2009). Consumptive emasculation: The ecological
 569 and evolutionary consequences of pollen theft. *Biological Reviews*, 84, 259–76.
 570 <http://doi.org/10.1111/j.1469-185X.2008.00074.x>.

571 Healy, S., & Rowe, C. (2010). Information processing: the ecology and evolution of
 572 cognitive abilities. In D. Westneat & C. Fox (Eds.), *Evolutionary Behavioral Ecology*.
 573 Oxford: OUP.

574 Heinrich, B. (2004). *Bumblebee Economics*. Harvard University Press.

575 Hofstede, F. E., & Sommeijer, M. J. (2006). Effect of food availability on individual foraging
 576 specialisation in the stingless bee *Plebeia tobagoensis* (Hymenoptera, Meliponini).
 577 *Apidologie*, 37(3), 387–397. <http://doi.org/10.1051/apido:2006009>

578 Jersáková, J., Johnson, S. D., & Kindlmann, P. (2006). Mechanisms and evolution of
 579 deceptive pollination in orchids. *Biological Reviews*, 81(2), 219–35.
 580 <http://doi.org/10.1017/S1464793105006986>

581 Leonard, A., Dornhaus, A., & Papaj, D. (2011a). Flowers help bees cope with uncertainty:
 582 signal detection and the function of floral complexity. *The Journal of Experimental*

583 *Biology*, 214, 113–121. <http://doi.org/10.1242/jeb.047407>

584 Leonard, A., Dornhaus, A., & Papaj, D. (2011b). Forget-me-not: complex floral displays,
 585 inter-signal interactions, and pollinator cognition. *Current Zoology*, 57, 215–224.

586 Leonard, A. S., & Masek, P. (2014). Multisensory integration of colors and scents: insights
 587 from bees and flowers. *Journal of Comparative Physiology A*, 200(6), 463–474.
 588 <http://doi.org/10.1007/s00359-014-0904-4>

589 Lewis, A. (1993). Learning and the evolution of resources: pollinators and flower
 590 morphology. In D. R. Papaj & A. Lewis (Eds.), *Insect Learning: Ecological and*
 591 *Evolutionary Perspectives* (pp. 219–242). Chapman & Hall, New York.

592 Mayntz, D. (2005). Nutrient-specific foraging in invertebrate predators. *Science*, 307(5706),
 593 111–113. <http://doi.org/10.1126/science.1105493>

594 Menzel, R. (2012). The honeybee as a model for understanding the basis of cognition. *Nature*
 595 *Reviews Neuroscience*, 13(11), 758–768. <http://doi.org/10.1038/nrn3357>

596 Menzel, R., & Giurfa, M. (2001). Cognitive architecture of a mini-brain: the honeybee.
 597 *Trends in Cognitive Sciences*, 5, 62–71.

598 Monsell, S. (2003). Task switching. *Trends in Cognitive Sciences*, 7(3), 134–140.
 599 [http://doi.org/10.1016/S1364-6613\(03\)00028-7](http://doi.org/10.1016/S1364-6613(03)00028-7)

600 Muth, F., Keasar, T., & Dornhaus, A. (2015). Trading off short-term costs for long-term
 601 gains: how do bumblebees decide to learn morphologically complex flowers? *Animal*
 602 *Behaviour*, 101, 191–199. <http://doi.org/10.1016/j.anbehav.2014.12.024>

603 Muth, F., Papaj, D. R., & Leonard, A. S. (2015). Colour learning when foraging for nectar
 604 and pollen: bees learn two colours at once. *Biology Letters*, 11(9), 20150628.
 605 <http://doi.org/10.1098/rsbl.2015.0628>

606 Muth, F., Papaj, D. R., & Leonard, A. S. (2016). Bees remember flowers for more than one
607 reason: pollen mediates associative learning. *Animal Behaviour*, *111*, 93–100.
608 <http://doi.org/10.1016/j.anbehav.2015.09.029>

609 Muth, F., Papaj, D. R., & Leonard, A. S. (2016b). Colour learning when foraging for nectar
610 and pollen: bees learn two colours at once. *Biology letters*, *11*(9), 20150628.

611 Nicholls, E., & Hempel de Ibarra, N. (2014). Bees associate colour cues with differences in
612 pollen rewards. *Journal of Experimental Biology*, *217*(15), 2783–2788.
613 <http://doi.org/10.1242/jeb.106120>

614 Nicolson, S. W. (2011). Bee food: the chemistry and nutritional value of nectar, pollen and
615 mixtures of the two: review article. *African Zoology*, *46*, 197–204.
616 <http://doi.org/10.3377/004.046.0201>

617 O'Donnell, S., Reichardt, M., & Foster, R. (2000). Individual and colony factors in bumble
618 bee division of labor (*Bombus bifarius nearcticus* Handl; Hymenoptera, Apidae).
619 *Insectes Sociaux*, *47*(2), 164–170. <http://doi.org/10.1007/PL00001696>

620 Page, R., Scheiner, R., Erber, J., & Amdam, G. (2006). The development and evolution of
621 division of labor and foraging specialization in a social insect (*Apis mellifera* L.).
622 *Current Topics in Developmental Biology*, *74*, 253–286. [http://doi.org/10.1016/S0070-](http://doi.org/10.1016/S0070-2153(06)74008-X)
623 [2153\(06\)74008-X](http://doi.org/10.1016/S0070-2153(06)74008-X)

624 Pashler, H. (1994). Dual-task interference in simple tasks: data and theory. *Psychological*
625 *Bulletin*, *116*, 220–244.

626 Pohl, M., Watolla, T., & Lunau, K. (2008). Anther-mimicking floral guides exploit a conflict
627 between innate preference and learning in bumblebees (*Bombus terrestris*). *Behavioral*
628 *Ecology and Sociobiology*, *63*, 295–302. <http://doi.org/10.1007/s00265-008-0661>

629 Raguso, R. A. (2004). Flowers as sensory billboards: progress towards an integrated
630 understanding of floral advertisement. *Current Opinion in Plant Biology*, 7(4), 434–440.
631 <http://doi.org/10.1016/j.pbi.2004.05.010>

632 Raine, N. E., & Chittka, L. (2008). The correlation of learning speed and natural foraging
633 success in bumble-bees. *Proceedings of the Royal Society of London, Series B:*
634 *Biological Sciences*, 275(1636), 803–808. <http://doi.org/10.1098/rspb.2007.1652>

635 Raubenheimer, D., & Tucker, D. (1997). Associative learning by locusts: pairing of visual
636 cues with consumption of protein and carbohydrate. *Animal Behaviour*, 54(6), 1449–59.
637 <http://doi.org/10.1006/anbe.1997.0542>

638 Renner, S. (2006). Rewardless flowers in the angiosperms and the role of insect cognition in
639 their evolution. In N. Waser & J. Ollerton (Eds.), *Plant-Pollinator Interactions: From*
640 *Specialization to Generalization* (p. 123–144.). Chicago, USA: University of Chicago
641 Press.

642 Russell, A. L., Golden, R. E., Leonard, A. S., & Papaj, D. R. (2015). Bees learn preferences
643 for plant species that offer only pollen as a reward. *Behavioral Ecology*, 27: 731-740.
644 <http://doi.org/10.1093/beheco/arv213>

645 Russell, A. & Papaj, D. (2016). Artificial pollen dispensing flowers and feeders for bee
646 behaviour experiments. *Journal of Pollination Ecology*, 18, 13–22.

647 Shettleworth, S. J. (2010). *Cognition, Evolution, and Behavior* (2nd ed.). Oxford, UK:
648 Oxford University Press.

649 Simpson, S. J., Sibly, R. M., Lee, K. P., Behmer, S. T., & Raubenheimer, D. (2004). Optimal
650 foraging when regulating intake of multiple nutrients. *Animal Behaviour*, 68(6), 1299–
651 1311. <http://doi.org/10.1016/j.anbehav.2004.03.003>

652 Simpson, S., & Raubenheimer, D. (2012). *The Nature of Nutrition: a Unifying Framework*
653 *from Animal Adaptation to Human Obesity*. Princeton University Press.

654 Sulikowski, D., & Burke, D. (2007). Food-specific spatial memory biases in an omnivorous
655 bird. *Biology Letters*, 3(3), 245–248. <http://doi.org/10.1098/rsbl.2007.0122>

656 Velthuis, H. H. W., & van Doorn, A. (2006). A century of advances in bumblebee
657 domestication and the economic and environmental aspects of its commercialization for
658 pollination. *Apidologie*, 37(4), 421–451. <http://doi.org/10.1051/apido:2006019>

659 Vergoz, V., Roussel, E., Sandoz, J.-C., & Giurfa, M. (2007). Aversive learning in honeybees
660 revealed by the olfactory conditioning of the sting extension reflex. *PLoS ONE*, 2(3),
661 e288. <http://doi.org/10.1371/journal.pone.0000288>

662 Waldron, E. M., & Ashby, F. G. (2001). The effects of concurrent task interference on
663 category learning: Evidence for multiple category learning systems. *Psychonomic*
664 *Bulletin & Review*, 8(1), 168–176. <http://doi.org/10.3758/BF03196154>

665 Waser, N. (1983). The adaptive nature of floral traits: ideas and evidence. In L. Real (Ed.),
666 *Pollination Biology* (pp. 241–285). Academic Press, Orlando, Florida.

667 Waser, N. (1986). Flower constancy: definition, cause, and measurement. *American*
668 *Naturalist*, 127, 593–603.

669 Weiss, M. R., & Papaj, D. R. (2003). Colour learning in two behavioural contexts: How
670 much can a butterfly keep in mind? *Animal Behaviour*, 65(3), 425–434.
671 <http://doi.org/10.1006/anbe.2003.2084>

672 Worden, B. D., Skemp, A. K., & Papaj, D. R. (2005). Learning in two contexts: the effects of
673 interference and body size in bumblebees. *The Journal of Experimental Biology*, 208(Pt
674 11), 2045–53. <http://doi.org/10.1242/jeb.01582>

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Figure1

Experiment 1:

Bee learns nectar association, with or without simultaneously collecting pollen

Treatment 1

One flower rewarding for nectar, no pollen

Treatment 2

One flower rewarding for nectar, pollen on all flowers

Experiment 2a:

Bee learns pollen association, with or without simultaneously collecting nectar

Treatment 1

One flower rewarding for pollen, no nectar

Treatment 2

One flower rewarding for pollen, nectar on all flowers

Experiment 2b:

Was the impairment to pollen learning in Expt 2a due to interference?

Treatment 1

Yellow flowers rewarding for pollen, no nectar

Treatment 2

Yellow flowers rewarding for pollen and nectar

Treatment 3

Yellow flowers rewarding for pollen, blue for nectar

Figure2

Figure3

Figure4

Table 1 ppt

Reward	(Nectar ● Pollen ●)	Examples	Reference
Nectar only		<i>Asclepias</i> Orchidaceae	Pleasants and Chaplin (1983) Sun, Huang, Yu, and Kou (2011)
Pollen only		<i>Pyrola</i> <i>Solanum</i> <i>Dodecatheon</i> <i>Papaver</i>	Knudsen and Olesen (1993) Buchmann (1983) Harder and Barclay (1994) Raine and Chittka (2007)
Various combinations of nectar and pollen		<i>Aster</i> Apiaceae Cucurbitaceae <i>Salix</i>	Niesenbaum, Patselas, and Weiner (1999) Langenberger and Davis (2002) Nepi, Guarnieri, and Pacini (2001) Mosquin (1971)
Transition from nectar and pollen to pollen only (protogyny)		<i>Campanula rotundiflora</i> <i>Erythronium grandiflorum</i>	Cresswell and Robertson (1994) Thomson (1986)
Transition from nectar and pollen to nectar only (protandry)	AM PM	<i>Lavandula stoechas</i> <i>Phacelia linearis</i> <i>Alstroemeria aurea</i> <i>Trachymene incisa</i>	Gonzalez et al. (1995) Eckhart (1991) Aizen and Basilio (1998) Davila and Wardle (2007)
One reward type becomes temporally available		<i>Rhus hirta</i> Sudworth <i>Aralia hispida</i> <i>Lavandula latifolia</i>	Greco, Holland, and Kevan (1996) Thomson, McKenna, and Cruzan (1989) Herrera (1990)