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Flower scent composition in *Dianthus* and *Saponaria* species (Caryophyllaceae) and its relevance for pollination biology and taxonomy

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Abstract

Floral fragrance compounds of seven *Dianthus* species (*D. arenarius*, *D. armeria*, *D. barbatus*, *D. deltoides*, *D. monspessulanus*, *D. superbus*, and *D. sylvestris*) and one *Saponaria* species (*S. officinalis*) (Caryophyllaceae) were studied using headspace adsorption technique followed by gas chromatography massspectrometry (GC-MS). The number of compounds (fatty acid derivatives, benzenoids, phenyl propanoids, isoprenoids, and nitrogen containing compounds) identified in the floral odors ranged from 18 to 51 but all were dominated by only 1–3 compounds. Most of the compounds identified in this study have been previously reported in floral scents from species of the closely related genus *Silene* L. However, the phenyl propanoids eugenol, methyleugenol, methylisoeugenol, *cis*-asarone, and *trans*-asarone have formerly not been found in *Silene*. Based on the measurement of Sørensen's index of similarity (I_s) nonmetric multidimensional scaling (MDS) was used to detect meaningful underlying dimensions and to visualize similarities between the investigated species. The MDS analysis showed three groups of species, (1) the diurnal *D. armeria*, *D. barbatus*, and *D. deltoides* are characterized by the predominance of fatty acid derivatives, (2) the closely related nocturnal *D. monspessulanus*, and *D. superbus* by high relative amounts of isoprenoids such as *cis*- β -ocimene and β -caryophyllene, and (3) the species *D. arenarius*, *D. sylvestris* and *S. officinalis* by a predominance of benzenoids, especially methylbenzoate. The results are discussed in relation to pollination, especially by butterflies, moths, and hawkmoths.

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Keywords: Caryophyllaceae; *Dianthus*; *Saponaria*; Flower scent; Pollination; Taxonomy

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1. Introduction

The genus *Dianthus* L. comprises about 300 species with a worldwide distribution but centred in the Mediterranean region; some species are cultivated as ornamentals (e.g., *D. caryophyllus* L., *D. chinensis* L.). The genus *Saponaria* L. comprises about 40 species in temperate Eurasia, chiefly in the Mediterranean and Irano-Turanean region (Bittrich, 1993). *Saponaria officinalis* is present from southern Europe to the middle of Scandinavia.

The flowers of *Dianthus* and *Saponaria* species with relatively long calyx tubes produce large amounts of nectar and scent and are often pollinated by various day- and night-active Lepidoptera (Bittrich, 1993). According to Vogel (1954) and Erhardt (1990, 1991) there is an evolutionary trend towards increased specialization from butterfly pollination to settling moth pollination and hawkmoth pollination in the genus *Dianthus*. The pink flower color and the narrow calyx tube of *Dianthus armeria*, *D. barbatus*, *D. deltoides* and *D. sylvestris* are characters typical for butterfly-pollinated (psychogamous) flowers (Erhardt, 1990). The whitish flower color and the strong scent emission of the long-tubed flowers of *D. arenarius*, *D. monspessulanus*, *D. superbus* and *S. officinalis*, especially during the night, indicate pollination by night-active flower visitors.

Floral scents are found in the vast majority of animal-pollinated plants with the exception of ornithophilous flowers (Proctor et al., 1996). The time of floral scent emission and the composition may be typical for certain groups of pollinators (Knudsen and Tollsten, 1993). However, floral scents may also reflect phylogenetic relationships of species and in a number of investigations (Dahl et al., 1990; Knudsen and Mori, 1996; Azuma et al., 1999; Nogueira et al., 2001) floral scent has been used as a tool in chemosystematic studies. Floral fragrances of moth-pollinated taxa are often described as strong, heavy, or being sweet perfumes present at night (Baker and Hurd, 1968; Grant, 1983) or being dominated by oxygenated terpenes and aromatic esters (Nilsson, 1983, 1985; Loughrin et al., 1990; Knudsen et al., 1993; Raguso and Pichersky, 1995; Jürgens et al., 2002). Moreover Knudsen and Tollsten (1993) found that the floral scent of highly specialized, sphingophilous, tropical flowers with a functional flower depth of more than 3 cm was readily distinguishable from that of phalenophilous flowers. Those sphingophilous flowers showed typical scent substances, such as geraniolic compounds, oxygenated sesquiterpenes, 1,8-cineole, linalool, and nitrogen containing compounds.

Information about the chemical composition of the floral aromas of *Dianthus* species have been reported by Erhardt and Jäggi (1995) for *Dianthus glacialis* Haenke. In a recent study of the floral scent composition in 13 nocturnal *Silene* species, benzenoids together with isoprenoids dominated the scent in all species (Jürgens et al., 2002). The floral scent composition in nocturnal *Silene* species was typical for species pollinated by settling moths (see Knudsen et al., 1993). Moreover, even in species with a relative long calyx tube and hawkmoths as regular flower visitors, Jürgens et al. (2002) found no indication for an specialization of floral scent composition towards hawkmoth pollination. In this paper we present data on the floral scent

composition of some *Dianthus* and *Saponaria* species. We discuss if the floral scent chemistry is related to the pollination syndrome or rather to the taxonomy of the species.

2. Materials and methods

Floral scent samples were collected from eight species (Table 1) grown from seeds in the Botanical Garden of the University of Ulm from 1994 to 1997.

Floral volatiles were sampled for 3–4 h from several flowers of one or two individuals of each species. Floral volatiles were sampled at the time of maximum odor production. Sampling time was at day-time for day-flowering species between 09:00 h and 13:00 h and at night for night-flowering species between 21:00 h and 01:00 h. Scent-containing air was sucked through glass cartridges with a 1:1 by weight (300 mg) mixture of Tenax^{TA}, mesh size 20–40, and CarbotrapTM, mesh size 20–40, with a battery-operated membrane pump. The flow rate through the cartridges was ca. 150 ml min⁻¹. Cartridges were conditioned by washing with acetone and heated out at 250 °C. The adsorbed scent substances were extracted with 1 ml of acetone (GC grade) into glass vials. In order to detect background contamination, a blank sample of the air was collected. The samples were analysed by coupled gas chromatography and massspectrometry (GC-MS) on a Varian 3400 gas chromatograph (GC) equipped with a CTC A200S autoinjector, and connected to a Finnigan Ion Trap Detector (Magnum ITD 800/ITS 40). The MS detector was operated at a scan range of 40–550 *m/z* at 1.0 s/scan. A nonpolar fused silica GC-column was used (DB1 30 m long, inner diameter 0.25 µm, thickness 0.25 µm). Helium was used as carrier gas, flowing at 39 cm/s. The GC was programmed for 1 min at 50 °C, increased by 3 °C/min for 55 min and maintained at 200 °C. Component identification was carried out using the NIST 98 mass spectral data base (NIST algorithm) and confirmed by comparison of retention times with published data (Jennings and Shibamoto, 1980; Davies, 1990).

Sørensen's index of similarity (I_s) with a quantitative modification for relative values was used for the comparison of scent profiles between taxa (Tollsten et al., 1994):

$$I_s = \frac{2M_w \times 100}{M_a + M_b}$$

where M_w is the sum of the smaller values of compounds present in both taxa, and M_a and M_b respectively, are the sums of all compounds found in the individual taxa. We used (nonmetric) multidimensional scaling (MDS) in the STATISTICA 5.1 program to detect meaningful underlying dimensions and to visualize similarities between species (see Borg and Lingoes, 1987). To evaluate how well (or poorly) the particular configuration produces the observed distance matrix the *raw stress* (or *raw Phi*) value (see Kruskal, 1964) and the coefficient of alienation K (see Guttman, 1968) are given. The smaller the stress value, the better is the fit of the reproduced distance matrix to the observed distance matrix.

Table 1
Taxa used for floral scent analysis, their systematic placement, flower visitors, mean calyx length [mm], and the origin of seeds (BG=Botanical Garden). The delimitation of the genus *Dianthus* into subgenera is according to Tutin et al. (1993)

Taxa/Species	Seeds ex BG:	Original locality	Pollination syndrome/Flower visitors ^{a,b}	Calyx length ^c
<i>Dianthus</i> L.				
Subgenus <i>Armeriastrum</i> Ser.	Neuchatel	Switzerland, pied de Jura, 500–700 m	Psy ¹ /RHO single observation ¹ , CO ¹¹	16.9±0.3
<i>D. armeria</i> L.	Berlin	Kroatien, Zlatar	Psy ¹ /RHO ^{1,7} , DSP ^{1,8}	15.5±0.4
<i>D. barbatus</i> L.	Berlin	Spain, Guadalajara, Sierra de Ayllon, 1400 m	Psy ^{1,2} /RHO ^{1,4} , HY ⁴ , DJ ¹ , ZYG ⁸	18.5±1.0
Subgenus <i>Dianthus</i>	Göttingen	Denmark, S. Vitemmöllo, Kristianstad	Sph ¹ -CO ¹	18.8±1.3
<i>D. deltoides</i> L.	Graz	Austria, Kämten, Koralpe, 1700–1800 m	DSP ¹	31.0±0.3
<i>D. arenarius</i> L.	Berlin	Germany, Berlin Spandau, Eiskeller	Sph ^{1,2} /DSP ¹ , NSP ⁵ , LEP ⁵ : ZYG ⁸ , RHO ⁷	24.4±0.4
<i>D. monopessulanus</i> L.	Gran Paradiso	Italy, Gollie, 1800 m	Psy ¹ /RHO ^{2,3} , DSP ^{1,2,3,10,11} , NSP ³ , LEP ^{3,10} , DJ ^{3,11}	23.4±0.7
<i>D. sylvestris</i> Wulfen	Ulm	Germany, Sachsen-Anhalt, Dessau	Sph ^{1,2} /NSP ^{1,2,6,8} , LEP ^{1,2,6,9} , DSP ^{1,8} , DJ ^{1,9} , HY ¹ , HYnr ⁹ , RHO ¹	22.5±0.9
<i>Saponaria</i> L.				
<i>S. officinalis</i> L.				

^a pha=phalophilous, psy=psychophilous, sph=sphingophilous, RHO=Rhopalocera (day-active Lepidoptera), DSP=day-active Sphingidae, NSP=night-active Sphingidae, ZYG=Zygenae, LEP=mostly Noctuidae and Geometridae (Lepidoptera except RHO, DSP, NSP, and ZYG), HY=Hymenoptera (mostly Apidae), nr=nectar robbing, DJ=Diptera (mostly Syrphidae), CO=Coleoptera.

^b Numbers indicate the following references: ¹Knuth (1898), ²Meusel and Mühlberg (1979), ³Erhardt (1988), ⁴Jennersten (1988), ⁵Erhardt (1991), ⁶Esche (1992), ⁷Ebert and Renwald (1993), ⁸Ebert et al. (1994), ⁹Jürgens et al. (1996), ¹⁰Collin et al. (2001), ¹¹Jürgens and Witt, personal observations (BG Ulm).

^c Based on 10 hermaphrodite flowers, female stage.

3. Results and discussion

3.1. Scent composition

A summary of the floral scent chemistry showing the relative amounts of volatiles, and their distribution among the five main chemical compound classes (i.e., fatty acid derivatives, benzenoids, phenyl propanoids, isoprenoids, and nitrogen-containing compounds) is given in Table 2. The number of components ranged from 18 in *D. sylvestris* to 51 in *D. arenarius*. The floral scent of all species studied is dominated by only 1–3 compounds: *D. arenarius* by the benzenoids methylbenzoate (42.1%), methyl-salicylate (14.5%), and dimethyl salicylate (8.9%); *D. armeria* by the fatty acid derivatives *cis*-3-hexenyl-1-acetate (29.8%), *n*-nonanal (11%), and the monoterpene α -limonene which accounts for 11.6% of the floral volatiles; *D. barbatus* by *n*-nonanal (14.7%) and the sesquiterpene β -caryophyllene (10.3%); *D. deltoides* by *cis*-3-hexenyl-1-acetate (27.1%), benzaldehyde (15%) and benzeneacetaldehyde (10.6%); *D. monspessulanus* and *D. superbus* by *cis*- β -ocimene (52.4%, 49.8%) and β -caryophyllene (18.5%, 23.5%); and *Saponaria officinalis* by methylbenzoate which accounted for 68.7% of the scent. Most of the compounds found in the investigated *Dianthus* and *Saponaria* species have previously been reported for nocturnal *Silene* species (Jürgens et al., 2002). However, the phenyl propanoids methyleugenol, methylisoeugenol, elemicin, *cis*-asarone, and *trans*-asarone (Table 2) may be specific for nocturnal *Dianthus* species.

According to the chemical compound classes the species can be divided into three groups, (1) *D. armeria*, *D. barbatus*, and *D. deltoides* are characterized by the predominance of fatty acid derivatives, (2) *D. monspessulanus*, and *D. superbus* have high relative amounts of isoprenoids, such as *cis*- β -ocimene and β -caryophyllene, and (3) *D. arenarius*, *D. sylvestris*, and *S. officinalis* show a predominance of benzenoids, especially methyl benzoate (Fig. 1). These groups are supported by the similarity index (Table 3) that is based on the comparison of single compounds of the species and the results of the MDS (Table 2 and Fig. 1). The highest similarity index was found for the closely related species *D. superbus* and *D. monspessulanus* (78.1%), the lowest between *D. deltoides* and *D. superbus* (2.8%) (Table 3).

3.2. Scent composition in diurnal butterfly-pollinated species

Dianthus armeria, *D. barbatus*, *D. deltoides*, and *D. sylvestris* all fit the butterfly pollination syndrome described by Faegri and van der Pijl (1979) in having red or pink, deep and tube-like flowers that exclude short-tongued flower visitors. The floral scent composition of three of these diurnal species (except *D. sylvestris*) was clearly distinct from that of the nocturnal species (Fig. 1, Fig. 2). Generally, the trend seems to be that the diurnal *Dianthus* species *D. armeria*, *D. barbatus*, and *D. deltoides* pollinated mainly by day-active butterflies, contained much higher amounts of fatty acid derived hydrocarbons than the mainly night-active, more or less white-colored *D. monspessulanus*, *D. superbus*, *D. arenarius*, and *S. officinalis*. Although butterfly-pollinated (psychophilous) flowers are typically brightly coloured with little scent

Table 2
Floral scent composition of seven *Dianthus* and one *Saponaria* species. Average relative amounts [in %] of floral volatiles emitted by *D. arenarius*=*D. are*, *D. armeria*=*D. arm*, *D. barbatus*=*D. bar*, *D. deltoides*=*D. del*, *D. monspessulanus*=*D. mon*, *D. superbus*=*D. sup*, and *S. officinalis*=*S. off*. The compounds are ordered in classes, which to some degree reflect their biosynthetic origin (see Knudsen and Tollsten, 1993). In each class, compounds are listed according to relative retention time order (RR_i). tr=trace amounts (<0.1 %). Unknowns were included when present with more than 5% in any sample

Number of samples	RR _i	<i>D. are</i>		<i>D. arm</i>		<i>D. bar</i>		<i>D. del</i>		<i>D. mon</i>		<i>D. syl</i>		<i>D. sup</i>		<i>S. off</i>	
		3	2	2	2	2	2	2	1	1	1	1	1	1	2	2	
FATTY ACID DERIVATIVES																	
Aldehydes																	
	204	0.4	tr	6.2	3.0	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	2.1	2.1
	335	0.3	1.0	6.9	1.2	0.1	0.1	tr	tr	tr	tr	tr	tr	tr	tr	1.8	1.8
	538	0.6	4.0	9.6	7.8	–	–	tr	tr	tr	tr	tr	tr	tr	tr	2.5	2.5
	793	1.1	11.0	14.7	3.5	–	–	tr	tr	tr	tr	tr	tr	tr	tr	4.3	4.3
	1069	0.9	8.2	6.7	2.0	0.2	0.2	tr	tr	tr	tr	tr	tr	tr	tr	2.6	2.6
Alcohols																	
	506	1.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Esters																	
	558	1.3	29.8	4.6	27.1	–	–	tr	tr	tr	tr	tr	tr	tr	tr	1.1	1.1
	577	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	0.1	0.1
	816	–	–	–	2.1	–	–	–	–	–	–	–	–	–	–	–	–
Ketones																	
	1835	3.9	0.5	–	–	–	–	–	–	–	–	–	1.7	–	–	–	–
	2311	1.4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
BENZENOIDS																	
	183	0.9	tr	5.5	5.2	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	3.1	3.1
	287	tr	tr	tr	1.8	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	–	–
	299	0.2	tr	4.6	2.3	0.1	0.1	tr	tr	tr	tr	tr	tr	tr	tr	1.2	1.2
	421	4.0	1.3	4.6	15.0	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	2.7	2.7
	480	0.1	–	0.5	0.3	–	–	tr	tr	tr	tr	tr	tr	tr	tr	0.2	0.2
	564	tr	–	tr	–	–	–	–	–	–	–	–	–	–	–	–	–
	575	–	0.7	1.5	–	–	–	–	–	–	–	–	–	–	–	–	–
	611	0.3	3.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	592	1.6	–	–	10.6	–	–	–	–	–	–	–	–	–	tr	–	–

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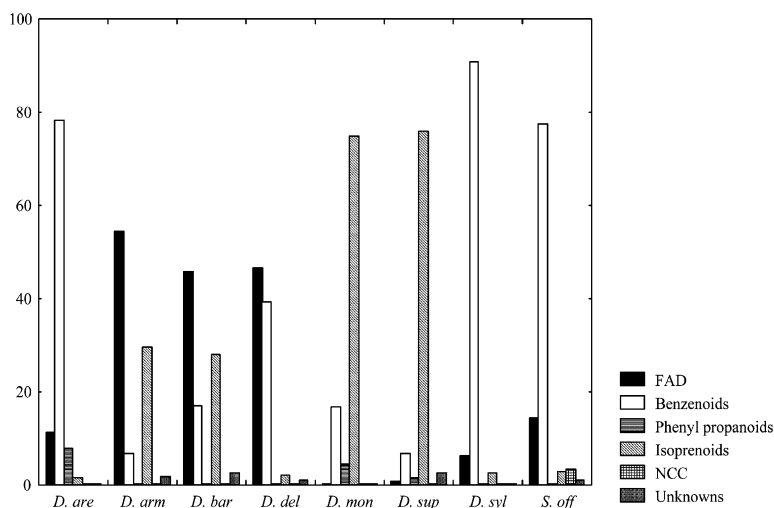


Fig. 1. Variation in the relative amounts of floral scent volatiles according to chemical compound classes. FAD=fatty acid derivatives, NCC=nitrogen-containing compounds.

Table 3

Sørensen's Index of similarity between floral scent profiles of seven *Dianthus* and one *Saponaria* species

	<i>D. are</i>	<i>D. arm</i>	<i>D. bar</i>	<i>D. del</i>	<i>D. mon</i>	<i>D. sup</i>	<i>D. syl</i>	<i>S. off</i>
<i>D. arenarius</i>	–	6.8	7.9	12.9	20.2	7.6	50.6	52.5
<i>D. armeria</i>	6.8	–	46.1	42.0	9.0	7.4	8.1	15.7
<i>D. barbatus</i>	7.9	46.1	–	39.8	17.9	15.4	6.9	24.5
<i>D. deltoides</i>	12.9	42.0	39.8	–	6.1	2.8	12.5	26.0
<i>D. monspessulanus</i>	20.2	9.0	17.9	6.1	–	78.1	7.2	7.2
<i>D. superbus</i>	7.6	7.4	15.4	2.8	78.1	–	4.5	4.5
<i>D. sylvestris</i>	50.6	8.1	6.9	12.5	7.2	4.5	–	72.1
<i>S. officinalis</i>	52.5	15.7	24.5	26.0	7.2	4.5	72.1	–

(Faegri and van der Pijl, 1979; Dobson, 1994), a number of different compounds have been found in the odor of all investigated diurnal taxa. The evidence of their use as olfactory cues in flower location and selection is rather meager compared to the numerous studies on moths (Dobson, 1994). Our results show that floral volatiles of day-flowering *Dianthus* species are typical for generalistic flowers, containing some of the most commonly reported scent compounds, namely octanal, nonanal, decanal, *cis*-3-hexenyl-1-acetate, benzaldehyde, methylbenzoate and limonene (Knudsen et al., 1993). An interesting case is *D. sylvestris* that originally has been classified as diurnal due to the pink petal color and the relatively weak floral fragrance. However, the flowers are clearly longer than those of the other red-flowered species, ranging between that of *D. arenarius* and *S. officinalis*, and the floral scent composition also shows the highest similarity index to *D. arenarius* and *S. officinalis*.

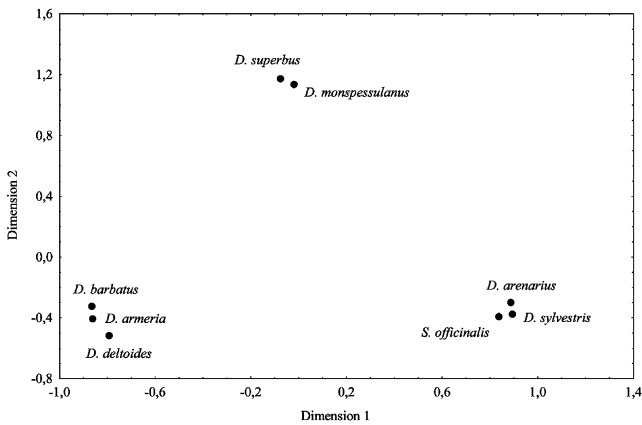


Fig. 2. Multidimensional scaling of seven *Dianthus* and one *Saponaria* species. Stress value $\Phi=0.001$ (see Kruskal, 1964); coefficient of alienation $K=0.007$ (see Guttman, 1968)

The scent of all these three species is dominated by methylbenzoate. Erhardt (1988) found syrphid flies, and the diurnal European hawkmoth *Macroglossum stellatarum* L., as main diurnal pollinators of *D. sylvestris*. However, recent results of Collin et al. (2001) show that nocturnal pollinators, probably moths of the genus *Hadena* Schrank, that also oviposit in flowers and whose caterpillars feed on developing fruits and seeds, were the main pollinators of *D. sylvestris*. Thus, our results of the floral scent composition of *D. sylvestris* support the assumption that this species is indeed adapted to nocturnal pollinators.

3.3. Scent composition in moth- and hawkmoth-pollinated species

The flowers of *D. arenarius*, *D. monspessulanus*, *D. superbus* and *S. officinalis* are whitish in color, have a long calyx tube, and are strongly scented, especially during the night, indicating pollination by night-active flower visitors. However, the flowers do not exclude diurnal flower visitors (compare Table 1) because, unlike some nocturnal *Silene* species, they do not close or wilt during the day following anthesis. Nevertheless, there are clear differences in the floral scent of diurnal butterfly-flowers and moth- or hawkmoth-pollinated (primarily) nocturnal species. The phenyl propanoids methyleugenol, methylisoeugenol, elemicin, *cis*-asarone, *trans*-asarone were only found in the nocturnal *Dianthus* species. *D. arenarius* and *S. officinalis* were predominated by methylbenzoate, whereas the isoprenoids, especially *cis*- β -ocimene β -caryophyllene, were the main components in *D. monspessulanus* and *D. superbus*. In previous studies of white flowers pollinated by moths, terpene alcohols (e.g., linalool, nerolidol, and farnesol), and the corresponding terpene hydrocarbons, accompanied by aromatic alcohols and their derived esters, salicylic acid esters and nitrogen-containing compounds have been reported to occur in relative high amounts (Knudsen and Tollsten, 1993). Linalool is often found in the floral fragrances of moth-pollinated taxa (Miyake et al., 1998; Raguso and Pichersky, 1999)

though it is not restricted to moth-pollinated flowers (Raguso and Pichersky, 1999) and occurs widely in many diurnal flowers pollinated by bees (Borg-Karlson et al., 1996), beetles (Thien et al., 1975), or butterflies (Honda et al., 1998). Accordingly, linalool was present in the diurnal *D. barbatus* (4.5%) and in trace amounts in the nocturnal *D. superbus*. Aromatic esters and esters of salicylic acid account for more than 65% of the volatile components in *D. arenarius* and *S. officinalis* thus suggesting that the floral fragrances of these species are clearly adapted to moths or hawkmoths. Additionally, the floral scent of *S. officinalis* contained small relative amounts of nitrogen-containing compounds. Nilsson (1985) suggested that nitrogen-containing fragrances are a specialization of tropical species pollinated by hawkmoths. In *D. monspessulanus* and *D. superbus* we found β -ocimene and β -caryophyllene as main compounds in the floral scent. These compounds are not typically found in nocturnal plant species pollinated by moths and hawkmoths. However, β -ocimene has been found also as a main compound in the floral fragrance of *Crinum asiaticum* L., a species that is pollinated mainly by nocturnal and diurnal hawkmoths and butterflies (Miyake et al., 1998). The diversity in the floral scent composition in the nocturnal species is in accordance with the findings of Miyake et al. (1998) in hawkmoth-pollinated flowers in Japan. Miyake et al. (1998) suggested that hawkmoths are attracted by various kinds of compounds and thus parallel evolution of hawkmoth-pollination in different plant lineages resulted in evolution of different sets of attractant compounds. From this we can hypothesize that in the investigated nocturnal moth- and hawkmoth-pollinated *Dianthus* and *Saponaria* species two different sets of attractant compounds evolved, (1) flowers dominated by aromatic esters, and (2) flowers dominated by terpenoids.

On the basis of our results, it seems that generally similarities in the floral scent composition of the investigated species can be explained by an adaptation to specific pollinator groups. Taxonomy seems to play a minor role. The high similarity in floral scent composition of *D. monspessulanus* and *D. superbus*, both belonging to the same section *Plumaria* (Opiz) A. et Gr. (Meusel and Mühlberg, 1979), and both being night-active, may be due to similar pollination syndromes, and phylogenetic constraints as well. However, *D. arenarius*, also belongs to the same section and is also nocturnal, but showed only a small similarity to *D. monspessulanus* and *D. superbus*. Far from it, its floral scent is very similar to that of a species of another genera, to the nocturnal *Saponaria officinalis*. Our results confirm the statement of Knudsen and Tollsten (1993), that the floral scent compounds in moth-pollinated taxa are often similar even if species belong to different taxonomical groups. However, to determine the pathways of adaptive evolution of the floral scent chemistry more information on floral scent composition, phylogenetic relationships, and pollination biology of more genera and species are needed. Superimposing information on floral scent composition and pollination ecology on a cladogram may suggest whether certain characters evolved within or among taxa (see Harvey and Pagel, 1991).

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