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Where are volatiles produced in the highly synorganised inflorescence of *Arum maculatum* L.?

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Abstract

Floral scents vary spatially within flowers and inflorescences, with effects on pollinator behaviour. Here, we restudied the spatial scent pattern of *Arum maculatum*, an Araceae, with a higher spatial resolution than previously. The inflorescence of this species consists of a spadix, whose distal part is a sterile osmophore called the appendix, whereas its basal part bears sterile and fertile male and female flowers. This spadix is surrounded by a spathe, which forms a floral chamber around the flowers. So far, it was known that the appendix is responsible for the emission of most of the volatiles, but some volatiles were specifically found within the floral chamber, with the organs(s) responsible for their emission being unclear. By repeatedly collecting (dynamic headspace) and analysing (gas chromatography / mass spectrometry) the scent of whole inflorescences and of inflorescences with the spathe and the various zones of the spadix sequentially removed, we determined and quantified all scent compounds of the major units of the inflorescence of *A. maculatum*. We confirm that the inflorescence scent is mainly composed of scents released by the appendix, and we for the first time provide evidence that the male flower zone of the spadix also contributes to the inflorescence scent. Future studies are needed to test whether, and if, how, this spatial scent pattern in *A. maculatum* affects the movement of pollinators within the inflorescence and their pollination effectiveness.

Keywords Araceae, dynamic headspace, gas chromatography-mass spectrometry, floral volatiles, floral ecology, bicyclogermacrene

1. Introduction

Most flowering plants are pollinated by animals (Ollerton et al., 2011), and they show inventive variations of visual and/or chemical (e.g. floral scent) signals in their flowers/inflorescences (Albre et al., 2003; Ollerton and Dafni, 2005; Baldwin, 2010). Floral scent cues enable pollinators to identify host plant species from a long distance and are key mediators in both diurnal and nocturnal pollination systems (Dormer, 1960; Dudareva and Pichersky, 2000; Raguso, 2008; Dötterl and Gershenzon, 2023). At short distances, visual cues as well as scent often direct the pollinators to rewarding flowers and within the flowers/inflorescences to the rewards (e.g. pollen, nectar) themselves (Dötterl and Jürgens, 2005; Dobson et al., 2005; Effmert et al., 2005). Many pollinators were shown to integrate visual and olfactory cues (Pellmyr and Thien, 1986; Kunze and Gumbert, 2001; Burger et al., 2010; Farré-Armengol et al., 2013), and both cues contribute to flower constancy of pollinators and guarantee effective pollen transfer among individuals within species (Dafni et al., 1997).

Visual and olfactory cues are known to vary temporally and spatially within flowers/inflorescences (Dobson et al., 1990; Knudsen and Gershenzon, 2006; Steenhuisen et al., 2010; Picazo-Aragonés et al., 2020). Temporal changes in visual and olfactory cues are well understood (Albre et al., 2003; Hoballah et al., 2005; Dötterl et al., 2012; Cordeiro et al., 2019; Szenteczki et al., 2021). Plants, e.g. change their colours and scents following pollination to direct pollinators to un-pollinated flowers (Jones and Buchmann, 1974; Weiss and Lamont, 1997; Ohashi et al., 2015; Lynch et al., 2020), and only release scents at day times when their pollinators are active (Albre et al., 2003; Jürgens et al., 2014; Marotz-Clausen et al., 2018.). Well documented are also visual (e.g. colour) spatial patterns within flowers/inflorescences as they help to position the pollinators in a way that they get in contact with the reproductive organs of the flowers while accessing the reward(s) (Willmer, 2011). Yet, comparably limited knowledge pertains to the importance of spatial scent patterns within flowers/inflorescences for pollinators, even though it is well-known that scent varies among pollen, nectar, and different organs or parts of a flower/inflorescence (e.g. Dobson et al., 1987; Pichersky et. al., 1994; Raguso, 2004; Dötterl and Jürgens, 2005; Flamini and Cioni, 2010; Heiduk et al., 2010; Martin et al., 2017; Jiang et al., 2020). It is also known that various volatiles released by a flower can trigger specific behavioural responses in pollinators (e.g. Dobson and Bergström, 2000; Haverkamp et al., 2016).

Most studies determining spatial floral scent patterns focused on species with flowers as a single pollination unit. Only a few studies, to the best of our knowledge all in Araceae,

determined the scent pattern of inflorescences forming a functional unit. So far, spatial scent patterns of inflorescences were studied in *Arum maculatum* (Kite, 1995), *Lagenandra ovata* (Buzgo, 1998), *Sauromatum guttatum* (Hadacek and Weber, 2002), *Homalomena propinqua* (Kumano and Yamaoka, 2006), *Schismatoglottis baangongensis* (Hoe and Wong, 2016), *Amorphophallus titanum* (Raman et al., 2017), *Philodendron adamantinum* (Gonçalves-Souza et al., 2017), and *Lysichiton americanus* (Pellmyr and Patt, 1986; Brodie et al., 2018). The inflorescences of these species are often quite large, and scent patterns might strongly influence the movement of flower visitors along the inflorescence, and thus also pollination effectiveness.

The Araceae comprise about 3700 species (Boyce and Croat, 2020). Their common features are an unbranched spadix carrying clusters of bisexual or unisexual flowers, and a bract named the spathe that often (partly) surrounds the spadix (Mayo et al., 1998). The arrangement of flowers along the spadix differs among genera, most obviously among genera such as *Philodendron* and *Arum*. In *Philodendron* species the whole spadix is covered with flowers and staminodes. The fertile staminate flowers occupy the distal part of the spadix, downwards followed by sterile staminate flowers reaching into the floral chamber, and, basally, by a small zone of fertile pistillate flowers within the floral chamber (Pereira et al., 2014; Gonçalves-Souza et al., 2017). In *P. adamantinum*, a species pollinated by cyclocephaline scarab beetles, fertile and sterile staminate zones of the spadix were shown to be the main organs of odour emission. The pistillate zone contributed only very modestly to the scent bouquet (Gonçalves-Souza et al., 2017).

In *Arum*, however, the spadix distally ends with an appendix, a sterile osmophore (Mayo et al., 1998; Gibernau et al., 2004b). Sterile male flowers, fertile male flowers, and female flowers are confined to the basal region, enclosed by the spathe forming a pollination chamber (Gibernau et al., 2004b). In *Arum maculatum*, the only species in this genus for which spatial scent patterns were studied before, Kite (1995) discriminated between the odour released by the spathe and the appendix, and odour available inside the chamber. He found that the inflorescence odour is predominantly emitted by the appendix (Kite, 1995). This was confirmed by Chartier et al. (2013, 2016) and by Szenteczki et al. (2021). The sesquiterpene bicyclogermacrene (wrongly identified as germacrene B in Kite, 1995; see Kite et al., 1998), however, was only recorded inside the chamber (Kite, 1995). Yet, it remained unknown so far which organ(s) within the chamber (e.g. fertile flowers (male or female) or sterile hair-modified flowers) predominantly release this volatile compound. Interestingly, a recent study found that the gene bicyclogermacrene synthase is expressed in male flowers but not in the appendix of *A. maculatum*, suggesting that bicyclogermacrene might be both produced and released by the male flowers within the chamber (Szenteczki et al., 2022). *A. maculatum* is a brood-site deceptive plant species pollinated by psychodid and other flies (Urru et al., 2011; Chartier et

al., 2013, 2016; Laina et al., 2022), and a recent electrophysiological study found that its pollinating flies respond to bicyclogermacrene (Gfrerer et al., 2022). Thus, this volatile compound might be involved in pollinator attraction, and in support of this hypothesis, it correlated with sex- and species-specific attraction of the main pollinators, i.e. *Psychoda phalaenoides* and *P. grisescens* (Szenteczki et al., 2022). Being released inside the floral chamber (whatever the – exact – floral organ), bicyclogermacrene might guide pollinators at short distances within the floral chamber.

The present study determines and quantifies the scents of the different major floral units of the inflorescence of *A. maculatum* (e.g. the spathe, the appendix, male flower zones – sterile and fertile floret zones separately –, and female flower zones altogether). Therefore, we first collected scent from the whole inflorescence, and then sequentially removed the spathe and the various zones of the spadix, until, finally, only the peduncle remained.

2. Results

2.1 Total absolute amount of scent

Sequential removal of inflorescence organs had strong effects on the total amount of scent released ($X^2_{N = 4,\ df = 5} = 16.00$, $p = 0.01$). Complete inflorescences and inflorescences from which the spathe had been removed released high amounts of scent (118–695 ng/5 min), with no significant differences between them. But removal of the appendix resulted in a nearly complete loss of scent, with only trace amounts still detectable (4–7 ng/5 min) (Figure 1). Removal of further zones of the spadix did not have further statistical effects on the amount of scent released.

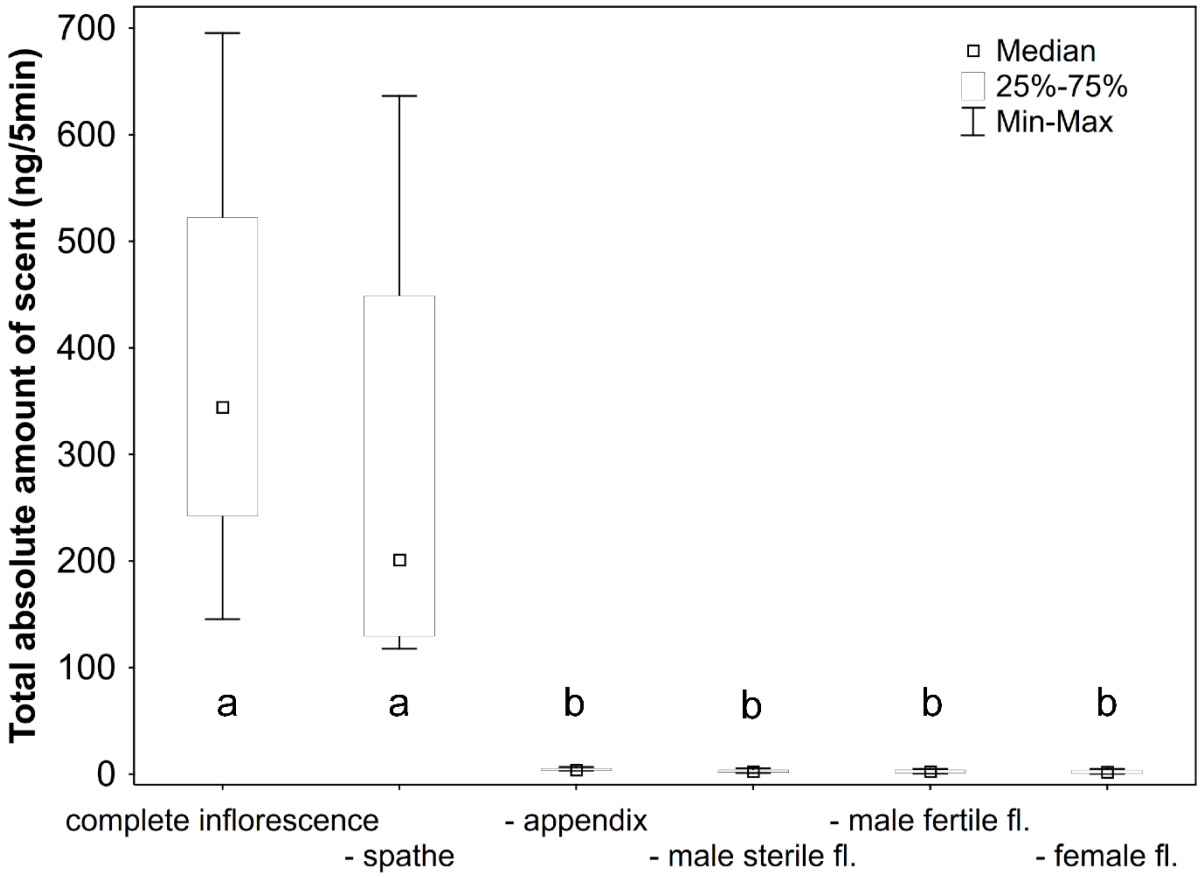

Figure 1. Total absolute amount of scent trapped (median, quartiles, Min–Max) from whole inflorescences of *Arum maculatum* and after sequential removal of their organs. fl.: flower zone of spadix. Different letters indicate significant differences according to Wilcoxon matched pairs post-hoc tests.

2.2 Number of substances and qualitative scent pattern

Sequential removal of inflorescence organs had strong effects on the number of compounds recorded ($X^2_{N = 4,\ df = 5} = 14.67$, $p = 0.01$). Complete inflorescences and inflorescences from which the spathe had been removed released between 32 and 47 compounds (59 overall), with no significant differences between them. Inflorescences from which any zone of the spadix had been removed, however, released only between two and eight compounds (Figure 2).

Complete inflorescences and inflorescences with the spathe removed released various aliphatic compounds, terpenoids, and unknowns, and also a few compounds of other compound classes (e.g. aromatics, nitrogen-bearing compounds). Forty-four compounds, especially terpenoids, but also many aliphatic, nitrogen-bearing, and all aromatic compounds were lost after removal of the appendix (Table 1).

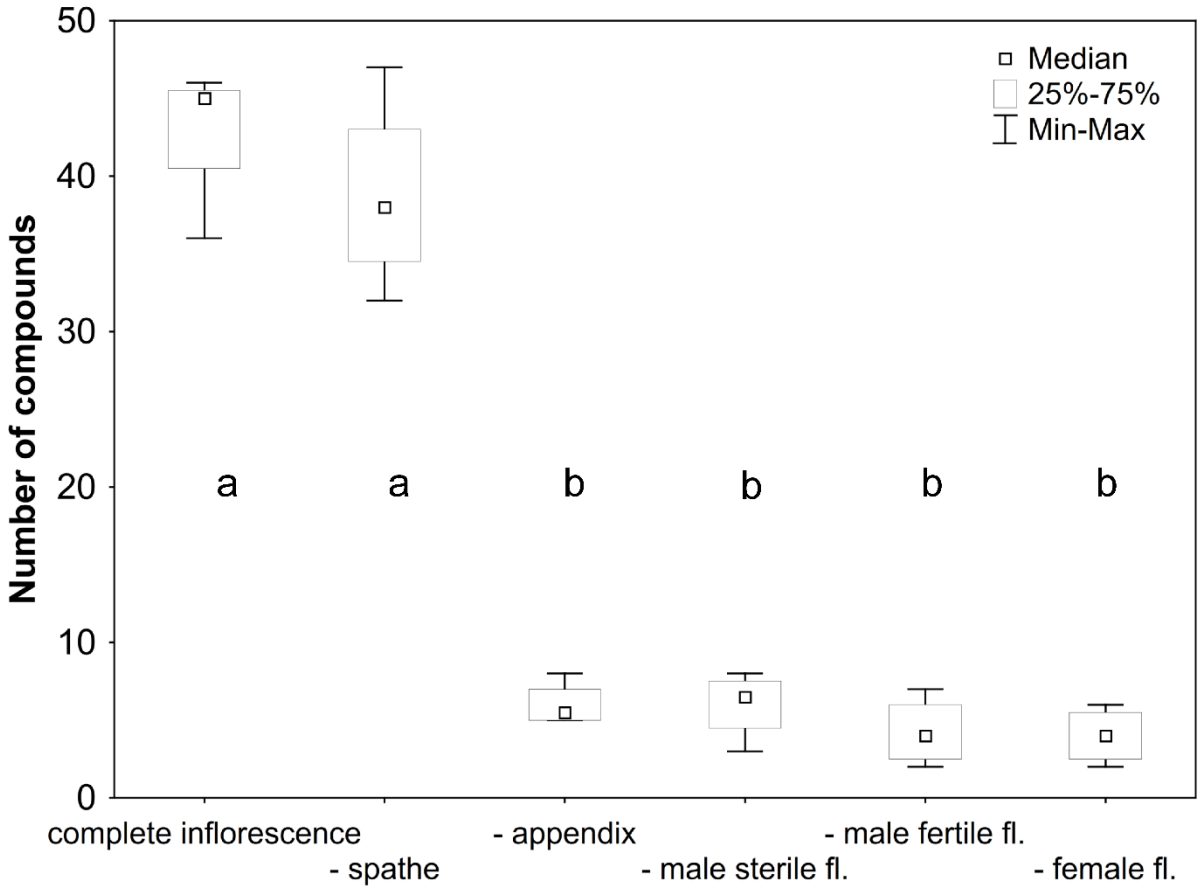

Figure 2. Number of compounds trapped from inflorescences of *Arum maculatum* during sequential removal of their organs. fl.: flower zone of spadix. Different letters indicate significant differences according to Wilcoxon matched pairs post-hoc tests.

Six compounds (butanoic acid, a methylheptanone isomer, hexanoic acid, 2-undecanone, indole, unknown RI 1353 with m/z: 121, 93, 107, 79, 91, 41) remained throughout all manipulations in at least one of the inflorescences. Others, such as 2-heptanol (aliphatic compound) and bicyclogermacrene (sesquiterpenoid) vanished with the loss of male flowers. 2-Nonanone (aliphatic compound) was no longer found after the female flower zone had been cut off (Table 2).

Table 1. Compounds that were lost due to the removal of the appendix. See Table S1 for IUPAC names of all the compounds.

Aliphatic compounds	3,7-Dimethyl-2,4-octadiene
1,3-Octadiene	(*Z*)-Sabinene hydrate
2,6-Dimethyl-1-heptene or 2-Methyl-1-octene	*allo*-Ocimene
2-Heptanone	*neoallo*-Ocimene
1-Octen-3-ol	Santolina alcohol
2-Nonanol	4-Terpineol

(*Z*)-3-Octenyl acetate	2,7- or 3,7-Dimethyloctanol
	β-Citronellol
Nitrogen-bearing compounds	
cf 1-Butyl pyrrole	**Sesquiterpenoids**
β-Lutidine	α-Copaene
	(*E*)-β-Caryophyllene
Aromatic compounds	α-Humulene
p-Cresol	δ-Cadinene
p-Creosol	
	Unknowns
Monoterpenoids	Unknown RI 1429, *mlz* 93, 41, 91, 69, 133, 79 (< 1%)
α-Citronellene	
3,7-Dimethyl-2-octene	18 Unknowns (17 thereof < 1%)
2,6-Dimethyl-2,6-octadiene	

2.3 Semi-quantitative scent pattern

Sequential removal of inflorescence organs had strong effects on the relative scent composition (Pseudo-$F_{5,15}$ = 3.72, $p < 0.001$). Pairwise PERMANOVA analyses showed that this effect was only due to the removal of the appendix ($p = 0.03$), whereas the removal of the spathe from the complete inflorescence ($p = 0.40$), and removal of flower-bearing zones of the spadix did not have significant effects ($p \geq 0.30$) on scent composition (Figure 3).

Scents of complete inflorescences and also inflorescences with the spathe removed were dominated by indole, β-citronellene, *p*-cresol, and 2-heptanone (Table 2, see also Figure 3). This scent composition strongly differed from scents of inflorescences with also the appendix removed, as they were characterised by high relative amounts of hexanoic acid, butanoic acid, and a methylheptanone isomer (Table 2, see also Figure 3). These volatile compounds were also released by the peduncle, though partly (a methylheptanone isomer) in smaller relative amounts. After removal of the appendix, two of the inflorescences were also emitting high relative amounts of bicyclogermacrene (≥ 35%) and an unknown compound (≥ 23%; RI 1353, Table 2). The (relative) amounts of these two compounds decreased strongly in one inflorescence and slightly in the other after removal of the male sterile flower zone. These two compounds disappeared from the scents of both inflorescences after the removal of the male fertile flower zone (Table 2). The other two inflorescences released these two compounds only in very small amounts (bicyclogermacrene: max. 1%; unknown RI 1353: max. 3%) without a clear pattern.

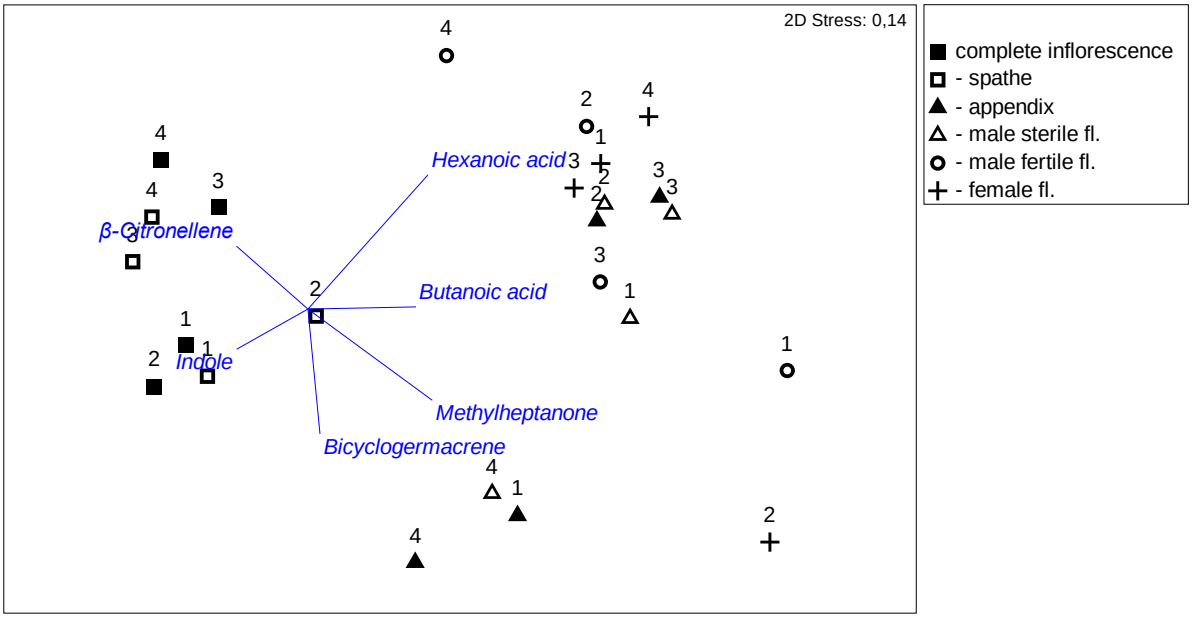

Figure 3. Non-metric multidimensional scaling (NMDS) to visualise similarities and dissimilarities in relative scent composition (based on pairwise Bray Curtis similarities) among the scent samples collected from complete inflorescences of *Arum maculatum* (N = 4) as well as from inflorescences after sequential removal of single organs/zones. The numbers indicate the four different individual inflorescences, and the compounds most correlated with the axes are also shown. fl.: flower zone of spadix.

Table 2. Median relative amounts and Min–Max (in brackets) of scent compounds (%) of complete and dissected inflorescences of *Arum maculatum* (N = 4); total amounts are also given (Median, Min–Max). RI: retention index. Unknowns with amounts < 1.0% are pooled. Tr: relative amount < 0.05%; 0.0: median of 0.0%, but compound was recorded in one individual; —: substance was not recorded in any plant individual. Values ≥ 5.0% are printed in bold. See Table S1 for IUPAC names of all the compounds.

	RI	Complete inflorescence	– spathe	– appendix	– sterile ♂ flowers	– fertile ♂ flowers	– ♀ flowers
Median (Min–Max) total absolute amount of scent (ng/5 min)		345 (152–695)	201 (118–636)	4 (4–7)	3 (3–5)	4 (<1–5)	2 (<1–5)
Aliphatic compounds							
Butanoic acid[E,S]	768	1.0 (0.1–1.7)	0.6 (0.0–3.6)	**9.2** (0.0–28.6)	**9.5** (0.2–30.9)	**18.3** (0.0–70.1)	**15.4** (1.4–18.0)
1,3-Octadiene	824	0.5 (0.0–1.9)	0.3 (0.0–2.7)	—	—	—	—
2,6-Dimethyl-1-heptene or 2-Methyl-1-octene	849	—	0.0 (0.0–0.0)	—	—	—	—
2-Heptanone[E,S]	890	**7.0** (0.0–14.4)	**13.1** (0.0–19.4)	—	—	—	—
2-Heptanol[E,S]	899	0.5 (0.0–1.9)	0.2 (0.0–0.7)	0.0 (0.0–0.1)	—	—	—
Methylheptanone isomer	954	0.1 (0.0–0.3)	0.1 (0.0–0.5)	**13.3** (0.0–19.1)	**19.7** (0.5–34.5)	**11.0** (0.0–29.9)	3.4 (0.0–98.6)
Hexanoic acid[S]	968	0.1 (0.0–6.3)	0.5 (0.0–17.5)	**32.3** (0.0–62.1)	**40.9** (20.9–64.6)	**22.2** (0.0–70.6)	**45.4** (0.0–86.8)
1-Octen-3-ol[E,S]	979	2.6 (0.0–6.0)	3.8 (0.0–11.9)	—	—	—	—
2-Nonanone[E,S]	1092	0.8 (0.0–0.9)	0.7 (0.0–1.4)	0.0 (0.0–9.4)	0.0 (0.0–17.4)	0.0 (0.0–12.7)	—
2-Nonanol [E,S]	1099	0.0 (0.0–0.1)	0.0 (0.0–0.0)	—	—	—	—
(*Z*)-3-Octenyl acetate	1279	0.1 (0.0–0.2)	0.1 (0.0–0.2)	—	—	—	—
2-Undecanone[S]	1294	0.3 (0.0–0.9)	0.1 (0.0–0.9)	4.2 (0.0–7.8)	0.0 (0.0–5.1)	4.4 (0.0–11.1)	2.0 (0.0–10.3)
C5-branched chain compounds							
2-Methylbutanoic acid[S]	839	0.0 (0.0–0.2)	0.0 (0.0–0.8)	—	0.0 (0.0–0.5)	—	—

	RI	Complete inflorescence	– spathe	– appendix	– sterile ♂ flowers	– fertile ♂ flowers	– ♀ flowers
Median (Min–Max) total absolute amount of scent (ng/5 min)		345 (152–695)	201 (118–636)	4 (4–7)	3 (3–5)	4 (<1–5)	2 (<1–5)
Nitrogen-bearing compounds							
cf 1-Butyl pyrrole	950	0.1 (0.0–0.2)	0.1 (0.0–0.2)	—	—	—	—
β-Lutidine[E]	960	0.1 (0.0–1.9)	0.0 (0.0–0.2)	—	—	—	—
Indole[E,S]	1303	**11.9** (5.7–58.6)	**22.0** (4.6–38.6)	3.1 (0.0–4.3)	0.0 (0.0–9.5)	0.8 (0.0-9.5)	0.9 (0.0–8.2)
Aromatic compounds							
p-Cresol[E,S]	1072	**8.2** (5.7–9.9)	**13.4** (0.0–26.7)	—	—	—	—
p-Creosol	1199	0.0 (0.0–0.01)	tr (0.0–0.08)	—	—	—	—
Monoterpenoids							
3,7-Dimethyloct-1-ene[E,S]	910	**5.3** (1.1–15.2)	4.5 (0.5–10.4)	—	—	0.0 (0.0–17.4)	—
α-Citronellene[E,S]	932	1.8 (0.5–4.4)	1.6 (0.0–3.3)	—	—	—	—
β-Citronellene[E,S]	946	**10.6** (6.2–35.1)	**9.1** (4.7–29.8)	—	—	0.0 (0.0–6.4)	—
3,7-Dimethyl-2-octene[E,S]	969	4.2 (2.3–10.8)	3.2 (0.0–8.2)	—	—	—	—
2,6-Dimethyl-2,6-octadiene[E,S]	1002	2.5 (1.3–3.7)	1.7 (0.2–3.3)	—	—	—	—
3,7-Dimethyl-2,4-octadiene	1026	0.3 (0.0–1.0)	0.3 (0.0–0.7)	—	—	—	—
(*Z*)-Sabinene hydrate	1073	0.0 (0.0–1.1)	0.0 (0.0–0.6)	—	—	—	—
allo-Ocimene[S]	1132	0.1 (0.0–1.7)	0.1 (0.0–1.9)	—	—	—	—
neoallo-Ocimene[E,S]	1145	0.0 (0.0–0.2)	0.0 (0.0–0.1)	—	—	—	—
Santolina alcohol	1174	0.0 (0.0–0.1)	0.0 (0.0–0.1)	—	—	—	—
4-Terpineol[E,S]	1187	tr (0.0–0.2)	tr (0.0–0.1)	—	—	—	—
2,7- or 3,7-Dimethyloctanol	1195	0.0 (0.0–0.1)	0.0 (0.0–0.1)	—	—	—	—
β-Citronellol[E,S]	1228	tr (0.0–0.3)	0.0 (0.0–0.0)	—	—	—	—

	RI	Complete inflorescence	– spathe	– appendix	– sterile ♂ flowers	– fertile ♂ flowers	– ♀ flowers
Median (Min–Max) total absolute amount of scent (ng/5 min)		345 (152–695)	201 (118–636)	4 (4–7)	3 (3–5)	4 (<1–5)	2 (<1–5)
Sesquiterpenoids							
Bicycloelemene	1343	0.1 (0.0–0.5)	0.1 (0.0–0.3)	0.0 (0.0–2.5)	0.0 (0.0–0.8)	—	—
α-Copaene[E,S]	1395	1.0 (0.3–3.7)	0.5 (0.3–1.4)	—	—	—	—
(*E*)-β-Caryophyllene[E,S]	1444	2.4 (0.9–9.0)	1.1 (0.7–3.1)	—	—	—	—
α-Humulene[E,S]	1479	1.6 (0.6–2.9)	0.5 (0.3–1.9)	—	—	—	—
Alloaromadendrene	1486	1.1 (0.4–3.8)	0.6 (0.4–1.6)	—	0.0 (0.0–1.3)	—	—
Bicyclogermacrene[E]	1521	1.5 (0.0–9.4)	3.5 (0.1–6.3)	**17.8** (0.0–47.6)	1.8 (0.0–44.9)	—	—
δ-Cadinene[E]	1542	0.3 (0.2–0.8)	0.1 (0.0–0.8)	—	—	—	—
Unknowns							
mlz 121, 93, 107, 79, 91, 41	1353	1.2 (0.0–7.9)	2.8 (0.1–5.0)	**11.6** (0.0–35.2)	2.9 (0.0–24.4)	—	—
mlz 93, 41, 91, 69, 133, 79	1429	0.8 (0.3–1.4)	0.8 (0.0–1.3)	—	—	—	—
19 Unknowns* < 1%		1.1 (0.1–11.4)	1.8 (0.0–7.2)	0.0 (0.0–0.0)	0.0 (0.0–2.0)	0.0 (0.0–0.1)	0.0 (0.0–4.8)

*One of the 19 Unknowns was present until the end of all manipulations. The other 18 Unknowns disappeared with the removal of the appendix.
E = electroantennographically active substance for psychodid and sphaerocerid flies, according to Gfrerer et al. (2022).
S = identification of compounds was verified by authentic standards

3. Discussion

Our study demonstrates that the inflorescence scent in *Arum maculatum* is released by the spadix. Not a single compound was exclusively released by the spathe as none disappeared from the profile after removal of the spathe. By far the highest amount of scent was released by the appendix, which was further the exclusive source of most of the recorded compounds. In two of the four inflorescences, two compounds, among them bicyclogermacrene, were released by male zones (sterile and/or fertile) of the spadix, as they disappeared after having removed these zones. We did not find evidence that the female flower zone of the spadix contributed significantly to the inflorescence scent. A few compounds were released by the peduncle after having removed the spathe and the spadix.

Our finding that the spathe does not significantly contribute to scent emission supports the findings of Kite (1995). We cannot exclude, though, that small amounts and a few compounds are released by this organ given that we recorded smaller median values for the scent amount and the numbers of compounds from inflorescences with removed spathe compared to complete inflorescences (Figures 1 and 2). If any, volatile compounds might be released actively or passively from the spathe, i.e. passive release may occur if volatile compounds emitted by spadix organs are ab- or adsorbed on the surface of the spathe (see also Boachon et al., 2019).

In Araceae, the spathe has been documented to contribute to scent emission but it seems not to be very frequent (Kevan, 1989; Buzgo, 1998; Gibernau et al., 1999; Vogel and Martens, 2000). In *Peltandra virginica*, the spathe is even the only scent-emitting organ (Patt et al., 1995). In *Lysichiton americanus*, the spathe (odour and colour in combination) was the most attractive to its pollinator, the rove beetle *Pelecomalium testaceum* (Staphylinidae), but only the spathe odour was sufficient to attract *P. testaceum* (Pellmyr and Patt, 1986). A gas chromatographic–electroantennographic detection (GC–EAD) study showed that three floral volatile compounds elicited antennal responses by the rove beetles, namely (*E*)-4 nonene, (*E*)-5-undecene, and indole (Brodie et al., 2018). Indole, emanating only from mature spathes, was the most attractive semiochemical being able to alone attract pollinators of *L. americanus* (Brodie et al., 2018). In other species, such as *Schismatoglottis baangongensis* (Hoe and Wong, 2016), *Amorphophallus titanum* (Raman et al., 2017), and *Homalomena propinqua* (Kumano and Yamaoka, 2006), the spathe emits volatiles different to those emitted by the spadix of the inflorescence.

As expected, most of the scent compounds, among them the most abundant ones of *A. maculatum*, are mainly (e.g. indole, β-citronellene) or only (e.g. *p*-cresol, 2-heptanone) released by the appendix (Table 1). The finding is in agreement with Kite (1995) (see also Diaz and Kite, 2002), though the inflorescence scent chemistry might differ among plant

individuals/populations. In both studies, more than 70% of the ca. 60 compounds recorded were appendix-specific, but only eight of them overlapped between the two studies (2-nonanol, 2-heptanone, *p*-cresol, 4-terpineol, α-copaene, β-caryophyllene, α-humulene, δ-cadinene), showing that the volatiles released by the appendix are quite variable in *A. maculatum*, as is true for the inflorescence scent overall (Chartier et al., 2013; Szenteczki et al., 2021; Gfrerer et al., 2021, and references therein). Several of the volatiles released by the appendix of *A. maculatum* are also released by the appendices of other *Arum* species (e.g. *A. italicum*, Diaz and Kite, 2002; Chartier et al., 2016) and non-*Arum* aroids (e.g. *Typhonium*, Sayers et al., 2020). Further, many of the appendix-specific volatiles emitted by *A. maculatum* were recently recorded to be physiologically active in the antennae of pollinating psychodid and sphaerocerid flies (Table 2; Gfrerer et al., 2022). Some of these volatiles (2-heptanone, α-humulene *p*-cresol) even attracted, individually or in mixtures, psychodid fly pollinators of *A. maculatum* and *Typhonium eliosurum* (Scheven, 1994; Kite et al., 1998; Sayers et al., 2020), underpinning the important role of the aroid appendix in deceiving the pollinators by olfactory means.

In addition to compounds exclusively released by the appendix, we identified compounds that were mainly released by the appendix, but in smaller (relative) amounts also by other organs (Table 2). Among these compounds is indole, a main compound in the scent of *A. maculatum*. It was even detected when only the peduncle was left (Table 2). This compound, an attractant to psychodid fly pollinators (Scheven, 1994; see also Sayers et al., 2020), has often been exclusively assigned to the appendix of *A. maculatum* (e.g. Kite, 1995; Kite et al., 1998) or *Sauromatum guttatum*, another Araceae (Chen and Meeuse, 1971; Skubatz et al., 1996). Recently, however, Szenteczki et al. (2022) found that genes likely responsible for the production of indole were expressed in both tissues studied, i.e. the appendix and male flowers, supporting our finding that indole is not exclusively originating from the appendix. Surprisingly, indole was not detected among the volatile compounds present in the pollination chamber (Kite 1995).

Some other compounds of the scent bouquet originated obviously, at least in some inflorescences, (mainly) from the male flower zones, among them bicyclogermacrene (see Table 2). This compound was absent after fertile male flowers had been excised, showing that the female flower zone, which was still present, did not contribute to the emission of this compound. Further, the removal of the spathe did not result in lower emission (both relative and absolute, see Table 2) of this compound, overall excluding the possibility that these two organs (the female flower zone, the spathe surrounding the flower zones) were the origin of bicyclogermacrene recorded by Kite (1995) in the floral chamber. Depending on the inflorescence, this compound seems to be mainly released, instead, by sterile or fertile male flower zones confirming the expression of bicyclogermacrene synthase exclusively in male florets of *A. maculatum* (Szenteczki et al., 2022). Interestingly, male flowers/spadix zones are

most responsible for the inflorescence scent of aroid species that do not have an appendix, such as in *Philodendron* spp. (Mayo et al., 1998; Pereira et al., 2014; Gonçalves-Souza et al., 2017), *Homalomena propinqua* (Kumano and Yamaoka, 2006), or *Dieffenbachia aurantiaca* (Etl et al., 2016).

4. Conclusions

Our data demonstrate that the inflorescence scent of *A. maculatum* is mainly composed of scents released by the appendix, and we provide, for the first time, evidence that also the male flower zone of the spadix contributes to the inflorescence scent. Further, we found that some compounds are only released by one organ/zone of the spadix, others by more organs/zones and some even by the peduncle. These findings altogether agree with a recent study that analysed the expression of genes involved in scent production of *A. maculatum* in the appendix and fertile male flowers (Szenteczki et al., 2022). It is obvious that the scent released by the appendix is key in attracting pollinators from distance. Still, it remains to be tested whether compounds released by the male flower zones, such as bicyclogermacrene, increase the attractiveness of the compounds released by the appendix and/or play a role in guiding attracted pollinators towards the floral chamber after their landing on the spathe or the appendix. A previous study found that compounds released by the male flower zone (i.e. stamens) during the female phase (bicyclogermacrene, UNK_1353; Table 2) are no longer released by inflorescences during the male phase (2nd day of flowering; Marotz-Clausen et al., 2018). Thus, these compounds appear to be only involved during the pollinator attraction phase and not to direct the pollinators to pollen-releasing stamens and/or to the exit of the floral chamber. Overall, our study shows that some compounds are released only by a single inflorescence organ, while others by several organs of *A. maculatum*, and that the scent strongly varies among inflorescence organs. This finding that the scent emission is not uniform within an inflorescence/flower is consiste-nt with studies on other plant species (e.g. Raguso, 2004; Dötterl and Jürgens, 2005; Martin et al., 2017). Yet, our knowledge on the importance of such patterns in pollination biology is strongly limited. This calls for future studies on *A. maculatum* and other plants to test whether and how these patterns are involved in manipulating pollinators. And/or to possibly discover other functions, such as promoting growth of specific organs or fighting against tissue-specific pathogens (Dötterl and Gershenzon, 2023).

5. Materials and Methods

5.1 Plant species and plant material

Arum maculatum is a European woodland monocot (Espíndola and Alvarez, 2011), mainly distributed in Western Europe, Central Europe and parts of Southern Europe (Espíndola and Alvarez, 2011). It shows thermogenesis and uses odour to communicate with its pollinators, e.g. psychodid and spaerocerid flies (Lack and Diaz, 1991; Kite, 1995; Espíndola et al., 2011; Laina et al., 2022). The deceptive strategy of monoecious *A. maculatum* focuses on 'brood site mimicry' (Willmer, 2011; Urru et al., 2011). During anthesis it releases an odour resembling the odour of dung, an oviposition site of deceived flies (Kite, 1995; Gfrerer et al., 2021).

Pollination of this protogynous plant is only possible within a short span of time. On day 1 (female phase) of anthesis female flowers are receptive. Odour, predominantly emitted during late afternoon and evening, attracts the flies (Marotz-Clausen et al., 2018; Gfrerer et al., 2021). When landing on the open upper part of the spathe or on the appendix, they finally glide into the inflorescence chamber remaining there overnight. If they carry pollen from a different plant, they potentially pollinate the receptive female flowers by moving around the inflorescence chamber looking for escape. On day 2 (male phase) of anthesis female flowers cease to be receptive, but fertile male flowers mature to release pollen. Pollen falls onto the flies, then being able to leave the chamber for eventually another deceiving inflorescence (Lack and Diaz, 1991; Ollerton and Diaz 1999; Gibernau et al, 2004b).

The plants of *Arum maculatum* used for the present study were collected in a riparian forest next to the Salzach river within the city limits of Salzburg, Austria (431 asl, 47°46'41.51"N, 13°44'20.40"E). After their excavation at the end of February, they were transferred to the Botanical Garden of the Paris Lodron University of Salzburg and potted (the volumes of the pots amounting to 7–10 litres). The pots were filled up with "Einheitserde Classic" (Einheitserde Werkverband E.V., Sinntal-Altengronau, Germany) and placed in a shady section of the Garden. The plants received regular watering until their flowering in April.

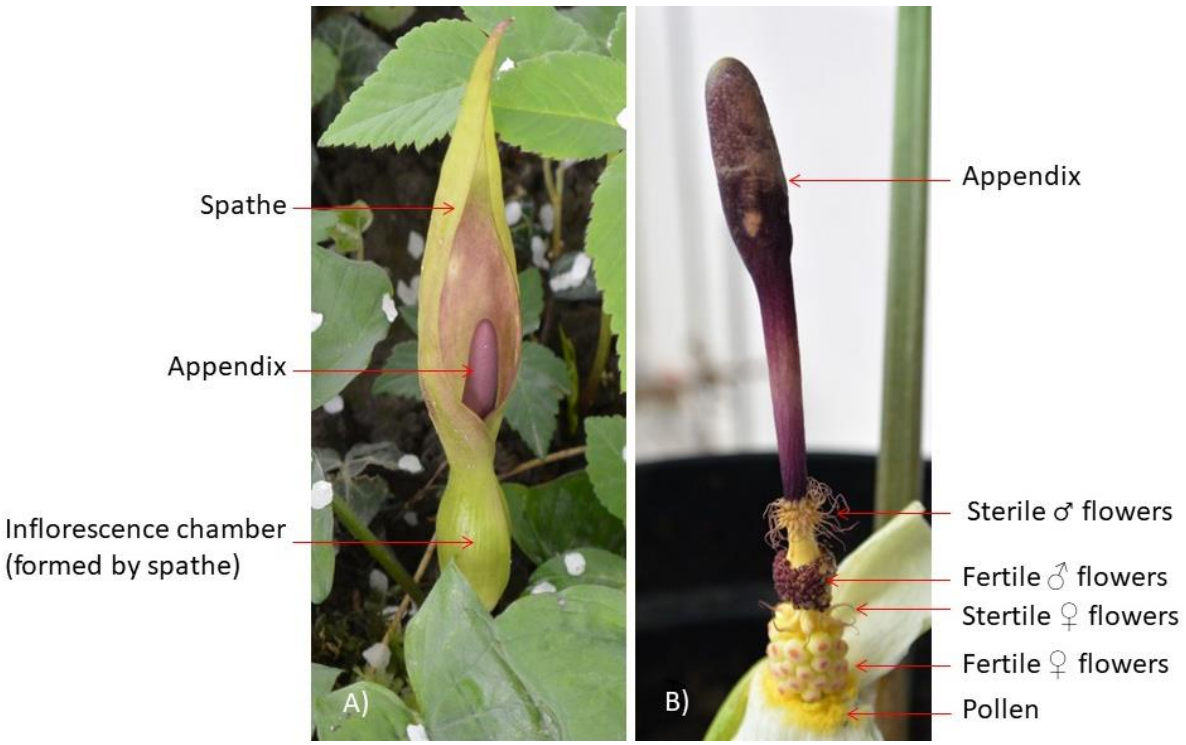

Figure 4. A) Complete inflorescence of *Arum maculatum* emitting scent during the female phase, and B) inflorescence with the spathe removed, making the different zones of the spadix visible. In B), the inflorescence was already in the male phase (note the pollen present at the bottom of the inflorescence chamber). (Photos G. Marotz-Clausen)

5.2 Collection of inflorescence scent

Samples were collected by dynamic headspace from four (dissected) female stage inflorescences between 17:00–21:00 – the time-span of highest scent production (Marotz-Clausen et al., 2018). Volatiles were collected first from a complete inflorescence. Parts of the inflorescence were then sequentially removed (spathe, appendix, zone of sterile male flowers, zone of fertile male flowers, sterile + fertile female flower zones), and volatiles were collected from the remaining 'inflorescence'. Thus, the final sample was collected from just the peduncle. Dudareva and Pichersky (2000) describe the sequential removal as most appropriate to determine the spatial scent pattern within a flower or inflorescence. The complete or dissected 'inflorescence' was enclosed within a polyester oven bag (31 x 12 cm; Toppits®, Minden, Germany). An adsorbent trap (quartz microvial of 2–3 cm length and an inner diameter of 2 mm) was inserted into the small hole cut on one upper edge of the bag. A mixture of 1,5 mg Tenax-TA (mesh 60–80) and 1,5 mg Carbotrap B (mesh 20–40; both Supelco, Germany) inside a trap was used to adsorb the volatiles. Glass wool retained this mixture in the tubes. Following Marotz-Clausen et al. (2018), for 2 min volatiles accumulated in the bag, thereafter they were trapped for 3 min with the help of a battery-operated vacuum pump (rotary vane pump G12/01 EB, Gardner Denver Austria GmbH, Vienna, Austria). Employing a power supply

and a flow meter (Cole-Parmer©, CA, USA) controlled the air flow through the trap and kept it at a flow rate of 200 mL/min.

Using the same method, three scent samples were also taken from green leaves (of three different plants). They served as negative controls and were used to discriminate between vegetative and inflorescence scents. Also, as removal of organs/parts of the inflorescence might cause injury volatiles to be released, we only considered compounds that were present in samples collected from complete inflorescences.

5.3 Chemical analyses of scent

Headspace samples were analysed with the help of an automatic TD (thermal desorption) system (TD-20, Shimadzu, Kyoto, Japan) coupled with a GC/MS (QP2010 Ultra EI, Shimadzu, Kyoto, Japan), the same as described by Marotz-Clausen et al. (2018). The GC/MS was equipped with a ZB-5 fused silica column (5% phenyl polysiloxane; 60 m long, inner diameter 0.25 mm, film thickness 0.25 µm, Phenomenex, Torrance, USA). The column flow (carrier gas helium) was adjusted to 1.5 mL/min. Beginning at 40 °C (split ratio 1:1) the GC oven temperature then increased by 6 °C per minute to 250 °C and was finally held for 1 minute. The MS interface worked at 250 °C. At 70 eV (in EI mode) mass spectra were taken from m/z 30 to 350. Processing of the GC/MS data was accomplished using the GCMSolution package, Version 2.72 (Shimadzu Corporation 2012).

Compounds were tentatively identified by applying the mass spectral libraries ADAMS (2007), FFNSC 2, W9N11, and ESSENTIAL OILS (available in MassFinder 3), and also considering linear retention indices sensu van Den Dool & Kratz (1963) of the compounds (based on n-alkane series). Some compounds were verified by comparing their mass spectra and retention times with those of standard compounds available in the reference collection of the Plant Ecology Lab of the University of Salzburg. For analysing VOCs quantitatively, 100 ng each of ca. 150 compounds available in the Plant Ecology Lab of the University of Salzburg – including monoterpenes, aliphatic and aromatic compounds – were injected into the GC/MS system. By using the mean of the peak areas (total ion current) of these compounds the total amount of scent available in the scent samples collected from *A. maculatum* was then assessed (see Etl et al., 2016; Marotz-Clausen et al., 2018).

5.4 Statistical analyses

To test for differences in the total amount of scent and the number of compounds among complete and dissected inflorescences, we calculated a Friedman ANOVA, followed by Wilcoxon matched pairs post hoc tests, both in STATISTICA 12 (StatSoft, Inc. 2012).

Differences in multivariate semi-quantitative aspects of floral scents among complete and dissected inflorescences were analysed by PERMANOVA in PRIMER 7.0.21 &

PERMANOVA+ 1 (PRIMER-E Ltd, http://primer-e.com) on pairwise Bray-Curtis similarities (relative amount of single scent compounds in relation to the total amount of scent in a sample). In these analyses, we used *plant individual* and *dissection stage* as fixed factors (9,999 permutations). The Bray-Curtis similarity matrix was further used to conduct a non-metric multidimensional scaling (NMDS), again in PRIMER, to visualise similarities and dissimilarities in scent among individual samples.

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Author contributions

Gertrud Marotz-Clausen: Conceptualisation; Formal analysis; Investigation; Methodology; Visualisation; Writing – original draft. Stefan Dötterl: Conceptualisation; Formal analysis; Supervision; Visualisation; Writing – review & editing. Marc Gibernau: Conceptualisation; Writing – review & editing.

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6. References

Adams, R.P., 2007. Identification of essential oil components by gas chromatography/mass spectrometry, fourth ed. Allured Publishing Corporation, Carol Stream, Illinois.

Albre, J., Quilichini, A., Gibernau, M., 2003. Pollination ecology of *Arum italicum* (Araceae). Bot. J. Linn. Soc.141, 205–214.

Baldwin, I.T., 2010. Plant volatiles. Curr. Biol. 20, 9, 392–397.

Boachon, B., Lynch, J.H., Ray, S., Yuan, J., Caldo, K.M.P., Junker, R.R., Kessler, S.A., Morgan, J.A., Dudareva, N., 2019. Natural fumigation as a mechanism for volatile transport between flower organs. Nat. Chem. Biol. 15, 583–588.

Boyce, P.C., Croat, T.B., 2020. The Überlist of Araceae, Totals for published and estimated number of species in aroid genera. Aroid society.

Brodie, B.S., Renyard, A., Gries, R., Zhai, H., Ogilvie, S., Avery, J., Gries, G., 2018. Identification and field testing of floral odorants that attract the rove beetle *Pelecomalium testaceum* (Mannerheim) to skunk cabbage, *Lysichiton americanus* (L.). Arthropod-Plant Interact. 12, 4, 591–599.

Burger, H., Dötterl, S., Ayasse, M., 2010. Host-plant finding and recognition by visual and olfactory floral cues in an oligolectic bee. Funct. Ecol. 24, 1234–1240.

Buzgo, M., 1998. Odor differentiation in *Lagenandra ovata* (Araceae). Plant Divers. 20, 10, 1–3.

Chartier, M., Pélozuelo, L., Buatois, B., Bessière, J-M., Gibernau, M., 2013. Geographical variations of odour and pollinators, and test for local adaptation by reciprocal transplant of two European *Arum* species. Funct. Ecol. 27, 6, 1367–1381.

Chartier, M., Liagre, S., Weiss-Schneeweiss, H., Kolano, B., Bessière, J-M., Schönenberger, J., Gibernau, M., 2016. Floral traits and pollination ecology of European *Arum* hybrids. Oecologia 180, 2, 439–451.

Chen, J., Meeuse, J.D., 1971. Production of free indole by some aroids. Acta Bot. Neerl. 20, 6, 627–635.

Cordeiro, G.D., Guimarães Fernandes dos Santos, I., da Silva, C.I., Schlindwein, C., Alves-dos-Santos, I., Dötterl, S., 2019. Nocturnal floral scent profiles of Myrtaceae fruit crops. Phytochemistry 162, 193–198.

Dafni, A., Lehrer, M., Kevan, P.G., 1997. Spatial flower parameters and insect spatial vision. Biol. Rev. 72, 239–282.

Diaz, A., Kite, G.C., 2002. A comparison of the pollination ecology of *Arum maculatum* and *A. italicum* in England. Watsonia 24, 171–181.

Dobson, H.E.M., Bergström, G., 2000. The ecology and evolution of pollen odors. Plant Syst. Evol. 222, 1–4, 63–87.

Dobson, H.E.M., Bergström, J., Bergström, G., Groth, I., 1987. Pollen and flower volatiles in two *Rosa* species. Phytochemistry 26, 12, 3171–3173.

Dobson, H.E.M., Bergström, G., Groth, I., 1990. Differences in fragrance chemistry between flower parts of *Rosa rugosa* Thunb. (Rosaceae). Israel J. Bot. 39, 143–156.

Dobson, H.E.M., Raguso, R.A., Knudsen, J.T., Ayasse, M., 2005. Scent as an attractant, in: Dafni, A., Kevan, P.G., Husband, B.C. (Eds.), Practical Pollination Biology. Enviroquest, Ltd., Cambridge, Ontario, Canada, pp. 197–230.

Dormer, K.J., 1960. The truth about pollination in *Arum*. New Phytol. 59, 3, 298–301.

Dötterl, S., Jürgens, A., 2005. Spatial fragrance patterns in flowers of *Silene latifolia*: lilac compounds as olfactory nectar guides? Pl. Syst. Evol. 255, 99–109.

Dötterl, S., David, A., Boland, W., Silberbauer-Gottsberger, I., Gottsberger, G., 2012. Evidence for behavioral attractiveness of methoxylated aromatics in a dynastid scarab beetle-pollinated Araceae. J. Chem. Ecol. 38, 1539–1543.

Dötterl, S., Gershenzon, J., 2023. Chemistry, biosynthesis and biology of floral volatiles: roles in pollination and other functions. Nat. Prod. Rep. DOI: 10.1039/d3np00024a.Dudareva, N., Pichersky, E., 2000. Biochemical and molecular genetic aspects of floral scents. Plant Physiol.122, 627–633.

Effmert, U., Große, J., Röse, U.S.R., Ehrig, F., Kägi, R., Piechulla, B., 2005. Volatile composition, emission pattern, and localization of floral scent emission in *Mirabilis jalapa* (Nyctaginaceae). Am. J. Bot. 92, 1, 2–12.

El-Sayed, A.M., 2020. The Pherobase: Database of Pheromones and Semiochemicals. http://www.pherobase.com (accessed 12 December 2021).

Espíndola, A., Alvarez, N., 2011. Comparative phylogeography in a specific and obligate pollination antagonism. PloS ONE 6, 12, e28662.

Espíndola, A., Pellissier, L., Alvarez, N., 2011. Variation in the proportion of flower visitors of *Arum maculatum* along its distributional range in relation with community-based climatic niche analyses. Oikos 120, 728–734.

Etl, F., Berger, A., Weber, A., Schönenberger, J., Dötterl, S., 2016. Nocturnal plant bugs use *cis*-jasmone to locate inflorescences of an Araceae as feeding and mating site. J. Chem. Ecol. 42, 300–304.

Farré-Armengol, G., Filella, J., Llusia, J., Peñuelas, J., 2013. Floral volatile organic compounds: between attraction and deterrence of visitors under global change. Perspect. Plant Ecol. Evol. Syst. 15, 56–62.

Flamini, G, Cioni, P.L., 2010. Odour gradients and patterns in volatile emission of different plant parts and developing fruits of grapefruit (*Citrus paradisi* L.). Food Chem. 120, 984–992.

Gfrerer, E., Laina, D., Gibernau, M., Fuchs, R., Happ, M., Tolasch, T., Trutschnig, W., Hörger, A.C., Comes, H.P., Dötterl, S., 2021. Floral scents of a deceptive plant are hyperdiverse and under population-specific phenotypic selection. Front. Plant Sci. 12, 719092.

Gfrerer, E., Laina, D., Wagner, R., Gibernau, M., Hörger, A.C., Comes, H P., Dötterl, S., 2022. Antennae of psychodid and sphaerocerid flies respond to a high variety of floral scent compounds of deceptive *Arum maculatum* L. Sci. Rep. 12, 5086.

Gibernau, M, Barabé, D, Cerdan, P, Dejean, A., 1999. Beetle pollination of *Philodendron solimoesense* (Araceae) in French Guiana. Int. J. Plant Sci. 160, 1135–1143.

Gibernau, M., Favre, C., Talou, T., Raynaud, C., 2004a. Floral odor of *Arum italicum*. Aroideana 27, 142–147.

Gibernau, M., Macquart, D., Przetak, G., 2004b. Pollination in the Genus *Arum* – a Review. Aroideana 27, 148–166.

Gonçalves-Souza, P., Schlindwein, C., Dötterl, S., Paiva, E.A.S., 2017. Unveiling the osmophores of *Philodendron adamantinum* (Araceae) as a means to understanding interactions with pollinators. Ann. Bot. 119, 533–543.

Hadacek, F., Weber, M., 2002. Club-shaped organs as additional osmophores within the *Sauromatum* inflorescence: odour analysis, ultrastructural changes and pollination aspects. Plant Biol. 4, 3, 367–383.

Haverkamp, A., Yon, F., Keesey, I.W., Missbach, C., Koenig, C., Hansson, B.S., Baldwin, I.T., Knaden, M., Kessler, D., 2016. Hawkmoths evaluate scenting flowers with the tip of their proboscis. eLife 5, e15039.

Heiduk, A., Brake, I., Tolasch, T., Frank, J., Jürgens, A., Meve, U., Dötterl, S., 2010. Scent chemistry and pollinator attraction in the deceptive trap flowers of *Ceropegia dolichophylla*. S. Afr. J. Bot. 76, 762–769.

Hoballah, M.E., Stuurman, J., Turlings, T.C.T., Guerin, P.M., Connéctable, S., Kuhlemeier, C., 2005. The composition and timing of flower odour emission by wild *Petunia axillaris* coincide with the antennal perception and nocturnal activity of the pollinator *Manduca sexta*. Planta 222, 141–150.

Hoe, Y.Ch., Wong, S.Y., 2016. Floral biology of *Schismatoglottis baangongensis* (Araceae) in West Sarawak, Borneo. Plant Syst. Evol. 302, 1239–1252.

Jiang, Y., Qian, R., Zhang, W., Wei, G., Ma, X., Zheng, J., Köllner, T.G., Chen, F., 2020. Composition and biosynthesis of scent compounds from sterile flowers of an ornamental plant *Clematis florida* cv. 'Kaiser'. Molecules 25, 1711.

Jones, C.E., Buchmann, S.L., 1974. Ultraviolet floral patterns as functional orientation cues in hymenopterous pollination systems. Anim. Behav. 22, 2, 481–485.

Jürgens, A., Glück, U., Aas, G., Dötterl, S., 2014. Diel fragrance pattern correlates with olfactory preferences of diurnal and nocturnal flower visitors in *Salix caprea* (Salicaceae). Bot. J. Linn. Soc. 175, 624–640.

Kevan, P.G., 1989. How honey bees forage for pollen at skunk cabbage, *Symplocarpus foetidus* (Araceae). Apidologie 20, 485–490.

Kite, G.C., 1995. The floral odour of *Arum maculatum*. Biochem. Syst. Ecol. 23, 4, 343–354.

Kite, G.C., Hetterscheid, W.L.A., Lewis, M.J., Boyce, P.C., Ollerton, J., Cocklin, E., Diaz, A., Simmonds, M.S.J., 1998. Inflorescence odours and pollinators of *Arum* and *Amorphophallus* (Araceae), in: Owens, S.J., Rudall, P.J. (Eds.), Reproductive biology. Royal Botanic Gardens, Kew, pp 295–315.

Knudsen, J.T., Gershenzon, J., 2006. The chemical diversity of floral scent, in: Dudareva, N., Pichersky, E. (Eds.), Biology of floral scent. Taylor & Francis Group, LLC, Boca Raton, Florida, pp 27–52.

Kumano, Y., Yamaoka, R., 2006. Synchronization between temporal variation in heat generation, floral scents and pollinator arrival in the beetle-pollinated tropical Araceae *Homalomena propinqua*. Plant Species Biol. 21, 173–183.

Kunze, J., Gumbert, A., 2001. The combined effect of color and odor on flower choice behavior of bumble bees in flower mimicry systems. Behav. Ecol. 12, 4, 447–456.

Lack, A.J., Diaz, A.,1991. The pollination of *Arum maculatum* L. – a historical review and new observations. Watsonia 18, 333–342.

Laina, D., Gfrerer, E., Scheurecker, V., Fuchs, R., Schleifer, M., Zittra, C., Wagner, R., Gibernau, M., Comes, H.P., Hörger, A., Dötterl, S., 2022. Local insect availability partly explains geographical differences in floral visitor assemblages of *Arum maculatum* L. (Araceae). Front. Plant Sci.13, 1, 1–17.

Lynch, J.H., Pichersky, E., Dudareva, N., 2020. Floral scent metabolic pathways and their regulation, in: Pichersky, E., Dudareva, N. (Eds.), Biology of Plant Volatiles. CRC Press, Boca Raton, Florida, pp 147–-164.

Marotz-Clausen, G., Jürschik, S., Fuchs, R., Schäffler, I., Sulzer, P., Gibernau, M., Dötterl, S., 2018. Incomplete synchrony of inflorescence scent and temperature patterns in *Arum maculatum* L. (Araceae). Phytochemistry 154, 77–84.

Martin, K.R., Moré, M., Hipólito, J., Charlemagne, S., Schlumpberger, B.O., Raguso, R.A., 2017. Spatial and temporal variation in volatile composition suggests olfactory division of labor within the trap flowers of *Aristolochia gigantea*. Flora 232, 153–168.

Mayo, S.J., Bogner, J., Boyce, P.C., 1998. Araceae, in: Kubitzki, K. (Eds.) Flowering Plants Monocotyledons. The families and genera of vascular plants, vol. 4. Springer, Berlin, Heidelberg, pp 26–74.

Ohashi, K., Makino, T.T., Arikawa, K., 2015. Floral colour change in the eyes of pollinators: testing possible constraints and correlated evolution. Funct. Ecol. 29, 1144–1155.

Ollerton, J., Diaz, A., 1999. Evidence for stabilising selection acting on flowering time in *Arum maculatum* (Araceae): the influence of phylogeny on adaptation. Oecologia 119, 340–348.

Ollerton, J., Dafni, A., 2005. Functional floral morphology and phenology, in: Dafni, A., Kevan, P.G., Husband, B.C. (Eds.), Practical Pollination Biology. Enviroquest, Ltd., Cambridge, Ontario, Canada, pp 1–26.

Ollerton, J., Winfree, R., Tarrant, S., 2011. How many flowering plants are pollinated by animals? Oikos 120, 321–326.

Patt, J.M., French, J.C., Schal, C., Lech, J., Hartman, T.G., 1995. The pollination biology of tuckahoe, *Peltandra virginica* (Araceae). Am. J. Bot. 82,10, 1230–1240.

Pellmyr, O., Patt., J.M., 1986. Function of olfactory and visual stimuli in pollination of *Lysichiton americanum* (Araceae) by a staphylinid beetle. Madroño 33, 1, 47–54.

Pellmyr, O., Thien, L.B., 1986. Insect reproduction and floral fragrances: keys to the evolution of the angiosperms? Taxon 35, 1, 76–85.

Pereira, J., Schlindwein, C., Antonini, Y., Maia, A.C.D., Dötterl, S., Martins, C., Navarro, D.M.D.A.F., Oliveira, R., 2014. *Philodendron adamantinum* (Araceae) lures its single cyclocephaline scarab pollinator with specific dominant floral scent volatiles. Biol. J. Linn. Soc. 111, 679–691.

Picazo-Aragonés, J., Terrab, A., Balao, F., 2020. Plant volatile organic compounds evolution: transcriptional regulation, epigenetics and polyploidy. Int. J. Mol. Sci. 21, 8956.

Pichersky, E., Raguso, R.A., Lewinsohn, E. Croteau, R., 1994. Floral scent production in *Clarkia* (Onagraceae). Plant Physiol. 106, 1533–1540.

Raguso, R.A., 2004. Flowers as sensory billboards: progress towards an integrated understanding of floral advertisement. Curr. Opin. Plant Biol. 7, 4, 434–440.

Raguso, R.A., 2008. Wake up and smell the roses: the ecology and evolution of floral scent. Annu. Rev. Ecol. Evol. Syst. 39, 549–569.

Raman, V., Tabanca, N., Demirci, B., Khan, I., 2017. Studies on the floral anatomy and scent chemistry of titan arum (*Amorphophallus titanum*, Araceae). Turk. J. Bot. 41, 63-74.

Sayers, T.D., Steinbauer, M.J., Farnier, K., Miller, R.E., 2020. Dung mimicry in *Typhonium* (Araceae): explaining floral trait and pollinator divergence in a widespread species complex and rare sister species. Bot. J. Linn. Soc. 193, 375–401.

Scheven, H.J., 1994. Gaschromatographisch/massenspektrometrische Untersuchungen des Appendixduftes blühender Pflanzen von *Arum maculatum* L. und *Arum italicum* MILLER; Nachweis der attraktiven Wirkung der Duftbestandteile Indol, Humulen und p-Kresol auf *Psychoda phalaenoides* L. PhD thesis, Philipps-Universität Marburg, Marburg, Germany.

Skubatz, H., Kunkel, D.D., Howald, W.N., Trenkle, R., Mookherjee, B., 1996. The *Sauromatum guttatum* appendix as an osmophore: excretory pathways, composition of volatiles and attractiveness to insects. New Phytol. 134, 631–640.

Steenhuisen, S.L., Raguso, R.A., Jürgens, A., Johnson, S.D., 2010. Variation in scent emission among floral parts and inflorescence developmental stages in beetle-pollinated *Protea* species (Proteaceae). S. Afr. J. Bot. 76, 779–787.

Szenteczki, M.A., Godschalx, A.L., Galmán, A., Espíndola, A., Gibernau, M., Alvarez, N., Rasmann, S., 2021. Spatial and temporal heterogeneity in pollinator communities maintains within-species floral odour variation. Oikos 130, 1487–1499.

Szenteczki, M.A., Godschalx, A.L., Gauthier, J., Gibernau, M., Rasmann, S., Alvarez, N., 2022. Transcriptomic analysis of deceptively pollinated *Arum maculatum* (Araceae) reveals

association between terpene synthase expression in floral trap chamber and species-specific pollinator attraction. G3, jkac175.

Urru, I., Stensmyr, M.C., Hansson, B.S., 2011. Pollination by brood-site deception. Phytochemistry 72, 1655–1666.

van Den Dool, H., Kratz, P.D., 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J. Chromatog. 11, 463–471.

Vogel, S., Martens, J., 2000. A survey of the function of the lethal kettle traps of *Arisaema* (Araceae), with records of pollinating fungus gnats from Nepal. Bot. J. Linn. Soc. 133, 61–100.

Weiss, M., Lamont, B.B., 1997. Floral color change and insect pollination: A dynamic relationship. Isr. J. Plant Sci. 45, 185–199.

Willmer, P., 2011. Pollination and floral ecology. Princeton University Press, Princeton, New Jersey.

Table S1. UPAC names (see https://pubchem.ncbi.nlm.nih.gov/) of the volatiles recorded in the present study (see also Table 2).

Aliphatic compounds	**IUPAC name**
Butanoic acid	butanoic acid
1,3-Octadiene	octa-1,3-diene
2,6-Dimethyl-1-heptene or 2-Methyl-1-octene	2,6-dimethylhept-1-ene 2-methyloct-1-ene
2-Heptanone	heptan-2-one
2-Heptanol	heptan-2-ol
Methylheptanone isomer	
Hexanoic acid	hexanoic acid
1-Octen-3-ol	oct-1-en-3-ol
2-Nonanone	nonan-2-one
2-Nonanol	nonan-2-ol
(*Z*)-3-Octenyl acetate	[(Z)-oct-3-enyl] acetate
2-Undecanone	undecan-2-one
C5-branched chain compounds	
2-Methylbutanoic acid	2-methylbutanoic acid
Nitrogen-bearing compounds	**IUPAC name**
cf 1-Butyl pyrrole	1-butylpyrrole
β-Lutidine	3-ethylpyridine
Indole	1*H*-Indole
Aromatic compounds	
p-Cresol	4-methylphenol
p-Creosol	2-methoxy-4-methylphenol
Monoterpenoids	
3,7-Dimethyloct-1-ene	3,7-dimethyloct-1-ene
α-Citronellene	2,6-dimethylocta-1,7-diene
β-Citronellene	3,7-dimethylocta-1,6-diene
3,7-Dimethyl-2-octene	3,7-dimethyloct-2-ene
2,6-Dimethyl-2,6-octadiene	2,6-dimethylocta-2,6-diene
3,7-Dimethyl-2,4-octadiene	3,7-dimethylocta-2,4-diene
(*Z*)-Sabinene hydrate	2-methyl-5-propan-2-ylbicyclo[3.1.0]hexan-2-ol
allo-Ocimene	(4*E*,6*E*)-2,6-dimethylocta-2,4,6-triene

neoallo-Ocimene	(4E,6Z)-2,6-dimethylocta-2,4,6-triene
Santolina alcohol	3-ethenyl-2,5-dimethylhex-4-en-2-ol
4-Terpineol	4-methyl-1-propan-2-ylcyclohex-3-en-1-ol
β-Citronellol	3,7-dimethyloct-6-en-1-ol
Sesquiterpenoids	
Bicycloelemene	3-ethenyl-3,7,7-trimethyl-2-prop-1-en-2-ylbicyclo[4.1.0]heptane
α-Copaene	1,3-dimethyl-8-propan-2-yltricyclo[4.4.0.02,7]dec-3-ene
(*E*)-β-Caryophyllene	4,11,11-trimethyl-8-methylidenebicyclo[7.2.0]undec-4-ene
α-Humulene	2,6,6,9-tetramethylcycloundeca-1,4,8-triene
Alloaromadendrene	1,1,7-trimethyl-4-methylidene-2,3,4*a*,5,6,7,7*a*,7*b*-octahydro-1*aH*-cyclopropa[e]azulene
Bicyclogermacrene	3,7,11,11-tetramethylbicyclo[8.1.0]undeca-2,6-diene
δ-Cadinene	4,7-dimethyl-1-propan-2-yl-1,2,3,5,6,8*a*-hexahydronaphthalene