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# Flower scent composition in diurnal *Silene* species (Caryophyllaceae): phylogenetic constraints or adaption to flower visitors?

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## Abstract

A comparative analysis of the flower volatiles of 10 day-flowering *Silene* species native to Central Europe was made to improve the understanding of the pollination biology and evolution of floral odours in the genus. Floral scent was collected by dynamic headspace adsorption and analysed via gas chromatography–mass spectrometry. In total, 60 compounds could be identified by their mass spectra as well as by their relative retention times. The number of compounds per species ranged between 16 in *Silene rupestris* and 40 in *S. viscaria*. Main compounds in most species were fatty acid derivatives (FADs, *cis*-3-hexen-1-ol, *cis*-3-hexenyl acetate, *n*-nonanal), benzenoids (benzaldehyde, phenylacetaldehyde, methyl benzoate), and monoterpenes (limonene, linalool), accompanied by sesquiterpenes, and nitrogen-containing compounds.

Nonmetric multidimensional scaling (CNESS, NMDS) based on relative amounts of single components leads to the same conclusion as visualization of similarities based on component classes reflecting to some degree biosynthetic pathways: differences in floral scent composition can be related to both the taxonomy and the pollination biology of the species investigated. In all but one species of the *Silene* group, and all species of the *Lychnis* group (*S. dioica*, *S. flos-cuculi*, *S. flos-jovis*, *S. pendula*), the dominating compound classes are benzenoids followed by FADs. The relatively high amounts of aromatic compounds (e.g. benzaldehyde, phenylacetaldehyde, methyl benzoate) are indicative of an adaptation towards butterfly pollination. Species of the *Viscaria* and *Eudianthe* groups showed high relative amounts of FADs but a lower content of benzenoids. Relatively high amounts of monoterpenes (>10%) were found in *S. alpestris*, *S. coeli-rosa*, *S. gallica*, and *S. viscaria*. It is suggested that the high relative content of the most volatile monoterpene alkenes (e.g.

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limonene) in *S. gallica* and *S. coeli-rosa* may be indicative of an adaptation to bees as pollinators in these species.

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*Keywords:* Caryophyllaceae; *Lychnis*; *Silene*; Day-flowering; Floral scent; GC-MS

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## 1. Introduction

The genus *Silene* L. with about 700 species is one of the larger genera of the world's flora (Greuter, 1995). Bees, bumblebees, butterflies, moths, hawkmoths, syrphid flies and mosquitoes are recorded as pollinators in the genus *Silene* (Brantjes and Leemans, 1976; Meusel and Mühlberg, 1979; Jürgens et al., 1996, 2002a). However, many species show adaptations typical for pollination by day- and night-active Lepidoptera and there seems to be an evolutionary trend to butterfly and moth pollination in the Caryophylloideae (Vogel, 1954; Erhardt, 1990, 1991). Remarkable is the high proportion of night-flowering species in the genera *Dianthus* and *Silene* (Friedrich, 1979). In an analysis of the anthophilous fauna of N.W. Europe, Ellis and Ellis-Adam (1993) found that dominant flower visitors of Caryophyllaceae, in particular, in the genus *Silene*, are moths (mainly Noctuidae, Geometridae, and Spingidae).

Unrelated plant species with similar pollinator types, such as birds or bees, moths or bats, often show similarities in floral characters due to convergent adaptations to the morphology, physiology, and behaviour of pollinators. This relationship between pollinating flower visitors and plant species is reflected in the widely adopted classification of flowers with different pollination syndromes (Faegri and van der Pijl, 1979). This long-standing notion is challenged on the basis of apparent widespread generalization in pollination systems. For example, Ellis and Ellis-Adam (1993) found that in N.W. Europe, the anthophilous fauna and the entomophilous flora are dominated by generalists. The view that specialization is the dominant evolutionary trend in the pollination systems of plants is questioned (Waser et al., 1996; Johnson and Steiner, 2000). However, together with visual cues, floral fragrance is an important character for the attraction of floral visitors (Proctor et al., 1996), and in a number of studies, correlations between the fragrance composition of flowers and the type of flower visitor have been found (Knudsen and Tollsten, 1993, 1995; Miyake et al., 1998; Andersson et al., 2002). It is well known that several butterflies utilize floral scents in their location of flowers (Honda et al., 1998; Ômura et al., 1999a, b). The results from Andersson et al. (2002) about the floral scent composition in butterfly-pollinated and/or butterfly-adapted plants demonstrate that a dominance of certain benzenoid compounds, monoterpenes and irregular terpenes may be typical plant adaptations matching the olfactory requirements of butterflies. Moreover, it has been shown that floral fragrances alone may function as isolating mechanisms (Williams and Dodson, 1972). Furthermore, fragrance chemistry has also been useful in confirming established taxonomic relationships (e.g. Gerlach and Schill, 1989; Dahl et al., 1990; Knudsen, 1994; Knudsen and Mori, 1996).

Information about the chemical composition of floral aromas of mostly moth-pollinated *Silene* species is given by Knudsen and Tollsten (1993) and by Jürgens et al. (2002a). However, data on the floral scent volatiles particular to diurnal *Silene* species are, to my knowledge, only available for *Silene flos-cuculi* (L.) Greuter & Burdet (Andersson et al., 2002). The objective of this paper is to provide a chemical description of floral scents of some day-flowering *Silene* species, and to relate the floral scent composition to the pollination biology and taxonomy. To characterize the pollination biology of the species, data on flower visitors from the literature were gathered and the calyx length and width were measured to characterize the nectar accessibility for potential pollinators. It was analysed if the floral fragrance chemistry gives evidence for the adaptation to butterflies. The results of the present study of the floral scent profiles of diurnal *Silene* species will be discussed in relation to our former results in nocturnal *Silene* species (Jürgens et al., 2002a). A comparison of the compounds of diurnal versus nocturnal species may give information about possible preadaptations in the floral scent composition of diurnal species that might have lead to changes in the pollination syndrome towards night activity.

## 2. Materials and methods

### 2.1. Plant material

Floral scent samples were collected from 10 species (Table 1) grown from seeds in the Botanical Garden of the University of Ulm from 1994 to 1997.

The generic delimitation and classification of the *Silene* is problematic, and DNA sequencing studies of Oxelman and Lidén (1995) and Desfeux and Lejeune (1996) support the assumption that *Silene* L., *Lychnis* L. and *Viscaria* Röhl. are unnatural assemblages (Greuter, 1995). Therefore, I tried to integrate the results of Greuter (1995), Oxelman and Lidén (1995), Desfeux and Lejeune (1996), and Oxelman et al. (1997) into the taxonomical subdivision of the “*Silenean* complex” (Table 1) and subdivided the “complex” into the *Eudianthe* group, *Viscaria* group, *Lychnis* group, and *Silene* group (compare with Lidén et al., 2001).

### 2.2. Flower morphology

Since calyx length and width are correlated with nectar accessibility for potential pollinators, these characters were used as additional indicators of plant specialization. For morphological measurement, calyces were slit longitudinally. Measurements were made on flowers in the second male phase (second day of anthesis) of male or hermaphrodite flowers. Each character was measured on five different flowers of several plants of each species. Calyx length was measured from base to apex. Calyx width was measured 50% from the base. All measurements were made under a dissecting microscope using an ocular micrometer (30× magnification).

Table 1  
Taxa used for floral scent analysis, their systematic placement, flower visitors, and mean calyx length (mm). Species were arranged according to Desfeux and Lejeune (1996), Greuter (1995), Oxelman and Lidén (1995), and Oxelman et al. (1997)

Taxa/species	Pollination syndrome/flower visitors	Calyx length <sup>a</sup>	Calyx length <sup>b</sup>	Calyx width <sup>a</sup>	Calyx width <sup>b</sup>
<i>Eudianthe group</i>					
<i>S. coeli-rosa</i> (L.) Godron	HY <sub>15</sub> ; DI <sub>15</sub>	25.3 ± 3.8	–	5.9 ± 0.4	–
<i>Viscaria group</i>					
<i>S. alpestris</i> Jacq.	–	6.8 ± 0.2	–	2.3 ± 0.2	–
<i>S. armeria</i> L.	Psy <sub>1,7</sub> /RHO <sub>1,17</sub> ; DSP <sub>1,7,13</sub> ; LEP <sub>1</sub>	17.0 ± 1.3	13.5 ± 0.3	3.1 ± 0.2	2.6 ± .01
<i>S. rupestris</i> L.	RHO <sub>1,7,12</sub> ; HY <sub>1,7</sub> ; LEP <sub>1,5</sub> ; DI <sub>1,7,18</sub>	4.7 ± 0.3	–	2.3 ± 0.3	–
<i>S. viscaria</i> (L.) Jessen	Psy <sub>1,7</sub> /RHO <sub>1,7,10,6,16</sub> ; HY <sub>1,7,9,10,6,16</sub> ; nr <sub>15,16</sub> ; LEP <sub>1,10</sub> ; NSP <sub>10</sub> ; DSP <sub>9,10</sub> ; CO <sub>1,7</sub> ; DI <sub>1,6,10</sub>	13.5 ± 0.2	–	5.3 ± 0.1	–
<i>Lychnis group</i>					
<i>S. flos-cuculi</i> (L.) Greut. & Burd.	Mel–Psy <sub>1,2,7</sub> /RHO <sub>1,2,4,7,12,17</sub> ; HY <sub>1,2,7,17</sub> ; DSP <sub>1,13</sub> ; LEP <sub>1,7,11,13,17</sub> ; DI <sub>1,2,7</sub>	9.2 ± 0.4	7.7 ± 0.3	5.0 ± 0.5	4.2 ± 0.8
<i>S. flos-jovis</i> (L.) Greut. & Burd.	Psy <sub>1</sub> /RHO <sub>1,17</sub> ; HY <sub>17</sub> ; DI <sub>1</sub> ; LEP <sub>17</sub>	15.6 ± 0.1	–	6.9 ± 0.8	–
<i>Silene group</i>					
<i>S. dioica</i> (L.) Clairv.	Psy <sub>1,5</sub> /RHO <sub>1,2,3,4,5,8,12</sub> ; HY <sub>1,3,8,14,15,17</sub> ; LEP <sub>1,11,14,15,17</sub> ; NSP <sub>11,13,15</sub> ; DI <sub>1,2,3,8,14,15,17</sub> ; CO <sub>2,3</sub>	14.8 ± 1.1	13.1 ± 1.0	5.5 ± .04	7.2 ± 0.8
<i>S. gallica</i> L.	S <sub>1,17,19</sub> /Psy <sub>4</sub> ; Hy <sub>1,17</sub>	9.7 ± 0.1	9.2 ± 0.4	4.4 ± 0.1	3.9 ± 0.2
<i>S. pendula</i> L.	HY <sub>17</sub> ; LEP <sub>17</sub>	16.1 ± 1.7	13.8 ± 0.8	6.9 ± 0.9	5.5 ± 0.4

<sup>1</sup>Knuth (1898–1905), <sup>2</sup>Willis and Burkill (1903), <sup>3</sup>Schulz (1905), <sup>4</sup>Vogel (1954), <sup>5</sup>Vogel (1975), <sup>6</sup>Blake et al. (1976), <sup>7</sup>Meusel and Mühlberg (1979), <sup>8</sup>Kay et al. (1984), <sup>9</sup>Dreisig (1985), <sup>10</sup>Jennersten (1988), <sup>11</sup>Esche (1992), <sup>12</sup>Ebert and Rennwald (1993), <sup>13</sup>Ebert et al. (1994), <sup>14</sup>Westerbergh and Saura (1994), <sup>15</sup>Jürgens et al. (1996), <sup>16</sup>Mustajärvi et al. (2001), <sup>17</sup>Jürgens and Witt personal observation, Botanical Garden Ulm, 1997–2000 (Germany); <sup>18</sup>Mayer personal observation; <sup>19</sup>Witt (2003).

Psy, psychophilous; Mel, melittophilous; RHO, Rhopalocera (butterflies); DSP, day-active Sphingidae; NSP, night-active Sphingidae; LEP, Lepidoptera (moths) except Rhopalocera and Sphingidae; HY, Hymenoptera (mainly Apidae); DI, Diptera (mainly Syrphidae); CO, Coleoptera; S, self-pollination; nr, nectar robbing. Index numbers refer to the reference numbers below.

<sup>a</sup> Hermaphrodite or male flowers.

<sup>b</sup> Female flowers.

### 2.3. Floral scent collection and analysis

As this study was part of a larger investigation on reproductive biology of more than 30 Caryophyllaceae species (data partly published in Jürgens et al., 2002a,b, 2003), it was not possible to go into detail by analysing intraspecific variation. Floral volatiles were sampled for 3–4 h from several flowers of one or two individuals of each species. Volatile samples were collected between 9.00 and 13.00 h. Several flowers of plants or inflorescences, or if possible whole inflorescences (e.g. *S. armeria*, *S. flos-jovis*) were enclosed with loose-fitting polyethylene bags. Contaminations found in blank samples from empty bags were omitted from analyses. With a battery-operated membrane pump, scent-containing air was sucked through glass cartridges (10 cm long, inner diameter 3 mm) filled with a 1:1 by weight mixture (ca. 200 mg) of Tenax<sup>TA</sup> and Carbotrap<sup>TM</sup>. The adsorbed scent substances were extracted with 1 ml of acetone (GC grade) into glass vials. The samples were analysed by coupled gas chromatography and mass spectrometry (GC-MS) on a Varian 3400 gas chromatograph (GC) equipped with a CTC A200S auto injector, and connected to a Finnigan Ion Trap Detector (Magnum ITD 800/ITS 40). Helium was used as carrier gas. A nonpolar fused silica GC-column was used (DB1 30 m long, inner diameter 0.25  $\mu\text{m}$ , thickness 0.25  $\mu\text{m}$ ). The GC temperature was programmed for 1 min at 50 °C, increased by 3 °C/min for 50 min and maintained at 200 °C for another 5 min. Component identification was carried out tentatively using the NIST 98 mass spectral data base (NIST algorithm) and confirmed by comparison of retention times with published data (Davies, 1990; Adams, 1995).

Relative abundance of compounds was achieved by integrating individual GC peak areas of the total ion chromatograms (TIC) using Varian's SATURN 5.2. software. For each species, these amounts were averaged across individuals and divided by the total average amount of volatiles produced.

### 2.4. Statistical analyses

Based on relative amounts of single components, the chord-normalized expected species shared (CNESS) distance index (Trueblood et al., 1994) was calculated using the updated version of the COMPAH program (Boesch, 1977) provided by Gallagher at UMASS/Boston (<http://www.es.umb.edu/edgwebp.htm>). COMPAH stands for combinatorial polythetic agglomerative hierarchical clustering. CNESS is a metric version of Grassle and Smith's (1976) NESS similarity index. We determined the best CNESS-m (m: sample size) using the method described in Trueblood et al. (1994). Nonmetric multidimensional scaling (NMDS) in the STATISTICA 5.1 program was used 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) is given. The smaller the stress value, the better is the fit of the reproduced distance matrix to the observed distance matrix.

### 3. Results and discussion

#### 3.1. Flower morphology and pollination biology

Many of the *Silene* species investigated show floral features that are typical for butterfly-pollinated plants, having long flower tubes with deeply hidden nectar (see Table 1), and a red flower colour (Faegri and van der Pijl, 1979). Especially, *S. armeria* and *S. flos-jovis* with their condensed, pink-flowered inflorescences offer ideal landing places for butterflies. Additionally, observations of flower visitors also indicate that in some species, butterflies seem to play an important role as pollinators (Table 1). However, a high diversity of flower visitors has been observed for many of the species and it can be assumed that none of the species in this investigation totally depends on butterflies as pollinators. There has been much discussion on the importance of butterflies as pollinators, particularly as long distance dispersers of pollen (Courtney et al., 1982; Tepedino, 1983; Courtney and Hill, 1983; Jennersten, 1984). Following Jennersten (1984), North European butterflies are, in general, comparatively unimportant as pollinators. Only in the case of two Caryophylloideae species, *Silene viscaria* (compare Table 1) and *Dianthus deltoides*, Jennersten (1984) found pollen loads indicating that skippers are important flower visitors. However, later studies (Kwak and Jennersten, 1986; Jennersten, 1988; Darmstad and Fry, 1995; Nielsen and Ims, 2000) revealed that bumblebees are the major pollinators of *S. viscaria*. Another well investigated case is *S. dioica*. Vogel (1975) observed the butterfly *Gonepteryx rhamni* L. (Pieridae) as a pollinator in *S. dioica*. In investigations of Kay et al. (1984) and Jürgens et al. (1996), however, *Bombus hortorum* L. was found to be the major pollinator of this species. In two other species studied, *S. alpestris* and *S. rupestris*, which both have white, and relatively short-tubed flowers (Table 1), nectar is accessible for insects with a relatively short tongue, e.g. small Hymenoptera and Diptera. Following E. Mayer (personal communication), at least *S. rupestris* is mainly visited and pollinated by flies. Nevertheless, in addition to Diptera and Hymenoptera, butterflies and moths have also been reported as flower visitors on *S. rupestris* (Knuth, 1898; Meusel and Mühlberg, 1979; Ebert et al., 1994). With respect to the observed flower visitors (Table 1), it seems obvious that even in the investigated species with typical features of butterfly-adapted plants pollination is achieved by “mixed” pollinator guilds. Furthermore, “mixed” pollination strategies might occur. Witt (2003) proved eight out of the 10 species investigated to be more or less self-compatible. Especially for *S. gallica*, high spontaneous selfing ability has been reported (Witt, 2003) and pollination by insects might not be that important for the reproduction of this species. Altogether, some diurnal *Silene* species seem to be more generalistic than thought at first glance.

#### 3.2. Scent composition in relation to pollination biology and taxonomy

The chemical composition of the floral scent of the 10 investigated diurnal *Silene* species is given in Table 2. The compounds are ordered in classes, which to some degree reflect their biosynthetic origin (see Knudsen et al., 1993). Although the

Table 2

Floral scent composition of 10 *Silene* species. Average relative amounts (%) of floral volatiles emitted by *Silene alpestris* (*S. alpes.*), *S. armeria* (*S. armer.*), *S. coeli-rosa* (*S. coeli.*), *S. flos-cuculi* (*S. cucul.*), *S. flos-jovis* (*S. Jovis.*), *S. dioica* (*S. dioic.*), *S. gallica* (*S. galli.*), *S. pendula* (*S. pendu.*), *S. rupestris* (*S. rupe.*), and *S. viscaria* (*S. visca.*). 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<sub>t</sub>). tr, trace amounts (<0.1%). Unknowns were included when present with more than 5% in any sample

Compound	RR <sub>t</sub>	<i>S. alpes.</i>	<i>S. armer.</i>	<i>S. coeli.</i>	<i>S. cucul.</i>	<i>S. dioic.</i>	<i>S. galli.</i>	<i>S. Jovis.</i>	<i>S. pendu.</i>	<i>S. rupe.</i>	<i>S. visca.</i>
Number of plants, <i>n</i>		2	1	1	1	2	2	1	1	1	3
Number of flowers per sample, <i>n</i>		16/30	15	5	19	12/28	8/7	59	48	30	14/15/35
Total number of compounds		27	18	29	28	30	29	25	24	16	40
<i>Fatty acid derivatives</i>											
<i>n</i> -Hexanal	204	2.3	1.4	4.7	8.2	4.0	5.5	3.6	8.6	–	4.3
<i>Cis</i> -3-hexen-1-ol	277	–	24.1	–	–	–	–	–	–	48.9	–
Unidentified <i>m/z</i> : 43, 67, 55, 82, 97 <sup>b</sup>	279	–	–	–	–	–	–	–	–	–	1.0
<i>n</i> -Octane	315	0.4	–	–	–	1.4	–	–	–	–	0.8
2-Heptanone	319	–	–	–	1.8	–	–	–	–	–	1.8
<i>n</i> -Heptanal	336	1.6	1.3	3.4	5.0	1.9	1.9	1.3	–	–	5.6
<i>n</i> -Pentyl-acetate	368	–	–	–	–	–	–	–	–	–	0.4
Unidentified <i>m/z</i> : 43, 57, 85, 70, 98, 111	375	–	–	–	–	–	1.7	–	–	–	–
<i>n</i> -Octanal	538	2.3	2.5	4.2	7.0	2.3	3.8	1.1	0.9	2.7	8.0
<i>Cis</i> -3-hexen-1-ol acetate	558	56.7	49.6	3.1	0.4	–	1.9	–	6.7	–	5.9
<i>n</i> -Hexyl acetate	577	1.3	0.6	0.6	–	–	–	–	–	0.8	0.8
<i>n</i> -Nonanal	793	5.3	8.7	14.5	13.4	9.6	13.6	10.5	5.7	9.0	15.3
<i>n</i> -Heptyl acetate	831	0.4	–	–	–	–	–	–	–	–	0.4
<i>n</i> -Decanal	1069	2.8	–	9.7	5.3	4.3	7.0	4.8	3.6	10.7	7.1
<i>Benzenoids</i>											
Ethylbenzene <sup>a</sup>	287	–	–	4.5	2.0	3.0	5.9	1.3	7.1	–	1.8
1,2-Dimethylbenzene <sup>a</sup>	299	0.9	0.6	5.9	3.9	5.4	6.5	3.2	17.1	–	3.6
Benzaldehyde	421	1.1	1.9	1.2	30.1	5.9	1.5	3.0	3.7	–	4.1

(continued on next page)

Table 2 (continued)

Compound	RR <sub>i</sub>	<i>S. alpes.</i>	<i>S. armer.</i>	<i>S. coeli.</i>	<i>S. cucul.</i>	<i>S. dioic.</i>	<i>S. galli.</i>	<i>S. jovis.</i>	<i>S. pendu.</i>	<i>S. rupes.</i>	<i>S. visca.</i>
Propylbenzene	448	0.2	0.1	0.4	0.6	0.8	1.9	0.3	1.9	—	0.9
Ethyltoluene <sup>a</sup>	464	—	—	—	—	2.2	—	—	—	—	—
1,2,3-Trimethylbenzene <sup>a</sup>	480	—	0.1	0.7	0.6	1.2	1.6	0.4	3.4	—	0.5
Phenyl acetate	564	—	—	0.1	0.5	2.4	—	0.5	1.0	—	0.3
Phenylacetaldehyde	592	—	1.4	—	0.8	—	—	56.3	—	—	—
Benzyl alcohol	603	—	—	—	—	—	—	—	—	—	1.1
<i>p</i> -Cymene	606	—	—	—	—	—	tr	—	—	—	—
Unidentified <i>m/z</i> : 105 <sup>a</sup> , 77, 51, 43, 63, 89	655	0.4	1.6	—	—	2.1	1.0	0.6	2.0	—	0.7
1,4-Diethylbenzene <sup>a</sup>	667	—	—	—	—	—	0.2	0.3	0.9	—	—
4-Methoxyphenol	730	—	—	—	—	—	—	1.7	—	—	—
Methyl benzoate	748	3.1	—	1.4	4.1	3.6	—	1.8	14.8	0.7	0.5
1,2-Dimethoxybenzene	873	—	—	—	—	—	—	—	—	—	0.1
1,4-Dimethoxybenzene	920	—	—	—	—	—	—	—	—	1.4	—
Benzyl acetate	922	—	—	tr	—	0.2	—	—	—	—	1.5
Methyl salicylate	1009	—	—	—	0.6	0.2	—	3.5	—	—	—
2-Phenylethyl acetate	1173	—	—	—	—	—	—	—	—	—	2.3
Phenyl benzoate	1254	—	—	—	0.8	—	—	1.2	—	—	0.2
Dimethyl salicylate	1368	0.4	—	—	1.1	—	—	0.2	—	—	—
<i>n</i> -Butyl benzoate	1381	—	—	—	—	—	—	0.1	—	—	—
Benzyl 3-methylbutanoate	1548	—	—	—	—	—	—	2.2	—	—	1.0
Unidentified benzenoid ester <i>m/z</i> : 105, 70, 77, 41, 51, 55, 123	1660	—	—	—	—	—	—	—	—	—	0.9
<i>Cis</i> -3-hexenyl benzoate	1987	—	—	—	—	—	—	—	—	4.7	—
Benzyl benzoate	2385	—	—	—	—	—	—	0.7	—	—	1.9
<i>Isoprenoids</i>											
<i>Monoterpenes</i>											
$\alpha$ -Thujene	415	—	—	0.7	—	—	0.3	—	—	—	—
$\alpha$ -Pinene	429	0.5	0.1	4.1	0.3	0.3	6.1	—	1.1	—	1.1
Camphene	452	0.4	—	4.2	—	tr	0.3	—	—	—	—



Table 2 (continued)

Compound	RR <sub>i</sub>	<i>S. alpes.</i>	<i>S. armer.</i>	<i>S. coeli.</i>	<i>S. cucul.</i>	<i>S. dioic.</i>	<i>S. galli.</i>	<i>S. jovis.</i>	<i>S. pendu.</i>	<i>S. rupes.</i>	<i>S. visca.</i>
$\alpha$ -Farnesene	1835	–	–	4.6	–	–	–	–	–	–	–
<i>Irregular terpenes</i>											
6-Methyl-5-hepten-2-one	579	–	–	–	–	0.9	–	–	–	1.4	–
<i>Nitrogen-containing compounds</i>											
Benzonitrile	456	–	tr	–	–	0.6	–	–	–	–	–
<i>n</i> -Acetyl-4(H)-pyridine	610	0.4	0.6	–	2.1	3.0	–	–	–	–	0.8
<i>Miscellaneous</i>											
Naphthalene <sup>b</sup>	975	0.2	–	1.1	0.3	0.2	1.2	0.7	–	–	0.8
<i>Unknowns</i>											
<i>m/z</i> : 236*, 205, 165, 41, 57, 165, 137, 180, 91, 122, 91, 77	1780	–	–	–	–	17.1	–	–	–	–	–
Fatty acid derivatives		73.1	88.2	40.2	41.1	23.5	35.4	21.3	25.5	72.1	51.4
Benzenoids		6.1	5.7	14.2	45.1	27.0	18.6	77.3	51.9	6.8	21.4
Isoprenoids		16.6	5.2	34.8	4.5	–	38.0	0.4	19.8	19.7	15.9
Nitrogen-containing compounds		0.4	0.6	–	2.1	3.6	–	–	–	–	0.8
Miscellaneous		0.2	–	1.1	0.3	0.2	1.2	0.7	–	–	0.8

<sup>a</sup> Compound might be of anthropogenic origin.

<sup>b</sup> Mass fragments for unknowns are listed with the molecular ion first (if known or inferred) marked by an asterisk, followed by the base peak and other fragments in order to decreasing abundance.

investigated plant species showed only a relatively weak fragrance to the human nose, all species contained a considerable number of compounds. The number of components ranged from 16 in *S. rupestris* to 40 in *S. viscaria*. Dominant compound classes were fatty acid derivatives (FADs), isoprenoids and benzenoids. Nitrogen-containing compounds were found only in low relative amounts with the highest content in *S. dioica* (3.6%). Similarities between species based on compound classes are shown in Fig. 1. Striking is the predominance of FADs in the *Viscaria* group (51.4% in *S. viscaria* to 88.2% in *S. armeria*). Contrary to this, there is a trend towards relatively high amounts of benzenoids in species of the *Silene* and the *Lychnis* group (up to 75.1% in *S. flos-jovis*). The proportions of other compound classes do not correlate with the taxonomy of the species. High proportions of isoprenoids are detected in several species belonging to different taxonomic groups. For example, we find relatively high amounts of monoterpenes in *S. alpestris* (15.9%), *S. coeli-rosa* (28.5%), *S. gallica* (34.7%), and *S. viscaria* (15.2%). *Silene rupestris* and *S. pendula* differ from the other species by their higher content of sesquiterpenes (Fig. 1).

For a more detailed analysis, similarities based on relative amounts of single components were visualized by nonmetric multidimensional scaling (CNESS, NMDS; Fig. 2). Again, the *Viscaria* group differs from the other species, but the NMDS also shows that the variability in this group is relatively high. While *S. viscaria* is near to all species of other taxonomic groups, the exceptionally small flowered alpine species *S. alpestris* and *S. rupestris* differ from all other species (Fig. 2). Furthermore, they differ clearly from each other by the high content of

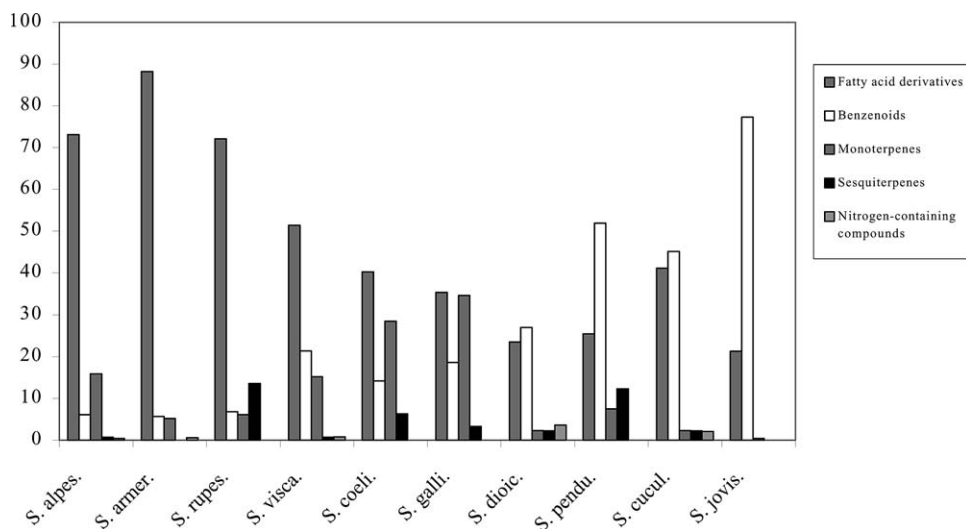


Fig. 1. Variation in the relative amounts of floral scent volatiles (%) according to chemical compound classes, which to some degree reflect their biosynthetic origin (see Knudsen and Tollsten, 1993). Abbreviation of species names as in Table 2.

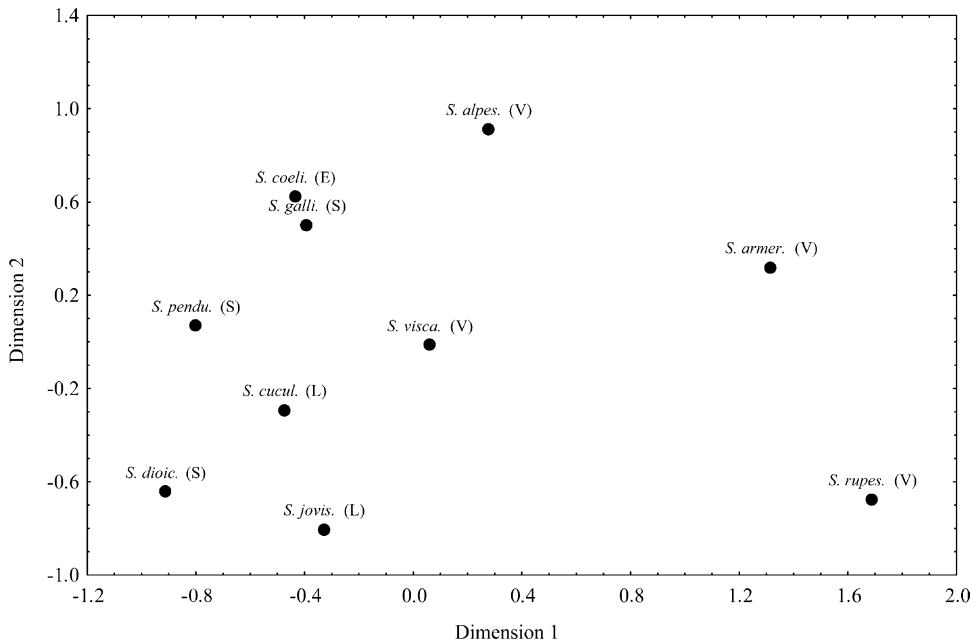


Fig. 2. Multidimensional scaling (CNESS followed by NMDS) of 10 *Silene* species based on relative amounts of single components. Stress value 0.111 (see Kruskal, 1964); coefficient of alienation 0.163 (see Guttman, 1968). Abbreviation of species names as in Table 2. E, *Eudianthe* group; L, *Lychnis* group; S, *Silene* group; V, *Viscaria* group.

*cis*-3-hexenol acetate and thujone in *S. alpestris*, and *cis*-3-hexen-1-ol and germa-crene D in *S. rupestris* (Table 2). Due to its high content of *cis*-3-hexen-1-ol and *cis*-3-hexenol acetate, the last member of the *Viscaria* group, the obviously butterfly-adapted *S. armeria* is also placed far from all other species. The more or less clustered species *S. viscaria*, *S. dioica*, *S. flos-cuculi*, *S. pendula*, and *S. flos-jovis* exhibit the highest degree of fragrances (benzenoid alcohols, -aldehydes, and -esters accompanied by linalool and lilac compounds) that are typically found in butterfly-adapted/-pollinated plants or that show antennal responses in butterflies (see Andersson et al., 2002; Andersson, 2003a, b). Andersson et al. (2002) found a predominance (in terms of both number and relative amount) of benzenoids in many of the scent blends of 15 European butterfly-pollinated species. As stated by these authors, it is likely that the benzenoids phenylacetaldehyde and 2-phenylethanol and the irregular terpene oxoisophorone serve as signals to attract pollinating butterflies. This assumption is supported by the studies of the chemical interactions between butterflies and flowers demonstrating that some floral scent compounds served as significant cues in the foraging behaviour of nymphalid, papilionid and pierid butterflies (Honda et al., 1998; Ômura et al., 1999a, b; Andersson, 2003b). Andersson (2003b) investigated the antennal responses in *Inachis io* L., *Aglais urticae* L. (Nymphalidae) and *Gonepteryx rhamni* (Pieridae), a flower visitor of *S. dioica*

(Vogel, 1975), to floral scents using combined gas chromatography and electro-antennographic detection (GC-EAD). She found high EAD responses to certain benzenoid compounds such as phenylacetaldehyde, monoterpenes such as linalool, and irregular terpenes such as oxoisophorone. However, in the present study, oxoisophorone was not detected in the floral scent of any species and the relative amount of benzenoids showed a high variation between the species. Species like *S. alpestris* and *S. rupestris*, which show no morphological adaptations to butterflies, had relatively low contents of benzenoids supporting the assumption that these species are not butterfly-adapted. Another interesting finding is the high similarity of *S. coeli-rosa* (*Eudianthe* group) and *S. gallica* (*Silene* group) (Fig. 2). Both species have high relative amounts of the most volatile monoterpene alkenes (e.g.  $\alpha$ -pinene,  $\beta$ -pinene, limonene; Table 2). Limonene is known to be perceived by bees (Fonta and Masson, 1984; reviewed by Dobson, 1994), and in combination with visual cues, it may be sufficient to attract bees from shorter distances. The fact that monoterpene alkenes showed low or nil antennal responses in temperate and tropical butterflies (Andersson, 2003b) supports the assumption that these substances, especially limonene, indicate adaptation of *S. coeli-rosa* and *S. gallica* to bees. Furthermore, both these two species have long flower tubes but in contrast to the other species with deeply hidden nectar (Table 1), their flower colour is not red or pink but more or less pale rose with red markings. This floral feature also corresponds better with bee-flowers, and indeed, no butterflies but Hymenoptera were observed visiting flowers of these species (Table 1).

### 3.3. *Floral scent composition of butterfly flowers as possible preadaptation to moth pollination*

Visual-olfactory synergism in floral characters eliciting feeding behaviour is common among flower-visiting Lepidoptera (Scherer and Kolb, 1987; Naumann et al., 1991; Honda et al., 1998; Raguso and Willis, 2002). In moth- and hawkmoth-pollinated flowers, fragrances play an important role for the attraction of moths over long distances. To the human nose, in diurnal species, the fragrance production is often weaker than in nocturnal flowers and optical signals may play a more important role in the attraction of pollinators. Nevertheless, floral fragrances can influence pollinator behaviour at shorter distances also and it has been shown already by Kugler (1932) that flower odours, even when weak to humans, can enhance landings by bees and that olfactory stimuli become increasingly important at closer range. The findings of Andersson (2003a) suggest that butterflies use floral scent as an important cue signal to initially identify and subsequently recognize and distinguish among rewarding plants. Moreover, she also demonstrated that floral scent constancy results from learning of odours. A high similarity in flower scent composition between diurnal, butterfly-pollinated *Silene* species, and nocturnal, moth-pollinated *Silene* species should be expected not only due to phylogenetic constraints, but also due to the adaptation to related pollinator groups with similar physiological properties. Despite these facts, the similarity between diurnal and nocturnal species in the genus is altogether relatively low. Only 32 of the 60

identified compounds, detected in one or more of the 10 diurnal species, were also present in the floral scent of one or more of 13 nocturnal species studied by Jürgens et al. (2002a). However, a closer look shows that with increasing relatedness, the similarity slightly increases: restricting comparison to species of the *Silene* group (3 diurnals, 13 nocturnals), 22 out of 36 identified compounds found in at least one of the diurnal species were also present in the floral scent of one or more nocturnal species. It is generally agreed that the diurnal *S. dioica* and the nocturnal *S. latifolia* share a recent common ancestry (Prentice, 1979; Desfeux and Lejeune, 1996). Comparing these two species shows that 17 out of 27 identified components occurring in *S. dioica* can also be found in *S. latifolia*. Altogether, the diurnal species differ from nocturnal species by having higher relative contents of FADs. In the nocturnal species, the relative amount of FADs never exceeded 20% (Jürgens et al., 2002a); in diurnal species, FADs were never below 20% and reached in some cases more than 50%. Even the close relatives *S. dioica* and *S. latifolia* differ clearly regarding their FAD proportions. This is in accordance with similar differences between nocturnal and diurnal species in the genus *Dianthus* (Jürgens et al., 2003). The higher content in FADs might be due to the lower floral odour production in diurnal plants, resulting in a higher relative amount of the green leaf volatiles (e.g. *cis*-3-hexen-1-ol, *cis*-3-hexenol acetate, octanal, nonanal, and decanal). Nevertheless, some of the FADs might play a role in the interaction with bees, flies and beetles that have been observed as flower visitors.

It can be assumed that in the genus *Silene*, night-flowering has been evolved several times independently from diurnal ancestors (Desfeux and Lejeune, 1996). We find several examples of closely related weakly scented diurnal or self-pollinated species and strongly scented nocturnal species in the genus *Silene*. This observation has also been made in other plant groups, leading to the conclusion that the ability to produce and emit floral scent is an easily acquired, and easily lost, trait (Dudareva et al., 1996). For example, the diurnal *S. dioica* and the nocturnal *S. latifolia* share a recent common ancestry and hybridisation between both species is a common phenomenon in areas where their habitats overlap, or are adjacent (Baker, 1947, 1948; Goulson and Jerrim, 1997). In two sympatric populations of Norg (The Netherlands), Biere and Honders (1996) reported the occurrence of interspecific hybrids to constitute more than 6%. These findings together with pollinator observations (Jürgens et al., 1996) demonstrate that there is an overlap in the pollinator spectra of these species. This raises the question if the switch from diurnal to nocturnal pollination is rather established by quantitative, or qualitative changes in the fragrance production. The nocturnal species in the genus *Silene* follow the general trend of floral scent compounds typical for moth-pollinated flowers (Knudsen and Tollsten, 1993; Kaiser, 1993; Jürgens et al., 2002a), i.e. flowers having acyclic terpene aldehydes and alcohols (e.g. lilac aldehyde, lilac alcohol, linalool), aromatic alcohols and aldehydes (e.g. benzyl alcohol, 2-phenylethanol, phenylacetaldehyde) and esters derived from them (e.g. benzyl acetate, benzyl benzoate) and small amounts of nitrogen-containing compounds (e.g. indole). However, some of these compounds that are typical for moth-pollinated flowers are also main compounds in some of the investigated diurnal *Silene* species. Phenylacetalde-

hyde dominated the floral scent of *S. flos-jovis*, and lilac aldehydes were found in minor relative amounts in *S. flos-cuculi*, and *S. viscaria*. These substances elicit strong antennal responses not only in butterflies but also in several moth species (Creighton et al., 1973; Heath et al., 1992; Plepys, 2001; Andersson, 2003b). Phenylacetaldehyde has been shown to attract a variety of noctuid moths to volatile baited field traps (Meagher, 2001, 2002). Therefore, in the more butterfly-adapted species (*S. dioica*, *S. viscaria*, *S. flos-cuculi*, *S. flos-jovis*), it seems likely that simply increasing the fragrance production may be sufficient to enhance the attractivity to moths.

### 3.4. Summary and perspectives

The investigated diurnal *Silene* species show differences in their floral scent composition that can be related to both the taxonomy and the pollination biology of the species. A predominance of FADs and a relatively low content of benzenoids were typical for species of the *Viscaria* group. Contrary to this, we find relatively high amounts of benzenoids and low amounts of FADs in the *Silene* group and the *Lychnis* group. The occurrence of benzenoid compounds, particularly phenylacetaldehyde, in some of the species (*S. dioica*, *S. flos-cuculi*, *S. pendula*, *S. flos-jovis*) suggests an adaptation towards butterfly pollination in these plants. The high relative amounts of most volatile monoterpenes alkenes (e.g.  $\alpha$ -pinene,  $\beta$ -pinene, limonene) in *S. coeli-rosa* and *S. gallica* may indicate an adaptation to bees.

In the genus *Silene*, the evolution of moth-pollination from diurnal ancestors seems to be an easy step, especially, in butterfly-adapted plants. Some of the diurnal species showed main compounds that are typically found in butterfly-adapted plants and these compounds have also been reported in moth-pollinated species or are well-known moth attractants (Meagher, 2001, 2002). Therefore, it is not surprising that some diurnal *Silene* species show overlapping pollinator spectra with nocturnal, moth-adapted plants.

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