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Chemical cues involved in the attraction of the oligolectic bee *Hoplitis adunca* to its host plant *Echium vulgare*

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ABSTRACT

Host recognition is a key process in oligolectic bees but the mechanisms through which they find and recognize appropriate pollen host plant are not entirely clear. *Hoplitis adunca* is a monolectic bee collecting pollen only from *Echium* spp. (Boraginaceae). We aimed to test whether *Echium vulgare* floral scent plays a major role in the attraction of *H. adunca* females, and to identify components of *E. vulgare* scent that may be involved in this specific attraction. We used a combination of behavioral and chemical (GC/GC–MS, PTR-MS) analyses. In order to identify the chemical cues likely to be involved in the specific attraction of *H. adunca*, we compared the scent of fresh flowers, nectar, pollen, and whole plants of *E. vulgare* and *Anchusa officinalis*, another Boraginaceae, which does not attract *H. adunca*. *H. adunca* females were attracted to the scent of *E. vulgare* flowers when offered against a blank or against the scent of *A. officinalis* flowers. However, *H. adunca* females were not attracted to the scent of *A. officinalis* flowers when offered against a blank. The emission spectra of the two plant species differed markedly, as did the emission spectra of various flower components (pollen, nectar and whole flowers) within a species. Pollen presented a low volatile release, but emitted significantly higher amounts of mass 55 (butanal, 1,3-butadiene, or other volatiles of molecular mass 54), and mass 83 (hexanal, hexenols, hexenyl acetate, or other volatiles of molecular mass 82) in *E. vulgare* than in *A. officinalis*. Nectar produced a particular emission spectrum with high emission rates of masses 109 and 123. Mass 109 may likely correspond to 1,4-benzoquinone, a volatile specifically measured in *E. vulgare* in parallel studies to this one. The flower emission spectrum was mainly a combination of the pollen and the nectar scents, although it also contained additional volatile compounds such as those of mass 63 or mass 81. As for terpenes, *E. vulgare* emitted limonene, longicyclene, junipene, trans-caryophyllene and α-humulene, that were not detected in *A. officinalis*, and the most emitted monoterpenes were α-pinene, junipene and limonene whereas the most emitted terpenoid by *A. officinalis* was α-pinene. After identifying these chemical cues, olfactory/behavioural assays with specific volatiles and combinations of volatiles are necessary to understand the chemical interactions of the *H. adunca*-*E. vulgare* system.

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1. Introduction

Pollinating insects in general, and bees in particular, use different information sources to locate their host plants, including olfactory and visual cues. The combination of both kinds of signals is at the base of plant-pollinator systems, in which pollinators choose between different flower species and plants need to attract (and sometimes compete for) pollinators while avoiding herbivores (Chittka and Raine, 2006). Both innate and learned behaviors are involved in the response of pollinators to olfactory and visual stimuli (Riffell et al., 2008; Praz et al., 2008).

Most bees are generalist in their use of pollen sources (polylecty), and provision their nests with pollen from a wide array of plant species belonging to various families (Cane and Sipes, 2006). However, an estimated 20–30% bee species (Westrich, 1989; Minckley and Roulston, 2006) are oligolectic, collecting pollen from members of a single plant family or even genus. Precise host recognition is therefore essential in these species. The mechanisms by which pollen-specific solitary bees find and recognize their appropriate pollen host plant seem to be related to floral scent (Dobson and Bergström, 2000; Dötterl et al., 2006) together with visual stimuli (Burger et al., 2008).

As for nectar, although most bee species collect this reward from a wide variety of plants, associations have been found between different pollinator groups (including bees) and sugar and amino acid composition (Baker and Baker, 1983; Petanidou et al., 2006). Other authors have found that a combination of olfactory and visual cues is necessary for successful foraging in solitary bees. Raguso (2008) suggested floral scent to function in concert with visual cues, attracting pollinators from a distance, and increasing approaches and landings. In fact, floral scent can also elicit distance attraction (Goyret et al., 2008).

Chemical signals involved in pollinator attraction include not only scents from the corolla, but also from other flower components, such the pollen and nectar themselves (Raguso, 2004a). Because of their causative association with the presence of rewards, such signals constitute a more honest signal than visual traits (Raguso, 2004a). There is ample evidence suggesting that bees are able to detect pollen in flowers via odor cues (Dobson, 1987; Dobson and Bergström, 2000; Goulson et al., 2001). Solitary bees in the genus *Osmia* bees are capable of directly detecting nectar presence via odor cues (Howell and Alarcon, 2007), and social bees are able to associate scent with the presence, quality and quantity of nectar (Wright and Schiestl, 2009, and references therein). Other studies have shown that specific pollen odor plays a key role in host recognition by oligolectic solitary bees (Dobson, 1987; Dobson and Bergström, 2000). Waelti et al. (2008) demonstrated that floral odor differences are important for maintaining reproductive isolation between closely related plant species.

In this study we investigate the role of scent signals of *Echium vulgare* (Boraginaceae) in the attraction of the solitary bee *Hoplitis adunca*. *H. adunca* is narrowly oligolectic (monolectic), collecting pollen solely from Echium spp. In a study conducted at the same time than ours Burger et al. (2008, 2010b) found that in flower-experienced *H. adunca*, a combination of both visual and olfactory cues was necessary to elicite landings on *E. vulgare* flowers. They conclude that bees are attracted from a distance by floral color and then recognize their host at a close range by way of their specific scent. In another parallel work, they identified the volatiles in the floral scents of this species (Burger et al., 2010a). Our objective in this study is to identify the components of *E. vulgare* scent that may be involved in this specific attraction but analyzing not only the volatiles from fresh whole flowers but also from different flower components, nectar and pollen. We use a combination of olfactometer bioassays and chemical (GC/GC–MS, PTR-MS) analyses. To identify the chemical cues likely to be involved in the specific attraction of *H. adunca*, we compared, as Burger et al. (2008, 2010a,b), the scent of *E. vulgare* with that of another Boraginaceae, *Anchusa officinalis*, with the same flowering period and growing areas than *E. vulgare*, but which does not attract *H. adunca*. Some pollinator species use the odor emitted by whole plants (or by vegetative parts) to locate their hosts, especially from long distances (Grison-Pigé et al., 2002; Dufay et al., 2003). Therefore, in addition to flower emissions, we analyzed the emissions of entire *E. vulgare* individuals.

2. Materials and methods

2.1. H. adunca *biology and sampling*

H. adunca (=*Osmia adunca*) is a Eurasian cavity-nesting solitary bee in the Megachilidae family. Throughout its distribution range, females provision their nests exclusively with *Echium* pollen (Westrich, 1989; Bosch et al., 2001; Banaszak and Romasenko, 2001; Amiet et al., 2004). Females of the closely related *Hoplitis anthocopides* caged with non-*Echium* floral resources do not engage in pollen collection and nest provision (Strickler, 1979). At an *E. vulgare* stand, we netted females, placed them in glass vials, and quickly took them to the laboratory. There, they were stored at 4 °C, until use in olfactometer bioassays.

2.2. *Olfactometer and experimental setup*

We used an olfactometer consisting of a glass Y tube (internal diameter = 1.6 cm, stem length = 18 cm, arms length = 10 cm), and two cuvettes connected to the arms of the Y tube by Teflon tubing. A peristaltic pump (SKC Inc., Eighty Four, PA) pumped charcoal filtered air at a constant flux of 300 ml min^{-1} through the system. Pitts-Singer (2007) used a similar set up to test the attraction of nest components to female Megachilid bees. Our cuvettes contained flower samples or were left empty to serve as blanks. Bees were placed at the stem entrance and observed until they contacted one of the two cuvettes. Bees that did not make a clear choice after 5 min, were discarded and scored as “no choice”. We used 30–50 females