Naturwissenschaften (2014) 101:523–526
DOI 10.1007/s00114-014-1188-9

SHORT COMMUNICATION

The innate responses of bumble bees to flower patterns: separating the nectar guide from the nectary changes bee movements and search time

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Received: 19 January 2014 / Revised: 16 May 2014 / Accepted: 20 May 2014 / Published online: 31 May 2014

Abstract Nectar guides can enhance pollinator efficiency and plant fitness by allowing pollinators to more rapidly find and remember the location of floral nectar. We tested if a radiating nectar guide around a nectary would enhance the ability of naïve bumble bee foragers to find nectar. Most experiments that test nectar guide efficacy, specifically radiating linear guides, have used guides positioned around the center of a radially symmetric flower, where nectaries are often found. However, the flower center may be intrinsically attractive. We therefore used an off-center guide and nectary and compared “conjunct” feeders with a nectar guide surrounding the nectary to “disjunct” feeders with a nectar guide separated from the nectary. We focused on the innate response of novice bee foragers that had never previously visited such feeders. We hypothesized that a disjunct nectar guide would conflict with the visual information provided by the nectary and negatively affect foraging. Approximately, equal numbers of bumble bees (*Bombus impatiens*) found nectar on both feeder types. On disjunct feeders, however, unsuccessful foragers spent significantly more time (on average 1.6-fold longer) searching for nectar than any other forager group. Successful foragers on disjunct feeders approached these feeders from random directions unlike successful foragers on conjunct feeders, which preferentially approached the combined nectary and nectar guide. Thus, the nectary and a surrounding nectar guide can be considered a combination of two signals that attract naïve foragers even when not in the floral center.

Keywords Nectar guides · Bee foraging · Orientation · Navigation · Pollination · Floral constancy

Introduction

Sprengel (1793) first suggested that contrasting floral patterns, such as dots or radiating lines surrounding the nectary, hereafter referred to as a “nectar guide”, assist pollinators in finding floral nectar. Multiple studies have found evidence for this hypothesis (reviewed in Dafni and Giurfa 1999). Nectar guides can benefit plant and pollinator, increasing plant fitness (Waser and Price 1983) and the processing speed and potential energetic gain of pollinators (Lunau 1991; Lunau et al. 2006; Leonard and Papaj 2011; Leonard et al. 2013).

Recent work has identified complex multi-component, visual and olfactory signaling in flower patterns (Dötterl and Jürgens 2005; Leonard et al. 2011). Even within a single modality, such as vision, attractive floral visual signals can have multiple components (Leonard et al. 2011). For example, the nectar guide often surrounds the nectary in radially symmetric flowers (Biesmeijer et al. 2005; Leonard and Papaj 2011), and both nectar guide and nectary can facilitate visual orientation. In addition, the center of a flower can be attractive to bees, particularly if the center is darker (Biesmeijer et al. 2005). This attraction has likely evolved because nectaries are usually in the center of radially symmetric (actinomorphic) flowers, a common morphology (83 and 72 % of dicot and monocot families, respectively, are actinomorphic; Neal et al. 1998).

Communicated by: Sven Thatje

Electronic supplementary material The online version of this article (doi:10.1007/s00114-014-1188-9) contains supplementary material, which is available to authorized users.

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Can the attraction of nectary and nectar guide be tested separately from the potential attraction of the floral center? Previous experiments focusing on linear nectar guides have not investigated this question because the nectar guide and nectaries (or dots representing the nectaries) were presented together, the nectar guide surrounded the floral center, or both conditions were present (Manning 1956; Free 1970; Dinkel and Lunau 2001; Leonard and Papaj 2011; Leonard et al. 2013).

We therefore tested if a radiating nectar guide around a nectary allows foragers to find the nectary more easily, even when these elements are not near the floral center. We made an artificial flower (feeder) in which these two visual elements competed for a forager's attention. We created "disjunct" feeders in which the nectar guide was separated from the nectar and "conjunct" feeders in which the radiating lines of the nectar guide surrounded the nectary (Fig. 1), as they often do occur in nature. To eliminate the potentially attractive effect of the flower center, the nectary and nectar guide were off-center on the conjunct feeder. On the disjunct feeder, these two elements were separated and off-center.

Methods

We tested the behavior of foragers from three successive *Bombus impatiens* colonies towards a 10-cm square feeder placed horizontally on the bottom of a foraging arena. On top, we attached a printed label: a blue circle, representing the flower, on a green background. The feeder was either conjunct or disjunct (Fig. 1), and the nectar guide, based upon Leonard and Papaj (2011), consisted of four white lines (each 10× 2 mm wide). The nectary was a 6-mm diameter well drilled into the plastic feeder and appeared dark against the blue flower because of shadows cast by the well wall (see Supplement). Into the nectary, we placed 1.5-M unscented analytical-grade sucrose solution, which bumble bees cannot smell (Kunze and Gumbert 2001). To eliminate social copying, we only used a naïve bee's first visit if it was made in the absence of other bees. After each visit, we cleaned the feeder to remove potential odor marks. We recorded the amount of time a naïve bee spent on the blue flower during its first floral visit and the direction from which it first crossed into the blue flower. Directly approaching the nectar guide was defined as 0°. We tested if the feeder treatment (conjunct or disjunct) influenced bee ability to find the nectary with a χ^2 test. We used circular statistics (Rayleigh's Z test and a V test for a unimodal circular distribution) to see if treatment or success influenced bee approach angle. Finally, we analyzed if treatment or success influenced time spent on the blue flower with a generalized linear model (GLM). Detailed methods are in the Supplement.

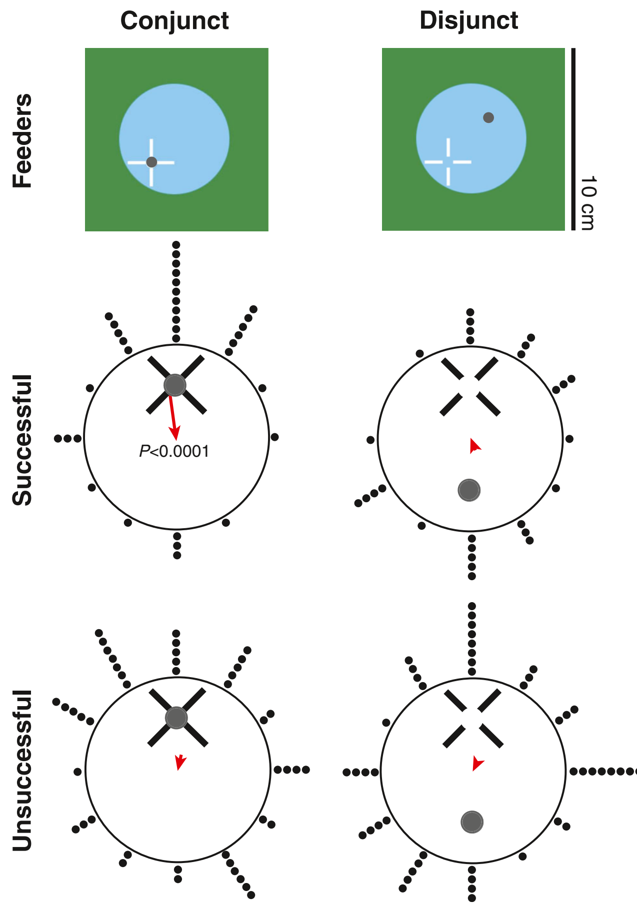

Fig. 1 The approach orientations of successful and unsuccessful bumble bee (*B. impatiens*) foragers towards conjunct and disjunct feeders. *White lines* on the feeders show the nectar guide, and the *gray circle* (see Supplement) is the nectary. *Each black dot* represents a different bee. The nectar guide was defined as the 0° position on conjunct and disjunct feeders. The *arrows* show mean vector magnitude (length relative to circle radius) and approach direction (Table S1). Only successful foragers approaching the conjunct feeders showed a distribution significantly different from random

Results

Bees took a variety of paths, but were often attracted to the nectar guide, circling and inspecting it closely (see Fig. S1). Most bees walked onto the feeder like bumble bees conducting walking nectar searches between large natural inflorescences (Pyke 1980; Thomson and Plowright 1980; Thomson 1986). On disjunct feeders, 96 % of bees approached the nectary or the nectar guide (crossed over the circular outer boundary defined by the nectar guide or an equally sized circle surrounding the nectary). Of these approaching bees (n=74), exactly 50 % approached the nectar guide first, demonstrating that both elements were equally attractive. Foraging success, however, was not affected by the spatial configuration of nectar guide and nectary. On the conjunct feeders, 43.8 % of foragers (n=80) successfully found nectar, while on the disjunct feeders, 35.1 % of foragers

(n=77) were successful (not a significant difference, χ_1^2= 1.03, P=0.31).

However, the spatial configuration of nectar guide and nectary affected bee approaches (Fig. 1). On the conjunct feeders, successful foragers crossed the blue floral circle at a mean angle of 352°. This approach distribution is non-uniform (P<0.0001, Table S1) and is significantly similar to 0° (V=3.95, 34 d.f., P<0.0001), the closest approach to nectary and nectar guide on the conjunct feeders. Unsuccessful foragers approached the conjunct feeders from random directions (P=0.41). On the disjunct feeders, successful and unsuccessful foragers approached the flower from random directions (P=0.20). Separating nectary and nectar guide evidently disrupted forager approaches.

We hypothesized that search times on a disjunct feeder would increase because the nectar guide should compete with the nectary for the bees' attention. Indeed, there is a significant interaction of feeder type × foraging success (GLM, χ_1^2= 4.50, P=0.03) on blue flower search times (feeder treatment has no overall effect, χ_1^2=1.93, P=0.16, although foraging success does, χ_1^2=8.11, P=0.004). There is no significant colony effect (χ_2^2=4.35, P=0.11). Essentially, bees that failed to find nectar on the disjunct feeder spent significantly more time searching on the flower as compared to all other groups (Fig. 2, contrast test, L-R χ_1^2=14.53, P=0.0001). On the conjunct feeders, there is no significant difference between the search times of successful vs. unsuccessful foragers (contrast test, L-R χ_1^2=0.34, P=0.56).

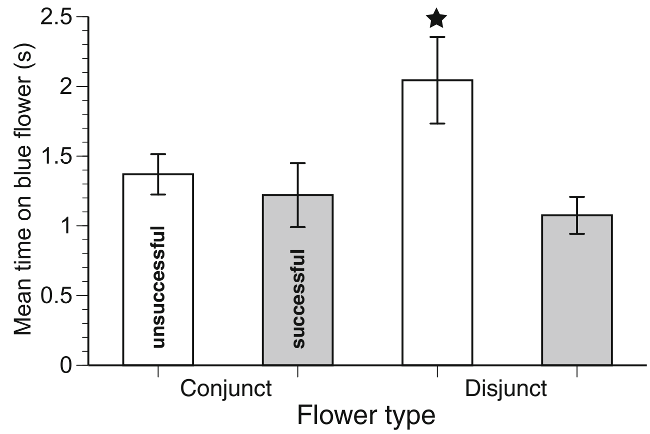

Fig. 2 The effect of feeder type on the amount of time that successful (*gray bars*) and unsuccessful bumble bee (*B. impatiens*) foragers (*white bars*) spent on conjunct and disjunct feeders. The *star* shows the group that is significantly different from all other groups. Each group consists of a different set of bees and the choice of each bee was tested only once. *Standard error bars* are shown

Discussion

Other studies have demonstrated the attraction of bees to a nectar guide consisting of radiating lines around the center of a flower relative to a flower without such lines (Manning 1956; Free 1970; Dinkel and Lunau 2001; Leonard and Papaj 2011; Leonard et al. 2013). Our study has three distinguishing features: (1) testing an off-center nectar guide, (2) separating the attraction of nectary and nectar guide by creating a disjunct feeder which provides information conflict, and (3) using bees that were not pre-trained and whose responses should thus indicate innate preferences (see Supplement). We demonstrate that such radiating lines can attract the attention of naïve bees even when not centered in the flower (an inherently attractive position; Biesmeijer et al. 2005) and not connected to a visible nectary (see Supplement about the visibility of the nectary in this and past research).

Our results show that the separation of nectar guide and nectary on the disjunct feeders confused bees in two ways. Successful bees did not directly approach the nectary (unlike their behavior on conjunct flowers), and unsuccessful bees spent significantly more time on the disjunct feeder than bees in any other situation. On the conjunct feeder, successful foragers surprisingly did not find the nectary faster than unsuccessful foragers. Overall success was also not affected by the spatial configuration of nectar guide and nectary. The stronger result of Leonard and Papaj (2011), who showed a significant increase in foraging success, may arise from the combination of a nectary and surrounding radial nectar guide in the floral center and the use of pre-trained foragers (see Supplement). Our results may also differ because, in our experiment, bees primarily walked onto the feeder. However, multiple bumble bee species are known to search by walking between large natural inflorescences, particularly when inflorescences are sufficiently close together (Pyke 1980; Thomson and Plowright 1980; Thomson 1986; see Supplement).

Considering nectary and the nectar guide as separately attractive floral elements may be useful because it expands our current understanding of multi-component floral signals and how they evolve. Usually, we think of such components as being in different sensory modalities (Hebets and Papaj 2005), i.e., the visual and olfactory components of nectar guides (Dötterl and Jürgens 2005; Leonard et al. 2011). Understanding multiple components of signals is important because combined information can influence the speed, accuracy, or both of pollinator decisions (Kulahci et al. 2008). Also, manipulating the elements of an intricate visual signal and testing the innate preferences of naïve bee may be useful for learning more about the complex phenomenon of floral nectar guides.

Acknowledgments We thank A. S. Leonard for her helpful comments during project conception, Tess Benjamin for her hard work on these experiments, and three anonymous reviewers for improving the manuscript. Stephen Mayfield kindly loaned us a light meter to measure nectary darkness. This research was supported by a Blasker Science and Technology grant from the San Diego Science Foundation and the 1000

Plan Recruitment Program from the People's Republic of China to EG, and by a UC Academic Senate Grant to JCN.

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