Floral scent composition in early diverging taxa of Asclepiadoideae, and Secamonoideae (Apocynaceae)

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Abstract

The Apocynaceae–Asclepiadoideae are well known for their specialized floral morphologies and pollination systems and many species have distinct floral aromas. However, our knowledge on the chemistry of floral volatiles in this plant family is relatively limited although it has been suspected that floral scent plays a key function for pollinator attraction. This is the third paper in a series of papers reporting on the floral odours of Asclepiadoideae. Floral odours of eleven species from seven genera (Cibirhiza, Fockea, Gymnema, Hoya, Marsdenia, Stephanotis and Telosma) of early diverging taxa of Apocynaceae–Asclepiadoideae, and two species of Secamone (Apocynaceae–Secamonoideae) were collected using headspace sampling and then analyzed via GC–MS. We detected 151 compounds, of which 103 were identified. The vast majority of chemicals identified are common components in flower odour bouquets of angiosperms. However, striking was the high relative amount of acetoin (97.6%) in the flower scent of Cibirhiza albersiana. This compound has rarely been reported as a flower scent component and is more commonly found in fermentation odours. Bray–Curtis similarities and Nonmetric-Multidimensional Scaling (NMDS) analyses showed that each of the species has a distinct odour pattern. This is mostly due to only twelve compounds which singly or in different combinations dominated the scent of the species: the benzenoids benzyl acetate, benzaldehyde, methyl benzoate, and 2-phenylethyl alcohol; the monoterpenoids (E)-ocimene, (Z)-ocimene, linalool, and eucalyptol; and the aliphatic compounds acetoin, and (E,Z)-2,6-nonadienal. The floral scent compositions are discussed in relation to tribal affiliations and their potential role for pollinator attraction, and are compared with the scent data available from other Asclepiadoideae species.

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1. Introduction

Floral scent has a key function for pollinator attraction in many angiosperm species (Jürgens, 2009; Raguso, 2008a,b). Unique and characteristic fragrance patterns in closely related species may indicate divergent evolution and different types of pollinators (Shuttleworth and Johnson, 2009), while a similar odour composition in unrelated species may indicate convergent evolution due to adaptations to pollinator types with similar physiology and behavioural preferences (e.g. Knudsen and Tollsten, 1993, 1995). Therefore, knowledge on the floral odour chemistry, particularly of highly specialized pollination systems, has provided insight into the ecology and evolution of their pollination systems (see Dobson, 2006). Good examples are the well-investigated orchids, where complex floral morphologies and pollen transfer via pollinaria together with specific odour signals are often associated with specific pollinator interactions (e.g. Schiestl, 2004).

The Apocynaceae–Asclepiadoideae are a group with comparably complex floral morphologies that have evolved also remarkably similar mechanisms for pollen transfer via pollinaria. The Asclepiadoideae (Worldwide) comprise approximately 3000 species (Meve, 2002) in 172 genera (Endress et al., 2007). Together with the subfamilies Secamonoideae (Old World) and Periplocoideae (Old World) they formerly represented the family Asclepiadaceae, the group of phylogenetic and taxonomic research of two of the authors of this paper (UM, SL-S). However, while the floral odour chemistry in orchids is one of the best investigated within the angiosperm families (see...
Knudsen et al., 2006), only few studies have investigated the floral odour composition of Apocynaceae–Asclepiadoideae. A systematic screening of the floral scent chemistry has been done only for the two most advanced tribes Ceropogieae (Old World, sapromyiophilous pollination syndrome and fetid odours, Jürgens et al., 2006) and Asclepiadeae (worldwide, sweet-scented day-flowering associated mainly with Hymenoptera, Lepidoptera, and Diptera as flower visitors, Jürgens et al., 2008) of subfam. Asclepiadoideae, the largest of the five subfamilies of Apocynaceae. No data are available for the basalmost tribe Fockeaeae (Old World); and in the fourth tribe, the Marsdenieae (worldwide), scent data of only two taxa, Hoya carnosa R. Br. (Altenburger and Matile, 1988; Kaiser, 1994; Matile and Altenburger, 1988) and Stephanotis floribunda Brongn. (Effmert et al., 2005; Kaiser, 1994; Mookherjee et al., 1990; Pott et al., 2002), were published earlier.

The existing data on the floral scent chemistry of just 32 species from two tribes of the Asclepiadoideae (Jürgens et al., 2006: Ceropogieae; Jürgens et al., 2008: Asclepiadeae) already show a relatively high chemical diversity of flower scents and suggest that a diverse range of different insects groups is involved in their pollination (see Jürgens et al., 2006, 2008). The data of the Ceropogieae species with fetid odours (Jürgens et al., 2006) indicate that there may be a number of different chemical strategies operating under the sapromyiophilous syndrome, including mimicry of carrion (characterized by oligosulphide emission), mimicry of carnivore faeces (characterized by emission of oligosulphides, phenol and skatole) and mimicry of herbivore dung. Floral scent data from, to the human nose, sweetly scented species of the tribe Asclepiadeae showed a different set of chemicals missing the main components found in the Ceropogieae.

However, many tribes and pollination syndromes of Asclepiadoideae have not been sampled and without these data any comparison with other plant families is biased. This study is the third in a series of studies reporting on the floral odour chemistry of Asclepiadoideae and it addresses two major objectives. First, to investigate the chemical diversity of floral scent in the basal groups within the family, and second, to compare in a meta-analysis the findings with the data on the other groups so far investigated and to discuss the data in relation to the pollination biology and tribal affiliations of the taxa.

2. Materials and methods

2.1. Plant material

All plants used for scent collection were or are in cultivation in the greenhouses at the Department of Plant Systematics at the University of Bayreuth (Germany). Voucher data of the plants investigated are given in Table 1. Either plants stem from field collections and related herbarium vouchers had been deposited when the living plant was taken into cultivation or the living plant in culture is the only existing specimen and serves as its own voucher. Most of the species were raised in the greenhouse from seeds collected in the field.

Volatile scents were sampled from intact flowers still attached to the living plants and from as many plant individuals as available (between 1 and 4 per species; see Table 2). For a selection of flower photographs of some of the sampled Asclepiadaceae see Fig. 1.

2.2. Volatile sampling

For the volatile sampling we used miniaturized thermodesorption tubes (micro-vials), similar to a packed capillary column, which can be loaded into a modified GC injector for thermal desorption (see Gordin and Amirav, 2000). The thermodesorption tubes were prepared from standard quartz sample vials (15 mm × 1.9 mm L.D., Varian, Inc., Palo Alto, CA, USA) that were opened on both sides. These micro-vials were filled with Tenax and Carbotrap (3–6 mm) and a piece of glass wool was added on both sides of the adsorption material to keep it in place (Döttler and Jürgens, 2005). The micro-vials were cleaned by washing with acetone and heated for 30 min at 250°C. Flowers (1–10, depending on species) were enclosed in polyacetate (oven) bags for volatile sampling. Scent-containing air was sucked through the micro-tube (flow rate 200 ml/min) with a battery-operated membrane pump (G12/01 EB, Rietschle Thomas, Memmingen, Germany).

2.3. Gas chromatography/mass spectrometry

The micro-vials were analyzed on a Varian Saturn 2000 System using a 1079 injector that had been fitted with the ChromatoProbe kit (see Dötterl and Jürgens, 2005 and references therein). This kit allows the thermal desorption of small amounts of solids or liquids contained in quartz microvials (Amirav and Dagan, 1997). A micro-vial was loaded into the probe, which was then inserted into the modified GC injector. A ZB-5 column (5% phenyl polysiloxane) was used for the analyses (60 m long, inner diameter 0.25 mm, film thickness 0.25 μm, Phenomenex, Torrance, CA, USA). An electronic flow control maintained a constant helium carrier gas flow of 1.8 ml/min. The GC oven temperature was held for 4.6 min at 40 °C, then increased by 6 °C per min to 260 °C and held for 1 min. The MS interface was 175 °C and the ion trap worked at 200 °C. The mass spectra were taken at 70 eV (in El mode) with a scanning speed of 1 scan/sec from m/z 30 to 350. The GC–MS data were processed using the Saturn Software package 5.2.1. Component identification was carried out using the NIST 2005 mass spectral data base and confirmed by comparison of relative retention times with the MassFinder 2.1 software and published data (Adams, 1995). Identification of individual components was confirmed by comparison of both mass spectra and GC retention times with those of authentic standards.

2.4. Statistical analyses

The variability of the individual floral scent samples collected from the 13 species in the present study was assessed using the Primer 6 program (Clarke and Gorley, 2006). Percentage data of compounds (= relative amounts with respect to total peak area) were used because the total amount of emitted volatiles varied greatly among different individuals. Compounds considered potential...
artefacts were excluded from the analysis. Non-metric multidimensional scaling (NMDS), based on Bray–Curtis similarities, was used to detect similarities and differences among samples. Data were square root transformed before calculating Bray–Curtis similarities. To evaluate how well a particular configuration produces the observed distance matrix, the stress value is given: The smaller the stress value, the better the fit of the reproduced ordination to the observed distance matrix (Clarke, 1993). The significance of differences in scent profiles between species was assessed by ANOSIM (Primer 6 programme; Clarke and Gorley, 2006) with 10,000 random permutations. To separate the effect of plant phylogeny (in terms of species belonging to the same tribe) from the effect of species differences, ANOSIM was calculated in a 2-way crossed layout (factors: species; plant tribe). SIMPER (factor: species) was used in Primer to identify the compounds responsible for dissimilarities among species (Clarke and Warwick, 2001).

To compare the floral scent data with those of other Asclepiadoideae species examined in two previous studies we conducted a similar analysis on a combined data set of 43 Asclepiadoideae and Secamonoideae species (13 Fockeeae, Marsdenieae and Secamoneae species from this study, 15 Ceropogeae and 15 Asclepiadeae species from Jürgens et al., 2006, 2008 respectively). The scent samples from all studies have been analyzed on the same instrument with the same analysis settings (compare this study with Jürgens et al., 2006, 2008).

Means for relative amounts were calculated from all samples collected for any species and analyzed via Bray–Curtis and NMDS. To determine whether scent differences between species were correlated with pollination systems, species of this larger data set were categorized into broad categories of pollination syndromes (hypothesized plant-pollinator associations) based on the ASCLEPOL database at (http://www.bio.uni-bayreuth.de/planta2/research/pollina/as_pol_d.html; and Jürgens et al., 2006).

### Table 1

Systematic affiliation of species investigated, abbreviated species names, voucher identification, subjective description of flowers and scent, and data on flower visitors (where available).

| Subfamily : tribe                      | Species | Abbr. | Vouchers (all in UBT) | Flower colour/morphology | Subjective description of floral scents | Flower visitors |
|---------------------------------------|---------|-------|-----------------------|--------------------------|----------------------------------------|-----------------|
| Asclepiadoideae: Fockeeae             | Cibirhiza albersiana Kunze, Meve and Liede | C. alb. | Tanzania, Specks 21460 ex hort. Frohning | Greenish spotted purplebrown | Slightly putrid | No data |
| Fockea angustifolia K. Schum.         | F. ang. | F. ang. ex hort. Frohning | Green petals, white tubular corona | Sweet | No data |
| Fockea edulis (Thunb.) K. Schum.      | F. edu. | F. edu. ex hort. | Green-yellow petals, white tubular corona | Slightly sweet | No data |
| Asclepiadoideae: Marsdenieae (clade I) | Hoya heuschkeliana Kloppenb. | H. heu. Philippines, Schmidt 96–96 | Bright red; urceolate | Slightly sweetish with caramel and (artificial) strawberry aromas | No data |
| Hoya increassata Warb.                | H. inc. | H. inc. Philippines, Meve 994 | Cream petals with brownish tips, corona white | Strongly sweet and musky | No data |
| Asclepiadoideae: Marsdenieae (clade II) | Gymnema sylvestre R. Br. | G. syl. | Cameroon, Meve 916 | Bright yellow | Sweetish, cheesy | Different Diptera species (pollinaria attached to the flies): Calliphoridae, Drosophilidae, Muscidae, Sarcophagidae, Sepsidae, Tachinidae, Tephritidae (Bhatnagar, 1986) |
| Marsdenia engleriana W. Rothe         | M. eng. Costa Rica, Voigt s.n. | Bright yellow | Sweet, similar to Jasminum and Syringa, predominantly released during the day |
| Marsdenia gillespieae Morillo         | M. gill. Guyana, Ollerton et al. 212 Cuba, Mangelsdorff 2285 | Ochre-yellow | Slightly sweetish | No data |
| Marsdenia linearis Decne.            | M. lin. ex. hort. | Rose | Sweetish | No data |
| Stephanotis floribunda (R. Br.) Brongn. | St. flo. Pakistan, Sultan s.n. | White, tubular corolla | Strongly sweet at evening and night | No data |
| Telosma pallida Wight                | T. pal. | Bright yellow, with long corolla tube | Sweet, subtle perfume, predominantly at evening | Different species of Noctuidae (Lepidoptera) (Bhatnagar, 1986) |
| Secamonoideae: Secamoneae             | Secamone afzelii (Roem. and Schult.) K. Schum. | S. afz. | Cameroon, Meve 922 | Yellow-orange | Sweet and musky | No data |
| Secamone parviflora S. Moore          | S. par. Tanzania, Liede and Meve s.n. | Yellow | Sweet | No data |
Table 2
Floral volatiles in 13 Asclepiadoideae (Apocynaceae) species. Order and abbreviations as in Table 1. Average relative amounts (in %) of floral scent compounds are listed according to compound class and Kovats retention index (KRI). tr = trace amounts (<0.1%). Unknowns which did not reach at least 5% of relative amount in any sample were pooled with the superscript digit indicating the number of pooled compounds. CAS # = CAS Registry Number.

| KRI | CAS #  | C. | F. | F. | H. | H. | G. | M. | M. | M. | St. | T. | S. | S. | par. |
|-----|--------|----|----|----|----|----|----|----|----|----|----|----|----|----|-----|
|     |        | alb.| ang.| edu.| heu.| inc.| syl.| eng.| gl.| lin.| flo.| pal.| afz.| par. |
|-----|--------|----|----|----|----|----|----|----|----|----|----|----|----|----|-----|
|     |        | 13 | 53 | 47 | 19 | 36 | 8  | 30 | 14 | 52 | 17 | 22 | 2  | 2   |
| Total number of compounds | | | | | | | | | | | | | | | |
| Number of samples collected | | 1 | 4 | 1 | 2 | 1 | 2 | 2 | 3 | 2 | 2 |

**Aliphatic compounds**

**Aldehydes**

- Hexanal
- Heptanal
- Octanal

**Esters**

- 4-Penten-1-ol
- (Z)-3-Hexen-1-ol
- (E)-2-Hexen-1-ol

**Ketones**

- Isobutyric acid
- 3-Methyl-butanoic acid
- Hexanoic acid
- Nonanoic acid
- Decanoic acid

**Acids**

- Isobutyric acid
- 3-Methyl-butyric acid
- 2-Methyl-butyric acid
- Hexanoic acid
- Nonanoic acid
- Decanoic acid

**Unknown fatty acid esters**

**Nitrogen containing compounds**

- Benzyl nitrite
- Indole
- Methyl anthranilate
- Unknown nitrogen containing compounds

**Benzenoids and phenylpropanoids**

- Benzaldehyde
- Phenol
- p-Methyl anisole
- Benzyl alcohol
- Phenylacetaldehyde
- Acetophenone
- Methyl benzoate
- 2-Phenylethyl alcohol
- Benzoic acid
- Benzyl acetate
- Ethyl benzoate
Table 2 (continued)

| KRI | CAS # | C. alb. | F. ang. | F. edu. | H. heu. | H. inc. | G. syl. | M. eng. | M. gil. | M. lin. | St. flo. | T. pal. | S. azf. | S. par. |
|-----|-------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
|     |       | 13      | 53      | 47      | 13      | 19      | 36      | 8       | 30      | 14      | 52      | 17      | 22      | 40      |
|     |       | 1       | 4       | 1       | 2       | 1       | 2       | 2       | 3       | 2       | 2       | 2       | 2       | 2       |

**Benzenoids and phenylpropanoids**

Methyl salicylate 1351 606-45-1
2-Phenylethyl acetate 1224 103-45-7
Benzy1 isobutanoate 1306 103-28-6
Unidentified aromatic ester<sup>b</sup> m/z: 104, 105, 103, 91, 102, 106, 63, 39, 65, 77
Dimethyl salicylate 1351 606-45-1
Eugenol 1385 97-53-0
Benzy1 isovalerate 1396 10361-39-4
Benzy1 valerate 1402 103-38-8
Isoamyl benzoate 1476 94-46-2
Benzy1 benzoate 1790 120-51-4

**Monoterpenoids**

α-Thujene 934 2867-05-2
α-Pinene 954 80-56-8
Camphene 951 79-92-5
Thuja-2,4(10)-diene 958 36262-09-6
β-Phellandrene 1021 555-10-2
α-3-Carene 1025 13466-78-9
Eucalyptol 1030 470-82-6
p-Cymene 1039 99-87-6
Limonene 1043 138-86-3
Thuja-2,4(10)-diene 958 36262-09-6
α-3-Carene 1025 13466-78-9
Eucalyptol 1030 470-82-6

**Sesquiterpenoids**

Z-Linalool oxide furanoide 1084 5989-33-3
E-Linalool oxide furanoide 1098 34995-77-2
Terpinolene 1099 586-62-9
Linalool 1104 78-70-6
(Z)-Rose oxide 1105 876-16-8
E,E)-2,6-Dimethyl-1,3,5,7-octatetraene<sup>c</sup> 1125 460-01-5
Lilac aldehyde A 1155 53447-45-3
Lilac aldehyde B+C 1163 53447-46-4
Lilac aldehyde B+C 1163 53447-47-5
p-Menthone 1169 89-80-5
Lilac aldehyde D 1178 53447-48-6
α-Terpinel 1190 98-55-5
Myrtenol 1194 515-00-4
Unidentified monoterpenoid<sup>b</sup> m/z: 81, 95, 137, 121, 136, 82, 93, 80, 109, 96

**Unknown monoterpenoids**

Unidentified monoterpenoid<sup>b</sup> m/z: 107, 135, 91, 151, 39, 150, 105, 109, 79, 122

**β-Cyclocitrall**

Unidentified monoterpenoid<sup>b</sup> m/z: 753

**Sexoterpeneoids**

α-3-Elemene 1352 20307-84-0
α-Cubebene 1367 17699-14-8
α-Ylangene 1393 14912-44-8
α-Copaene 1397 3856-25-5
β-Bourbonene 1409 5208-59-3
β-Gurjunene 1412 489-40-7
α-Cubebene 1416 13744-15-5
Unidentified monoterpenoid<sup>b</sup> m/z: 2.7<sup>2</sup>

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Table 2 (continued)

| KRI | CAS # | C. alb. | F. ang. | F. edd. | H. heu. | H. inc. | G. syl. | M. eng. | M. gir. | M. lin. | St. flo. | T. pal. | S. azf. | S. par. |
|-----|-------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| Total number of compounds | | 13 | 53 | 47 | 13 | 19 | 36 | 8 | 30 | 14 | 52 | 17 | 22 | 40 |
| Number of samples collected | | 1 | 4 | 1 | 2 | 1 | 1 | 2 | 2 | 3 | 2 | 2 | 2 | 2 |

Sesquiterpenoids

Seychellene 1458 20085-93-2 – – 0.1 – – – – – – – – – – –
(Z)-β-Farnesene 1461 28973-97-9 – 0.2 0.1 – – – – – – – – – – –
α-Humulene 1481 6753-98-6 – 0.2 – – – – – – – – – – –
(E)-β-ionone 1487 79-77-6 – – – – – – – – 3.1 – – – –
γ-Murolene 1496 30021-74-0 – – 0.3 – – – – – – – – – – –
Valencene 1508 4630-07-3 – – 1.4 – – – – – – – – – – –
(E,E)-α-Farnesene 1514 502-61-4 – – – – 0.1 – – – – – – – – –
Calamene 1546 483-77-2 – – tr – – – – – – – – – – –
β-Calacorene 1547 50277-34-4 – – 0.1 – – – – – – – – – – –
(E)-Nerolidol 1548 40716-66-3 – – 0.4 – – – – – – – – – – –
(E,E)-Farnesyl acetate 1579 4128-17-0 – – 1.9 – – – – – – – – – – –
Unknown sesquiterpenoids – 1.53 0.21 tr 2.31 0.15 0.13
Irregular terpenes

4-Oxoisophorone 1158 1125-21-9 – – – – 7.7 – – – – 5.4 8.3 –

Unknowns

Unknown1: m/z: 94, 153, 67, 81, 95, 135, 39, 43, 53, 93

1174 – – – – 5.4 – – – – – – – – –

Unknown2: m/z: 41, 39, 55, 67, 57, 43, 69, 81, 56, 82

1181 – – 0.2 – – – 8.7 – – – – – 1.0

Other unknowns 0.27 2.91 0.64 3.84 6.82 0.41 3.53 1.03 3.12 5.01 7.65

Total percentage of identified compounds 99.3 88.1 96.5 98 45.5 85.1 100 88.4 80.6 87 95.1 94.6 90.8

* Compound might be of anthropogenic origin.

1 Mass fragments for unknowns are listed with the base peak first, followed by other fragments in order of decreasing abundance.

2 Possibly an artefact built from (E)-ocimene (see Kaiser, 1993).

...and references therein for sapromyiophilous species or plant-pollinator associations based on the ASCLEPOL database for Cynanchum formosum, Jonkers (1990) for Desmedorchis flava, Bhatnagar (1986) and Rahman (1995) for Telosma pallida, Bathnagar (1986) for Gymnema sylvestre, Krombein et al. (1979) for Funasus cyanthochooides, and inferred from Wolff et al. (2008) observations on Orthosia ellemannii for O. scoparia, and personal observations by U. Meve and S. Dötterl for Vincetoxicum hirundinacea. For species with no data plant-pollinator associations were hypothesized based on floral morphology, and time of floral scent emission: (1) predominantly Diptera-pollinated, (2) predominantly Hymenoptera-pollinated systems (including wasps and bees), (3) predominantly Lepidoptera-pollinated, (4) beetle-pollinated, and (5) generalists. These categories were mapped on the NMDS plot to find out if species of the same category show similarities in their odour composition. A statistical analysis using ANOSIM and SIMPER was not conducted as the categorization into plant-pollinator associations was mostly hypothetical.

3. Results

3.1. General overview and compound class patterns

In the flowers of 13 species of three investigated tribes, 151 volatile compounds were detected, of which 33 were identified to compound class and 103 to compound (Table 2). Only 15 compounds remained unknown and unidentified. Altogether, the investigated species show a wide variety of volatile compounds, including fatty acid derivatives, benzenoids and phenylpropanoids, monoterpenoids, sesquiterpenoids, and nitrogen containing compounds (Table 2). More than 48% of the occurring compounds were isoprene derivatives, including 42 sesquiterpenoids with diverse hydrocarbon skeletons, 30 monoterpenoids, and one irregular terpenoid (4-oxoisophorone) (Table 2). Also present in our study species are 21 benzenoids and phenylpropanoids and 38 aliphatic compounds (including hydrocarbon esters, alcohols, ketons, acids, and aldehydes), of which several are products of the lipoxygenase cascade (Croft et al., 1993), and 5 nitrogen containing volatiles (e.g. indole) (Table 2). Most of the compounds were detected in only small relative amounts, and only 21 compounds (8 benzenoids, 6 aliphatic compounds, 6 monoterpenoids, and one sesquiterpene) reached a relative amount higher than 10% in any species.

Although sesquiterpenoids comprised the highest number of different compounds, they played only a minor role in terms of the relative (percentage) amount that they contributed to the total scent of any given species. Species can roughly be grouped according to the dominance of either of three other compound classes (Fig. 2): (1) Monoterpenoids dominated the scent in Secamone azelii (81.7%), S. parviflora (59.0%), Hoya incrassata (89.2%), and H. heuschkeliana (63.2%); (2) Benzenoids dominated in Marsdenia engleriana (93.2%), St.
floribunda (79.3%), G. sylvestre (57.0%), Fockea edulis (53.8%), and T. pallida (49.7%). (3) Aliphatic compounds were prominent in Fockea angustifolia (65.2%), and Cibirhiza albersiana (98.5%). Only the two Marsdenia species M. linearis and M. gillespieae showed no clear dominance with a single compound class contributing about 50% or more.

3.2. Species-specific patterns of scent compounds

The number of scent compounds varied markedly among species, ranging from 8 in Marsdenia englerianna to 53 compounds in F. angustifolia (Table 2). Sample sizes are quite low due to the limited number of plants in cultivation, but all species for which several individuals could be sampled show high similarity among samples (see e.g. grouping of four samples of F. angustifolia and three samples of St. floribunda in Fig. 3). Overall the studied species showed a high diversity of distinct species-specific patterns of volatile compounds, clear differences in floral scent composition were found even in closely related species, and consequently there is a good separation of species in odour space (Fig. 3: ANOSIM Global R_{species} = 0.79; p < 0.01; 2D stress value 0.15). In six species a single compound (a different one for each species) contributed about 50% or more to the scent bouquet but also in species with a less clear dominance of a single compound, odour composition was individual and distinct (all chemicals that contributed more than 20% to total scent are mapped onto

Fig. 1. Flowers of some of the sampled Apocynaceae species (for vouchers see Table 1): a) Cibirhiza albersiana. b) Fockea angustifolia. c) Gymnema sylvestre. d) Hoya incrassata. e) Marsdenia englerianna. f) Marsdenia gillespieae. g) Secamone afzelii. h) Telosma pallida. All photographs by U. Meve.
Fig. 2. Floral scent composition of 13 Asclepiadoideae and Secamonoideae species ordered by dominance of compound classes. Species with >50% monoterpenoids (H. inc–S. par) on the left, >50% aliphatic compounds (F. ang–C. alb) on the right; Species with intermediate composition (M. lin and M. gil) frame the benzenoids-dominated species (T. pal–M. eng) in the centre. For species abbreviations see Table 1. MT = monoterpenoids, AC = aliphatic compounds, BC = benzenoids, NCC = nitrogen-containing compounds, ST = sesquiterpenoids, UNK = unknowns, Others = e.g. irregular terpenoids, sulphur containing compounds.

Fig. 3. Non-metric multidimensional scaling (NMDS) based on Bray–Curtis similarities of the odour composition (151 compounds) of all samples from 13 species (Fockeeae, Marsdenieae, and Secamoneae). 2D stress value = 0.15; ANOSIM Global Rspecies = 1; p < 0.01.
Fig. 3; for more details see also Table 2): *C. albersiana* was dominated by acetoïn (2-hydroxy-3-butanone), which made up 97.6% of the total scent in this species. Benzaldehyde was the dominating compound in *M. engleriana* (81.2%) and (E)-ocimene in *S. azelii* (74.8%). Two moth-pollinated species, *T. pallida* and *St. floribunda*, were dominated by methyl benzoate (49.2% and 62.1% respectively) but *T. pallida* differed clearly from *St. floribunda* by its exceptionally high eucalypt content (21.1%). Benzaldehyde was predominant in *F. edulis* (52.7) in combination with (E)-ocimene (34.3%), whereas *F. angustifolia* emitted 33.0% of (E,Z)-2,6-nonadienal. In *M. linearis* 37.2% of (E)-ocimene were combined with the highest contents of acetophenone (14.2%) and (E)-geranyl acetone (12.7%) found in any species. Both *Hoya* species had high relative amounts of monoterpénoids, but while the scent of *H. incrassata* was dominated by (Z)-ocimene (26.4%) and an unidentified monoterpénoid (34.0%), the scent of *H. heuschkeliana* was dominated by (E)-ocimene (37.5%) in combination with linalool (20.9%). *S. parviflora* had also high proportions of linalool (24.0%) but differed regarding the composition of its other less dominant scent compounds. And finally, *G. sylvestre* was distinct due to its high content of 2-phenylethyl alcohol (27.1%).

While we found such a high diversity of patterns of volatile compounds among species, the species do not cluster according to their systematic relationships. For example species of the tribe Marsdenieae are widely distributed in odour space (Fig. 3) and even the three *Marsdenia* species do not cluster together. In addition, there are only a few instances in which a compound was exclusively present in relatively closely related species as such as (E,Z)-2,6-nonadienal in *Fockea* (although it was only found in traces in *F. edulis* compared to 33% in *F. angustifolia*). Consequently, no significant differences between the different tribes (*Fockeeae*, Marsdenieae, Secamoneae) were evident (ANOVSIM, tribe, R = 0.052; p = 0.297; Fig. 3).

### 3.3. Meta-analysis of combined data from this and two previous studies

The results of the statistical analysis of the scent, combining the data of 43 species from this study and two previous studies can been seen in Fig. 4. In total 237 compounds (median = 27 compounds per species) were included in the analysis. The NMDS based on Bray–Curtis similarities shows that species that have been classified as sapromyiophilous systems all group together and are separated from the others. The two moth-pollinated Marsdenieae species show similar odour patterns and are well separated from the sapromyiophilous stapeliads (Fig. 4). For the other species the picture is less clear. Most of the Asclepiadoïdes, Fockeeae, remaining Marsdenieae and their far relatives, the two Secamoneae species, take intermediate positions between the sapromyiophilous Ceroxepheae and the moth-pollinated Marsdenieae; only one Fockeeae and one Marsdenieae species have separate positions at the margins of the field (*C. albersiana* and *H. incrassata*). Many of the open access, primarily Diptera-pollinated species show an overlap with species for which Hymenoptera have been observed or are suggested as the primary pollinators.

### 4. Discussion

**4.1. This study—floral scent in relation to systematics and pollination biology**

Most of the 103 identified compounds found in the 13 species investigated are well-known scent compounds commonly found in flowers of many different angiosperm taxa (Knudsen et al., 2006). The most striking finding, regarding the floral odour composition in the analysed species, is the scent of the Fockeeae species *C. albersiana* (Fig. 1a) that consisted almost exclusively of the aliphatic ketone acetoïn (2-hydroxy-3-butanone). Unfortunately, no data exists on the pollination biology of *C. albersiana*. (A compilation of all published and some unpublished pollination data from Asclepiadoïdes is online with the ASCLEPOL database at [http://www.bio.uni-bayreuth.de/planta2/research/pollina/as_pol_d.html](http://www.bio.uni-bayreuth.de/planta2/research/pollina/as_pol_d.html); see Ollerton and Liede, 1997). The flower colour patterns of *C. albersiana* resemble those of sapromyiophilous stapeliads (compare Fig. 1a with Jürgens et al., 2006), suggesting that the species might belong to the same pollination syndrome, although it takes a distinct position (Fig. 4) due to its particular acetoïn content, a compound typically found as a result of microbial fermentation. Acetoïn is, as demonstrated by sniffing experiments, responsible for the, to the human nose, slightly putrid scent of this species. It is a rarely reported floral scent compound (Knudsen et al., 2006) and is typically found, often in combination with aliphatic alcohols, as a product of sugar fermentation by bacteria and yeast (Goodrich et al., 2006 and references therein), and has also been recorded in headspace samples of rotting meat (Johnson and Jürgens, 2010). However, Goodrich et al. (2006) and Goodrich and Raguso (2009) found high relative amounts of acetoïn in the floral scent of *Asimina triflora*, and *Asimina parviflora* (Annonaceae) that are probably pollinated by flies or beetles suggesting that fermentation odours are used by flowers to attract specific pollinators. These observations, together with our data suggest that the occurrence of floral fermentation odours seems to be a more widespread phenomenon in flowering plants. Fermentation odours have been shown to elicit strong behavioural responses in fruit flies and some beetles. Acetoïn for example has been shown to be attractive to *Drosophila melanogaster* in behavioural bioassays (Stensmyr et al., 2003) and nitidulid beetles were attracted by the fermentation odours produced by yeasts in a study by Nout and Bartelt (1998). Further studies are needed to identify whether flies or beetles are the target of the emitted fermentation odours.

The two other Fockeeae species included in our study differ in scent composition and morphology clearly from *C. albersiana* (Figs. 1, 2 and 3; Tables 1 and 2), and for both these sweetly scented *Fockea* species with tubular coronas it can at least be assumed that their nectar can be accessed only by long-tongued insects. Summing up some of the compounds that are often correlated with butterfly- and moth-pollination for the species investigated here (benzaldehyde, benzyl alcohol, linalool, methyl benzoate, (E)- and (Z)-ocimene, 4-oxoisophorone, phenylacetaldehyde, 2-phenylethyl alcohol e.g. Guédot et al., 2008; Knudsen and Tollsten, 1993), these attractants reach in *F. edulis* 88.1% but only 16.1% in *F. angustifolia*.
Pollinator observations may confirm different pollination syndromes for these two species.

It has been hypothesized by Ollerton and Liede (1997) that fly pollination is primitive within the Asclepiadoideae and in the tribe Marsdenieae, to which 8 of the 13 investigated species of the current study belong to, open access flowers, which are typical for fly-pollination (see Ollerton and Liede, 1997), predominate (Fig. 1). However, the only Marsdenieae in our study of which flower visitor and pollinator data are available are two Marsdenieae species with tubular corolla, *Telosma pallida* and *St. floribunda*, for which moths have been observed or suggested as pollinators (Bhatnagar, 1986). Accordingly, the compounds that correlate with butterfly- and moth-pollination as listed above sum-up to 81.5% in *St. floribunda* and 69.2% in *T. pallida*. In *St. floribunda* the white, tubular corollas and the emission of scent in the evening correspond to the classic diagnostic features of a moth-pollinated plant (Faegri and Van der Pijl, 1979; Matile and Altenburger, 1988) and the odour chemistry of this species fits well into that picture. It has been shown in earlier studies that the flowers of *St. floribunda* emit primarily methyl benzoate, 1-nitro-2-phenyl ethane, linalool and methyl salicylate and that the rhythm of emission has a maximum for linalool and methyl benzoate around midnight, while 1-nitro-2-phenyl ethane emission reaches highest levels in the morning (Matile and Altenburger, 1988; Pott et al., 2002). Methyl benzoate was the dominant component in *St. floribunda*, accompanied by (E)-ocimene, and benzylalcohol, however, linalool and 1-nitro-2-phenyl ethane were missing from our samples, which were collected in the late afternoon. Both compounds, methyl benzoate and linalool, are commonly reported in the floral odours of moth-adapted plant species (e.g. Dobson, 2006; Jürgens et al., 2003; Knudsen and Tollsten, 1993; Raguso and Pichersky, 1999). The attractiveness of methyl benzoate and linalool for noctuid moths has been demonstrated in upwind flight experiments in wind-tunnel systems (Dötterl et al., 2006; Plepys et al., 2002).

Interestingly *T. pallida* (Fig. 1h), the other Marsdenieae species that appears to be pollinated by different noctuid moths (Bhatnagar, 1986), has also methyl benzoate as the dominant component in its scent. Although moth pollination is apparently only confirmed...
for St. floribunda and T. pallida, the flowers of all other investigated species were also mostly sweetly scented to the human nose (see Table 1). Only, G. sylvestre emits a smell where an unpleasant cheesy component (likely due to its especially high content of 2- and 3-methyl-butanolic acid) is added to the sweetish scent. So it is not surprising that flies belonging to many different families are attracted by G. sylvestre flowers (Bhatnagar, 1986).

Diurnal insects, like butterflies, but possibly also nocturnal insects, might be attracted by the yellow, nectar-rich and open flowers of M. engleriana (Fig. 1e) whose scent is sweet and reminiscent to Jasminum. However, none of the Marsdenia species emits a particularly high proportion of the typical butterfly and moth attractants. Any prediction with respect to possible pollinators is difficult to make especially in the genus Marsdenia, where on different species different flies (Bhatnagar 1986; Forster, 1992) but also beetles have been so far observed as pollinators only (Forster, 1989). Similarly difficult is an interpretation of the data of the two Secamone species. While in S. afzelii the selection of moth- and butterfly attractants reached 88.5%, it was only 30.2% in S. parviflora, and no data on flower visitors are published to date.

4.2. Meta-analysis

In the combined analysis of floral scent data of 43 Asclepiadoideae and Secamonoideae species that include data from the present and two previous studies (Jürgens et al., 2006, 2008), all species of the Asclepiadoideae–Ceropegiae form a separate group at the top of Fig. 4. It is possible that the similarities in the scent composition of the Ceropegiae reflect, to some extent, the phylogenetic relatedness of these species. However, the pollination biology seems to be a more important factor and better explains the patterns found in the NMDS (see Fig. 4). This is supported by the fact that for all other species tribal affiliation seems to play no role to explain their position in Fig. 4. The Ceropegiae share a distinct odour composition with pungent odours indicating protein degradation and nitrogen rich food sources, and belong to the same pollination syndrome (sapromyiophil) (Jürgens et al., 2006). The NMDS positions Sarcostemma socotrumanum (#23 in Fig. 4) close to this group of sapromyiophilous species as its scent contains several of the carboxylic acids (e.g. hexanoic acid and nonanoic acid) which are typically found in faeces and urine of different animals, especially after degradation by bacteria (see Arnould et al., 1998; Smith et al., 2000), and in the floral scent of sapromyiophilous stapeliads (Jürgens et al., 2006; Ollerton and Raguso, 2006) such as the two Echidnopsis species included here (#29 and #30 in Fig. 4; Jürgens et al., 2006). Other compounds typically reported from sapromyiophilous flowers are skatole (3-methyl-indole), and indole (e.g. Jürgens et al., 2006; Ollerton and Raguso, 2006), but only indole was found in the scent of species studied here (M. engleriana #9 and St. floribunda #4 in Fig. 4). However, the separation of the sapromyiophilous stapeliads (Ceropegiae) from other Asclepiadoideae as demonstrated by NMDS (Fig. 4) is mainly due to the occurrence of the oligosulfides dimethyltrisulfide, and dimethylthiosulfide.

Moth-pollinated species are fairly separated from the others species (Fig. 4; but see also Fig. 3), but there are no clear clusters or patterns for the other species, which more or less evenly fill the transition zone between these two syndromes. For many of these mostly open access species pollination by wasps, bees, or flies is suspected but unconfirmed.

4.3. Conclusions and summary

According to Ollerton and Liede (1997) it seems likely that fly pollination has been lost subsequently and regained a number of times throughout the family. It might be assumed that the high potential to produce different volatiles is an important feature that pre-adapts a plant group for relatively fast and thus frequent switches between different pollinator types. The variety of scent compounds emitted by the different species of the Asclepiadoideae studied supports this view. The variety can be interpreted both ways: either as an advantageous pre-adaptation for the shift towards fly-pollination, or as the result of repeated gain and loss of fly-pollination in the past. This is because a wide range of different scent compounds from different biosynthetic pathways are potential fly attractants. Flies are an enormously diverse group in terms of life-strategies. Diptera pollinators of “asclepiads” include such different groups as Calliphoridae, Drosophilidae, Empedidae, Muscidae, Sarcoptagidae, Sepsidae, Tachinidae, and Tephritidae (Meve and Liede, 1994). It is likely that the chemical diversity in the floral scent of Asclepiadoideae reflects to some extent olfactory and behavioural differences of the fly groups associated with the plants. While some of the chemicals may exploit the searching behaviour of flies for carbohydrate sources (e.g. nectar and fruits), others may exploit their searching behaviour for protein rich sources (e.g. dung, carcasses and bacterial protein degradation) as food and/or brood sites. Interestingly, the data of Heiduk et al. (2010; data not included in the meta-analysis) suggest that flowers of another Asclepiadoideae, Ceropegia dolichophylla, mimic odours released from the food of the kleptoparasitic fly pollinators, i.e. secretions of insects. There is probably a good chance that the floral bouquet of any Asclepiadoideae species includes at least one or several compounds that attract some kind of fly, allowing for further selection and evolution of the interaction towards a specialised fly-pollination syndrome.

However, when comparing the combined scent data of the Asclepiadoideae with the list of floral scent compounds from Knudsen et al. (2006) for seed plants (991 species having in total 1791 compounds), it seems that the family Apocynaceae shows a diversity of volatiles very similar to other plant families, with 4.5% (43) of species encompassing 13.2% (237 compounds) of the total number of volatile compounds found in seed plants. Although it is not easy to compare data from different studies that have used different instruments and sampling techniques, the numbers of identified compounds for the Apocynaceae and three other families are relatively similar when considering the species numbers: Araceae (55 species, 349 compounds), Magnoliaceae (26 species, 141 compounds) and Arecales (40 species, 209 compounds) (see Knudsen et al., 2006). Thus the number of scent compounds per species ranges between 5.2 and 6.3 in these families. For the Orchidaceae (835 compounds identified in 417 species/subspecies), this value is only 2.0 whereas in the Rosaceae
(264 compounds identified in 24 species/subspecies) this value is with 11 much higher. Considering the small number of plant families of which floral scents of species have been analyzed, it is probably premature to draw any conclusions on whether these differences reflect research gaps and bias rather than truly differing family-specific capacities for the production of a wide diversity of chemicals. The relatively low compound/species value for orchids, for example, may be due to a saturation effect because orchids have been extensively investigated. Thus the likelihood to discover new compounds is probably much lower for Orchidaceae than for other plant groups for which our knowledge is still very limited, thus underlining the importance of studies such as the present one. The Apocynaceae are now mostly represented by Asclepiadoideae and including more species from other subfamilies is likely to increase the scent diversity in this family further.

In summary, since Knudsen et al. (2006) published their updated checklist on floral scent, some progress has been made with regard to the analysis of odours of Apocynaceae and especially its subfamily Asclepiadoideae, which is now one of the better-investigated groups among the angiosperms. It shows a wide range of chemicals and mixtures representing different pollination syndromes, such as sweet scents of the moth pollination syndrome or pungent scents representing the carrion fly syndrome. The chemicals emitted by the flowers seem to reflect different resource types, where the pungent odours of carrion fly systems mimic food sources rich in nitrogen, amino acids, or proteins whereas the sweet scents of the moth-pollinated species reflect carbohydrate resources (nectar). The finding that the floral scent composition of many Hymenoptera- and open access Diptera-pollinated flowers are located in a two-dimensional ordination between these two more clearly defined pollination syndromes seems to indicate that they signal (to a different degree) a combination of resources. Nevertheless, there are still wide gaps in our knowledge regarding the pollination biology of this plant group with most of our pollinator projections being hypothetical, based on floral morphology and scent chemistry. To truly test the correspondence between scent chemistry and pollinator type, more data are needed documenting pollinators. This will then allow for a better understanding of the floral scent patterns in an ecological and evolutionary context.

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