Host location by visual and olfactory floral cues in an oligolectic bee: innate and learned behavior

Paulo Milet-Pinheiro, a,b Manfred Ayasse, b Clemens Schlindwein, c Heidi E. M. Dobson, d and Stefan Dötterl

aDepartment of Botany, Federal University of Pernambuco, Av. Prof. Moraes Rego, s/n, 50670-901, Recife, Brazil, bInstitute of Experimental Ecology, University of Ulm, Albert-Einstein-Allee 11, 89069 Ulm, Germany, cDepartment of Botany, Federal University of Minas Gerais, Av. Antônio Carlos, 6627, 31270-901 Belo Horizonte, MG, Brazil, dDepartment of Biology, Whitman College, 345 Boyer Ave, Walla Walla, WA 99362, USA, and eDepartment of Plant Systematics, University of Bayreuth, Universitätstrasse 30, 95447 Bayreuth, Germany

Oligolectic bees collect pollen from only a few related plant taxa, and our understanding of both the bees’ innate and learned behavior in host-plant recognition is incomplete. For the oligolectic bee Chelostoma rapunculi, whose host plants are within Campanula, we conducted choice tests on foraging-naïve individuals to investigate the bee’s innate preference for visual and olfactory floral cues of its host plants over those of nonhost plants. In addition, we tested both foraging-naïve and foraging-experienced individuals to determine the relative importance of these 2 sensory modalities in the bee’s innate and learned host-flower location. Visual and olfactory cues of Campanula trachelium flowers, both separately and combined, attracted significantly more foraging-naïve bees than equivalent cues of nonhost plants. Furthermore, for both foraging-naïve and -experienced bees, the visual cues of host plants were more attractive than the olfactory ones, and the 2 cues combined attracted more bees than either alone. In foraging-naïve bees, visual and olfactory cues alone elicited almost exclusively approaches, whereas after the bees gained foraging experience, landings became more frequent in response to visual cues but not to olfactory cues; in both bee groups, the combination of visual and olfactory cues was most effective in promoting landings. We conclude that Ch. rapunculi has an innate preference for the floral cues of its host plants over those of nonhost species and that both foraging-naïve and foraging-experienced bees integrate visual and olfactory cues to find their host flowers, with a slightly greater reliance on visual cues in bees with foraging experience.

Key words: Campanula, Chelostoma, floral cues, foraging behavior, host location, specialized bees.

INTRODUCTION

Bees are the main pollinators of flowering plants and visit flowers to feed on nectar (for energy) and pollen (for protein) and to collect both as provisioning for their offspring (Michener 1974; Roubik 1989). Although some bee species are polylectic and collect pollen from plants of different families, oligolectic bees restrict their pollen gathering (used to feed the larvae) to species in the same genus or family (Robertson 1925; Cane and Sipes 2006). The phenomenon of oligolecty raises several interesting questions related to the ecology, evolution, and physiology of bees (Wcislo and Cane 1996), including the fundamental question: which floral cues do oligolectic bees use to find and recognize their host flowers?

Visual and olfactory cues have been shown to be important for attracting generalist bees, such as the honey bee or bumble bees, to flowers (Chittka and Raine 2006; Wright and Schiestl 2009). In terms of visual cues, bees are known to rely first on the green contrast between background and target in order to detect a flower (Giurfa and Lehrer 2001; Spaethe et al. 2001). Only at close range (up to 1 m away, depending on flower size), they can detect color, color patterns, and flower shape, all of which can elicit flight toward the flower followed by landing responses (Lunau 1991, 1992, 1995, 2000; Lunau and Maier 1995; Giurfa and Lehrer 2001; Simonds and Ploewright 2004; Chittka and Raine 2006). Olfactory cues, on the other hand, can attract bees both at close range (Dobson and Bergström 2000) and from far away, in some cases over more than 1 km, such as in male euglossine bees (Dressler 1982). Olfactory cues are often of major importance to foraging-naïve generalist bees or bees searching for new food plants (Dötterl and Vereecken 2010). For example, honey bee workers foraging for the first time cannot be attracted by color stimuli alone and instead rely on olfactory stimuli (Giurfa et al. 1995). Experienced generalist bees, however, typically seem to rely on a combination of visual and olfactory cues to locate their (known) host plants (Dötterl and Vereecken 2010).

To date, only a few studies have been conducted to evaluate which signals are used by oligolectic bees to find their specific pollen host-plant species (Dötterl and Vereecken 2010). Dobson and Bergström (2000) reported that in unpublished studies, foraging-naïve individuals of the narrowly oligolectic bee Chelostoma floridosumne (Megachilidae), which is specialized on yellow-flowered Ranunculus species (Ranunculaceae), did...
not respond to olfactory cues without an accompanying yellow visual stimulus. The bees in fact relied on a combination of visual cues and the uncommon floral scent compound protanemonin, which dominates the pollen odor, to recognize their host flowers. However, after gaining foraging experience, the bees became more responsive to the complex volatile blend of the whole flower, thus displaying a sharp contrast to the innate preference of foraging-naïve individuals for one particular scent compound. In another oligolectic bee, Hoplitis adunca (Megaschilidae), Burger et al. (2010) showed that visual cues of the host flower Echium vulgare were attractive to foraging-naïve bees, whereas olfactory cues alone were not. However, bees could not discriminate host from nonhost plants based on visual cues alone; olfactory cues were necessary for them to distinguish flowers of E. vulgare from those of a closely related nonhost plant, Anchusa officinalis. In the oligolectic Macropsis fulvipes (Melittidae), which is specialized on Lysimachia, olfactory but not visual floral cues alone were attractive to Lysimachia-naïve females, although the 2 cues combined elicited the greatest attraction (Dötterl et al. 2011). After gaining foraging experience, however, the bees’ behavior changed in that they responded to both visual and olfactory cues alone; the combined cues remained more attractive than the single ones. Collectively, these 3 studies show that a combination of visual and olfactory floral cues seems to be most attractive to oligolectic bees; however, the relative importance of the different cues varies among species. Although Ch. florismet and H. adunca responded only to olfactory cues in the presence of visual ones, it was vice versa in M. fulvipes, which responded only to visual cues in the presence of olfactory ones. This suggests that each oligolectic bee–flower association needs to be investigated within its own biological and ecological context (Dobson and Bergström 2000).

Chelostoma rapunculi is one of several narrowly oligolectic bees that gather pollen only from flowers of Campanula species (Westrich 1989; Schlindwein et al. 2005). However, there is a lack of information concerning both the bee’s innate preferences for floral cues of Campanula species over those of nonhost plants and the nature of the floral cues used by the bee to locate its host flowers. In this study, we addressed these 2 aspects of host-flower location by rearing Ch. rapunculi bees and performing several behavioral dual-choice experiments within a large flight cage. First, to test the hypothesis that Ch. rapunculi has an innate preference for the olfactory and/or visual cues of Campanula trachelium over those of the nonhost plants E. vulgare and Potentilla recta, we offered foraging-naïve bees various pairwise combinations of the flowers. Second, to determine the relative attractiveness of visual and olfactory floral cues to the bee, we investigated whether visual and olfactory cues of Ca. trachelium, when offered singly or combined, elicited different behavioral responses, in both foraging-naïve and foraging-experienced bees. This also allowed us to clarify the extent to which any innate behavior in foraging-naïve bees is modified by learning when bees gain foraging experience on their host flowers.

**MATERIALS AND METHODS**

**The bee**

Chelostoma rapunculi (Lepeletier 1841) (Figure 1A) is a univoltine solitary bee species that is widespread in central Europe and active from early June to late August. It nests in preexisting cavities in wood and readily accepts wood or twig trap nests. This bee is a pollen specialist on Campanula; although flowers of Campanula species are the only pollen sources for females, both females and males visit mainly Campanula for nectar (Westrich 1989; Schlindwein et al. 2005). Bees used for this study were acquired from trap nests placed in natural habitats in Göttingen and Knüllwald, Germany.

**The plants**

Campanula trachelium L. (Campanulaceae) is a European perennial herb that blooms from June to September (Schmeil and Fitschen 2009). The hermaphroditic blue to violet flowers are protandrous and self-incompatible and display secondary pollen presentation, where pollen is presented to flower visitors on the outer surface of the style, which is covered with hairs (Figure 1A) (Shtetl 1979). Echium vulgare L. (Boraginaceae) is a biannual herb that flowers in central Europe between June and October (Schmeil and Fitschen 2005). This species is outcrossing and has hermaphroditic (eventually unisexual) blue flowers that are disposed in large cymose inflorescences (Figure 1B) (Klinkhamer et al. 1991; Rademaker et al. 1997). Potentilla recta L. (Rosaceae) is a perennial herb that blooms in central Europe in June and July (Schmeil and Fitschen 2005). It has hermaphroditic self-incompatible flowers that are yellow and disc shaped (Figure 1C) (Werner and Soule 1976; Batra 1979).

All plant taxa used in this study are pollinated primarily by bees. Campanula trachelium is visited by polylectic social species, such as bumble bees and honey bees, and various solitary bees, including oligoletes of the genus Chelostoma, Andrena, and Melitta (Knuth 1906). Echium vulgare has polylectic pollinators that are similar to those on Ca. trachelium but is visited by distinct oligolectic visitors of the genus Hoplitis (Westrich 1989). Finally, P. recta is visited by polylectic visitors, including various bees and flies (Batra 1979). In spite of the sympatric occurrence and synchronization in flowering period of E. vulgare and P. recta with Ca. trachelium, there is no record of Ch. rapunculi visiting flowers of either of these 2 nonhost species (neither for nectar- nor for collecting pollen).

**Flight cage**

From early June to end July 2009 and 2010, the responses of male and female Ch. rapunculi bees to visual and olfactory cues of the host plant Ca. trachelium, and of the nonhost plants E. vulgare and P. recta, were studied in a flight cage (7.2 × 3.6 × 2.2 m; the same as described in Dötterl and Schäffler.
situated inside a glass greenhouse (6.5 × 17 m), located in the Ecological Botanical Garden of the University of Bayreuth, Germany. The side windows (2 × 15 m²) and roof (2 × 20 m²) of the greenhouse were kept open, allowing natural light to enter without being filtered through glass. In the experimental flight cage, which consisted of a wooden frame with a fine mesh, bees were free flying, and we provided them conditions as similar as possible to those found in nature.

Foraging-naïve bees

To obtain foraging-naïve bees, which we define here as inexperienced with respect to foraging on the host and nonhost flowers used in the bioassays, trap nests filled with preimaginal Ch. rapunculi bees were placed in the cage at the beginning of June, so that all bees emerged directly in the flight cage. Newly emerged bees were given no contact with the host and nonhost flowers used in the bioassays (Ca. trachelium, E. vulgare, and P. recta). Instead, they were offered sugar water (30%, fructose and glucose 1:1), presented in saturated beige sponge feeders, and nonhost nectar flowers of species that were not used in the bioassays (Geranium pratense: Geraniaceae; Lythrum salicaria: Lythraceae; Sinapis arvensis and Brassica sp.: Brassicaceae). The foraging-naïve bees were observed foraging for nectar on both nonhost flowers and sponge feeders and mated freely.

Foraging-experienced bees

After completion of the bioassays on foraging-naïve bees, these same bees were allowed to become foraging experienced by introducing Ca. trachelium host plants into the flight cage. As soon as Ca. trachelium plants were placed in the cage, both males and females restricted their foraging to these host flowers and completely neglected the nonhost ones; the now foraging-experienced bees gathered pollen and nectar and built nests in the trap nests (cylindrical pots containing cut stems of Phragmites australis) that we provided. Thus, bees initiated nesting only in the presence of Ca. trachelium; conversely, foraging-experienced bees promptly ceased nest provisioning whenever Ca. trachelium plants were temporarily removed from the flight cage to carry out behavioral experiments.

Design of behavioral bioassays

To establish whether Ch. rapunculi has innate preferences for the visual and olfactory cues (alone or combined) of its host plants over those of nonhost species and to determine the relative importance of visual and olfactory cues in host-flower location by both foraging-naïve and foraging-experienced bees, dual-choice bioassays were conducted. Bees were offered a choice of inflorescences of 2 different species, 2 different inflorescence cues (visual, olfactory, visual + olfactory) of the same species, or 1 inflorescence and 1 negative (nonflower) control. All plant samples, which consisted of single stems bearing about 10 flowers each, were covered with quartz glass cylinders (29 cm height, 10 cm diameter; Figure 2). Quartz glass was used because of its ultraviolet (UV) transparency. The cylinders were mounted on a black Polyvinyl Chloride disc (diameter 11 cm), which was attached to a square wooden table.

Figure 2

Overview of the quartz glass cylinders (29 cm height, 10 cm diameter) and experimental setup used for behavioral assays. Two identical cylinders each were used to test olfactory (A), visual (B), or olfactory + visual (C) cues of Campanula host plants versus those of nonhost plants and also to test the single or combined cues of Campanula versus respective negative controls (empty cylinders). Two different types of cylinders each were used to test olfactory versus visual cues (D) and the combined cues versus either olfactory (E) or visual (F) cues of Campanula. Arrows indicate an air flow (1 l min⁻¹), which was generated by a membrane pump.
To offer the bees visual and olfactory cues of the tested plant species singly or in combination, different kinds of quartz glass cylinders were used for the 3 bioassays. 1) Attraction of bees to olfactory cues alone: we used black cylinders with 60 small holes (diameter 0.2 cm). The cylinders were painted with semi-matte varnish and dried for 1 week at 50 °C to eliminate any scent emission from the varnish. Air containing floral scent from the enclosed flowering stems was blown out of the holes by a membrane pump (G12/01 EB; Rietschle Thomas, Puchheim, Germany) at a flow rate of 1 l/min. 2) Attraction of bees to visual cues alone: we used transparent solid cylinders, without holes. 3) Attraction of bees to olfactory and visual cues combined: we used transparent cylinders with small holes. Scented air was blown from the cylinders as described for the olfactory cues alone.

**Host versus nonhost flowers**

Dual-choice bioassays, in which bees were offered a choice of host versus nonhost flowers, were performed on foraging-naive bees only and in the following order: 1) olfactory cues (Figure 2A), 2) visual cues (Figure 2B), and 3) olfactory + visual cues (Figure 2C). In each case, a flowering stem of Ca. trachelium was offered first against a flowering stem of E. vulgare and then against one of P. recta.

**Visual versus olfactory cues of host flowers**

To determine the relative importance of visual and olfactory cues in the attraction of both foraging-naive and foraging-experienced bees to Ca. trachelium inflorescences, we conducted 6 different dual-choice bioassays. The biotests were carried out in the following order: 1) olfactory cues versus empty control (Figure 2A), 2) visual cues versus empty control (Figure 2B), 3) olfactory + visual cues versus empty control (Figure 2C), 4) olfactory versus visual cues (Figure 2D), 5) olfactory + visual versus olfactory cues (Figure 2E), and 6) olfactory + visual versus visual cues (Figure 2F).

**Bioassay protocol**

The bioassays were conducted on sunny days between 10:00 and 15:00 h, when the bees were most active. On each test day, all nonhost plants, as well as Campanula plants in the case of foraging-experienced bees, were removed from the flight cage in the early morning (07:00–08:00 h) and were put back again after the tests were completed.

Each bioassay lasted 30 min; the position of the paired cylinders, which were disposed 1.5 m apart, was exchanged after the first 15 min. The behavioral responses of the bees were recorded as either 1) approaches: flights toward the cylinder, to a distance closer than 5 cm, without landing or 2) landings: approaches followed by landing on the cylinders. All responding bees were promptly collected, using nets, after they responded in order to prevent them from interfering in the attraction of other individuals; we never observed a responding bee being “followed” by another bee. Approaching bees were collected when they flew away from the cylinders, landing bees after they landed. All responding bees were given test-specific markings using permanent color markers and were stored in an icebox until the end of the experiment, at which time they were released back into the flight cage and could participate in subsequent tests. Thus, an individual bee could respond only once in each specific bioassay. Tests on foraging-experienced bees were initiated only after the cage-reared and field-collected bees had foraged for at least 3 days on host flowers in the cage.

The number of individual bees tested (i.e., present in the flight cage) varied among experiments (25–80 bees) and decreased over time due to stochastic factors, such as mortality. In order to begin the bioassays on foraging-experienced bees with a similar number of individuals as used in the foraging-naive bee tests, we collected free-flying bees foraging on Campanula flowers in the Ecological Botanical Garden and placed them in the flight cage. Thus, bioassays on experienced bees included both wild-collected bees and cage-reared bees used previously in the tests on foraging-naive bees.

**Statistical analyses**

To test for differences in total bee responses (approaches and landings, pooled) between the paired treatments in each behavioral bioassay and for differences in landing responses between the paired treatments involving host versus nonhost flowers, observed versus expected chi-square tests were performed. We used 2 × 3 chi-square contingency tables (based on behavioral responses obtained from bioassays where floral cues, alone or combined, were offered against empty controls) to test whether the ratios of approaches versus landings by foraging-naive and foraging-experienced bees depend on the Ca. trachelium cues offered (olfactory cues, visual cues, visual + olfactory cues). Fisher’s Exact tests were used as post hoc tests for 2 × 3 contingency table tests and to compare, separately for each distinct cue of Ca. trachelium, behavioral responses (i.e., approaches vs. landings) between foraging-naive and foraging-experienced bees. All tests were performed with Statistica (StatSoft 2004), except the 2 × 3 contingency table tests, which were performed on the website http://www.physics.csbsju.edu/stats/contingency_NROW_NCOLUMN_form.html (Kirkman 1996). Responses of male and female bees were pooled, as individuals of both sexes responded equally in all bioassays (Fisher’s Exact tests: 0.17 < P < 0.73).

**RESULTS**

**Behavioral assays**

**Host versus nonhost flowers**

In the dual-choice bioassays, foraging-naive Ch. rapunculi bees showed a clear preference in their total responses (approaches + landings) for cues of their host plant Ca. trachelium over those of nonhost plants. Olfactory and visual cues, alone and in combination, of Ca. trachelium flowers were significantly more attractive to foraging-naive bees than those of either of the nonhost flowers, E. vulgare (Figure 3A) and P. recta (Figure 3B).

When testing the attractiveness of the olfactory inflorescence cues of Ca. trachelium against those of E. vulgare, no bees landed on the cylinders. Visual cues, on the other hand, triggered landings in 19% (n = 7) of the attracted (approaching) bees, and all were on Ca. trachelium. When testing visual + olfactory cues, 30% of attracted bees landed on the cylinders, with a strong preference for Ca. trachelium (12 vs. 0 bees; chi square = 12.00, degrees of freedom [df] = 1, P = 0.001).

Similarly, the bioassays testing the olfactory cues of Ca. trachelium against those of P. recta, elicited no landings by bees. Visual cues triggered landings in 24% (n = 11) of the attracted bees, 8 on Ca. trachelium and 3 on P. recta (chi square = 2.27, df = 1, P = 0.132). Finally, when testing visual + olfactory cues, 40% of attracted bees landed on the cylinders, with a strong preference for Ca. trachelium (13 vs. 2 bees; chi square = 8.07, df = 1, P = 0.005).

**Visual versus olfactory cues of Ca. trachelium**

The foraging-naive (Figure 4A) and foraging-experienced (Figure 4B) bees showed similar response patterns in the bioassays using only Ca. trachelium floral cues. The olfactory and visual cues alone were each more attractive, in terms of total responses (number of approaches + landings), than the empty
control to both foraging-naïve and foraging-experienced bees. However, both bee groups preferred the visual cues over olfactory cues when tested against each other, and the combination of both cues was more attractive than either floral cue alone.

When the total bee responses were separated into approaches and landings, the floral cues were shown to trigger significantly different behavioral responses, as expressed in the relative proportion of approaches and landings. Trends across the cues were generally similar in the foraging-naïve and foraging-experienced bees, with the caveat that experienced bees exhibited proportionately more landings throughout (Figure 5). In foraging-naïve bees, olfactory cues alone triggered exclusively approaches (22.7% of bee responses) amidst the predominance of approaches. When both cues were offered together, landings became proportionally more frequent (59.4% of responses) and comparable to the approaches. In foraging-experienced bees, olfactory cues alone elicited mainly approaches, but also some landings (10% of responses), visual cues alone resulted in equal frequencies of approaches and landings, and both cues in combination led to a predominance of landing responses (77.4%). Taken together, these data show that olfactory cues alone elicited similarly low landing frequencies in foraging-naïve and foraging-experienced bees; visual cues alone elicited landing frequencies in experienced bees (51.6%) that were significantly greater than in naïve bees (22.7%); and finally, both cues combined elicited similarly high landing frequencies in the 2 bee groups.

DISCUSSION

Our results show that Ch. rapunculi bees can discriminate between floral cues of host and nonhost plants and have an innate preference for the olfactory and visual flower cues of their host plant Ca. trachelium over co-flowering and co-occurring nonhost plants. These findings have implications to the possible mechanisms proposed to underlie host-plant specificity in bees (see below). Furthermore, our finding that both foraging-naïve and foraging-experienced bees are more attracted to the combination of visual and olfactory cues compared with the single cues underlines the reliance of bees on the integration of multiple floral cues, of different modalities, to locate their host plants, as has been suggested by a few earlier studies (Dobson and Bergström 2000; Burger et al. 2010; Dötterl et al. 2011).
The clear preference displayed by foraging-naïve bees of *Ch. rapunculi* to the visual and olfactory floral cues of their host flower *Ca. trachelium* over those of 2 co-occurring non-host flowers adds a new species to the list of oligolectic specialist bees that demonstrate an innate preference for their host flowers. This evidently has genetically based preferences to flower cues. Both cues alone or in combination were more attractive than the empty control (no floral cues) to both foraging-naïve and foraging-experienced bees to each cue were assessed using Fisher’s exact tests and are shown at the right-hand side of the bars; n.s. indicates no significant difference, *P* > 0.05.

Our behavioral assays testing the relative attractiveness of only visual and only olfactory cues revealed different responses by *Ch. rapunculi* to each cue, suggesting that these 2 floral cues play differing roles in the attraction of the bees and in their recognition of host flowers. Both cues alone or in combination were more attractive than the empty control (no floral cues) to both foraging-naïve and foraging-experienced bees, which is not surprising given that the control provided no floral cues at all. More important was the observation that the visual cues alone were more attractive than the olfactory ones when tested against each other. In oligolectic bees, there is only little information concerning the specific visual cues that they use to find flowers (Dötterl and Vereecken 2010). The few behavioral studies performed to date on other oligolectic bees found an innate preference for the color of the host flowers (Dobson and Bergström 2000; Burger et al. 2010), and field observations suggested that this was also the case in *Ch. rapunculi* (Nilsson 1983). Our findings, however, put into question the ability of *Ch. rapunculi* to discriminate host from nonhost flowers based on floral color alone. Whereas the color differences between the flowers of *Ca. trachelium*, which are bee UV-blue, and those of the nonhost *E. vulgare*, which are UV-green (Milet-Pinheiro 2011), are sufficient to explain the higher attractiveness of the host flowers when bees were given this choice, this does not apply to when the bees preferred *Ca. trachelium over E. vulgare*. Indeed, the flowers of *E. vulgare* and *Ca. trachelium* are similarly colored (color hexagon distance of 0.044; based on data given in Arnold et al. 2010), which suggests that *Ch. rapunculi*’s discrimination between these 2 species using visual cues alone may have been based on other, noncolor, visual parameters of the flowers/inflorescences (e.g., shape, size).

Our observations that both foraging-naïve and foraging-experienced *Ch. rapunculi* bees displayed a lesser attractiveness for olfactory cues compared with visual cues should not be interpreted as meaning that flower scents play a minor role
in host-flower location and recognition. Indeed, the 2 bee
groups were significantly more attracted to the combined cues
than to either one alone, revealing that both cues play essential
roles in host-flower recognition. Furthermore, the bees
discriminated host from nonhost flowers using olfactory cues
alone and did so to the same extent as with visual cues alone.
Olfactory cues, as encompassed in floral scents, are species
specific and can be pivotal factors in flower discrimination
by insects (Ayasse 2006; Dobson 2006; Knudsen et al. 2006;
Raguso 2008). The potentially infinite diversity of flower
cents, made possible by variations in both the chemical iden-
tities and relative proportions of individual compounds in
a floral odor blend (Williams 1983; Knudsen et al. 2006),
suggests that olfactory cues can provide a highly specific ident-
fier for flowers. In plant species associated with oligoleptic
bees, taxon-specific flower and/or pollen scents are believed
to play key roles in host-flower recognition (Dobson 1987;
Dobson and Bergström 2000; Dötterl and Vereeken 2010).
Among the few studies investigating the role of floral scents in
host location by oligoleptic bees, in 2 cases unusual, distinctive
compounds have been reported to be central in attracting
foraging-naive oligoleptic bees, namely protoanemonin in
Ranunculus flowers that are visited by Ch. florissonae (see
Dobson and Bergström 2000) and 1,4-benzoquinone in
Echium/Potentillum species visited by H. adunca (Burger et al.
2011). Analyses of floral scents of Ca. trachelium and other
Campanula species, coupled with electrophysiology and behav-
ioral bioassays, are needed to reveal if any species- or genus-
specific volatiles are similarly used by Ch. rapunculi as key host-
flower recognition chemicals.

Differences in the behavioral responses to floral cues by
foraging-naive Ch. rapunculi bees, on the one hand, and
foraging-experienced ones, on the other, indicate that learn-
ing of floral cues occurs in this specialized bee at least to some
extent. After foraging-naive bees visited Ca. trachelium flowers
and thereby became foraging experienced, they remained
similarly attracted (in terms of total responses) to single and
combined cues of Campanula flowers, but the proportion of
landings compared with approaches increased significantly
for visual cues. This increased reliance on visual cues as a land-
ing stimulus in experienced bees might lead to faster host
location, thereby increasing foraging efficiency, as was also
suggested for the ectopic oligolege Colletes fulgidus longipilosus
(Dobson 1987) and oligolectic M. fulvipes (Dötterl et al.
2011). Interestingly, when we combined visual and olfactory
cues in our bioassays on Ch. rapunculi, there was no enhance-
ment of landing responses following the acquisition of forag-
ing experience, showing that these bees rely on a multimodal
set of floral cues to recognize their host flowers, whether or
not they have foraging experience.

In conclusion, the innate preference by the oligolege
Ch. rapunculi to both visual and olfactory cues of its host flowers
over those of nonhost flowers adds a new species to the list
of specialist bees that display at least some genetically based
sensory orientation to host plants. Although visual floral cues
alone appear to attract the bees more strongly than olfactory
cues alone, the demonstration that visual and olfactory stimuli
in combination result in more effective host-flower location
than either cue alone, as expressed both in the higher numbers
of attracted bees and in the greater proportion of landing
responses in both foraging-naive and foraging-experienced
bees, indicates that this bee relies on the integration of multi-
modal stimuli to recognize its host flowers. Furthermore, the
slight increase in the relative impact of visual cues on the bee's
attraction to its host flowers as a result of learning during
flower visitation together with the concurrent unchanged at-
traction when both cues are combined indicates that Ch. rapun-
culi's search image of its host flower changes little over time as
bees become foraging-experienced, possibly reflecting con-
straints inherent in the floral cues themselves.

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**REFERENCES**

FReD: the floral reflectance database—a web portal for analyses of

Ayasse M. 2006. Floral scent and pollinator attraction in sexually
deceptive orchids. In: Dudareva N, Pichersky E, editors. Biology of

Batra SWT. 1979. Insects associated with weeds in the northeastern
United States. H. Cinquefoils, Potentilla norvegica and *P. veta* (Rosaceae).
J N Y Entomol Soc. 87:216–222.

Burger H, Dötterl S, Ayasse M. 2010. Host-plant finding and recogni-
tion by visual and olfactory floral cues in an oligoleptic bee. Funct
Ecol. 24:1234–1240.

arthropod deterrent attracts specialised bees to their host plants.

Cane JH, Sipes S. 2006. Characterizing floral specialization by bees:
analytical methods and revised lexicon for olfactory. In: Waser NM,
Ollerton J, editors. Plant-pollinator interactions: from specialization
to generalization. Chicago (IL): The University of Chicago Press.
p. 99–121.


Dobson HEM. 1987. Role of flower and pollen aromas in host-plant

Dobson HEM. 2006. Relationship between floral fragrance composi-
tion and type of pollinator. In: Dudareva N, Pichersky E, editors.

Dobson HEM, Bergström G. 2000. The ecology of pollen odors. Plant

differences in host finding of a specialized bee species. J Comp Physiol
A Sens Neural Behav Physiol. 197:1119–1126.

Dötterl S, Schäffler I. 2007. Flower scent of floral oil-producing
Lysimachia punceata as attractant for the oil-bee Macropis fulvipes. J Chem

Dötterl S, Vereeken JN. 2010. The chemical ecology and evolution of
88:668–697.


Giurfa M, Lehrer M. 2001. Honeybee vision and floral display: from
detection to close-up recognition. In: Chittka L, Thompson JN, editors.
Cognitive ecology of pollination. Cambridge: Cambridge
University Press. p. 61–82.

Giurfa M, Nunez J, Chittka L, Menzel R. 1995. Color preferences of
flower-naive honeybees. J Comp Physiol A Sens Neural Behav Physiol.
177:247–259.

Kirkman TW. 1996. Statistists to use [Internet]. [cited 2010 Nov 23].
Available from: http://www.physics.csbsju.edu/stats/.

Klinkhamer PGL, de Jong TJ, Wesselingh EA. 1991. Implications of
differences between hermaphrodite and female flowers for attractiv-
ness to pollinators and seed production. Neth J Zool. 41:130–143.
distribution of floral scent. Bot Rev. 72:1–120.
Lunau K. 1991. Innate flower recognition in bumblebees (Bombus
terrestris, B. lucorum; Apidae): optical signals from stamens as land-
Lunau K. 1992. Innate recognition of flowers by bumble bee orien-
J Comp Physiol A Sens Neural Behav Physiol. 177:1–19.
Michener CD. 1974. The social behavior of the bees. Cambridge
(MA): Harvard University Press.
Milet-Pinheiro P. 2011. Abelhas oligolecticas e plantas hospedeiras:
ecologia cognitiva e da polinizacao [thesis]. [Recife (Brazil)]: Uni-
versidade Federal de Pernambuco. p. 111.
Nilsson LA. 1983. Mimesis of bellflower Campanula by the red helle-
Praz CJ, Müller A, Dorn S. 2008a. Host recognition in a pollen-
Praz CJ, Müller A, Dorn S. 2008b. Specialized bees fail to develop on
non-host pollen: do plants chemically protect their pollen. Ecology.
89:795–804.
Raguso RA. 2008. Wake up and smell the roses: the ecology and
York: Cambridge University Press.
Schlindwein C, Wittmann D, Martins CF, Hamm A, Siqueira JA,
Schiffer D, Machado IC. 2005. Pollination of Campanula
rapunculus L. (Campanulaceae): how much pollen flows into
pollination and into reproduction of oligolectic pollinators? Plant
Syst Evol. 250:147–156.
Schmeil O, Fiteschen J. 2005. Flora von Deutschland und angrenzen-
den länder. Ulm (Germany): Quelle and Meyer.
Sedivy C, Praz CJ, Müller A, Widmer A, Dorn S. 2008. Patterns of
host-plant choice in bees of the genus Chelostoma: the constraint
hypothesis of host-range evolution in bees. Evolution. 62:
2487–2507.
Shetler SG. 1979. Pollen-collecting hairs of Campanula (Campanula-
Simonds V, Prewright CMS. 2004. How do bumblebees first find flow-
ers? Unlearned approach responses and habituation. Anim Behav.
bumblebees: flower size and color affect search time and flight
(Germany): Eugen Ulmer.
Williams NH. 1983. Floral fragrances as cues in animal behavior. In:
Jones EC, Little RJ, editors. Handbook of experimental pollination
Wright GA, Schiestl FP. 2009. The evolution of floral scent: the in-
fluence of olfactory learning by insect pollinators on the honest