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Multiple rewards have asymmetric effects on learning in bumblebees

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

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 multifaceted 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 are due to learning a second association generally, or learning a second association that conflicts with the first (i.e. that a given stimulus is 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 resource versus multiple resources is

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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 that plants offer pollinators (e.g. resins, oils, oviposition sites, etc.; Armbruster, 2011; Renner, 2006), 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 laboratory-based studies.

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

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

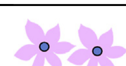
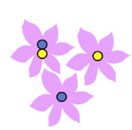
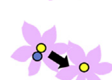
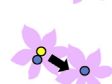
If collecting multiple types of reward impairs learning, then we expected that bees collecting two reward types would find it more difficult to learn an association between a given reward type and a floral feature than bees learning this association in isolation. Alternatively, since individual bumblebees 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 (experiment 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 11 colonies of *B. impatiens* (Koppert Biological Systems, Howell, MI, U.S.A.) represented equally across treatments within each experiment, with at least 11 subjects per treatment included in the final analysis (for sample sizes see Appendix, Table A1). Colonies were connected to a central foraging arena (L × W × H: 122 × 59 × 59 cm) where all

Table 1
Examples of plant strategies for offering nectar and pollen as pollinator rewards

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

The majority of research on bee cognition approximates the 'nectar only' reward scenario.