Importance of olfactory and visuel cues of *Echium* for host-plant finding of the oligolectic bee *Osmia adunca* (Megachilidae)

Hannah Burger[1], Stefan Dötterl[2], Manfred Ayasse[1]

[1]Institut für Experimentelle Ökologie, Universität Ulm
[2]Lehrstuhl für Pflanzensystematik, Universität Bayreuth

Abstract: Bienen besuchen Blüten, um u. a. Pollen zu sammeln, der hauptsächlich der Versorgung der Larven dient. Einige Bienenarten sammeln Pollen von nur einer oder wenigen Pflanzengattungen und werden als Oligolekten bezeichnet. Der Anlockung von Bienen dienen olfaktorische und visuelle Blütensignale. Wir postulierten, dass die eng auf *Echium* (Boraginaceae) spezialisierte Mauerbiene *Osmia adunca* (Hymenoptera: Megachilidae) gattungsspezifische visuelle und olfaktorische Blütensignale benutzt, um ihre Wirtspflanzen zu finden und zu erkennen. Mittels chemischer Analysen (GC) konnten wir einen *Echium*-spezifischen Duft nachweisen, der sich deutlich von einer nah verwandten *Anchusa* Art unterscheidet. Biotests in einem Flugkäfig mit blütenerfahrenen, nestbauenden Weibchen ergaben weiterhin, dass *Echium*-Blütenduft bei der Erkennung der Pflanzen im Nahbereich eine große Rolle spielt, während visuelle Signale wahrscheinlich bei der Fernanlockung von Bedeutung sind. Die mit einem Reflexionsspektrometer analysierten visuellen Signale dreier *Echium* Arten ähnelten sich sehr und könnten daher vermutlich eine Funktion zur spezifischen Anlockung von *O. adunca* haben. Weitere Tests müssen nun zeigen, welche Blütensignale naive Tiere für die Wirtspflanzenerkennung nutzen.

Key words: *Osmia adunca*, *Echium*, floral signals, optic, scent, host-plant recognition, oligolectic bees

H. Burger, Institut für Experimentelle Ökologie, Universität Ulm, Albert-Einstein-Allee 11, 89069 Ulm, E-mail: Hannah.Burger@uni-ulm.de

Solitary bees are important pollinators of flowers. Besides nectar they collect pollen at flowers mainly to provide their larvae with food. Many bee species collect pollen only on a few closely related plant species (oligolecty) (MÜLLER & al. 1997). Little is known about the visual and olfactorial signals they use for host-plant finding (WCISLO & CANE 1996). However, bees can olfactorily distinguish between different pollen species (VON FRISCH 1923), and a species-specific chemistry of pollen odour is known for some plant species (BERGSTRÖM & al. 1995, DOBSON & al. 1999). Further, it was shown that naïve oligolectic bees recognize their host-plant on the basis of pollen volatiles (DOBSON & BERGSTRÖM 2000) and that flower-experienced bees could use pollen odours to assess pollen availability (DOBSON & al. 1999). Besides scent, also visual cues are of relevance for host-plant finding, and bees orientate especially on spectral contrasts. Biotests with dummy flowers revealed that colour contrast and not intensity and dominant wavelengths of flowers are influencing innate behavioural responses (LUNAU 1990). Further it was shown that naïve bumblebees were most motivated to land on a flower when visual stimuli from the antheres are combined with olfactorial stimuli from the pollen (LUNAU 1992).

We choose *Osmia adunca* P., which is highly specialized on *Echium* L., as a model to investigate the importance of floral cues for an oligolectic bee. Because bees learn to associate odours with reward more rapidly than visual cues (MENZEL 1985), we hypothesize that floral scent plays a major role in attraction of flower-experienced *O. adunca* females. We used gas chromatography to compare the scent of three *Echium* species with the scent of a closely related *Anchusa* species, and a spectrometer to compare the colour of the three *Echium* species. Additionally we conducted a biotest to determine the importance of visual and olfactorial signals of *Echium* for host-plant finding of experienced *O. adunca* females.

Material and Methods

Chemical Analyses*:* Floral scent was analysed from *E. plantagineum*, *E. vulgare*, *E. italicum* and *Anchusa officinalis*, all growing in the the Ecological-Botanical Garden of the University of Bayreuth. For each sample pollen and petals from 30 *Echium* flowers and 30 flowers from *A. officinalis* were extracted in pentane (Sigma-Aldrich 99%) for 24 h, respectively. Chemical analyses were performed by gas chromatography (Hewlett Packard, type 5890 series II). The samples were analyzed on a DB-5 column (J & W Folsom, USA; length 30 m; inner diameter 0.25 mm). The flow rate of the carrier gas (hydrogen) was 2 ml/min. 1 µl of each sample was injected splittless at 50°C. After 1 min the splitter was opened and the oven temperature increased with a rate of 10°C/min to 310°C. The injector and detector temperature was 310°C. The relative amounts of the more volatile compounds of the samples (until C_{22}) were used for statistical analyses. At first, the Bray-Curtis similarity index was determined, and based on this data the similarities and differences among samples were visualized by multidimensional scaling. Further, it was tested by analysis of similarities, whether there is a difference in scent of *Echium* and *Anchusa* (one-way ANOSIM), the different *Echium* species, and pollen and petals among the *Echium* species (two-way ANOSIM in the crossed design: factors *species* and *flower part*). All statistical tests were performed with the Primer (version 6.1.1) program.

Spectral Measurements: The spectral reflection (300-700 nm) of flowers of the three *Echium* species was recorded with the spectrometer SpectraUSB 2000 (Ocean Optics). Three parts of the flowers were measured: the top of the margin, the site between two connected petals, and pollen. As white standard we used a MS20 ceramic and as black standard an open black box of films. The measure area had a diameter of 3 mm. The recordings were analysed with the program OOIBase32 Vers. 1.0.2.0 (Ocean Optics, Inc. 1999).

Behavioural Experiment: The attractiveness of visual and olfactorial signals of *Echium* on *O. adunca* was tested using a quarz glass flask which was put over some inflorescences of *E. plantagineum*. To examine the importance of floral scent, the flask was covered with a cardboard to hide visual cues. Air, enriched with floral scent of the inflorescence, left the flask through an opening on top by using a membrane pump. To test the attractiveness of the visual cues of flowers the openings of the flask were closed that the bees could see, but not smell the plant. For the combination of optical and olfactorial signals, scent was pumped out of an uncovered flask, so that the bees could see and smell the plant. As a control, an empty flask was used. Each design was offered to *O. adunca* females in a flight cage (7.2 x 3.6 x 2.2 m) for 30 min on two different days. The behavioural responses of bees were reported as approaches (distance max. 10 cm) and landings. All landed bees were caught. The behavioural responses were compared among the different set-ups using a Chi-Square test comparing expected with observed events (the number of available bees was the same for all experiments).

Results

Chemical Analyses*:* In total 95 substances were found in the scent samples. There was a higher similarity in the scent of different *Echium* species (ANOSIM: R=0.523 p<0.001) and between pollen and petals of each *Echium* species (ANOSIM: R=0.554 p<0.001) (Fig. 1) than in a comparison of *Anchusa* and *Echium* (ANOSIM: R=0.831 p<0.001) (Fig. 1).
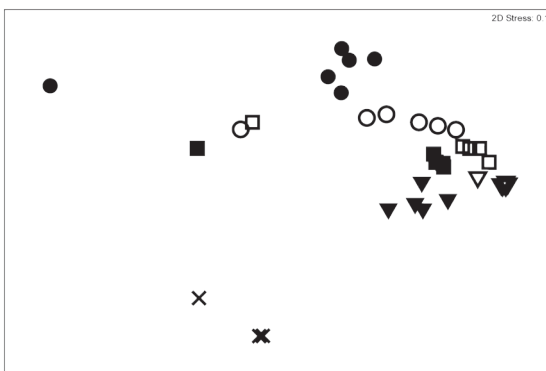

Fig. 1: Multidimensional scaling of individual pollen and petal samples of *E. plantagineum* (petals ▼ and pollen ▽), *E. vulgare* (petals ■ and pollen □) and *E. italicum* (petals ● and pollen ○) and of whole flowers of *A. officinalis* (✕) based on Bray-Curtis similarities

Spectral Measurements: The reflectance measurements of different *Echium* species and flower parts revealed a high similarity among species, however, also some differences especially among different flower parts. The margin of all flowers had the highest reflection in the blue range (Fig. 2). Because of the missing red-receptor of the most hymenoptera we considered only wavelengths between 300 and 600 nm. Pollen formed an optical contrast to the outer parts of the flowers. The maximum was shifted in a region with longer wavelength and showed a higher reflection of green light (Fig. 2). A significant UV-reflection was only measured in flowers of *E. plantagineum* (Fig. 2). Further, in *E. italicum* flowers a higher green-reflection was measured compared to the other species (Fig. 2).
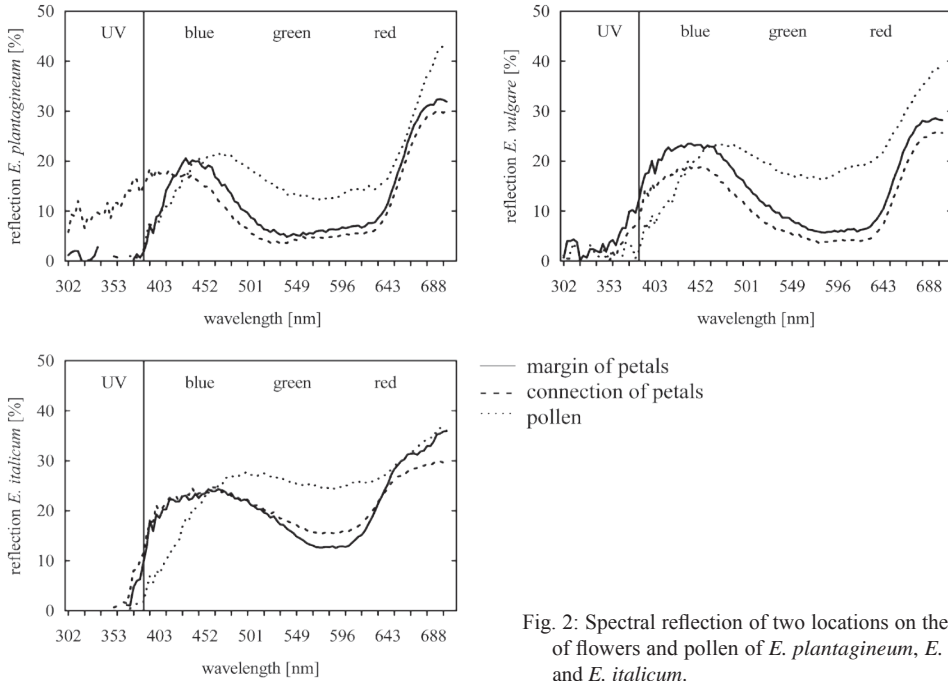

Fig. 2: Spectral reflection of two locations on the margin of flowers and pollen of *E. plantagineum*, *E. vulgare* and *E. italicum*.

Behavioural Experiment: The bioassay revealed that the combination of optical and olfactorial signals of the host plant were most attractive for *O. adunca*. The bees were most motivated to land on the treatment "optic + scent" (Chi^2 obs. vs. exp: χ^2=42.26 p<0.001) (Fig. 3). The optical cues elicited more approaches compared to the control and the scent treatment. (Chi^2 obs. vs. exp.: χ^2=178.57 p<0.001) (Fig. 3).
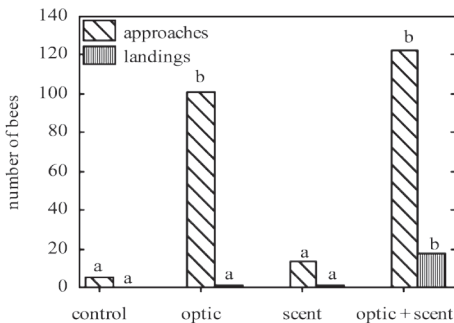

Fig. 3: Comparison of the attractiveness of different floral signals. Different set-ups of a quarz glass flask were offered to *O. adunca* females in a flight cage. The letters indicate significant differences of the behaviour between the test groups (Chi^2-test, p<0.05).

Discussion

The results provided a clear evidence for a genus specific odour profile in *Echium* that was different from the floral scent of *Anchusa*. Newly emerged *O. adunca* females could use this *Echium* specific odour for host-plant recognition. Naïve bees of another oligolectic species, *Chelostoma florisomne*, rely on pollen odour, and more specifically on its major component protoanemonin, to recognize its host *Ranunculus* (Dobson & Bergström 2000). A key chemical in the floral scent of *Salix* plays also a central role in attraction of *Andrena vaga*, a willow-specialist (Dötterl & al. 2005).

Besides scent, also visual cues are of relevance for host-plant finding. The spectral measurements showed a similar pattern among the *Echium* species. Colour could have a function as attractant from distance. It was shown that naïve bumblebees were attracted from a distance elicited by the large corolla and guided towards a site for closer inspection and landing elicited by floral guides (Lunau & al. 2006).

The bioassay revealed a great importance of optical cues for flower-experienced *O. adunca*. Nevertheless, the key signal elicited landings was a combination of visual and olfactory cues. This demonstrated that floral scent plays a central role in attraction of *O. adunca* by *Echium* flowers. For naïve bumblebees it was shown that they are most motivated to land on a flower when visual stimuli from the anthers are combined with olfactorial stimuli from the pollen (Lunau 1992). Pollen volatiles increase the number of landings on flowers of experienced bumblebees, too (Dobson & al. 1999). After few visits of flowers, bees have learned to associate floral scent with food. This allows *O. adunca* to restrict visits on rewarded flowers and optical cues can help them to localize flowers and form a landing site.

Further examinations have to reveal how naïve *O. adunca* females can find their host and which volatiles elicit this behaviour. We hypothesize that they are attracted from a distance by floral colour and recognize their host at close range based on *Echium* specific chemicals.

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