

Floral Scents of Hawkmoth-Pollinated Flowers in Japan

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Similarity among the floral scents of hawkmoth-pollinated plants was investigated with headspace samplings. Six of seven plant species belonging to different families were found to be rich in isoprenoids, among which linalool was the most common compound. Linalool showed rhythmicity with a nocturnal increase in *Lonicera japonica*. These findings suggest that linalool is a common attractant for nocturnal hawkmoths. However, the composition of other isoprenoids, benzenoids and fatty acid derivatives varied markedly among the plant species examined. There was a significant correlation between species composition of flower-visiting hawkmoths and specific floral scents, suggesting that attractiveness to each hawkmoth species is dependent upon floral scent.

Key words: Floral volatile compounds — Nocturnal scent emission — Pollinator composition — Sphingophilous flowers

Introduction

There are several lines of circumstantial evidence regarding the function of floral scents as attractants for pollinators. First, intraspecific or intrageneric differences in floral scents correspond to differences in pollinator composition, as seen in *Cycnoches* (Orchidaceae) pollinated by euglossine bees (Gregg 1983), *Cimicifuga simplex* (Ranunculaceae) pollinated by bumble bees and butterflies (Pellmyr 1986), and *Cypripedium calceolus* (Orchidaceae) pollinated by *Andrena*, *Ceratina*, or halictid bees (Bergström *et al.* 1992). Secondly, a post-pollination change in floral scents occurs, as has been reported in *Platanthera bifolia* (Orchidaceae) (Tollsten and Bergström 1989). Thirdly, some floral scents are characteristic of particular pollination syndromes (van der Pijl 1961, Wyatt 1983). For example, scent compounds of bat-pollinated flowers are unique and quite distinct from those of most species pollinated by other animals: they include sulphur-containing compounds (Kaiser and Tollsten 1995, Knudsen and Tollsten 1995). Unpleasant aminoid odors containing monoamines and diamines are characteristic of sapromyophilous flowers in that the odors mimic the flies' normal food and oviposition sites (Dobson 1993, Harborne 1993).

Most hawkmoth-pollinated (sphingophilous) flowers, on the other hand, have a scent which is heavy-sweet to the

human nose (van der Pijl 1961, Wyatt 1983). Although there has been a number of studies on floral scents in sphingophilous flowers (e.g. Morgan and Lyon 1928, Kaiser 1991, Knudsen and Tollsten 1993, Raguso and Pichersky 1995), common structures or common metabolisms among the compounds have not been identified to any satisfactory degree. In addition, most studies lack field observations of actual pollinator composition. Many sphingophilous plants are pollinated not only by several species of hawkmoths, but also by diurnal insects (Grant 1983, Haber and Frankie 1989, Ghazoul 1997). This diversity in pollinator composition may affect the diversity in floral scents of sphingophilous plants. In the present study, we investigated the pollinator composition and the floral scents of several plant species belonging to different families. These plants were classified as sphingophilous based on their characteristics (see van der Pijl 1961, Wyatt 1983). Although many plant species in Japan have been thought to be sphingophilous, there are as yet few studies on their pollinators or scents (Kawano *et al.* 1995).

In this study, we focused on the following questions:

- 1) Do different sphingophilous species emit the same floral scent compounds?
- 2) If so, do they show rhythmicity, with a nocturnal increase?
- 3) Is there any correlation between pollinator composition and scent composition?

Materials and Methods

Plants

Plant species from which floral scents were collected are listed in the Appendix. All species have narrow corolla tubes or a spur or brush-like shape. Materials were all obtained from native habitats in Fukuoka, Japan, except for *Platanthera mandarinorum*, which was in Yamaguchi, adjacent to Fukuoka.

Observations of flower-visitors

We observed flower-visitors to each of the species arbitrarily during the daytime and during the evening in a field in Fukuoka in 1995, with the exception of *Crinum asiaticum* whose flower-visitors were observed in Yakushima Island in 1997. We did not observe flower-visitors to *Trichosanthes kirilowii* and *P. mandarinorum*.

Sampling of floral scents

The flowers borne on rooted plants or cut branches (see Appendix) were enclosed in a polyethylene bag. Transport of volatiles from the headspace to an absorption tube (7 mm i.d.) was achieved with passive pressure from an air pump at a rate of approximately 200 ml min⁻¹. Fifty mg of Tenax TA (Shimadzu, Kyoto, Japan) were packed within the absorption tube as an absorbent, whilst the tube was plugged at both ends with glass wool. The sampling periods differed depending on the size of the flower samples, ranging from 5 to 24 hr. As a control, air through a polyethylene bag was collected over the same period at a distance of one or two meters from the flower samples. After sampling, the absorption tubes were sealed and stored in a refrigerator.

Time course for the sampling of floral scents of *Lonicera japonica*

Among the floral scents studied here, linalool was found in most species (see Results). We investigated the rhythmicity of linalool with regard to *L. japonica*, in which the relative amount of linalool was the highest. Twenty flowers of *L. japonica* picked from the plant were enclosed in a glass chamber. Transport of volatiles from the headspace to the absorption tube was achieved as described above. Entering air was purified with charcoal and Molecular Sieves® 5A 1/8 (Nacalai Tesque Inc., Kyoto, Japan). Floral volatiles were sampled for 30 min. Flowers were newly picked at three-hour intervals just before sampling to minimize the influence of picking. Thirty mg of Tenax TA were packed within the absorption tube as an absorbent.

Analysis of floral scents

The compounds trapped by the absorption tube were eluted with 2 ml of diethyl ether for pesticide residue analysis (Diethyl Ether 1000®, Wako Pure Chemical Industries, Osaka, Japan). The eluate was concentrated to the limit by the passing of N₂ across its surface. Twenty µl of hexane for pesticide residue analysis (Hexane 1000®, Wako) were added to the residue and 2 µl of it were injected for analysis. For the quantitative analysis, 10 µl of hexane and 10 µl of a 100 ng µl⁻¹ solution of *n*-eicosane (internal standard) were added to the residue and 2 µl of it were injected.

The Shimadzu (Kyoto, Japan) GC-14A system with a Shimadzu CR-6A integrator was used for gas chromatography (GC). A fused silica capillary column (25 m×0.25 mm) coated with a 0.25-µm film of OV-1 (Shimadzu) was used. Conditions for GC comprised: carrier gas=He; detector=flame ionization detector (FID); temperature of injector=240 C; temperature of detector=300 C; temperature program=5 min at 40 C followed by a 40–300 gradient (10 C min⁻¹) and 10 min at 300 C. For mass spectroscopy (MS), Shimadzu GC-MS-QP1000EX was employed with the ion source temperature at 300 C and the ionization energy at 70 eV.

As for the analysis for time course samplings of floral scents of *L. japonica*, a Hitachi (Tokyo, Japan) G 5000 system with a Hitachi D-2500 integrator was used for GC. The fused silica capillary column (30 m×0.25 mm) coated with a 0.25-µm film of TC-WAX (corresponding to DB-WAX)(GL

Sciences, Tokyo, Japan) was used. Conditions for GC comprised: carrier gas=He; detector=FID; temperature of injector and detector=240 C; temperature program=5 min at 40 C followed by a 40–240 gradient (10 C min⁻¹) and 10 min at 240 C. For GC-MS, a Hewlett-Packard (Palo Alto, Calif., USA) 5890 Series II with 5971A for MS with DB-WAX column (30 m×0.25 mm, 0.25 µm film, J & W Scientific, Calif. USA) was employed with the ion source temperature at 280 C.

Statistical analysis

Sørensen's index of similarity (Tollsten and Knudsen 1992, Knudsen and Tollsten 1993, Tollsten *et al.* 1994) of the floral scent composition and the species composition of flower-visiting hawkmoths was calculated between plant species. Using elements of the two similarity matrices obtained (Table 3), significance of correlation between the corresponding elements was tested by Mantel test (Manly 1991). The distribution of the *r*, correlation coefficient calculated for the two matrices using the corresponding elements, was calculated with 10,000 trials of randomization. The observed value of correlation coefficient was compared with this distribution to examine significance.

Results

Floral volatile composition

The relative amounts of floral volatile compounds present in the seven studied taxa are listed in Table 1. The compounds are ordered in classes reflecting their biosynthetic origin: fatty acid derivatives (products of the malonic acid pathways), benzenoids (products of the shikimic acid pathways) and isoprenoids (products of the mevalonic acid pathways). Generally, fatty acid derivatives were present in low proportions. Benzenoids were more common, but we detected only traces of them in *Oenothera stricta* and *Lonicera japonica*. Benzyl alcohol and phenyl acetaldehyde were present at the largest proportion in *P. mandarinorum* and *T. kirilowii*, respectively. Except for *P. mandarinorum*, a large amount of isoprenoids was present in all the plant species examined. Linalool was one of the most common compounds and it occupied the largest proportion in *L. japonica*, *O. stricta*, *Clerodendrum trichotomum* and *Albizia julibrissin*. Ocimene, which was not commonly present, occupied the largest proportion in *Crinum asiaticum*.

Composition of visitors to flowers

Table 2 shows the flower-visitors observed in the field by ourselves and by Inoue (1983). Most species were visited not only by nocturnal hawkmoths as expected by the pollination syndrome, but also by diurnal hawkmoths, butterflies and bees. Except for *Diachysia intermixta* on *L. japonica*, the bodies of these flower-visitors were observed to touch the anthers or to carry pollen. Even visitor compositions within the family Sphingidae differed among plant species (Table 2). The correlation between a matrix of similarity indices of sphingid visitors and a matrix of similarity indices of floral scent composition (Table 3) was significant (Mantel test, *n*' = 15, *p* < 0.05).

Table 1. Scent composition of seven species of sphingophilous plants

compounds	A. jul.	Cl. tri.	Cr. asi.	T. kir.	L. jap.	O. str.	P. man.
Fatty acid derivatives							
butyl acetate	10.9						
pentadecanoic acid						4.3	
hexadecanoic acid						9.3	
isopentyl acetate	1.9						
nonanal (RI)							1.7
decanal (RI)					0.7		
n-undecane (RI)							0.5
n-dodecane (RI)							1.9
n-tetradecane (RI)							1.1
hexenyl tiglate (RT)					2.5		
cinnamic aldehyde (RI)							0.5
unknown (sum)	3.0		1.5				
Benzenoids							
benzaldehyde (RT)		0.5		2.9			
benzyl acetate (RT)		7.4					
benzyl alcohol (RT)							45.9
benzyl benzoate (RT)							2.2
methyl benzoate (RT)		15.1	12.6			t	
methyl salicylate (RT)		6.1	2.9				
phenyl acetaldehyde (RT)				38.9			
Isoprenoids							
linalool (RT)	26.3	60.4	8.4	23.0	81.7	56.1	
linalool oxide (RT)	20.9						
ocimene (RI)	5.6		44.6				
nerolidol (RT)			8.0		4.4		
β -myrcene (RI)			0.5				
germacrene D (RT)					3.7		
α -cubebene (RI)		0.5					
Miscellaneous							
indole (RT)					1.8		

Relative amounts (in %) of floral volatiles collected from *Albizia julibrissin* (A. jul.), *Clerodendrum trichotomum* (Cl. tri.), *Crinum asiaticum* var. *japonicum* (Cr. asi.), *Trichosanthes kirilowii* var. *japonica* (T. kir.), *Lonicera japonica* (L. jap.), *Oenothera stricta* (O. str.), and *Platanthera mandarinorum* var. *mandarinorum* (P. man.). t: trace amounts. RT, RI: an identification has been confirmed by GC-retention time of authentic compounds (RT) or by GC-retention index (RI) (Jennings and Shibamoto 1980).

Rhythmicity in the quantity of volatile compounds

Figure 1 illustrates the rhythmicity of floral volatile emission in *L. japonica*. During the flowering period of two days, three compounds showed typical cycles: linalool, germacrene D and a compound eluted with a retention time of 18.43 min (RT 18.43; unidentified). Changes in other compounds were either constant, or else drifted with no rhythmicity. Linalool, which occupies the largest proportion (Table 1), demonstrated the maximal emission at 0.44 $\mu\text{g flower}^{-1}$ 30 min^{-1} at the first midnight. The amounts of maximal emission of germacrene D and RT 18.43 were smaller by one digit.

Discussion

Floral volatile compounds in hawkmoth-pollinated plants

Fatty acid derivatives were not found, or were found in only small amounts in the sphingophilous species studied (Table 1). This supports the finding of Knudsen and Tollsten (1993). Methyl benzoate was present in *Cl. trichotomum* and *T. kirilowii*. Phenyl acetaldehyde, which has been reported to be an attractant for many species of moths (Creighton *et al.* 1973, Cantelo and Jacobson 1979, Haynes *et al.* 1991, Heath *et al.* 1992; but not reported for hawkmoths), was present in the highest proportion in *T. kirilowii*. Large amounts of acyclic terpenes (linalool, linalool oxide, ocimene, nerolidol and β -myrcene) were found in *Cl. trichotomum* (60.4%), *O. stricta* (56.1%), *L. japonica* (86.1%), *Cr. asiaticum* (61.5%), and

Table 2. A list of flower-visitors to six species of sphingophilous plants

Flower-visitors	A. jul.	Cl. tri.	Cr. asi.	L. jap.	O. str.	P. man.
Sphingidae						
<i>Theretra japonica</i>	○	○	○	○	○	○ ^b
<i>T. nesus</i>			○ ^a			
<i>T. oldenlandiae oldenlandiae</i>	○	○	○	○	○	
<i>Deilephila elpenor lewisii</i>			○			
<i>Rhagastis mongoliana mongoliana</i>	○					
<i>Meganoton increta</i>			○			
<i>Agrius convolvuli</i>		○	○	○	○	
<i>Kentorchrysalis consimilis</i>	○					
<i>Macroglossum</i> spp. (diurnal)		○				
Noctuidae						
<i>Diachysia intermixta</i>				○		
Papilionidae						
<i>Papilio</i> spp. (diurnal)	○	○	○ ^a			
Pieridae						
<i>Hebomoia glaucippe shirozui</i> (diurnal)			○ ^a			
Anthophoridae						
<i>Tetralonia nipponensis</i> (diurnal)				○		
Haliictidae						
<i>Lasioglossum</i> sp. (diurnal)				○		

^a Observation on Yakushima Island.

^b Observation in Yamaguchi by Inoue (1983).

Table 3. Sørensen's indices of similarity of the floral scent composition (the lower triangular part) and the species composition of flower-visiting hawkmoths (the upper triangular part)

	A. jul.	Cl. tri.	Cr. asi.	L. jap.	O. str.	P. man.
A. jul.		0.500	0.400	0.571	0.571	0.400
Cl. tri.	0.332		0.667	1.000	1.000	0.500
Cr. asi.	0.176	0.327		0.667	0.667	0.286
L. jap.	0.324	0.677	0.139		1.000	0.500
O. str.	0.380	0.703	0.105	0.686		0.500
P. man.	0.000	0.000	0.000	0.000	0.000	

A. julibrissin, (52.8%), with a considerable amount in *T. kirilowii* (23.0%). We found germacrene D and nerolidol only in a couple of species and then at low proportions. α -Farnesene was not detected in any of the flowers studied. Schlotzhauer *et al.* (1996) reported the presence of large amounts of germacrene D and α -farnesene in a solvent extract of *L. japonica*. This nonconformity with our results was due to a difference in sampling techniques; these sesquiterpenes (Mw=204) do not readily vaporize. Nitrogen-containing compounds were not found except for indole in *L. japonica* (Table 1). 2- and 3-Methylbutyraldoxime formerly reported to be present in *L. japonica* (Kaiser and Lamparsky 1980, Kaiser 1991) were not detected in this study. However, the presence of nitrogen-containing compounds needs to be carefully confirmed by NPD (a detector for

nitrogen and phosphor). Nilsson (1985) suggested that nitrogen-containing fragrances are a specialization for tropical hawkmoths (see Knudsen and Tollsten 1993, for further comments).

White flowers visited by moths often contain acyclic terpene alcohols (e.g., linalool, nerolidol and farnesol), the corresponding hydrocarbons, accompanied by aromatic alcohols, especially benzyl alcohol and 2-phenylethanol, and the esters derived from them, together with the esters of salicylic acid, in addition to nitrogen-containing compounds (Dobson 1993, Knudsen and Tollsten 1993). These account for more than half of the volatile components present in *L. japonica*, *O. stricta*, *Cl. trichotomum*, *A. julibrissin* and *Cr. asiaticum*.

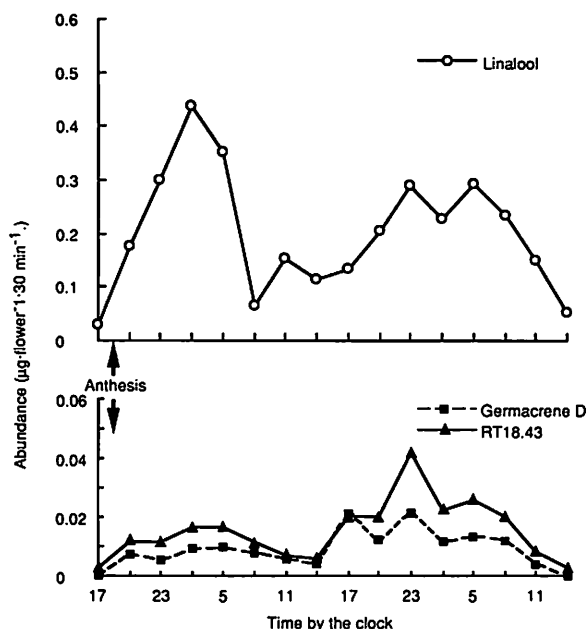


Fig. 1. Rhythmic emission of floral scent compounds in *Lonicera japonica*. The compound eluting with a retention time of 18.43 has not been identified.

Flower-visitor compositions

Most flowers were visited by diurnal hawkmoths, butterflies or bees, in addition to the nocturnal hawkmoths which are expected by the pollination syndrome (Table 2). The bodies of these flower-visitors, except for *Diachysia intermixta*, are observed to touch the anthers or to carry pollen (Inoue 1983, Miyake and Yahara unpublished, Miyake personal observation), suggesting that they are effective as pollinators. Diurnal pollinators have been reported in other plants whose flowers open at dusk (Haber and Frankie 1989, Jürgens *et al.* 1996, Ghazoul 1997). However, our recorded diurnal pollinator compositions do not seem to explain the scent profiles of flowers (Tables 1, 2). Even though diurnal butterflies also visit the flowers of *Ci. trichotomum*, *A. julibrissin* and *Cr. asiaticum*, common characteristics within their floral compounds do not seem to exist, except for linalool.

It is worth noticing that a pierid butterfly, *Hebonaria glaucippe* which visited *Cr. asiaticum* in Yakushima Island (Table 2), secretes ocimene and that the male butterflies demonstrated a high EAG (electroantennogram) response to ocimene (Hayashi *et al.* 1985). Unfortunately, we did not record the sex of the butterflies visiting *Cr. asiaticum*. Ocimene may play a role in attracting the pierid butterfly in *Cr. asiaticum*, whose flowers emit ocimene in the highest proportion (Table 1).

Within Sphingidae, pollinator compositions differed among the plant species (Table 2). Significant correlation between the hawkmoth composition and the floral scent composition suggests that the attractiveness to each hawkmoth species is based on specific floral scent composition. The difference in the composition of hawkmoths visiting nocturnal flowers, as seen in a Costa Rican dry forest (Haber and

Frankie 1989), may have been due to the floral scent composition.

Rhythmicity and possible attractants of floral scents

In a number of plant species, temporal changes in the floral scent composition or in the quantity of certain compounds have been reported recently using a night vs. day comparison (Loughrin *et al.* 1990, Mookherjee *et al.* 1990, Kite and Smith 1997) or using time-course sampling (Matile and Altenburger 1988, Loughrin *et al.* 1990, Loughrin *et al.* 1991, Jakobson and Olsen 1994). Although some of these studies imply an adaptive emission pattern for nocturnal or diurnal pollinators, they all lack information about the pollinators, except for the report of Heath *et al.* (1992), which revealed quantitative correspondence between the major compounds of the floral scent of jessamine and the visitation numbers of a cabbage looper moth inside a greenhouse.

In the present study, rhythmic emission with regard to the quantity of linalool, germacrene D and RT 18.43 was detected in the case of *L. japonica* (Fig. 1), with both diurnal and nocturnal pollinators being observed (Table 2). All were emitted in higher amounts during the night, which suggests an adaptation to nocturnal hawkmoths regarding the emission pattern. A change in the quantity of linalool, which is dominant (80% of total scent) within the floral scent of *L. japonica*, will affect the total amount of the floral scent or the strength of it. The rhythmicity of linalool is consistent with the night vs. morning comparison of the compounds of solvent extraction from *L. japonica* as reported by Schlotzhauer *et al.* (1996). Not all the compounds shown by Schlotzhauer *et al.* (1996), however, behaved in the same way in our study, probably because their data regarding solvent extraction do not always reflect the headspace (Altenburger and Matile 1990, Knudsen *et al.* 1993).

Biological assay using the pollinators must be the most direct way of identifying the attractants within floral scents, however comparative surveys on common compounds found within the pollination syndrome groups and temporal changes of emission corresponding to their pollinators can often reveal the possible attractants within the floral scents (Dobson 1993, Harborne 1993, Kaiser and Tollsten 1995, Knudsen and Tollsten 1995).

In this manner, one would predict that linalool is a common attractant for hawkmoths (Table 1, Fig. 2). However, linalool has also been reported to be an attractant within floral fragrances for beetles (Ikeda *et al.* 1993) and honeybees (Dobson 1993, Blight *et al.* 1997). Moreover, we found that *P. mandariorum* lacked linalool in its floral scent (Table 1). These findings show that sphingophilous flowers do not seem to share common and unique characteristic compounds. In previous studies, acyclic monoterpenes and benzenoids have been reported to occur in a high proportion in white flowers, most of which are pollinated by moths (Dobson 1993, Knudsen and Tollsten 1993). However, they are not unique to the sphingophilous flowers, nor do they originate via the same metabolism pathway, as can be seen the case in bat-pollinated or sapromyophilous flowers (Knudsen *et al.* 1993). Thus, it is too impetuous to conclude that

they are the attractants specific for hawkmoths as is the case of sulphur-containing compounds in bat flowers and amines in sapromyophilous flowers.

We suggest 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. To test this hypothesis, further comparative studies on ancestral species pollinated only by diurnal pollinators and derivative sphingophilous species are essential.

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References

- Altenburger, R. and Matile, P. 1990. Further observations on rhythmic emission of fragrance in flowers. *Planta* **180**: 194-197.
- Bergström, G., Birgersson, G., Groth, I. and Nilsson, L.A. 1992. Floral fragrance disparity between three taxa of lady's slipper *Cypripedium calceolus* (Orchidaceae). *Phytochem.* **31**: 2315-2319.
- Blight, M.M., Le Métayer, M., Delègue, M.H.P., Pickett, J.A., Marionpoll, F. and Wadhams, L.J. 1997. Identification of floral volatiles involved in recognition of oilseed rape flowers, *Brassica napus* by honeybees, *Apis mellifera*. *J. Chem. Ecol.* **23**: 1715-1727.
- Cantelo, W.W. and Jacobson, M. 1979. Phenylacetaldehyde attracts moths to bladder flower and to blacklight traps. *Environ. Entomol.* **8**: 444-447.
- Creighton, C.S., McFadden, T.L. and Cuthbert, E.R. 1973. Supplementary data on phenylacetaldehyde: an attractant for Lepidoptera. *J. Econ. Entomol.* **66**: 114-115.
- Dobson, H.E.M. 1993. Floral volatiles in insect biology. In E.A. Bernays, ed., *Insect-Plant Interactions*, vol. 5, CRC Press, Florida, pp. 47-81.
- Ghazoul, J. 1997. The pollination and breeding system of *Dipterocarpus obtusifolius* (Dipterocarpaceae) in dry deciduous forests of Thailand. *J. Nat. Hist.* **31**: 901-916.
- Grant, V. 1983. The systematic and geographical distribution of hawkmoth flowers in the temperate North American flora. *Bot. Gaz.* **144**: 439-449.
- Gregg, K.B. 1983. Variation in floral fragrances and morphology: Incipient speciation in cycnoches? *Bot. Gaz.* **144**: 566-576.
- Haber, W.A. and Frankie, G.W. 1989. A tropical hawkmoth community: Costa Rican dry forest Sphingidae. *Biotropica* **21**: 155-172.
- Harborne, J.B. 1993. *Introduction to Ecological Biochemistry*. Academic Press, London.
- Hayashi, N., Nishi, A., Murakami, T., Maeshima, K., Komae, H. and Sakao, T. 1985. The scent substances of pierid butterflies (*Hebomoia glausippe* Linnaeus) and the volatile components of their food plants (*Crataeva religiosa* Forst.). *Z. Naturforsch.* **40c**: 47-50.
- Haynes, K.F., Zhao, J. and Latif, A. 1991. Identification of floral compounds from *Abelia grandiflora* that stimulate upwind flight in cabbage looper moths. *J. Chem. Ecol.* **17**: 637-646.
- Heath, R.R., Landolt, P.J., Dueben, B. and Lenczewski, B. 1992. Identification of floral compounds of night-blooming jessamine attractive to cabbage looper moths. *Environ. Entomol.* **21**: 854-859.
- Ikeda, T., Ohya, E., Makihara, H., Nakashima, T., Saitoh, A., Tate, K. and Kojima, K. 1993. Olfactory responses of *Anaglyptus subfasciatus* Pic and *Demonax transilis* Bates (Coleoptera: Cerambycidae) to flower scents. *J. Jpn. For. Soc.* **75**: 108-112.
- Inoue, K. 1983. Systematics of the genus *Platanthera* (Orchidaceae) in Japan and adjacent regions with special reference to pollination. *J. Fac. Sci. Univ. Tokyo III* **13**: 285-374.
- Jakobson, H.B. and Olsen, C.E. 1994. Influence of climatic factors on emission of flower volatiles *in situ*. *Planta* **192**: 365-371.
- Jennings, W. and Shibamoto, T. 1980. *Qualitative analysis of flavor and fragrance volatiles by glass capillary gas chromatography*. Academic Press, London.
- Jürgens, A., Witt, T. and Gottsberger, G. 1996. Reproduction and pollination in Central European populations of *Silene* and *Saponaria* species. *Bot. Acta* **109**: 316-324.
- Kaiser, R. 1991. Trapping, investigation and reconstruction of flower scents. In P.M. Müller and D. Lanparsky, eds., *Perfumes: Art, Science and Technology*, Elsevier Applied Science, London, pp. 213-250.
- Kaiser, R. and Lamparsky, D. 1982. Constituants azotés en trace de quelques absolues de fleurs et leurs head-spaces correspondants. In FEDAROM, ed., *Proc. 8th Internat. Congress of Essential Oils, Cannes, 1980, Grasse*, pp. 287-294.
- Kaiser, R. and Tollsten, L. 1995. An introduction to the scent of Cacti. *Flavour and Fragrance J.* **10**: 153-164.
- Kawano, S., Odaki, M., Yamaoka, R., Oda-Tanabe, M., Takeuchi, M. and Kawano, N. 1995. Pollination biology of *Oenothera* (Onagraceae). The interplay between floral UV-absorbancy patterns and floral volatiles as signals to nocturnal insects. *Pl. Sp. Biol.* **10**: 31-38.
- Kite, G.C. and Smith, S.A.L. 1997. Inflorescence odour of *Senecio articulatus*: temporal variation in isovaleric acid levels. *Phytochem.* **45**: 1135-1138.
- Knudsen, J.T. and Tollsten, L. 1993. Trends in floral scent chemistry in pollination syndromes: floral scent composition in moth pollinated taxa. *Bot. J. Linn. Soc.* **113**: 263-284.
- Knudsen, J.T. and Tollsten, L. 1995. Floral scent in bat-pollinated plants: a case of convergent evolution. *Bot. J. Linn. Soc.* **119**: 45-57.
- Loughrin, J.H., Hamilton-Kemp, T.R., Andersen, R.A. and Hildebrand, D.F. 1990. Volatiles from flowers of

- Nicotiana sylvestris*, *N. otophara* and *Malus x Domestica*: headspace components and day/night changes in their relative concentrations. *Phytochem.* **29**: 2473-2477.
- Loughrin, J.H., Hamilton-Kemp, T.R., Andersen, R.A. and Hildebrand, D.F. 1991. Circadian rhythm of volatile emission from flowers of *Nicotiana sylvestris* and *N. suaveolens*. *Physiol. Plant.* **83**: 492-496.
- Manly, B.F.J. 1991. Randomization and Monte Carlo methods in biology. Chapman and Hall, London.
- Matile, P. and Altenburger, R. 1988. Rhythms of fragrance emission in flowers. *Planta* **174**: 242-247.
- Mookherjee, B.D., Trenkle, R.W. and Wilson, R.A. 1990. The chemistry of flowers, fruits and spices: live vs. dead a new dimension in fragrance research. *Pure Appl. Chem.* **62**: 1357-1364.
- Morgan, A.C. and Lyon, S.C. 1928. Notes on amyl salicylate as an attractant to the tobacco hornworm moth. *J. Econ. Entomol.* **21**: 189-191.
- Nilsson, L.A. 1985. Characteristics and distribution of intermediates between *Platanthera bifolia* and *P. chlorantha* (Orchidaceae) in the Nordic countries. *Nord. J. Bot.* **5**: 407-419.
- Pellmyr, O. 1986. Three pollination morphs in *Cimicifuga simplex*; incipient speciation due to inferiority in competition. *Oecologia* **68**: 304-307.
- Pijl, L. van der. 1961. Ecological aspects of flower evolution. II. Zoophilous flower classes. *Evolution* **15**: 44-59.
- Raguso, R.A. and Pichersky, E. 1995. Floral volatiles from *Clarkia breweri* and *C. concinna* (Onagraceae): recent evolution of floral scent and moth pollination. *Pl. Syst. Evol.* **194**: 55-67.
- Schlotzhauer, W.S., Pair, S.D. and Horvat, R.J. 1996. Volatile constituents from flowers of Japanese honeysuckle (*Lonicera japonica*). *J. Agric. Food Chem.* **44**: 206-209.
- Tollsten, L. and Bergström, J. 1989. Variation and post-pollination changes in floral odours released by *Platanthera bifolia*. *Nord. J. Bot.* **9**: 359-362.
- Tollsten, L. and Knudsen, J.T. 1992. Floral scent in dioecious *Salix* (Salicaceae)-a cue determining the pollination system? *Pl. Syst. Evol.* **182**: 229-237.
- Tollsten, L., Knudsen, J.T. and Bergström, G. 1994. Floral scent in generalistic *Angelica* (Apiaceae)-an adaptive character? *Biochem. Syst. Ecol.* **22**: 161-169.
- Wyatt, R. 1983. Pollinator plant interactions and the evolution of breeding systems. In L. Real, ed., *Pollination Biology*, Academic Press, New York. pp. 51-96.

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Appendix

List of taxa

List of taxa from which floral scents were collected. Floral scent collection is indicated as being from flowers on cut branches or from flowers borne on rooted plants.

- Lonicera japonica* (Caprifoliaceae): Perennial semievergreen woody vine. Flowering in May and June. Corolla, initially white colored, but later turning cream. Flowers open in the evening. The floral scent was collected from a rooted plant in a field between 19 : 30 and 9 : 30 on 8/9 July, 1995.
- Oenothera stricta* (Onagraceae): Perennial herb originally distributed in South America. Flowering from May to July. Corolla, yellow colored. Flowers open in the evening. The floral scent was collected from a rooted plant in a field between 19 : 00 and 9 : 00 on 6/7 June, 1995.
- Clerodendrum trichotomum* (Verbenaceae): Shrub, 2-3 m in height. Flowering from July to September. Corolla, white to pink colored. The floral scent was collected from a cut branch in the laboratory between 17 : 50 and 17 : 50 on 17/18 August, 1995.
- Crinum asiaticum* var. *japonicum* (Amaryllidaceae): Perennial herb, 50-80 cm in height. Flowering in July and August. Corolla, white colored. Flowers open in the early evening. The floral scent was collected from a rooted plant in a field between 19 : 30 and 10 : 30 on 30/31 July, 1995.
- Albizia julibrissin* (Leguminosae): Tree. Corolla white to pink colored with brush-like shape. Flowering in July and August. Flowers open in the evening. The floral scent was collected from a rooted plant in a field between 21 : 00 and 12 : 00 on 30 September/1 October, 1995.
- Trichosanthes kirilowii* var. *japonica* (Cucurbitaceae): Perennial vine herb. Flowering in June to September. Corolla, white colored. Flowers open in the late evening. The floral scent was collected from a rooted plant in a field between 20 : 00 and 9 : 30 on 25/26 September, 1995.
- Platanthera mandarinorum* var. *mandarinorum* (Orchidaceae): Herb, 20-40 cm in height. Flowering in May. Corolla, light yellowish green colored with long spur. The floral scent was collected from a rooted plant in a field between 19 : 15 and 0 : 15 on 30/31 May, 1996.