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An Aromatic Volatile Attracts Oligolectic bee Pollinators in an Interdependent bee-Plant Relationship

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Abstract Chemical signals emitted by the plant frequently mediate host-plant localization in specialized animal – plant associations. Studying the interdependent highly specialized association of the narrowly oligolectic bee pollinator *Protodiscelis palpalis* (Colletidae, Neopasiphaeinae) with *Hydrocleys martii* (Alismataceae) in ephemeral aquatic water bodies in semi-arid Caatinga of Brazil, we asked if specific volatile compounds produced by the flowers mediate pollinator attraction. The yellow *Hydrocleys* flowers are the sole pollen and nectar resources, and mating sites for the bees. We analyzed the floral scents of this species and of the closely related *H. nymphoides*, which is not visited by *P. palpalis,* and tested the main volatile compounds of both species under field conditions to evaluate their attractiveness to bees of *P. palpalis*. Methoxylated aromatics were the dominant floral scent components in both species, but each species exhibited a characteristic scent profile. Dual choice bioassays using artificial flowers made of yellow and blue adhesive paper clearly revealed that ρ-methylanisole alone, the dominant volatile of *H. martii,* attracted significantly more bees than unbaited flowers. This compound represents an olfactory communication channel used by the plant that lures its effective oligolectic pollinators to its flowers. Yellow artificial flowers baited significantly more bees than blue ones. Our study reinforces the recent findings that specific compounds in complex floral scent bouquets are crucial for host-plant location in oligolectic bees.

Keywords *Protodiscelis* · Neopasiphaeinae · Colletidae · Floral scents · ρ-methylanisole · Pollination · Caatinga · Host plant location · Brazil

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Introduction

Specialized relationships between insect pollinators and their host-plant flowers are commonly mediated by specific volatile compounds (Milet-Pinheiro et al. 2013; Raguso 2008; Schiestl et al. 2011). Recent studies showed that in some specialized pollination systems only one or a few specific floral volatile compounds mediate communication with specific pollinator species. In cases where only the intended receiver (specific pollinator) but not other flower visitors available in the habitat perceive this compound well, the signal was termed private communication channel (Raguso 2008). The mutualistic interaction between a fig tree species and its fig-wasp pollinator was suggested to be mediated by such a private channel (Chen et al. 2009). Private signaling also may occur between cantharophilous flowers of Araceae / Annonaceae and beetles of Cyclocephalini (Scarabaeidae) (Dötterl et al. 2012; Maia et al. 2012, 2013) as well as in deceptive pollination systems that benefit only the plant, like

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in many orchids (e.g., Ayasse et al. 2011; Raguso 2003; Schiestl et al. 2003).

In many other pollination systems, pollinators are attracted by a blend of widespread volatile compounds (Carvalho et al. 2012; Dobson 2006; Dressler 1982; Roubik and Hanson 2004; Schiestl and Marion-Poll 2002; Schiestl et al. 1999; Stensmyr et al. 2002).

The most important pollinators are bees (Ollerton et al. 2011) and, depending on region, 15-60 % of them are oligolectic (Minckley and Roulston 2006), i.e., they are specialized to collect pollen on plant species of the same genus or family (Cane and Sipes 2006; Robertson 1925). Some recent studies have shown that volatiles are key chemical cues and allow host plant location and recognition by bees (Burger et al. 2010, 2013; Dobson 1987; Dötterl and Vereecken 2010; Dötterl et al. 2011; Milet-Pinheiro et al. 2012, 2013). Interestingly, in the few studies available so far, oligolectic bees responded mostly to uncommon compounds (but see Dötterl et al. 2005) and not to specific mixtures of widespread compounds (Burger et al. 2012, 2013; Dobson and Bergström 2000; Milet-Pinheiro et al. 2013). Some of these studies also indicate that, besides olfactory cues, visual cues are involved in host flower localization (Burger and Dötterl 2010; Burger et al. 2010b; Dobson and Bergström 2000; Dötterl et al. 2011; Milet-Pinheiro et al. 2012).

To date, the studies on signaling in interactions between oligolectic bees and their host plants were all on bees from the northern hemisphere, despite the diverse fauna of oligolectic bees in the southern hemisphere (Cane and Sipes 2006; Schlindwein 1998). A bee group, where almost all of the 420 described species are suggested to be oligolectic, is the colletid subfamily Neopasiphaeinae formerly included in Paracolletini (Almeida and Danforth 2009; Almeida et al. 2012; Michener 2007; Schlindwein 1998, 2004; Schlindwein and Wittmann 1997; Silveira 2009). It occurs mainly in South America and Australia. The narrowly oligolectic *Protodiscelis palpalis* (Ducke, 1908) (Colletidae, Neopasiphaeinae) forms a highly specialized association with its aquatic host-plant *Hydrocleys martii* Seub. (Alismataceae, sensu APGIII The Angiosperm Phylogeny Group 2009) (Carvalho and Schlindwein 2011). Bee and plant species are common, but highly seasonal in ephemeral water bodies that arise after heavy rain falls in the semiarid Caatinga, a dry tropical forest with abundant thorny shrubs and succulents in northeastern Brazil. The *Hydrocleys* flowers are the sole pollen and nectar resources for the bees (Carvalho and Schlindwein 2011), and mating occurs exclusively on the flowers of these plants (Oliveira et al. 2012, 2013). Given that both plant and bee species show reproductive interdependence, this association is a good candidate for investigating the role of floral scents in host-finding by oligolectic bees.

Hydrocleys nymphoides (Willd.) Buchenau that mainly occurs in the Atlantic Rainforest is the sister species of *H. martii* (Haynes and Holm-Nielsen 1992) and is pollinated by polyletic bees (*Bombus brevivillus*, *Trigona spinipes, Apis mellifera*; Apidae) (Carvalho and Schlindwein, unpublished). Thus, it is interesting to test for differences in the olfactory cues between these closely related, but differently pollinated species.

We investigated the floral odor bouquets of *H. martii* and *H. nymphoides,* and tested the effectiveness of the main volatile compounds of the two species in attracting females of *P. palpalis* in natural habitats in the Caatinga region. We asked: 1) Which volatile compounds are produced by flowers of *H. martii* and *H. nymphoides*? 2) Does the scent profile of the species differ? 3) Are the main compounds produced by flowers of *Hydrocleys* involved in the attraction of *P. palpalis* bees? We expected that *H. martii* emits uncommon compounds used by *P. palpalis* for host location, different from *H. nymphoides*. We tested the main compounds produced of both species using artificial flowers of two different colors.

Materials and Methods

Studied Plant Species and Study Sites Hydrocleys martii is distributed in the region of the *Caatinga*, northeastern Brazil (Haynes and Holm-Nielsen 1992), which is characterized by a highly seasonal climate with a long severe drought and a short rainy season with strong rainfalls that varies drastically in its magnitude year by year (Andrade-Lima 1981; Prado 2003). *Hydrocleys nymphoides* has a much wider distribution in Brazil and occurs mainly in the Atlantic Rainforest, Pantanal and Amazon basin (Haynes and Holm-Nielsen 1992).

Plants of both species of *Hydrocleys* occur in shallow water bodies. Flower buds develop under the water surface, rise above it to bloom for 6–8 h, and then submerge again to develop mature fruits under water. The flowers of *H. martii* are somewhat smaller than those of *H. nymphoides* and are bright yellow colored, while those of *H. nymphoides* have pale beige colored petals and reddish staminodes. The flowers of *H. martii* show a staminodal cone that covers the fertile stamens, and only bees of *Protodiscelis palpalis* get access to the pollen chamber and nectaries hidden by the staminodes. In the flowers of *H. nymphoides*, staminodes are short and narrow and do not cover the stamens (Carvalho and Schlindwein 2011).

Individuals of *H. martii* used for odor collections were obtained from a pond in the municipality of Pedra, in the Farm *Lagoa do Chá*, (8°41’6”S and 36°54’48”W; five samples from five different individuals), and a pond in the municipality of Venturosa in the Carrapateira village (8°36’42”S and 36°51’25”W, five samples from five different individuals). *Hydrocleys nymphoides* plants were from Jaboatão dos Guararapes (08°05’ 46”S and 35°03’ 20”W, eight samples

from five different individuals). All obtained individuals were cultivated until flowering in a common garden in Recife (Pernambuco) in 20 L aquarium tanks with 10 L of a substrate composed of 50 % sand and 50 % garden soil.

The biotests were performed from June to August 2010 in the two ponds, from which plants of *H. martii* were obtained for scent collection, and additionally in a pond in the municipality of Pedra, on the Farm *Caboclo* (8°42'35"S and 36°53'8"W) and a pond in the municipality of Venturosa on the Farm *Areias* (8°21'33" S and 36°44'20"W). Besides *H. martii, Echinodorus palaefolius* (Alismataceae), a species pollinated by *Protodiscelis alismatis* (Ducke, 1908) and a few polylectic bees (Carvalho 2012), occurred and were flowering in these four ponds.

Sampling of Floral Volatiles and Chemical Analyses Floral scent was collected from the cultivated individuals in the experimental garden, using standard dynamic headspace methods (Dötterl and Dötterl and Jürgens 2005) to establish the scent profile emitted by the flowers (*H. martii*, *N* =10; *H. nymphoides*, *N*=8) and to determine the scent released by artificial flowers (see below). Natural (1 to 4 flowers per sample, depending on the availability) and artificial (*N*=1, enclosed 10 min after adding the volatile mixture) flowers were bagged in polyester bags (Toppits Bratschlauch®, 10 × 10 cm). Volatiles were adsorbed in a glass tube with 0.2 g of a 1:1 mixture of Tenax-TA 60–80 and Carbotrap 20–40 (both Supelco, 6 samples of *H. martii*; 4 samples of *H. nymphoides*), or 0.2 g of SuperQ (Supelco, 4 samples of *H. martii*; 4 samples of *H. nymphoides*). The air inside the bag was sucked out for 4 h through the absorbent tube, using a vacuum pump (G12/01 EB, Thomas, Puchheim, Germany). We used 100 μl of acetone (SupraSolv, Merck KgaA, Germany) and a 9:1 mixture of hexane: acetone (SupraSolv, Merck, Germany) to elute volatiles trapped in the Tenax/Carbotrap and SuperQ tubes, respectively. The samples were stored in a freezer at −20 °C until analysis at the Department of Plant Systematics, University of Bayreuth, Germany.

To identify the compounds emitted by the natural and artificial flowers, 1μl of the solvent samples was analyzed on a Varian Saturn 2000 mass spectrometer coupled to a Varian 3800 gas chromatograph fitted with a 1079 injector (Varian Inc., Palo Alto, CA, USA). The ZB-5 column and settings were as described in Dötterl et al. (2005). Component identification was carried out using the NIST 08 mass spectral data base, or MassFinder 3, and confirmed by comparison of retention times with published data (Adams 2007). Identification of individual components was confirmed by comparison of both mass spectrum and GC retention data with those of authentic standards. For quantification of compounds, 1 μg of 3-chloro-4-methoxytoluene was added as internal standard to samples collected from natural and artificial flowers.

Biotests with Artificial Flowers and Synthetic Compounds We mounted artificial flowers with scentless yellow or light blue plastic adhesive tape (BioTrap ®, Biocontrol, São Paulo, Brazil), used in greenhouses to trap pest insects. Conic artificial flowers with clusters of small strips of yellow and blue silk paper, resembling staminodes, were fixed at the end of a wooden stick in the center of the cone (Fig. 1). The long end of the stick penetrated a rectangle of Styrofoam (Isopor, 25 × 10 cm) that swam on the water surface with the artificial flowers oriented upward like flowers of *Hydrocleys*. Only on the upper face of the artificial flower, the sticky surface of the plastic tape was exposed. The artificial flowers had the size of the flowers of *H. martii* (28–30 mm diam).

We performed dual-choice experiments with two artificial flowers (distance: 20 cm): a scented one impregnated with the test-substance and a scentless artificial flower as control. Both artificial flowers were fixed to the Styrofoam rectangle. Sampling units were placed in the central area of the pond, next to patches of flowering *H. martii* (≈60–150 flowers per m^2) and about 1 m distant from each other. The sampling units were inspected every 30 to 50 min (6 to 7 times per day), and *P. palpalis* bees that adhered to the adhesive surface were transferred to collection vials containing ethyl acetate. After inspection, the sampling units were repositioned at least 1.5 m distant from the previous sampling point. Each Styrofoam rectangle was rotated so that the artificial flowers stayed opposed to the previous position. Experiments began at 9:00 and finished at 13:00 h.

In the biotests, we used standard compounds of *p*-methylanisole (≥99 %), 2-methoxy-4-methylphenol (≥98 %), 3,4-dimethoxytoluene (≥97 %), and 3,4,5-trimethoxytoluene (≥97 %), all purchased from Sigma-Aldrich. We applied 1 μl of a pure compound on the staminode imitation consisting of silk paper in the center of blue or yellow artificial flowers and tested it against a negative control (a scentless artificial flower). The sampling units were installed at the pond surface 10 min after adding the substances. Only for *p*-methylanisole, the concentration was consistent with natural concentrations release by a patch of natural flowers (see Table 1).

The amount of scent released from artificial flowers during the bioassays was determined by dynamic-headspace and GC/MS analyses, using the method described above (Table 1).

Data Analysis The exact test for goodness-of-fit was performed to test the null hypothesis that each compound attracted in total the same number of bees as its paired control. We also used this test to compare the effect of color on the number of attracted bees, separately for the two-choice assays with the different compounds. Bees attracted by both the controls and the synthetic compounds were considered for this analysis, and we weighed for the number of artificial flowers tested. The null hypothesis was that the choice

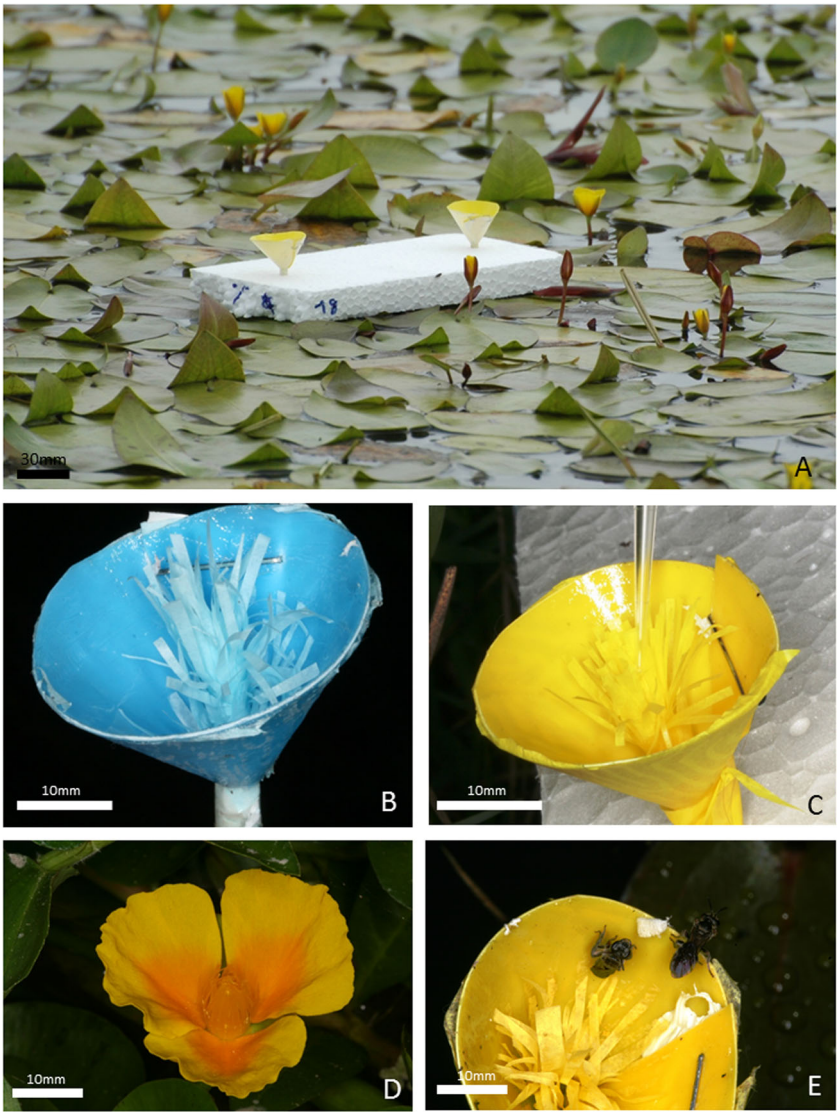

Fig. 1 **a** Sample unit, a rectangular piece of styrofoam with two artificial flowers. **b** Blue artificial flower. **c** Yellow artificial flower. **d** *Hydrocleys martii* natural flowers from Pedra, Pernambuco, Brazil. **e** Bees of *Protodiscelis palpalis* glued to yellow artificial flowers

experiments with a specific compound attracted the same number of bees independently of the color when correcting for the number of artificial flowers tested (McDonald 2009). To address the qualitative (presence/absence of compounds) and semi-quantitative (relative amount of compounds with respect to total peak area) differences in floral scent composition between the two *Hydrocleys* species, we performed analyses of similarities (ANOSIM) based on qualitative Sorensen similarities and (semi-) quantitative Bray-Curtis similarities, respectively, using Primer6 (Clarke and Gorley 2006).

Results

Floral Scents The flowers of both *Hydrocleys* species emitted a similar absolute amount of scent (0.2 μg/h and 0.3 μg/h in

Table 1 Amount of scent emitted by artificial flowers per tested compound given as flower equivalents (the number of natural flowers) for *Hydrocleys martii* and *H. nymphoides*

	p-Methylanisole	2-Methoxy-4-methylphenol	3,4-Dimethoxytoluene	3,4,5-Trimethoxytoluene
H. martii	74	–	20.684	91.300
H. nymphoides	344	2.091	530	30.214

H. martii and *H. nymphoides*, respectively), which consisted of aromatic and N-bearing compounds as well as terpenoids (Table 2). Flowers of *H. martii* produced 22 compounds, and those of *H. nymphoides* 13. Some compounds were exclusive to one or the other species, and each species had its characteristic profile of compounds (qualitative scent pattern: ANOSIM: R =0.98, P<0.001). The relative amounts of compounds emitted by each species also differed (semiquantitative scent pattern: ANOSIM: R =1.00, P<0.001). In both plant species, methoxylated aromatics were dominant, contributing to the total scent by 88 % in *H. martii* and 91 % in *H. nymphoides.* The compounds *p*-methylanisole and 3,4-dimethoxytoluene were most abundant in flowers of *H. martii* (80,7 %) and *H. nymphoides* (65,5 %), respectively.

Biotests In the two-choice tests, both yellow and blue artificial flowers impregnated with *P*-methylanisole attracted significantly more bees than the scentless control flowers. Only a few bees were collected on the scented artificial flowers that tested other abundant compounds found in *Hydrocleys* flowers, i.e., 2-methoxy-4-methylphenol, 3,4-dimethoxytoluene, and 3,4,5-trimethoxytoluene (Table 2 and Fig. 2).

The proportion of bees attracted to the flowers (treatment+control) differed between the two colors for *p*-methylanisole (yellow: 52 bees on 42 traps; blue: 11 bees on 28 traps; P<0.001) and 3,4-dimethoxytoluene (yellow: 9 bees on 22 traps; blue: 1 bee on 20 traps; P=0.023), but not for 2-methoxy-4-methylphenol (yellow: 9 bees on 22 traps; blue: 2 bees on 20 traps; P= 0.069). In both cases with significant effects, the number of bees trapped in experiments with yellow flowers was higher than expected indicating that yellow flowers were more attractive than blue flowers.

Table 2 Mean percentage (%± SE) and total amount of scent compounds in flower scent samples of *Hydrocleys martii* and *H. nymphoides* using dynamic headspace technique. Tr: trace amount (<0.005 %)

	H. martii N=10	*H. nymphoides* N=8
Amount of floral scent per flower [µg/h]	0.21±0.08 (N=4)	0.26±0.03 (N=4)
Aromatics		
p-Methylanisole*[1]	80.73±2.45	7.12±1.65
Methyl benzoate*	0.21±0.10	1.62±0.75
2-Methoxy-4-methylphenol*[2]	–	11.12±4.25
Methyl salicylate*	0.01±0.01	0.62±0.32
3,4-Dimethoxytoluene*[3]	2.68±0.69	67.5±6,65
2,4-Dimethoxytoluene	5.04±0.65	–
3,4,5-Trimethoxytoluene*[4]	0.01±0.01	2.37±1.87
3,4-Dimethoxybenzaldehyde	–	0.25±0.25
Elemicin[a]	–	0.25±0.16
N-bearing compounds		
Benzeneacetonitrile*	0.44±0.18	Tr
Indole*	–	Tr
Terpenoids		
α-Pinene*	3.02±1.77	Tr
β-Myrcene*	0.30±0.21	1.75±1.27
(*E*)-β-Ocimene*	0.97±0.38	–
Linalool*	0.18±0.10	–
(*Z*)-Ocimene oxide	0.25±0.10	–
(*E*)-Ocimene oxide	2.29±0.71	–
(*E*)-4,8-Dimethyl-1,3,7-nonatriene*	0.24±0.15	0.42±0.42
β-Bourbonene+other sesquiterpene	0.79±0.22	–
β-Caryophyllene*	0.57±0.17	–
unknown, m/z: 161,105,91,119,133,79	0.03±0.02	–
γ-Muurolene	0.19±0.06	–
α-Muurolene	1.38±0.36	0.76±0.76
unknown, m/z: 119,161,91,105,79,39	0.05±0.05	1.08±1.08
δ-Cadinene	0.04±0.04	5.13±1.13
(*E*)-Nerolidol*	0.47±0.2	–

* Identification based on authentic standards

[1–4] Numbers indicate compounds tested in bioassays with artificial flowers

[a] 1,2,3-Trimethoxy-5-prop-2-enylbenzene

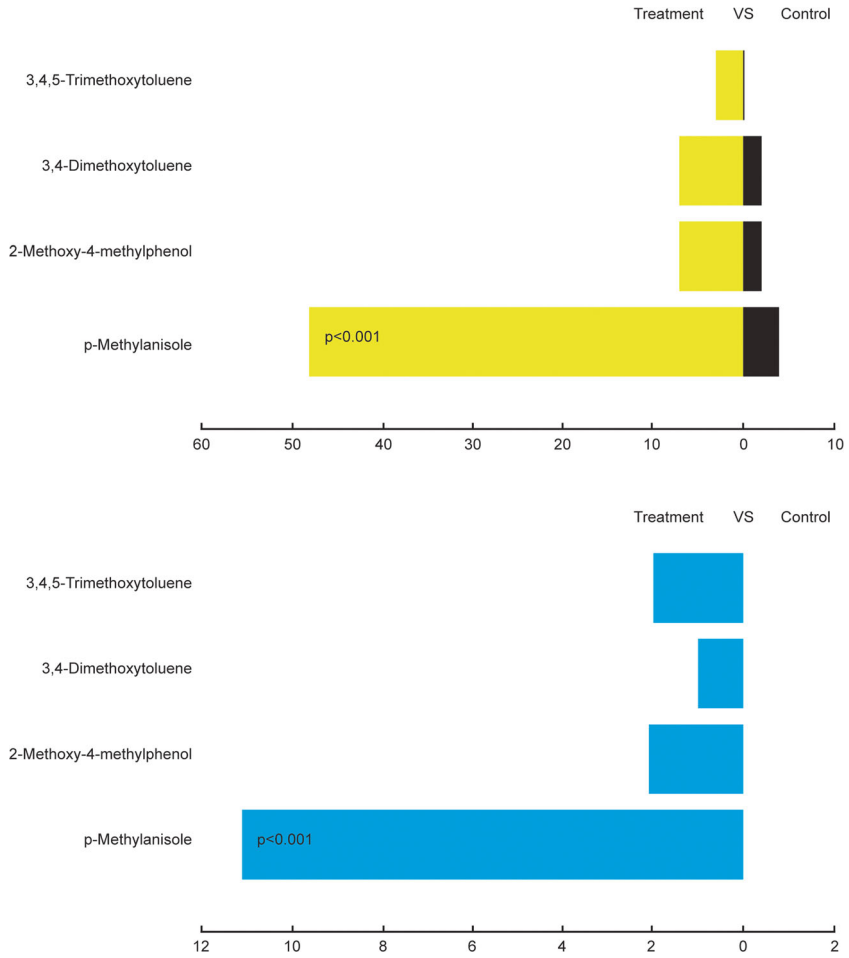

Fig 2 Number of females of *Protodiscelis palpalis* trapped on yellow (*top*) and blue (*below*) artificial flowers. Differences in attractiveness between treatment and control flowers were assessed by an exact test for goodness-of-fit. We exposed overall 88 artificial blue (total time of exposure: 1,760 min) and 106 artificial yellow (total time of exposure: 1,860 min) flowers

Discussion

Our results show that flowers of *Hydrocleys martii* and *H. nymphoides* produce volatiles in similar total amounts, but with different qualitative and semiquantitative properties. The aromatic compound *p*-methylanisole, which was most abundant in *H. martii*, but also present in lower amounts in flowers of *H. nymphoides*, attracted females of *P. palpalis* in field bioassays. Both yellow as well as blue artificial flowers impregnated with *p*-methylanisole lured the oligolectic bees, whereas yellow flowers attracted more bees than blue flowers. These results show that *p*-methylanisole is used by bees of *P. palpalis* to locate host flowers. Moreover, visual stimuli also influence the bees' behavior. The finding that bees use both visual and olfactory cues when they search for food is consistent with other studies focusing on the cue modalities involved in host finding of oligolectic bees (Burger et al. 2010; Dötterl et al. 2011; Milet-Pinheiro et al. 2012). In our study, *P. palpalis* bees did not respond to the other compounds tested nor to pure scentless yellow or blue artificial flowers. However, only *p*-methylanisole was used in concentrations naturally perceived by the bees (emission rate resembled the scent released by a small patch of *H. martii*), and we thus cannot rule out that the other compounds also are involved in host plant location of *P. palpalis*.

This first analysis of floral scents in *Hydrocleys* revealed that the main compounds are methoxylated aromatics. All of them are of restricted occurrence in flower bouquets. 3,4,5-Trimethoxytoluene has been found so far only in *Narcissus* (Amaryllidaceae) and 3,4-dimethoxytoluene in Malvaceae, Orchidaceae, and Valerianaceae (El-Sayed 2011; Knudsen et al. 2006). 2-Methoxy-4-methylphenol (p-creosol) is more widespread and has been found in some Amaryllidaceae, Annonaceae, Araceae, Asteraceae, Oleaceae, and Orchidaceae (Braun et al. 2011; El-Sayed 2012; Knudsen et al. 2006). The compound is biologically active and has repellent properties for TseTse flies (genus *Glossina*, Diptera, Glossinidae) (Saini and Hassanali 2007).

P-methylanisole, the main floral scent compound of *H. martii* that attracted its pollinator species, also was found in low quantities in floral bouquets of several species (Knudsen et al. 2006), but only a few species emit this compound in high relative amounts. In sycones of *Ficus semichordata* (Moraceae) in China, nevertheless, this aromatic compound represents more than 90 % of the scent

production (Chen et al. 2009), and it also is common in flowers of *Cymbopetalum brasiliense* (Annonaceae) in the Atlantic rain forest of Brazil (Braun et al. 2011) and inflorescences of *Phytelephas* palms (Arecaceae) in Ecuador and Colombia (Ervik et al. 1999). While the function of the volatile compound has not yet been studied in the beetle pollinated Annonaceae, Chen et al. (2009) demonstrated that it is unusual in *Ficus,* and functions in *F. semichordata* as the only attractant of the specialized wasp pollinator *Ceratosolen gravelyi* Grandi 1916 (Chalcidoidea, Agaonidae). Therefore, the authors considered it as a private communication channel in this specialized interaction. In *Phytelephas* palms, *p*-methylanisole has been found to attract *Drosophila* flies, *Trigona* stingless bee, and beetle pollinators (Ervik et al. 1999). *P*-methylanisole, thus, does not only lure oligolectic *P. palpalis* pollinators to *H. martii* flowers in the Caatinga, but also is a mediator in other plant-pollinator interactions involving quite different plant and insect taxa, and may generally be well perceived by a wide array of insects. Following the definition given by Raguso (2008), the substance may not be classified as a private communication channel in any of the above cited plant-pollinator associations because it is bioactive in several systems. Even though these are highly specialized and *p*-methylanisole was otherwise absent in the habitat of the plants involved.

Bees of a second congeneric, *Protodiscelis alismatis* (Ducke, 1908), oligolectic on species of *Echinodorus* (Alismataceae) (Carvalho 2012; Carvalho and Schlindwein 2011), were common at the study sites, but not attracted by any of the compounds used in the luring experiments. This points to the specificity of the chemical communication channel in the water ponds. Indeed, the blend of odors produced by flowers of *Echinodorus palaefolius* did not contain any of the main substances produced by *Hydrocleys* flowers (Carvalho, Dötterl, and Schlindwein, unpublished).

There is only limited information on the flower scent compounds involved in the attraction of oligolectic bees to their host-plants, but studies available so far demonstrate that bees either respond to compounds quite widespread among floral scents or to uncommon compounds. Females of *Andrena vaga* Panzer 1799 (Andrenidae, Andreninae), oligolectic on flowers of *Salix* (Salicaceae), and oligolectic squash bee *Peponapis pruinosa* (Say 1836) (Apidae, Eucerini) respond to widespread compounds (e.g., 1,4-dimethoxybenzene 2-phenylethanol in *Andrena*, Dötterl et al. 2005; Dötterl and Vereecken 2010; 1,2,4-trimethoxybenzene in *Peponapis*, Andrews et al. 2007). Females of the oligolectic megachilid *Hoplitis adunca* (Megachilidae, Osmiini) (specialized on *Echium* (Boraginaceae) are attracted by uncommon 1,4-benzoquinone (Burger et al. 2010), and those of another megachilid, *Chelostoma florisomne* (Megachilidae, Osmiini) (specialized on *Ranunculus*), by uncommon protoanemonine (Dobson and Bergström 2000). In the interaction between *Campanula* (Campanulaceae) and its oligolege *Chelostoma rapunculi* (Lepeletier 1841), unusual spiroacetals have been identified as key signals (Milet-Pinheiro et al. 2013).

This is the first study on scent-mediated attraction of an oligolectic species to its host-plants in the southern hemisphere and the first of a Neopasiphaeinae bee. It identifies *p*-methylanisole as a compound used by *P. palpalis* to find flowers of *H. martii*. It is found as a floral scent compound in several plant families (Knudsen et al. 2006) and elicits behavioral responses in several orders of insects. Nevertheless, in some of the interactions that are mediated by this compound, such as between *H. martii* and *P. palpalis,* which occurs in ephemeral ponds with very low species numbers of flowering plants and pollinators, the compound functions as a reliable signal in these specialized pollination systems.

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