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Osmia bees (Hymenoptera: Megachilidae) can detect nectar-rewarding flowers using olfactory cues

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Nectar-foraging bees frequently face the choice of which flowers to visit and which to avoid. One possible mechanism by which bees could discriminate between flowers before visiting them is by detecting nectar via its odours. To test this idea, we observed visits by solitary bees in the genus *Osmia* (Megachilidae) to flowers of *Penstemon caesius* (Scrophulariaceae) in the San Bernardino Mountains of southern California. We observed that free-foraging *Osmia* bees visited flowers containing nectar seven times more frequently than they visited nectar-depleted flowers. To test whether bees could detect the presence of nectar via odour cues, we compared floral preferences between trials where we blocked the olfactory capabilities of bees by coating their antennae with nontoxic silicone and where bees foraged with uncovered antennae. We randomly assigned the order of the silicone treatments and attempted to test 32 bees at *P. caesius* arrays containing nectar-depleted flowers, nectar-depleted flowers with added water and nectar-rewarding control flowers. Bees with uncovered antennae visited more than twice as many control flowers as they did either group of nectar-depleted flowers. In contrast, bees foraging with silicone-covered antennae visited all treatment flowers equally. Bees that completed both trials visited nectar-rewarding control flowers twice as frequently while foraging with uncovered antennae as they did while foraging with silicone-coated antennae. These results are consistent with the idea that solitary *Osmia* bees are capable of perceiving nectar volatiles to identify nectar-rewarding *Penstemon* flowers.

Keywords: direct detection; nectar; nectar foraging; nectar scent; olfactory cue; *Osmia*; *Penstemon*; solitary bee

There is an inherent conflict of interest between the species involved in mutualistic interactions. Participants frequently attempt to reap the benefits of such interactions by exploiting their partners while minimizing the costs of providing a reward or service (Bronstein 2001). In pollination, this conflict has undoubtedly influenced the evolution of 'honest signals', indicating the presence of rewards, and the sensory and cognitive machinery pollinators require to perceive them (Raguso 2004a). To attract pollinators, plants display 'sensory billboards', which are context-dependent combinations of visual, olfactory, gustatory or tactile signals that direct foraging animals to flowers and rewards (Raguso 2004a). These signals are extremely important for bees (Hymenoptera, Apoidea), since most species completely rely on pollen and nectar to provision offspring and feed themselves (Michener 2000). Bees must frequently decide which flowers to visit given the heterogeneity of nectar volumes, nectar concentrations and pollen quantities available within a patch (Heinrich 1979). If a bee could identify and avoid unrewarding flowers before visiting them, it could increase its fitness by simultaneously reducing foraging costs and spend more time protecting its nest from predators and parasites (Goodell 2003).

Although it has been suggested that honeybees (*Apis mellifera*) and bumblebees (*Bombus* spp.) are capable of discriminating between rewarding and nonrewarding flowers within a patch, the mechanisms by which this is accomplished are not entirely clear. Some evidence suggests that bees can visually detect the presence of nectar or pollen in flowers (Thorp et al. 1975) or perceive changes in flower colour associated with rewards (Weiss 1991; Nuttman et al. 2006). However, a growing number of studies

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suggest that honeybees and bumblebees primarily rely on olfactory cues. There are two ways that olfactory signals are thought to guide bees to nectar-rewarding flowers: direct detection, in which an individual 'smells' the presence of rewards, and scent marking, where a bee deposits a volatile substance onto a flower that conveys information about the quality of the reward.

It is now recognized that nectar is frequently scented, and in some cases, emits unique compounds compared to other floral tissues (Raguso 2004b). However, there are only a few reports of bumblebees or honeybees directly detecting the presence of nectar through smell (Heinrich 1979; Marden 1984; Goulson et al. 2001). Most reports instead suggest that bumblebees and honeybees use deterrent and/or attractive scent marks on flowers. Deterrent scent marks are short-term, volatile signals that discourage bees from probing recently visited flowers, with the signal sometimes wearing off about the time when nectar has been replenished (Corbet et al. 1984; Wetherwax 1986; Giurfa & Nuñez 1992; Giurfa 1993; Goulson et al. 1998; Stout et al. 1998; Williams 1998). Attractive scent marks are typically composed of long-lasting volatiles that allow bees to relocate highly rewarding flowers (Cameron 1981; Schmitt & Bertsch 1990). There is some evidence suggesting that honeybees and bumblebees are capable of using both short-term deterrent and long-term attractive signals when foraging on a particular resource (Free & Williams 1983; Kato 1988; Stout & Goulson 2001). For example, Stout & Goulson (2001) report that honeybees avoid visiting *Melilotus officinalis* (Fabaceae) flowers within 40 min following a bumblebee or honeybee visit, but they are more likely to visit the same flower 24 h later than they are to visit a flower that has never been visited before.

Compared to honeybees and bumblebees, significantly less is known about the foraging behaviour and sensory capabilities of solitary bees, including how they locate nectar-rewarding flowers within a patch. Some solitary bees do deposit deterrent scent marks on recently visited flowers when foraging for nectar (Frankie & Vinson 1979; Gilbert et al. 2001; Gawleta et al. 2005), but it is unlikely that all solitary species use such scent marking, given their diverse behaviours and biology (Linsley 1958). Although there is some evidence that honeybees and bumblebees can detect nectar via olfactory cues, to our knowledge, there is no evidence of nectar detection via olfaction for solitary bees. There is, however, evidence suggesting that solitary bees are capable of detecting pollen in flowers via odour cues (Dobson 1987; Dobson & Bergström 2000; Goulson et al. 2001). Given that nectar is frequently scented, and solitary bees are capable of directly detecting pollen via odour, it seems highly probable that some solitary bee species are able to detect the presence of nectar via odour cues. To test this idea, we first observed the foraging behaviour of free-flying solitary *Osmia* bees (Hymenoptera, Megachilidae) at flowers in which we had manipulated the presence of nectar, then we manipulated the olfactory capabilities of bees to determine whether they used nectar odour cues to discriminate between nectar-rewarding and nonrewarding flowers.

METHODS

Study System

This study was conducted near Bluff Lake (34°13.2′N, 116°57.5′W; ~2300 m) in the San Bernardino Mountains of southern California, in a clearing within the yellow pine forest (*Pinus jeffreyi* and *P. ponderosa*). The focal plant, *Penstemon caesius* Gray (Scrophulariaceae), is a mat-like perennial herb that grows in patches on sandy/rocky soils (Hickman 1993). Plants often produce multiple inflorescences with horizontally oriented, tubular, blue-purple flowers ($\overline{X} \pm$ SE corolla length $= 14.9 \pm 0.2$ mm; corolla width $= 5.1 \pm 0.1$ mm; corolla height $= 4.7 \pm 0.3$ mm, $N = 16$). The protandrous flowers stay open for 3–4 days, and typically remain in the male phase for the first 2 days (personal observation). We calculated the mean ± SE volume of nectar, which is located at the base of the corolla, for unvisited male-phase flowers (0.97 ± 0.06 μl, $N = 17$ plants).

Bees are the primary visitors to *P. caesius* flowers, although in patches adjacent to meadows and riparian zones, they are frequently visited by the generalist hawkmoth *Hyles lineata* Fabricius (Sphingidae; personal observation). Honeybees and small bumblebee workers rarely visit flowers because the corolla aperture is generally too narrow for them to enter to gather nectar. Thus, most visits were from solitary bees in the genera *Osmia* and *Heriades* (Megachilidae). *Osmia* and *Heriades* bees visited *P. caesius* flowers primarily for nectar by entering the corolla and crawling to the base. In the few instances that we observed *Osmia* bees collect pollen, they did not crawl into the flower, but instead stood at the entrance and harvested pollen from anthers located just below the dorsal lip of the corolla. For this study, we decided to focus on the nectar foraging behaviour of female *Osmia* bees because they were abundant in 2002 and easy to observe; male *Osmia* bees were rarely encountered visiting *P. caesius* flowers. At least seven species of *Osmia* visited *Penstemon* flowers at our study site: *O. bella* Cresson, *O. bruneri* Cockerell, *O. calla* Cockerell, *O. juxta* Cresson, *O. laeta* Sandhouse, *O. nifoata* Cockerell and *O. pusilla* Cresson.

2002 Experiments

To explore whether *Osmia* bees can detect nectar-depleted flowers, we allowed free-flying bees to visit nectar-manipulated flowers during focal plant observations. In early July 2002, we caged 10 plants, each containing several inflorescences with unopened buds, using wire cages covered with nylon mesh to prevent insect visits. When two or more male-phase flowers were available on a plant, we randomly assigned them to receive either the control or nectar-depleted treatment. Depleted flowers had their nectar removed using a microsyringe or 2-μl capillary tubes and filter paper wicks (Whatman's no. 1). Control flowers were also handled in a similar fashion, in that they were probed using the microsyringe or capillary tubes and wicks but nectar was not removed.

We performed nectar manipulations just before each observation period, and we discarded the flowers at the