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Scent chemistry and pollinator attraction in the deceptive trap flowers of *Ceropegia dolichophylla*

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Abstract

Ceropegia species (Apocynaceae, Asclepiadoideae) have pitfall flowers and are pollinated by small flies through deception. It has been suggested that these flies are attracted by floral scent. However, the scent that is emitted from *Ceropegia* flowers has not been studied using headspace and gas chromatography mass spectrometry methods. It has also been unclear whether or not the flowers are mimics of particular models that attract flies. In the present study, we determined the composition as well as the spatial and temporal patterns of floral scent emitted by *C. dolichophylla*. Furthermore, we determined the pollinators in the native (China) and non-native (Germany) range of this species, and tested the capability of the floral scent to attract flies in the non-native range. Our data demonstrate that the floral scent, which is emitted from morning until evening, primarily from the tips of the corolla lobes, consists mainly of spiroacetals and aliphatic compounds. Milichiid flies were common visitors/pollinators in the native as well as non-native range, and were attracted by floral scent in bioassays performed in the non-native range. The compounds emitted by *C. dolichophylla* are unusual for flowers, but are well known from insect pheromones and occur in the glandular secretions of insects. The milichiid flies that visit and pollinate the flowers are kleptoparasites that feed on the prey (haemolymph or other secretions) of predatory arthropods, e.g. spiders, to which they are attracted by scent. Our data thus suggest that the floral scent of *C. dolichophylla* mimics the feeding sites of kleptoparasitic flies.

Keywords: Bioassay; Floral scent; Fly-pollination; Headspace GC–MS; Kleptoparasites; Milichiidae; Spiroacetals

1. Introduction

Plants advertise their flowers by visual (e.g. shape and colour) and olfactory (scent) cues (Chittka and Thompson, 2001), however, the specific cues (e.g. scent compounds) responsible for attraction of pollinators are understood for just a few pollination systems (e.g. Dötterl et al., 2006; Schiestl et al., 1999). In general, the olfactory display of flowers is considered to be more specific than the visual one (Dobson, 1994). Attraction of specific pollinators in specialized systems can depend on the intensity, composition and emission time of scent (Raguso, 2008).

In the present paper we describe the chemistry of floral scent in a *Ceropegia* L. species (Apocynaceae, Asclepiadoideae) and its role in attraction of pollinators. *Ceropegia* comprises more than 180 species, all restricted to the Old World. The plants are found in tropical and subtropical habitats from Canary Islands and West-Africa as far as Australia, with main distribution areas in East-Africa, India, Madagascar and China (Meve and Liede-Schumann, 2007). Characteristic for all *Ceropegia* species is their floral Bauplan of so called pitfall flowers which can assume astonishing forms and functions. The corolla of *Ceropegia* flowers is fused resulting in a basally inflated tube. The corolla lobes are fused at their tips forming a cage like

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structure which restricts access to the flower (Vogel, 1961). The great variety in shape, size, colour, ornamentation and scent has attracted the attention of biologists for a long time (e.g. Vogel, 1961). All pollinators identified thus far are small dipterans (<3 mm in length) which belong to at least 26 genera in 20 families (Ollerton et al., 2009). The complicated pollination process, which has been described in detail by Vogel (1961), starts with the landing of the fly pollinator on the flower tip. From there the insect plunges into the slippery tube and finally slides into the inflated base. Escape from there is prevented by the presence of hairs forming a barrier between the tube and its inflated base. While being trapped within the flower for about 24 h, the fly explores its jail (for food) and comes in touch with the gynostegium, a structure formed by the fused androecium and gynoecium. The pollinaria, two discrete pollen masses (pollinia) interconnected by a mechanical clip (i.e. the corpusculum), consequently become attached to the mouthparts of the fly. If the fly carried pollinaria from a previous flower visit, one or more pollinia can be inserted into the five guide-rails on the flanks of the gynostegium. In *Ceropegia*, anthesis lasts between one to five days (Vogel, 1961; own obs.). As it withers, the flower turns downwards, obtaining at least a horizontal position (Vogel, 1961). During this process, the hairs blocking the way out of the inflated base of the tube collapse and the fly can escape. Though the flowers produce a small amount of nectar, they are considered deceptive flowers (Vogel, 1961, 1993). This is because the primary reason for flies to visit the flowers is unlikely to be the small amount of nectar they contain. The majority of fly species that visit flowers of *Ceropegia* feed either in the larval or adult stage on animals or animal secretions, and find these food sources using odour cues (Vogel, 1961, 1993). *Ceropegia* may therefore mimic animal-related odours, though other possibilities are mimicry of rotting plant material, because it is used as food substrate by larvae of some flies, and mimicry of male sex pheromones, because flies attracted are mostly female (Ollerton et al., 2009; Vogel, 1961). To date, odour of *Ceropegia* flowers, though discernable to the human nose, has not been analysed with modern analytical techniques, and the compounds emitted are thus unknown. Vogel (1961) suggested that scent is emitted from the distal corolla lobes of the flowers. The period of scent emission begins at anthesis and lasts, depending on species, for a few hours to a few days (Vogel, 1961). Interactions between *Ceropegia* flowers and flies have been assumed to be mediated by floral scent (Vogel, 1961). Indeed, observations and experiments conducted in the lab point towards a function of floral scent for attracting flies from a distance and also for eliciting landing behaviours. Visual cues may play a secondary role in short-distance attraction (Vogel, 1961).

Ceropegia dolichophylla Schltr., the subject of this paper, is native to South China. We have cultivated a few individuals of this plant since 2007 in a greenhouse in Bayreuth. These plants regularly produce fruits with fertile seeds indicating that there are insects successfully transferring pollinia in the greenhouse.

As a first step to understanding the pollination systems in *Ceropegia*, we determined the pollinators of *C. dolichophylla*, and analysed its floral scents. We specifically asked, 1) which flies are pollinators/flower visitors in the native range in China, and in the greenhouse in Bayreuth? 2) which scent compounds are emitted by the flowers? 3) what is the temporal and spatial pattern of scent emission? and 4) is scent responsible for attraction of flies in the non-native range?

2. Methods and materials

2.1. Flower visitors and pollinators

To get information about the flower visitors and pollinators of *C. dolichophylla* in its native range, 100 flowers were collected in a natural habitat in the Chinese province of Guizhou on 9th July 2008 (UBT, for voucher details see Plant material). Picked flowers were immediately transferred into ethanol and subsequently dried before shipment to Bayreuth for further investigation. In Bayreuth, flowers were opened carefully and every fly present therein was classified as far as possible, and analysed for the presence of pollinaria.

To identify the flies visiting and pollinating the flowers in a greenhouse of the University of Bayreuth, we collected 100 flies inside the flowers during summers of 2007 and 2008, determined them to genus level, and 23 thereof to species level. We also checked these 23 flies for the presence of pollinaria. The abundance and occurrence of flies strongly varied during summer, and we did not determine the proportion of flowers that contained flies or that were pollinated.

2.2. Plant material

All investigations in the non-native range (scent, flower visiting flies) are based on only one accession: China, Guizhou, Fanjing Mt. (27° 55′ N, 108° 47′ E), 7th October 2007, *Y. Zhou* sub *H. Kong* 0674, (UBT). Living plants were raised from seeds collected at the original locality and grown in the greenhouse of the Dept. of Plant Systematics, University of Bayreuth.

2.3. Volatile collection

Floral volatiles were collected from cultivated *Ceropegia dolichophylla* (Fig. 1A) during daytime using dynamic headspace methods (Dötterl et al., 2005). For that purpose, individual, newly opened flowers were enclosed in polyester oven bags (5 cm × 6 cm, Toppits®, Germany) and their emitted scent was trapped by sucking the air from the bag into an adsorbent tube. Two different types of tubes were used. One type, the small sized tube, was made of ChromatoProbe quartz microvials of Varion Inc. (length: 15 mm, inner diameter: 2 mm), from which the closed end was cut off. They were filled with a mixture of 1.5 mg Tenax-TA (mesh 60–80) and 1.5 mg Carbotrap (mesh 20–40), which was fixed by using glass wool. The other and bigger type of tubes consisted of glass capillaries (length: 8 cm, inner diameter: 2.5 mm) filled with 15 mg Tenax-TA (mesh 60–80) and 15 mg Carbotrap (mesh 20–40).

The air was sucked through the tubes using a membrane pump (G12/01 EB, Rietschle Thomas Inc., Puchheim, Germany) driven by a power supply; the flow rate was adjusted to 200 ml/min

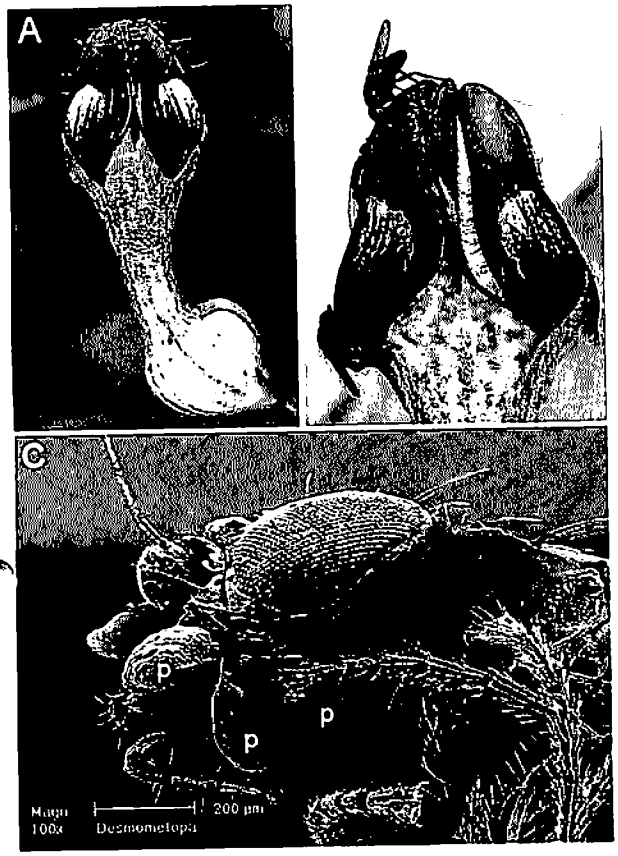

Fig. 1. (A) Flower of *Ceropegia dolichophylla*. (B) Flower tip of *C. dolichophylla* with individuals of the milichiid fly *Desmometopa sordida*. After landing, the flies crawl around on the corolla lobes extend their proboscis and probe the surface. (C) SEM of a head of *Desmometopa sordida* with pollinaria of *C. dolichophylla* attached to the base (rostrum) of its mouth parts. p = pollinium. The fly carries two pollinaria with three pollinia indicating that one pollinium was already successfully inserted into the stigmatic chamber.

(small tubes) or 100 ml/min (bigger tubes) by the use of a flow meter. To distinguish between floral and ambient air compounds, the surrounding air was collected simultaneously.

To determine the scent emitted by the flowers, individual flowers were enclosed *in situ* in the bags for 5 min followed by 2 min of scent collection into the small tubes. To analyse the spatial pattern of scent emission, five individual flowers were removed from two different plants, and cut into the four pieces 'corolla lobe tips', 'corolla lobe bases', 'corolla tube' and 'basal inflation'. For each flower these parts were enclosed separately in bags (4 cm × 5 cm) for 10 min and scent was subsequently collected for 2 min, again into the small adsorbent tubes. For the analysis of temporal scent emission, six individual flowers (two and four from different plant individuals, respectively) were separately enclosed *in situ* in oven bags for 9 h from 9 am to 6 pm, and the air was constantly sucked out using the same pumps as described above. Every hour the pumps were shut off for 10 min to allow accumulation of the floral scent, which was subsequently trapped into small adsorbent tubes for 2 min. The percentage amount of compounds was similar among the samples collected at different times (Heiduk and Dötterl, unpubl. data), and here we focus only on the total amount of scent.

To get a scent sample used for the bioassays (see below), we again enclosed individual flowers *in situ* in separate oven bags as described above. The scent was trapped using the larger adsorbent tubes and the air was sucked through the tubes for 7 h during daytime. The trapped volatiles were eluted from each adsorbent tube with 60 µl of acetone (SupraSolv, Merck KgaA, Germany). In total, we collected scent from 11 flowers, and all samples were pooled.

2.4. Chemical analysis

The volatile samples were analysed by GC–MS using a Varian Saturn 3800 gas chromatograph (GC) and a Varian Saturn 2000 mass spectrometer (MS). The GC was fitted with a 1079 injector and a ZB-5 column (5% phenyl polysiloxane, length 60 m, inner diameter 0.25 mm, film thickness 0.25 µm, Phenomenex). To allow thermal desorption of the volatiles trapped in the quartz microvials, the injector was fitted with the ChromatoProbe kit (Micro-SPE, Amirav and Dagan, 1997; see also Dötterl et al., 2005).

To flush any air from the system, the injector split vent was opened and the injector heated at 40 °C for 2 min. Then the split vent was closed, the injector heated at 200 °C/min and stayed at 200 °C for 4.2 min. The split vent was then opened again and the injector cooled down. Electronic flow control was used to maintain a constant helium carrier gas flow rate (1.8 ml/min). The GC oven temperature was held for 7 min at 40 °C, then increased by 6 °C/min to 260 °C and held at this temperature for 1 min. The mass spectra were taken at 70 eV with a scanning speed of 1 scan/s from m/z 30 to 350.

Processing of the data was performed by the help of the Saturn Software package 5.2.1. Tentative identification of floral scent components of the GC–MS spectra was carried out using the mass spectral data bases NIST 08, Wiley 8, MassFinder 3, and Adams (2007).

Scent samples were used to determine the compounds emitted from flowers or flower parts, and to determine the total amount of scent as well as the contribution of the single compounds to the total scent (percentage amount). To determine the total amount of scent, known amounts of monoterpenoids, benzenoids, and fatty acid derivatives were injected, and the mean peak area of these compounds was used for quantification.

2.5. Statistical analysis

To test whether the total amount of scent emitted differs during daytime, and among different flower parts, data were analysed using Repeated Measures ANOVAs (StatSoft, Inc., 2008). For graphical display of the temporal variation in scent during daytime (9 am to 6 pm), the total amount of scent was calculated in relation to the maximum amount of scent emitted by a specific flower. This standardisation was necessary as the total amount of scent emitted varied among flowers (Table 1).

Table 1
Total amount of scent and percentage amounts of the compounds emitted by six flowers (A–F) of two different plant individuals of *Ceropegia dolichophylla* at 9 am. KRI = Kovats retention index; tr: the amount was less than 0.05%. Values of more than 5.0% are printed in bold.

	KRI	Plant 1				Plant 2	
		A	B	C	D	E	F
Total amount trapped per min (ng)		56.9	19.1	46.1	10.1	16.0	28.6
N-bearing compounds							
N-3-Methylbutylacetamide	1141	2.2	tr	0.3	2.7	0.2	1.4
Spiroacetals							
m/z: 112,115,69,114,97,43	1152	**10.0**	**7.3**	**11.4**	2.6	**5.8**	**15.5**
m/z: 115,112,97,69,55,125	1319	**41.4**	**18.6**	**47.5**	**36.7**	**30.7**	**26.0**
m/z: 83,129,55,126,111,84	1331	**11.9**	**6.8**	**15.7**	**9.3**	**8.0**	**7.5**
Further unknown spiroacetals[a]		3.0[9]	1.6[9]	4.1[9]	2.9[9]	2.1[7]	4.2[9]
Aliphatics							
a Tridecene	1288	0.6	1.0	0.3	1.7	2.6	3.0
a Tridecene	1292	0.6	1.1	0.8	0.9	1.8	1.5
Tridecane	1300	**14.9**	**26.9**	**12.5**	**24.5**	**25.5**	**19.4**
a Pentadecadiene	1479	4.1	**10.1**	1.3	3.1	3.5	7
a Pentadecene	1483	**8.5**	**21.6**	3.2	**9.2**	**13.5**	**11.3**
a Pentadecene	1488	tr	tr	tr	0.1	0.5	0.1
Pentadecane	1500	0.3	1.1	0.4	0.9	0.9	0.7
2-Acethoxytridecane	1715	1.6	2.4	0.8	4.9	3.2	0.9
Irregular terpenes							
α-Ionone	1444	tr	0.1	0.2	0.1	tr	0.2
Unknowns[a]		0.7[2]	1.3[2]	1.5[2]	0.6[2]	1.6[2]	1.1[2]

[a] Unknown spiroacetals with a percentage amount of less than 1.0% and other unknowns were pooled with the superscript digit giving the number of pooled compounds.

2.6. Bioassay

To test whether flies can be attracted by the floral scent, the acetone scent sample (see above), representing the scent emitted during 7 h from three flowers (c. one fourth of the pooled sample), was used.

The bioassays were conducted in the field (Ecological-Botanical Garden of the University of Bayreuth). The acetone scent sample was offered in a small glass vial tucked into the soil and tested against a glass vial containing a similar amount of acetone only. The distance between the two vials was 30 cm. Bioassays took place twice (2 pm and 3 pm) on one day (September 2009; temperature: 24 °C, weather condition: full sun) lasting 40 min each. The position of scent sample and control was exchanged after 20 min each. Every fly approaching the vials within a range of 5 cm was caught (when sitting) using Eppendorf® tubes (1.5 ml).

3. Results

3.1. Flower scent

The floral scent of *Ceropegia dolichophylla*, as detectable by the human nose, can be described as sour-sweet with musky and sourish-metallic components.

The amount of floral scent emitted strongly differed among various flower parts (Fig. 2). The highest amount of scent was emitted by the very tip of the flower (lobe tips). The amount of scent emitted by the lower parts of the lobes, the lobe bases, was reduced to one sixth related to the very tip. The tube and the inflation emitted only trace amounts of scent.

During the period of measurement the total amount of scent seemed to depend on daytime, however, variation among individual flowers was high, and overall no significant differences in the scent emitted among different times were found (Fig. 3).

The total amount of scent trapped varied among flowers and was between 10 and 60 ng/min (Table 1). The flowers emitted one nitrogen bearing compound (N-3-methylbutylacetamide), spiroacetals, aliphatics, one irregular terpene (α-ionone), and a few compounds of unknown class. Spiroacetals were identified using their molecular ion combined with the characteristic pair of pronounced peaks built by retro-cleavage of the ring system

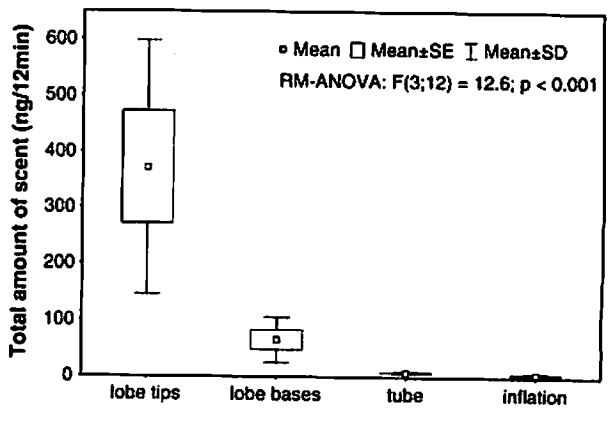

Fig. 2. Total amount of scent emitted by different floral parts of *Ceropegia dolichophylla* (five flowers from two plant individuals were used).

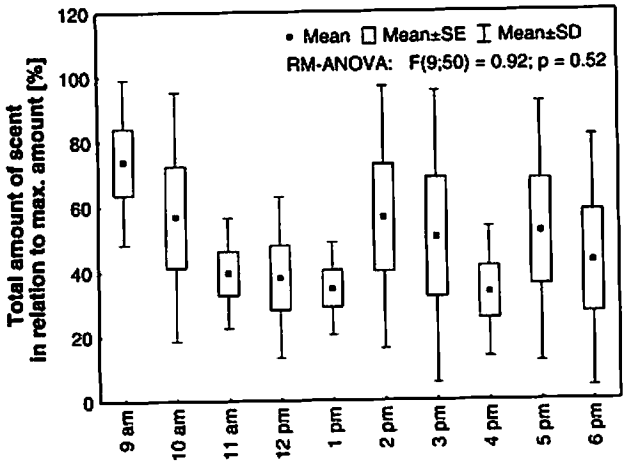

Fig. 3. Temporal pattern of floral scent emission in *Ceropegia dolichophylla*.

(see Francke and Kitching, 2001). Qualitative variation in scent was low, and most of the compounds were found in the samples of all flowers studied. Spiroacetals and aliphatics were the most abundant compound classes in all flowers. Spiroacetals contributed 34% to 79% to the total amount of scent emitted. Tridecane, one pentadecadiene, and one pentadecene were the most abundant aliphatics contributing 17% to 59% to the total amount of scent.

3.2. Flower visitors

Five insects (all flies) were found in 100 flowers collected in the native range of *C. dolichophylla* in China. These comprised a female *Desmometopa m-nigrum* (Milichiidae), two female *Neophyllomyza* sp. (Milichiidae), an unidentified species belonging to the Sciaridae and an individual insect in such bad condition that further identification was impossible (Table 2).

The 100 flies collected from flowers of *C. dolichophylla* in the greenhouse in Bayreuth, Germany, all belonged to *Desmometopa* (Milichiidae). Of these, 23 were sexed and identified to species level. All were females of *D. sordida*, and six thereof carried pollinaria of *C. dolichophylla* (Fig. 1C). Behavioural observations revealed that flies approached the flowers in the greenhouse in a zigzag manner, and landed mostly on the lobe tips. After landing many flies crawled around on the lobes, extended their proboscis and probed the lobe tips (Fig. 1B).

3.3. Bioassay

A flower scent sample (in acetone) of *C. dolichophylla* attracted 15 flies in one bioassay, and in a second biossay 12 flies during 40 min of observation each. No fly individual was attracted by the acetone controls. The first individuals approached within the first min after opening the extract tubes. All flies approached the tubes in a zigzag manner, against the direction of wind. All attracted flies were Milichiidae, and with the exception of one, all were *D. sordida*. One individual was a *Neophyllomyza acyglossa* female (Table 2).

4. Discussion

This study is the first in which scent emitted from *Ceropegia* flowers was analysed using dynamic headspace and GC-MS methods. The results show that scent in *C. dolichophylla* is mainly emitted from the corolla tips and from the morning until evening. The floral scent consisted mainly of spiroacetals and aliphatic compounds. The milichiid flies visiting the flowers in the native range in China and in a greenhouse in Germany are closely related. Bioassays with floral scent performed in the non-native range effectively attracted flies suggesting the importance of floral scent as pollinator attractant in *C. dolichophylla*.

Investigations of *C. dolichophylla* flowers collected in the native habitat revealed that female milichiliid flies (*Desmometopa m-nigrum* and *Neophyllomyza* sp.) and an unknown sciarid species are flower visitors and therefore potential pollinators. Species of both fly families and even of the genera *Desmometopa* and *Neophyllomyza* are already known visitors and potential pollinators for several *Ceropegia* species, but were not known as visitors of *C. dolichophylla* (Endress, 1996; Knuth, 1898-1905; Masinde, 2004; Vogel, 1961, 1993). The plants of *C. dolichophylla* cultivated in our greenhouse in Bayreuth, although far away from their native habitat, are regularly visited by females of *D. sordida* (Table 2). Furthermore, some of these flies also carried pollinaria clipped to their mouth parts, which suggests that they successfully act as pollinators of *C. dolichophylla* in the greenhouse (Fig. 1C). Indeed, the plants regularly set fruit, most likely as a result of geitonogamy or xenogamy (Meve, unpubl. data). The flies

Table 2
Number of dipterans found in *Ceropegia dolichophylla* flowers collected from plants in the native range (China) or from plants grown in Bayreuth, and number of dipterans attracted to floral scent in two bioassays. nd = not determined.

Family	Genus	Species	Sex	China	Bayreuth	Bioassays (Bayreuth)
Milichiidae	*Desmometopa* LOEW 1866	sp.	nd		76	
Milichiidae	*Desmometopa*	*m-nigrum* (ZETTERSTEDT 1848)	♀	1		
Milichiidae	*Desmometopa*	*sordida* (FALLÉN 1820)	♀		23	26[a]
Milichiidae	*Neophyllomyza*	*acyglossa* (VILLENEUVE 1920)	♀			1
Milichiidae	*Neophyllomyza* MELANDER 1913	sp.	♀	2		
Sciaridae			nd	1		
Unknown			nd	1		

[a] 15 and 11 flies, respectively were attracted in the two bioassays.

collected in the native range did not carry pollinaria, and we therefore do not know whether they act as pollinators. However, the *ex situ* pollinator *Desmometopa* has also been found in flowers collected in the native range and is most likely an *in situ* pollinator, too.

Sciaridae and Milichiidae both have worldwide distributions. The milichiid genus *Desmometopa* consists of 55 species, and the small black flies can easily be identified by an "M" on their frons. The two very similar species *D. m-nigrum* and *D. sordida* occurring as flower visitors in the greenhouse and natural habitat, respectively, are both cosmopolitan (Sabrosky, 1983). The milichiid fly genus *Neophyllomyza* consists of nine species distributed in all biogeographic regions (Brake, 2000, 2010). The family Sciaridae comprises 1700 described species. Milichiidae and Sciaridae are suggested to be saprophagous or phytophagous (only Sciaridae) food specialists (Vogel, 1961) or otherwise depend on carrion, fungal substrates, rotting plant or decaying organic material during the larval stages (Daly et al., 1998; Ollerton et al., 2009). Milichiid flies also have the noteworthy trait of kleptoparasitism — stealing food from other animals. They are known to feed on the prey (haemolymph or other secretions) of predatory arthropods, e.g. spiders (Eisner et al., 1991; Robinson and Robinson, 1977; Sabrosky, 1983; Sivinski, 1985; Sivinski and Stowe, 1980; Sivinski et al., 1999). Interestingly, with a few exceptions, only females are found to exploit such prey items (Sivinski, 1985; Sivinski et al., 1999). Volatile organic compounds from prey defense secretions, such as (E)-2-hexanol, hexyl butyrate, (E)-2-hexenyl butyrate, 2,4-hexadienyl hexanoate, and 2,4-hexadienyl butyrate are known to be responsible for the attraction of kleptoparasitic flies, including species of *Desmometopa* and *Neophyllomyza* (Aldrich and Barros, 1995; Beavers et al., 1972; Eisner et al., 1991; Sivinski et al., 1999; Zhang and Aldrich, 2004). Large amounts of glandular secretions are released from dead and injured insects, or from insects devoured by a predator (Zhang and Aldrich, 2004).

Volatile organic compounds, and specifically floral scents, are also suggested to be the main mode of attraction of fly pollinators in *Ceropegia* (Ollerton et al., 2009; Vogel, 1961). Our bioassay demonstrated that floral scent of *C. dolichophylla* alone is capable of attracting the fly pollinator *D. sordida*, as well as *N. acyglossa*, in the non-native range. We did not have the opportunity to test the attractiveness of the scent on the native pollinators. However, we assume that the identified potential milichiid pollinators are also attracted by the scent of the flowers in the native range, since all *Desmometopa* and all *Neophyllomyza* species have a very similar biology.

Our scent analyses demonstrate in a quantitative manner for the first time that the distal part of the flower (lobe tips) is mostly responsible for scent emission in a *Ceropegia* species, whereas other flower parts emit only very small amounts of these compounds (Fig. 2), and no other compounds (A. Heiduk, unpubl. data). This finding is consistent with the observations of Vogel (1961). He found in *Ceropegia* species other than *C. dolichophylla* that flower scent is produced by special epithelia ("osmophores") at the very tip of the flower and sniffing experiments allowed him to conclude that this flower part is also responsible for sent emission.

The flowers of *C. dolichophylla* open in the morning (between 4 and 5 am, in July) shortly before sunrise and start to wither and turn upside-down in the evening of the same day (around 8 pm) at sunset (A. Heiduk, unpubl. data). We measured scent emission from 9 am to 6 pm and results reveal that scent is continuously emitted during that time (Fig. 3). The presence of flies in some flowers already at 9 am (such flowers were not used for determining scent rhythmicity) and the occurrence of landings on flowers in the evening before sunset point towards an emission of floral scent throughout the whole time of anthesis.

C. dolichophylla flowers did not emit compounds which are known attractants for kleptoparasitic *Desmometopa* and *Neophyllomyza* (see above) or Sciaridae. Instead, flowers emit mainly three unknown spiroacetals, tridecane, a pentadecene, and a pentadecadiene (Table 1). Spiroacetals are unusual floral scent compounds with only six described so far (Knudsen et al., 2006): (E)-/(Z)-chalcogran (in few Orchidaceae, a Rubiaceae and a Solanaceae species), (E)-/(Z)-conophthorin (in [illegible] families), 8,8-dimethyl-4-methylene-1-oxospiro[2.5]oct-5-ene (in *Osmanthus fragrans* Lour., Oleaceae), and spiro[4.5]dec-1-ene (in *Hedychium coronarium* König, Zingiberaceae). These six spiroacetals typically occur only in minor amounts in the scents, but (E)-conophthorin was an abundant compound in the scent of *Chelyocarpus ulei* Dammer (Arecaceae; pollinators unknown; Knudsen et al., 2001) and *Dorstenia turnerifolia* Fisch. & C. A. Mey (Moraceae; pollinators unknown; Kaiser, 2000). Tridecane is a widespread floral scent compound, while pentadecenes and pentadecadienes are not that widespread, and typically are only minor compounds in floral scents. It is unknown whether these spiroacetals and aliphatic compounds play a role in the communication between plants and pollinators. N-3-Methylbutylacetamide and 2-acetoxytridecane, with relative abundance of up to 3% and 5% in *C. dolichophylla* scent, respectively, were not described in floral scents before. 2-Acetoxytridecane, however, is already known as a secondary metabolite in plants, and occurs in trace amounts in the essential oil of leaves of members of the Rutaceae (Ivanova et al., 2004).

Interestingly, the spiroacetals, N-3-methylbutylacetamide, and 2-acetoxytridecane are all well known insect pheromones or occur at least in glandular secretions of insects. Spiroacetals occur e.g. in beetles, wasps, bees, ants, bugs, and fruit flies, and several of them have pheromonal functions (Francke and Kitching, 2001). N-3-Methylbutylacetamide occurs as an alarm pheromone in cockroaches (Farine et al., 2002) and wasps (Keeling et al., 2004), as a male sex pheromone in fruit flies (e.g. Wee and Tan, 2005), and was found in prothoracic glandular secretions of lacewings (Aldrich et al., 2009; Zhang et al., 2006). 2-Acetoxytridecane is a female sex pheromone in midges (Hillbur et al., 2000). Therefore, these compounds are widespread among insects, and insects of several orders, including Diptera, have olfactory capabilities to detect these compounds. We assume that kleptoparasitic *Desmometopa* and *Neophyllomyza* flies perceive these compounds, and that they play a role in finding appropriate feeding sites. *Desmometopa* flies, including *D. sordida* and *D. m-nigrum* are frequently recorded to be attracted to arthropods preying on honey bees

(Landau and Gaylor, 1987; Lopez, 1984; Sabrosky, 1983), and it would be possible that one of the compounds attracting these flies to *Ceropegia* flowers is present in the alarm pheromone of honey bees. However, the compounds emitted from *C. dolichophylla* are not known from honey bees though they might be present in other prey items attractive to *D. sordida* and *D. m-nigrum*. However, no data exists about the prey of these flies in the native range of *C. dolichophylla*.

Fly-pollinated trap flowers comparable to *Ceropegia* also occur in *Aristolochia* (Aristolochiaceae) and *Arisaema* (Araceae) species, and plants of these genera also attract their flies by specific scents (Barriault et al., 2010; Sakai, 2002). Scents of these plants can be described as faint earthy, meaty (resembling carrion), and mushroom like (see also Trujillo and Sérsic, 2006; Johnson and Jürgens, 2010). In contrast to *Ceropegia*, however, these plants are suggested to mimic brood sites of the visiting flies (Proctor et al., 1996).

Vogel (1961) suggested that the scent of *Ceropegia* could be an imitation of either food sites, breeding sites or sexual pheromones of flies, and our study supports the idea that *Ceropegia* (at least *C. dolichophylla*) mimics food sites, i.e. dead insects, of the pollinating flies. Our observations and those of Vogel (1961) indicating that flies scan the plant surface with their proboscides after landing, most likely in search for food, also support this hypothesis. In contrast, our data do not support the hypothesis that *Ceropegia* flowers imitate breeding sites (dung or rotting plant material; see above), as compounds found in the present study are not known from the flies' breeding substrates or from plants mimicking such substrates (e.g. dung, see Johnson and Jürgens, 2010; Jürgens et al., 2006). The hypothesis that the flowers mimic male sex pheromones cannot yet be evaluated as the sexual pheromones of these flies are unknown. In order to further understand the myiophilous *C. dolichophylla* pollination system, it is necessary to identify the unknown spiroacetals and aliphatic compounds, and to determine their attractiveness to fly pollinators in the natural habitat.

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