



Interspecific Variation in Floral Fragrances within the Genus *Narcissus* (Amaryllidaceae)

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Key Word Index—*Narcissus*; Amaryllidaceae; daffodils; flower volatiles; chemotaxonomy; headspace analysis; GC-MS; pollination; entomophily; Spain.

Abstract—To improve our understanding of the floral biology, pollination, and systematics of the genus *Narcissus*, a comparative study was made of flower volatiles from nine species native to southern Spain using headspace collection and GC-MS analysis. The species fell into three fragrance types based on the identity of their major volatiles. In all but one species the fragrances consisted mainly of monoterpene isoprenoids mixed with benzenoids: in six species *trans*- β -ocimene occurred in high proportions, in two others it was lacking; the last species had a fragrance dominated by fatty acid derivatives, mixed with terpenoids. Two of the species showed marked intraspecific variation in many of their volatiles. When the volatile data matrix of all species was subjected to cluster analyses and the resulting phenetic trees compared with currently recognized taxonomic groups, there was no congruence at the subgeneric level. However, there was considerable agreement at the sectional level, although in most sections we studied only a single species. This apparent agreement was stronger when the volatiles were analyzed according to shared biosynthetic pathways rather than treated individually, pointing to the higher value of using biosynthetic pathways for uncovering and confirming phenetic, and probable evolutionary, relationships among species. In terms of possible selective pressures from pollinators in shaping fragrance chemistry, available information on the pollination of our species suggested an association between fragrance and types of pollinators. Two pollinator-fragrance groups were apparent: (1) species pollinated by insects that include butterflies and moths displayed fragrances containing volatiles typical of moth-pollinated flowers, most particularly indole combined with high amounts of esters, and (2) species visited exclusively by insects other than butterflies and moths, especially by bees and flies, had fragrances lacking this combination of volatiles. *Narcissus assoanus* was unusual among our species in having both fragrance chemotypes. Future pollination studies of *Narcissus* in the field are needed to test the reliability and predictability of the proposed fragrance-pollinator associations. © 1997 Elsevier Science Ltd

Introduction

The genus *Narcissus* is estimated to comprise generally between 35–70 species (see Barrett *et al.*, 1996), the majority of which are considered native to the western Mediterranean area, with the center of diversity located in the southern Iberian Peninsula and northern Morocco (Fernandes, 1951). The phylogenetic relationships within *Narcissus* are little known, and both the status and boundaries of many of the taxa are unclear. This is due in part to the group's long history of cultivation as an ornamental (including extensive artificial hybridization), which makes it often difficult to ascertain whether populations, and even taxa, growing in the wild are native or represent naturalized escaped ornamentals (Webb, 1980). The frequent occurrence of natural hybridization and polyploidy complicates the picture even further (Fernandes, 1951). While species

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circumscriptions vary, there is currently general agreement in recognizing the genus to comprise two subgenera and ten sections (excluding those of clearly hybrid origin) (see reviews by Fernandes, 1951, 1975; Blanchard, 1990).

Surprisingly little is known about the pollination biology of this horticulturally important and popular group of plants. The flowers of *Narcissus* species are characterized by having a corona and a perianth tube, but vary in their morphology, as well as in other features such as flower size, orientation, and number, and occurrence of style polymorphisms (a topic of controversy) and true heterostyly (see Barrett *et al.*, 1996). Most species bloom in early spring (otherwise in autumn), have white, yellow, or orange perianths, and some are highly scented; most investigated species are self-sterile, thus requiring cross-pollination for seed set. Various aspects of the reproductive biology, especially in reference to stylar polymorphism, have been examined in some species, but pollination information is sparse (Knuth, 1898; Dulberger, 1964, 1967; Lloyd *et al.*, 1990; Arroyo and Dafni, 1993, 1995; Herrera, 1995; Barrett *et al.*, 1996). The few observations of flower visitors indicate that the pollinators are insects, primarily bees and moths.

Flower fragrances in general have been shown to influence the behavior and flower visitation patterns of pollinators (Faegri and van der Pijl, 1979; Robacker *et al.*, 1988; Dobson, 1994), and can thereby play an important role in plant evolution and speciation. Consequently, comparative studies of fragrance chemistry are predicted to be valuable in taxonomic studies of closely related species. When different species are pollinated by the same group of animals, their flowers tend to show convergent evolution in various morphological and ecological features, which together define the characteristic "pollination syndrome" associated with a particular pollinator group (Faegri and van der Pijl, 1979; Wyatt, 1983). Recently, flowers falling into certain pollination syndromes have been shown also to display some uniform properties in their fragrance chemistry. These fragrance syndromes, which can be valuable for predicting a plant's pollination biology, have been demonstrated most clearly in flowers pollinated by night-flying moths, bats, carrion- and dung-seeking insects, and male euglossine bees (Faegri and van der Pijl, 1979; Williams and Whitten, 1983; Vogel, 1990; Gerlach and Schill, 1991; Kaiser, 1993a,b; Knudsen and Tollsten, 1993, 1995; Kaiser and Tollsten, 1995). Up to present, fragrance chemistry has been most useful in confirming established taxonomic relationships and clarifying uncertain positions of taxa for genera in which the species both share the same pollination syndrome and have fragrances that serve as strong reproductive barriers. Examples include *Magnolia* (Magnoliaceae) (Thien *et al.*, 1975), Pyrolaceae (Knudsen and Tollsten, 1991), and Orchidaceae pollinated by male Hymenoptera (e.g. Kullenberg, 1961; Hills *et al.*, 1972; Gregg, 1983; Williams and Whitten, 1983; Gerlach and Schill, 1989; Borg-Karlson, 1990; Paulus and Gack, 1990). When congeneric species differ in both pollination and fragrance syndromes (e.g. Kaiser, 1993a), the strong convergence in fragrance chemistry that accompanies each syndrome can make any large differences in fragrance chemistry of marginal value for elucidating phylogenetic relationships among the species. One approach that might overcome these drawbacks is to examine the fragrance chemistry from the perspective of the representative biosynthetic pathways, which would be more effective for revealing genetically-based relationships (Croteau and Gershenzon, 1994), rather than on the basis of individual volatiles.

Several chemical studies of floral volatiles have been conducted in *Narcissus*, but these have been largely restricted to only a few taxa and cultivars that are valued for their perfumery properties (Joulain, 1986, 1993; Loo and Richard, 1988; van Dort *et al.*, 1993; Surburg *et al.*, 1993), and most of the genus remains uninvestigated. In the present study we analyzed the flower volatiles from nine of the fourteen *Narcissus* species that grow wild in western Andalusia, Spain, with the following three goals: (1) to characterize the composition of flower volatiles in each species and identify any major patterns among species; (2) to compare species groupings obtained from cluster analyses of the fragrance data with current systematic phenetic classifications within the genus; (3) to establish if there is any relation between fragrance chemistry and the currently known or predicted pollinators. We selected the species on the basis of their accessibility in the field and taxonomic affiliations in an attempt to obtain a broad sample of the genus; they represent both subgenera and seven of the ten sections, following the nomenclature of Valdés *et al.* (1987). Variation shown among the species in floral morphology, flower color, and blooming phenology is fairly representative for the genus.

Materials and Methods

Flowering plant specimens were collected whole in the field from wild populations in Andalusia during the spring (1990 and 1991) and fall (1990). They were put into pots and kept on the roof of the Biology building at the University of Sevilla. Fragrance samples were collected from the potted plants, using headspace sorption techniques. Population locations and sample sizes of specimens studied from each of the 9 species are listed in Table 1. Voucher specimens are deposited in the herbarium of the University of Sevilla.

To collect flower volatiles, transparent oven bags (25×40 cm; Reynolds®) were placed over 1–3 pots of plants and tied loosely enough around the pot rims so that air could enter from below. For some samples of tall plants (i.e. two samples of *N. bugei* and one sample of *N. papyraceus*), flowering stems were cut and put in a water-filled container, and the whole covered with a bag. A glass cartridge (5 cm long, 7 mm id) filled with 150 mg of solvent-cleaned adsorbant Porapak® Q (80–100 mesh) was inserted into a hole at the top of the bag (see Dobson, 1991). Volatiles were collected by drawing air through the bag and cartridge, using a battery-run mini suction pump, at a rate of 100 ml min⁻¹ for 7–9 h, beginning around mid-morning (9:00) except for one night sample of *N. serotinus*. Used cartridges were wrapped in aluminum foil and kept at –20°C until desorption, in Sweden. Volatiles were desorbed from the Porapak with 1 ml of high grade diethyl ether, and the liquid samples stored in vials at –20°C. Prior to analysis, samples were concentrated to 0.3–0.5 ml by slow evaporation of the solvent at room temperature.

All samples were analyzed by GC on a Hewlett Packard 5880A instrument equipped with a CP-Wax 52CB column (25 m × 0.25 mm id, Df 0.2 μm); temperature programming was set at 50°C for 5 min followed by an increase of 5°C min⁻¹ to 250°C, then isothermal for 20 min. Selected samples of each species were also analyzed on a GC Varian 3400 coupled to a Finnigan ITD 700 (on-column injection), with a similar column as used above; temperature programming was set at 40°C for 5 min, then increased 8°C min⁻¹ to 230°C. All but two volatiles were identified by comparison of both their retention times and mass spectra with a personal library and reference compounds. Percent composition of each fragrance sample was calculated from the relative GC peak areas of constituent volatiles.

To determine the phenetic similarities in fragrance chemistry among species, the volatile data matrix was subjected to two cluster analyses. In one analysis, all 84 compounds detected from the nine species were treated individually; in the other, the identified compounds were treated in 16 sets grouped according to shared biosynthetic pathways. Prior to analysis, mean percent values from the volatile composition of each species were arcsine transformed. All pairs of species were compared by product-moment correlations, and the resulting similarity matrix submitted to a UPGMA clustering (see Sneath and Sokal, 1973). The goodness-of-fit of trees to the data sets was determined by computing a cophenetic similarity matrix for each tree and testing its correlation with the original data matrix. Since the matrices are not independent and hence exclude the application of a typical Mantel test, significance levels for cophenetic correlations were obtained after 1000 permutations for each matrix pair. A cluster analysis of individual compounds was also carried out at the intraspecific level for the five samples of *N. assoanus*. All analyses were performed with a NTSYS-pc v. 1.8 package (Rohlf, 1993).

TABLE 1. SPECIES OF *Narcissus* STUDIED, WITH GEOGRAPHIC ORIGINS (GROWING IN THE WILD) AND SAMPLING DETAILS. All provinces are in SW Spain, except Huesca (NE Spain)

Species	Province: Location	No. flowers per sample	No. samples ¹	Date
I. Subgenus <i>Narcissus</i>				
Section <i>Apodanthi</i>				
<i>N. cantabricus</i> Fernández-Casas, Lainz & Ruiz Rejón	Cádiz: Villaluenga	14	1	IV-91
Section <i>Bulbocodium</i>				
<i>N. bulbocodium</i> L. subsp. <i>bulbocodium</i>	Huelva: El Rompido	1-7	3	III-90
Section <i>Ganymedes</i>				
<i>N. triandrus</i> L. subsp. <i>pallidulus</i> (Graells) Rivas Goday ex Fernández-Casas	Badajoz: Calera de León	3-4	3	III-90
Section <i>Jonquillae</i>				
<i>N. assoanus</i> Dufour subsp. <i>assoanus</i>	Huesca (Pirineés): Jaca, El Boaler	3	1	III-90
subsp. <i>praelongus</i> A. Barra & G. López pop. 1	Cádiz: Villaluenga	12	1	III-91
subsp. <i>praelongus</i> pop. 2	Córdoba: NE of Cabra, Nuestra Señora de la Sierra	8-18	3	III-90
<i>N. gaditanus</i> Boiss. & Reuter	Huelva: El Rompido	15	3	III-90
<i>N. jonquilla</i> L.	Sevilla: La Puebla de Los Infantes	15-60	2	III-90
Section <i>Pseudonarcissi</i>				
<i>N. bugei</i> (Fernández-Casas) Fernández-Casas	Córdoba: NE of Cabra, La Nava	9-18	3	III-90
II. Subgenus <i>Hermione</i>				
Section <i>Serotini</i>				
<i>N. serotinus</i> L.	Sevilla: Montequinto, campus of the University	7-12	2	XI-90
Section <i>Tazettae</i>				
<i>N. papyraceus</i> Ker-Gawler	Sevilla: Aznalcázar, La Tiesa	4-29	5	III-90

¹Each sample was from different plants.

Results

Fragrance chemistry: interspecific comparisons

General. Fragrance compositions of the nine *Narcissus* species, expressed as mean percent values, are shown in Table 2. A total of 84 volatiles were detected in all species. The volatiles were grouped as much as possible according to biosynthetic pathways, following Croteau and Karp (1991) and Gershenzon and Croteau (1993), to more clearly reveal genetically based patterns. While the biosynthesis of different isoprenoids is relatively well understood, that of many benzenoid compounds is problematical. Consequently, benzenoids were grouped according to the methoxylation of the phenyl group. A total of 19 volatiles were unidentified, of which we were able to assign nine to compound classes.

In general, the fragrances consisted mainly of monoterpene isoprenoids (up to 99% of the volatiles in one species), with benzenoids making up the remainder; in *N. jonquilla* these two chemical classes were equally represented. Fatty acid derivatives were identified in only a few species; in the fragrance of *N. cuatrecasasii* they were the dominant constituents, making this species a notable exception to the general fragrance pattern observed in the genus.

All species contained monoterpenes formed through basic pathways from the general precursor geranyl pyrophosphate; the most common were limonene, myrcene, and *trans*- β -ocimene. Derivatives of these basic compounds, however, were more selectively distributed among the taxa and contributed to the species specificity of fragrance profiles. Most frequent were the derivatives of β -ocimenes, some of which might represent by-products of the oxidative decomposition of ocimenes incurred during fragrance collection (Surburg *et al.*, 1993). Benzenoid compounds were detected in all but two species and varied considerably among species. Most were non-methoxylated, but a few species (especially *N. bugei*) contained methoxylated forms (i.e. ethers).

Among the other groups of compounds, the nitrogen-containing volatile indole occurred in five of the nine species and was generally associated with high representations of esters. Small amounts of isopentenoids of undetermined biosynthetic origin were detected in most of the species. Sesquiterpenes were sparse. Also, several compounds, which were present in low representations across species, were not identified.

In each species some compounds were clearly dominant quantitatively, and in some cases a single volatile could comprise over 50% of the fragrance. Species had either few (6–13) or many (23–31) total compounds in their fragrance; the proportion of volatiles detected as minor components (<1.0% of fragrance) ranged from 0 to 85% and was markedly greater in species with many volatiles.

Descriptive groupings of species. The nine species can be divided into three fragrance groups based on their dominant volatiles and other distinctive attributes. The major features of each group and its included species are described below.

GROUP 1. Fragrance dominated by fatty-acid derived volatiles; few compounds.

(1) *Narcissus cuatrecasasii* fragrance showed a dominance of fatty acid derived acetates found exclusively in this species, and the strong presence of the otherwise rarely detected β -ionone; no benzenoids were detected.

GROUP 2. Fragrance dominated by monoterpenes, but lacked *trans*- β -ocimene; few compounds.

(1) *Narcissus bulbocodium* fragrance comprised mainly myrcene and its derivatives (>66%); the sesquiterpene longifolene was detected exclusively in this species.

TABLE 2. MEAN PERCENT COMPOSITION (AND RANGE) OF FLORAL FRAGRANCES COLLECTED BY HEADSPACE SORPTION FROM EACH *Narcissus* SPECIES (*n* = NUMBER OF SAMPLES). Volatiles are grouped by biosynthetic pathways, and listed in the order of their retention times. * indicates compounds that have been reported in headspace of *N. jonquilla*, *N. poeticus*, *N. pseudonarcissus*, and *N. tazetta* (Joulain, 1986, 1993; Mookherjee *et al.*, 1989; Surburg *et al.*, 1993); compound synonyms used in other *Narcissus* fragrance studies are given in footnotes

COMPOUND	<i>assanus</i> n = 5 (%)	<i>bugei</i> n = 3 (%)	<i>bulbocodium</i> n = 3 (%)	<i>cutreacasasi</i> n = 1 (%)	<i>gaditanus</i> n = 3 (%)	<i>jonquilla</i> n = 2 (%)	<i>papyraceus</i> n = 5 (%)	<i>serotinus</i> n = 2 (%)	<i>triandrus</i> n = 3 (%)
FATTY ACID DERIVATIVES									
2-undecyl acetate			30.7						
2-tridecyl acetate			14.8						
Unidentified:									
1									
2			18.7		6.1 (0-16.4)				
3					2.2 (tr-3.8)				
4									
5		0.2 (0.1-0.3)				<0.1 (tr-0.1)	0.9 (0.2-2.0)		
Total %	0	0.2	0	64.2	8.3	0.1	0.9	15.9	0
MISCELLANEOUS ISOPENTENOIDS									
2-methyl-3-buten-2-ol	0.1 (0-0.4)	0.2 (0-0.5)				0.1 (0.1)	0.1 (tr-0.4)		
*3-methyl-3-buten-1-ol	0.2 (0-0.6)		2.7 (tr-7.0)			0.7 (0.6-0.8)			
methyl 2-methylbutanoate						0.8 (0.7-0.8)			
methyl 3-pentenoate ¹	0.4 (0-1.8)					0.2 (0.2-0.3)			
3-methylbutyl acetate									
methyl 3-methyl-2-butenate	0.4 (0-1.9)					1.1 (1.1-1.2)			
*3-methyl-3-butenyl acetate ²	0.5 (0-1.6)					0.7 (0.6-0.8)			
Total %	1.6	0.2	2.7	0	0	3.6	0.1	0.3	0
ISOPRENOIDS C₁₀: monoterpene									
<i>Geranyl pyrophosphate derivatives:</i>									
α -pinene									18.4 (13.1-22.5)
camphene									12.2 (9.7-17.2)
β -pinene									7.8 (6.7-9.8)
myrcene	0.5 (tr-2.6)	11.0 (6.3-20.0)	36.3 (19.9-62.7)	0.9		0.8 (0.6-1.1)	0.7 (0.4-1.0)	0.3 (0.2-0.5)	
limonene	0.3 (0-1.2)	0.1 (0.1)	9.0 (0-23.6)	16.9	1.4 (1.1-1.6)	tr ³ (0-tr)	0.1 (0-0.2)	0.7 (0.7-0.8)	7.6 (5.0-9.0)
<i>cis</i> - β -ocimene	2.7 (2.1-4.2)	2.0 (1.0-2.7)			2.6 (1.9-3.2)	0.7 (0.6-0.8)	2.6 (2.2-3.3)	1.9 (1.4-2.4)	
* <i>trans</i> - β -ocimene	64.4 (42.9-86.8)	59.3 (54.7-64.4)			67.9 (48.9-86.0)	25.6 (24.6-26.6)	69.6 (42.0-89.0)	27.1 (21.4-32.9)	
1,8-cineole	0.1 (0-0.2)							0.4 (0.1-0.7)	
*linalool	1.7 (0-8.7)	0.6 (0.4-0.8)			3.9 (1.1-5.7)	15.0 (10.2-19.8)	0.1 (0-0.2)	8.3 (7.1-9.4)	

TABLE 2—CONTINUED

COMPOUND	<i>assarius</i> n = 5 (%)	<i>bugei</i> n = 3 (%)	<i>bulbocodium</i> n = 3 (%)	<i>cuatrecasasi</i> n = 1 (%)	<i>gacitanus</i> n = 3 (%)	<i>jonquilla</i> n = 2 (%)	<i>papyraceus</i> n = 5 (%)	<i>serotinus</i> n = 2 (%)	<i>triandrus</i> n = 3 (%)
C₁₅: sesquiterpenes									
longifolene			11.9 (3.2–28.8)						
β -bisabolene	<0.1 (0–0.1)	<0.1 (0–0.1)							
* <i>trans</i> - α -farnesene	<0.1 (0–0.1)	1.2 (0.5–2.6)				0.1 (<0.1–0.2)			
Total %	70.5	75.6	88.6	35.8	75.8	43.5	75.0	65.9	95.3
BENZENOIDS (based on phenyl group methoxylation)									
<i>No methoxy groups</i>									
benzaldehyde	0.6 (0–1.7)						0.1 (0–0.2)	0.6 (0.4–0.7)	0.6 (0–2.0)
phenylacetaldehyde	2.7 (0–5.0)							0.9 (0.3–1.4)	
*methyl benzoate	15.2 (0–41.1)					46.4 (37.2–55.5)			
*benzyl acetate	0.6 (0–1.6)					0.6 (0.3–1.0)	16.7 (4.0–33.8)	8.0 (7.4–8.6)	
2-phenylethyl acetate	2.9 (0–6.1)							3.7 (3.5–3.9)	
3-phenylpropyl acetate					1.9 (0–3.5)				
*3-methyl-3-butenyl benzoate ⁶	0.1 (0–0.4)					0.9 (0.6–1.2)			
*3-methyl-2-butenyl benzoate ⁷	1.0 (0–2.1)					2.8 (1.6–3.9)			
*methyl cinnamate						<0.1 (<0.1)			
*benzyl benzoate	3.8 (0.4–11.5)					1.4 (0.8–2.1)			
2-phenylethanol	0.4 (0–1.2)								
2-phenoxyethanol			8.7 (0–25.7)		2.0 (0–3.7)				
<i>p-Hydroxylated/methoxylated</i>									
*4-methylphenol									
*4-methylanisole ⁸									
estragol ⁹		0.2 (0–0.5)							
*1,4-dimethoxybenzene		3.3 (3.0–3.8)							
<i>m-Methoxylated</i>		0.3 (0–0.8)			1.6 (tr–4.9)				
*1,3-dimethoxy-5-methylbenzene ¹⁰		19.2 (11.4–25.9)							
<i>p- and m-Methoxylated</i>									
methyl Eugenol		0.2 (0.2)							
1,2,3-trimethoxy-5-methylbenzene ¹								3.4 (2.3–4.4)	
Unidentified					1.2 (0–3.6)				0.6 (0.5–0.8)
9									<0.1 (tr–0.1)

Total %	27.3	23.2	8.7	0	6.7	52.1	21.4	17.2	0.6
NITROGEN COMPOUNDS									
*indole	0.2 (0-0.7)				tr (0-tr)	0.2 (0.1-0.4)	0.2 (0-1.2)	<0.1 (tr-0.1)	
OTHER UNIDENTIFIED									
10		0.1 (0.1)				<0.1 (tr-0.1)	0.2 (0.1-0.3)		
11	0.5 (0-2.1)	0.3 (0.2-0.4)				0.2 (0.2)	0.5 (0.3-0.8)		
12		0.1 (tr-0.2)				<0.1 (tr-0.1)	0.2 (0.1-0.4)		
13		tr (tr)					0.1 (tr-0.2)		
14		0.3 (0.2-0.4)					0.8 (0.3-1.4)		
15						0.2 (0.2-0.3)			
16		0.3 (0.1-0.6)				0.1 (tr-0.1)	0.6(0.2-1.6)	0.5 (0.3-0.7)	
17									
18					1.4 (0-4.1)				
19 ¹¹					7.9 (0-15.4)				
Overall total %	100	100	100	100	100	100	100	100	100
Total number of compounds	23	27	8	6	13	31	26	27	12

¹Tentatively identified based on mass spectra only.

²Isoprenyl acetate.

³tr = trace quantities detected after search for characteristic ions in the GC-MS profile.

⁴(Z)- and (E)-2,6-dimethyl-3,5,7-octatrien-2-ol.

⁵For all liliac compounds, stereoisomers are distinguished by letters following Kaiser (1993a, p.178).

⁶Isoprenyl benzoate.

⁷Prenyl benzoate.

⁸1-Methoxy-4-methylbenzene.

⁹1-Methoxy-4(2-propenyl)benzene.

¹⁰3,5-Dimethoxy toluene.

¹¹Suspected to be an artefact.

(2) *Narcissus triandrus* fragrance showed a predominance of several monoterpenes that were detected exclusively in this species, namely camphene, α -pinene, and their derivatives, and derivatives of bornyl pyrophosphate.

GROUP 3. Fragrance dominated by monoterpenes and mainly *trans*- β -ocimene (26–70%), which was restricted to this group; also found only in these species were linalool and usually also indole, the latter occurring together with esters; mostly many compounds.

(1) *Narcissus assoanus* fragrance comprised mainly *trans*- β -ocimene (64%), a high diversity of benzenoid esters, with methyl benzoate being most abundant, and indole.

(2) *Narcissus bugelii* fragrance contained mainly *trans*- β -ocimene (59%), a high abundance of benzenoid ethers (23%), especially 1,3-dimethoxy-5-methyl-benzene, but lacked indole and esters.

(3) *Narcissus gaditanus* fragrance comprised mainly *trans*- β -ocimene (68%), indole, and small amounts of benzenoid alcohols and esters; it had few compounds.

(4) *Narcissus jonquilla* fragrance was dominated by methyl benzoate (46%) and only partly by *trans*- β -ocimene (26%), but with total isoprenoids and benzenoids being equally abundant; it contained indole.

(5) *Narcissus papyraceus* fragrance comprised mainly *trans*- β -ocimene (70%), was characterized by a relatively high abundance of benzyl acetate, and contained indole.

(6) *Narcissus serotinus* fragrance was characterized by the exclusive presence of various linalool-derived lilac compounds (27%), which shared a dominance with *trans*- β -ocimene; it also contained indole and a relatively high abundance of esters.

Cluster analyses. The cluster trees generally followed the descriptive fragrance groupings above, with some deviations. The tree based on all detected compounds treated individually (Fig. 1) showed two main clusters: one with species of groups 1 and 2, the other with species of group 3. The similarity coefficient among members of the first cluster was very low, while the species of group 3 formed a more cohesive cluster, in which subclusters could also be separated by the representation of several volatiles, including *trans*- β -ocimene, linalool, indole, and various methoxylated benzenoids. The

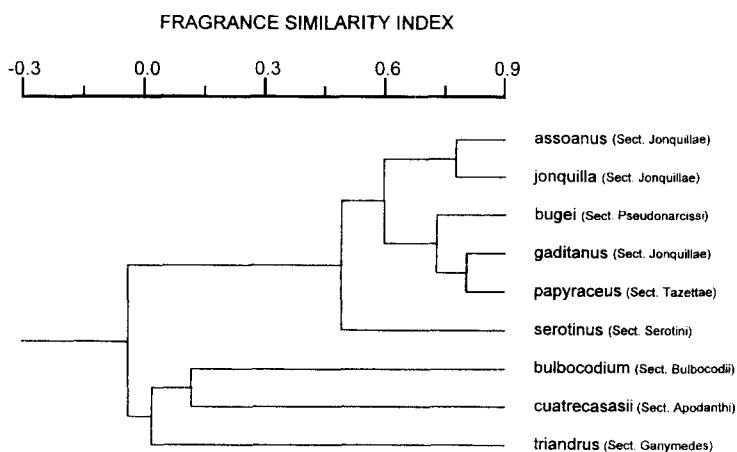


FIG. 1. SIMILARITY TREE FOR THE 9 *Narcissus* SPECIES OBTAINED FROM CLUSTER ANALYSIS OF ALL 84 VOLATILES DETECTED, TREATED INDIVIDUALLY. Goodness-of-fit of tree to data matrix, $r = 0.970$; after 1000 permutations of matrix, $p = 0.001$. *Narcissus papyraceus* and *N. serotinus* are in the subgenus *Hermione*, the remainder species in subgenus *Narcissus*.

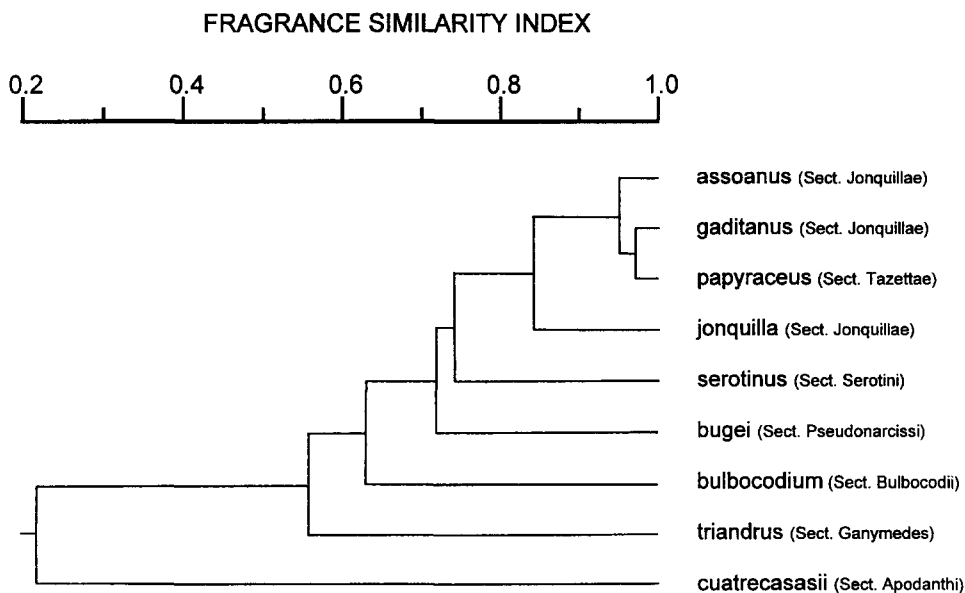


FIG. 2. SIMILARITY TREE FOR THE 9 *Narcissus* SPECIES OBTAINED FROM CLUSTER ANALYSIS OF THE 16 GROUPS OF IDENTIFIED VOLATILES (SEE TABLE 2), WHICH ARE BASED ON COMMONNESS OF BIOSYNTHETIC PATHWAYS. Goodness-of-fit of tree to data matrix, $r=0.924$; after 1000 permutations of matrix, $p=0.001$.

cluster tree based on the 16 biosynthetic-pathway sets of compounds (see Table 2), also showed a similar 2-parted cluster pattern (Fig. 2), but the delimitation of the two clusters was less clear and there were some repositionings among species. The first cluster comprised only *N. cuatrecasasii* (=group 1 fragrance with fatty acid derivatives and no benzenoids), while the other cluster comprised species of groups 2 and 3 (fragrances high in isoprenoids: 35–99%, low in fatty acid derivatives: 0–16%, and variable in benzenoids). Resolution of species in the second cluster was loose, with the two most outlying species, *N. triandrus* and *N. bulbocodium* (=group 2), separating out in having comparatively few volatiles but several distinctive isoprenoid pathways.

Species of group 3 clustered together in both analyses, although less tightly in the tree based on biosynthetic pathways. *Narcissus bugei* stood apart in the biosynthetic pathway analysis in its lack of indole and benzenoid esters, and high presence of variously methoxylated benzenoids (23%). *Narcissus serotinus*, the only fall-blooming species, clustered out in the total volatile analysis and more subtly in the biosynthetic pathway tree by being the only taxon having diverse linalool-derived volatiles, notably lilac compounds. The four remaining species, which included all three species of section *Jonquilla*, the only section represented here by more than one species, showed varying overlaps in their volatiles. In the biosynthetic pathway tree, *N. jonquilla* separated out from the others in its very high representation of benzenoid esters and presence of linalool derivatives. The surprisingly close similarity of *N. gaditanus* to *N. papyraceus* lies in that all of its few volatiles were also detected in the volatile-diverse fragrance of *N. papyraceus*.

Fragrance chemistry: intraspecific variation

Descriptive. In most species, marked variation among the different samples was generally restricted to single volatiles (e.g. *trans*- β -ocimene in *N. gaditanus*, myrcene in *N.*

bugei). In *N. papyraceus*, as many as three volatiles varied (i.e. *trans*- β -ocimene, 4-methylphenol, and benzyl acetate). More widespread variation among the fragrance components was observed in *N. bulbocodium* and *N. assoanus*. In *N. bulbocodium*, most volatiles fluctuated widely in relative abundance among the three samples and with no evident pattern.

In *N. assoanus* (Table 3), the five samples were from three different populations representing two subspecies: subsp. *assoanus* from northern Spain and subsp. *praelongus* from southern Spain. There was high variation both between and within populations, with no clear differences along subspecific lines. Within subsp. *praelongus*, the single sample of population 1 was very similar to sample 2a except for the detection of indole and linalool, and both were distinctive among all samples in having high amounts of methyl benzoate, markedly less *trans*- β -ocimene, and no detected phenylacetaldehyde.

TABLE 3. PERCENT COMPOSITION OF FLOWER FRAGRANCES IN 5 SAMPLES OF *N. assoanus*, REPRESENTING 2 SUBSPECIES AND 3 POPULATIONS (SUBSP. *assoanus*, AND SUBSP. *praelongus* POPULATIONS 1 AND 2). Number of flowers in each sample is shown in parentheses

COMPOUND	Subsp. <i>assoanus</i> (3 fls)	Subsp. <i>praelongus</i>			
		1 ¹ (12 fls)	2a (8 fls)	2b (18 fls)	2c (15 fls)
MISC. ISOPENTENOIDS					
2-methyl-3-buten-2-ol	---	0.1	0.4	---	---
3-methyl-3-buten-1-ol	0.2	0.2	0.6	---	---
methyl 3-pentenoate	---	1.8	tr	---	---
methyl 3-methyl-2-butenate	---	1.9	tr	---	---
3-methyl-3-butenyl acetate	tr	1.6	0.8	---	0.2
ISOPRENOIDS					
Monoterpenes					
myrcene	tr	< 0.1	2.6	tr	tr
limonene	tr	1.2	tr	---	0.4
<i>cis</i> - β -ocimene	4.2	2.1	2.2	2.1	2.8
<i>trans</i> - β -ocimene	80.4	42.9	45.4	86.8	66.8
<i>trans</i> -ocimene epoxide	tr	0.1	0.8	0.4	2.9
linalool	---	---	8.7	tr	---
Sesquiterpenes					
<i>trans</i> - α -farnesene	---	0.1	tr	---	---
BENZENOIDS					
benzaldehyde	tr	tr	---	1.4	1.7
phenylacetaldehyde	5.0	---	---	4.6	3.6
methyl benzoate	---	41.1	34.7	---	---
benzyl acetate	1.6	0.8	0.6	tr	---
2-phenylethyl acetate	6.0	---	---	2.4	6.1
3-methyl-3-butenyl benzoate	---	0.1	0.4	---	tr
3-methyl-2-butenyl benzoate	0.3	2.1	1.9	---	0.7
benzyl benzoate	1.8	3.2	0.4	1.9	11.5
2-phenylethanol	0.6	---	---	0.4	1.2
NITROGEN COMPOUNDS					
indole	---	0.7	---	---	---
OTHER UNIDENTIFIED					
compound 11	---	tr	0.5	---	2.1
Total summed %	100	100	100	100	100
Total number of compounds	14	19	18	11	14

¹ Non-typical population, showing characters intermediate with those of *N. fernandesii* G. Pedro (= *N. cordubensis* Fernández-Casas).

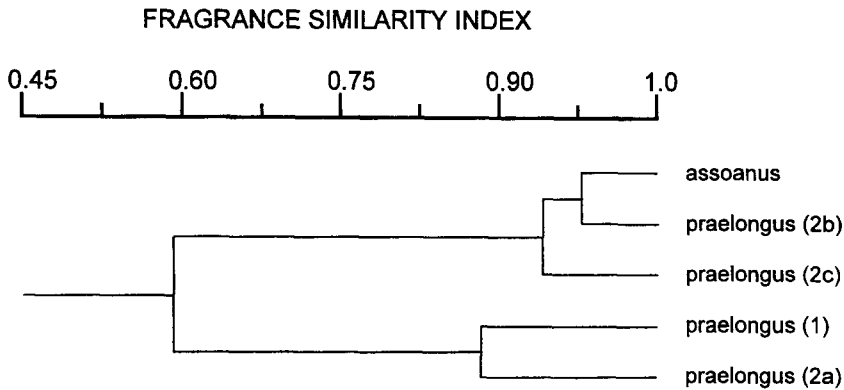


FIG. 3. SIMILARITY TREE FOR THE 5 SAMPLES OF *N. assoanus* (TWO SUBSPECIES AND THREE POPULATIONS) OBTAINED FROM CLUSTER ANALYSIS OF ALL INDIVIDUAL VOLATILES. POPULATIONS: (1) SUBSP. *assoanus*, (2) SUBSP. *praelongus* 1, SHOWING UNUSUAL MORPHOLOGY, (3) SUBSP. *praelongus* 2 (3 SAMPLES), SHOWING TYPICAL MORPHOLOGY. Goodness-of-fit of tree to data matrix, $r = 0.987$; after 1000 permutations of matrix, $p = 0.05$.

Cluster analysis. Analysis of all volatiles (treated individually) at the intraspecific level in *N. assoanus* yielded two clusters that crossed over subspecies and populations, thus showing no segregation along taxonomic or geographic boundaries (Fig. 3).

Other variation. With respect to methodological effects on fragrance composition, three are worthy of note. Firstly, collecting fragrances from flowers on cut stems as opposed to those on rooted plants yielded no differences in either *N. bugei* or *N. papyraceus*. Secondly, in a sample of *N. jonquilla* that suffered water stress and wilted during volatile collection (not included in data here), certain volatiles differed markedly in their relative representations. Most notable were the very low amount of the otherwise dominant *trans*- β -ocimene, substitution of methyl benzoate with 3-methyl-3-butenyl benzoate, and higher contents of 2,6-dimethyl-3,7-octadien-2,6-diol and several unidentified compounds. Thirdly, the single sample collected at night in the white-flowered *N. serotinus* (not shown in Table 2) differed little from the day collections, although it had a lower representation of benzenoid esters (but more benzenoid ethers) and lacked indole.

Pollinators

The few reports of insects visiting flowers of the *Narcissus* species studied here, based on observations made by one of us (J.A.) and any other sources we located, are summarized in Table 4. No records were located for *N. cuatrecasasii*. The greatest diversity of visitors has been observed on *N. papyraceus*, which has also been the most intensively studied. Some patterns between pollinator types and flower fragrance chemistry could be discerned in these limited data, based on whether or not Lepidoptera (i.e. moths and butterflies) are included among the pollinators. Accordingly, two general pollinator-fragrance complexes were apparent (Table 5).

First, all species that include Lepidoptera among their visitors, namely *N. assoanus*, *N. gaditanus*, *N. papyraceus*, and *N. serotinus*, belonged to fragrance group 3, and had volatile profiles that contained indole in combination with usually high representations of esters; in addition, they contained linalool and high amounts of *trans*- β -ocimene

TABLE 4. RECORDS OF INSECT VISITORS IN SOUTHERN SPAIN FOR EIGHT OF THE NINE *Narcissus* SPECIES STUDIED. All observations were made by authors (J.A.) unless indicated

Insects	<i>assoanus</i>	<i>bugei</i>	<i>bulboc.</i>	<i>gaditan.</i>	<i>jonquil.</i>	<i>papyrac.</i>	<i>serotin.</i>	<i>triandrus</i>
LEPIDOPTERA (diurnal)								
Sphingidae:	+ ¹				p ²			
<i>Macroglossum stellatarum</i>						+	+	
Other				+		+		
HYMENOPTERA: APOIDEA								
Apidae:								
<i>Bombus</i> spp.								+
<i>Apis mellifera</i>						+		
Anthophoridae:								
<i>Xylocopa</i> spp.						+		
<i>Anthophora</i> spp.	+ ¹	+, + ³			+ ¹	+		+
Other (small solitary bees)		+, + ³	+	+		+		
DIPTERA								
Syrphidae:								
<i>Eristalis tenax</i>		+ ³				+		
Bombyliidae:								
<i>Bombylius</i> spp.						+		
Other		+ ³				+		
COLEOPTERA								
Nitidulidae		+ ³						

¹Based on observations by L. Harder and S. C. H. Barrett (personal communication).

²p = predicted only (Knuth, 1898).

³Observations for *N. pseudonarcissus* (of which *N. bugei* is a microspecies) in Knuth (1898).

(Table 5). We will refer to this particular mixture of volatiles as the "Lepidoptera odor". Moth Lepidoptera have been observed visiting each species except *N. gaditanus*, for which there are only butterfly records. *Narcissus gaditanus* had a lower representation of both indole and esters than the other species with a Lepidoptera odor. *Narcissus jonquilla*, which also fits into the Lepidoptera odor category, has been predicted but not observed to be pollinated by Lepidoptera (Knuth, 1898). All these species are visited by other insects, especially bees, in addition to Lepidoptera.

Second, all species visited mainly by Hymenoptera (bees) and some Diptera (flies) to the exclusion of Lepidoptera, namely *N. bugei*, *N. bulbocodium*, and *N. triandrus*, had fragrances that lacked the "Lepidoptera odor". The first species belonged to fragrance group 3, where it stood out in having diverse benzenoid ethers, while the other two species belonged to group 2, which typically showed fragrances having an abundance of various terpenoids.

Discussion

Narcissus fragrances are characterized by comprising volatiles that fall mainly into two chemical classes, monoterpene isoprenoids and benzenoids (*N. cuatrecasasii* being an exception), and the genus can be subdivided into major fragrance groups based on the predominant volatiles in each species. The presence of species groups within a general genus-wide fragrance framework has also been reported in other genera (e.g. Thien *et al.*, 1975; Williams and Whitten, 1983; Pellmyr *et al.*, 1987; Patt *et al.*, 1988; Borg-Karlson, 1990; Dahl *et al.*, 1990; Loughrin *et al.*, 1990; Knudsen and Tollsten, 1991; Tollsten and Bergström, 1993; Toyoda *et al.*, 1993; Knudsen and Ståhl, 1994). In *Nar-*

TABLE 5. SUMMARY OF FLOWER FRAGRANCE CHEMISTRY (% COMPOSITION) OF ALL *Narcissus* SPECIES IN RELATION TO AVAILABLE POLLINATOR DATA, WITH COMPOUNDS TYPICAL OF THE "LEPIDOPTERA ODOR" MIXTURE INDICATED IN BOLD ITALICS

COMPOUND	<i>assoanus</i> n = 5	<i>bugei</i> n = 3	<i>bulbocodium</i> n = 3	<i>cutreacasasi</i> n = 1	<i>gaditanus</i> n = 3	<i>jonquilla</i> n = 2	<i>papyraceus</i> n = 5	<i>serotinus</i> n = 2	<i>triandrus</i> n = 3
FATTY ACID DERIVATIVES (<i>esters</i>)		0.2		64.2 45.5	8.3	0.1	0.9	15.9	
MISC. ISOPENTENOIDS	1.6	0.2	2.7			3.6	0.1	0.3	
ISOPRENOIDS: MONOTERPENES (<i>trans-β-ocimene</i>)	70.4	74.3	76.7	35.8	75.8	43.4	75.0	65.9	99.3
(<i>linalool</i>)	64.4	59.3			67.9	25.6	69.6	27.1	
(<i>esters</i>)	1.7	0.6			3.9	15.0	0.1	8.3	9.8
ISOPRENOIDS: SESQUITERPENES	<0.1	1.3	11.9			0.1			
BENZENOIDS (<i>esters</i>)	27.3	23.2	8.7		5.5	52.1	21.4	17.2	0.6
	23.6				1.9	52.0	16.8	11.7	
NITROGEN COMPOUNDS (<i>indole</i>)	0.2				<i>tr</i>	0.2	0.2	0.1	
OTHER UNIDENTIFIED	0.5	1.1				0.7	2.3	0.5	
Total number of compounds	23	27	8	6	13	31	26	27	12
POLLINATORS ¹	L,H	H,D,C	H	?	L,H	(L) ² ,H	L,H,D,C	L,D	H
Descriptive fragrance group	3	3	2	1	3	3	3	3	2

¹ L = Lepidoptera (moths and butterflies), H = Hymenoptera (bees), D = Diptera (flies), C = Coleoptera (beetles).

² L predicted, but not yet observed (see Table 4).

cissus, the species diverge in terms of both the particular volatiles they contain within each chemical class and the occurrence of certain of the major biosynthetic pathways recognized here. While the fragrance chemistry shows some patterns that follow recognized phenetically-based taxonomic groupings, the data suggest that other selective pressures are involved in shaping floral fragrance, particularly pollination ecology.

Our chemical data both confirm and supplement earlier fragrance studies of *Narcissus*, which were restricted mainly to cultivars of *N. tazetta*, *N. poeticus*, *N. jonquilla*, and *N. pseudonarcissus* (Loo and Richard, 1988; van Dort *et al.*, 1993). Many of the volatiles we found have been reported previously in *Narcissus* flower odors, especially those analysed using headspace as opposed to extracting methods (Joulain, 1986; Mookherjee *et al.*, 1989; Surburg *et al.*, 1993). Furthermore, for the two species in which previous headspace odor analyses have been conducted, our results are similar to the published data: in *N. jonquilla*, Joulain (1993) found the same major compounds, including a dominance of *trans*- β -ocimene and methyl benzoate, and in *N. pseudonarcissus*, of which *N. bugei* is a microspecies (Blanchard, 1990), Surburg *et al.* (1993) report a fragrance that resembled that of *N. bugei*, and contained *trans*- α -farnesene and various benzenoid ethers. Over half of our species had a fragrance characterized by a high representation of *trans*- β -ocimene, which has been pointed out as being frequently predominant in *Narcissus* fragrances (Loo and Richard, 1988). However, the results of our survey, which emphasizes wild-growing species, indicate that the genus *Narcissus* is more chemically diverse than suggested by the previous studies, which focused on plants of horticultural and perfume-related importance. While van Dort *et al.* (1993) list several volatiles as being common to most *Narcissus* varieties in their review of *Narcissus* fragrance chemistry, limonene is the only volatile that occurred in all of our species, pointing to greater divergence among species than earlier reported. Our analyses also uncovered some volatiles that have hitherto not been described in *Narcissus* flowers. Among them are the major components of *N. cuatrecasasii*, namely two aliphatic acetates and β -ionone, which have been reported in the headspace of only a very few plant species (Knudsen *et al.*, 1993).

Fragrance chemistry and taxonomy of the genus Narcissus

The nine *Narcissus* species studied here fall into three main fragrance types, described by their major volatiles (1. fatty acid derivatives, 2. isoprenoids and benzenoids without *trans*- β -ocimene, and 3. isoprenoids and benzenoids with *trans*- β -ocimene). These groupings are supported by the cluster analyses performed on the volatiles, in which the compounds were treated either individually or grouped by biosynthetic pathways. Each *Narcissus* species tends to show a characteristic dominance of certain isoprenoid pathways, but most pathways are common across species, suggesting that fragrance differences might be due mainly, but not exclusively, to differences in the regulation of enzyme activity, as documented in *Clarkia* flowers (Pichersky *et al.*, 1994; Raguso and Pichersky, 1995), rather than to presence or absence of specific enzymes. However, some species have volatiles produced by pathways not found in other species (e.g. *N. triandrus*, *N. bulbocodium*, and *N. serotinus*), or show an absence of particular pathways, such as that for indole. *Narcissus cuatrecasasii* is exceptional in being dominated by fatty acid derived esters and in not having any volatiles in common with other species (except the ubiquitous limonene and the near-ubiquitous myrcene).

Cluster analyses of the volatiles in each *Narcissus* fragrance, treated individually or grouped by biosynthetic pathways, gave mixed support of currently delimited taxonomic groupings. Confirmation at the sectional level was suggestively stronger than at the subgeneric level, although each section except *Jonquillae* is represented by only a single species. In his extensive studies, Fernandes (1951, 1967, 1975) divides the genus on the basis of chromosomal differences and morphological, ecological, and geographical characteristics. The two subgenera of *Narcissus*, distinguished by their different chromosome base numbers (Fernandes, 1975), do not cluster apart by fragrance chemistry: the two species of subgenus *Hermione* are more similar to species of subgenus *Narcissus* than to each other. Furthermore, lilac volatiles, which we found exclusively in *N. serotinus* subgenus *Hermione*, have been reported also in the fragrance of *N. poeticus* subgenus *Narcissus* (Joulain, 1986), a taxon considered to be phenetically distantly related (Fernandes, 1951). The fragrance data do, however, concur with some of the phenetic taxonomic groupings recognized within the subgenus *Narcissus*. Thus, placement of *N. cuatrecasasii*, *N. triandrus*, and *N. bulbocodium* by Fernandes (1951, 1967) into three separate sections is supported by each having a comparatively low coefficient of similarity with other species, especially when all volatiles are treated individually; each also includes volatiles from biosynthetic pathways not found in the other species studied. Stronger support comes from the three species of section *Jonquillae*, which fall within the same fragrance cluster and show a high coefficient of similarity, especially in terms of biosynthetic pathways. The closer relationship suggested by Fernandes (1967, 1975) to exist between *N. gaditanus* and *N. assoanus* than either to *N. jonquilla* is also supported by cluster analyses using biosynthetic pathways. Within section *Tazettae*, our fragrances for *N. papyraceus* are similar to published ones for *N. tazetta* (Mookherjee *et al.*, 1989; Surburg *et al.*, 1993) in that both species have fragrances containing a high diversity of volatiles and biosynthetic pathways (particularly for benzenoids) that are, however, not species specific. Extension of these fragrance studies to include a greater number of species would allow us better to characterize fragrance patterns in *Narcissus*. The fragrance-based phenetic groupings could then be more precisely compared not only with recognized groups based on classical taxonomy, but also with phylogenetic classifications, which, although not yet available for *Narcissus*, are currently underway (S. Graham and S. C. H. Barrett, personal communication).

Intraspecific variation is low in most of the species studied. The reason behind the seemingly random intersample variation in *N. bulbocodium*, where the relative quantities of most volatiles varied widely, is unclear, but it could be related in part to the highly variable ploidy number in this species (Fernandes, 1967). Such high interplant fragrance variation within populations has been reported in other plant species as well (e.g. Tollsten *et al.*, 1994). In contrast to *N. bulbocodium*, the variation displayed among the samples of *N. assoanus* shows patterns, and appears to be associated with pollinators, as discussed below.

Fragrances and pollination ecology

Fragrance data available for *Narcissus* point to a probable close association between the types of pollinators of a species and the volatile profile of its flower fragrance. Furthermore, the pollination ecology of *Narcissus*, like its fragrance biochemistry, shows patterns that follow recognized taxonomic groupings. The records of pollinators on

Narcissus, however, are generally scarce, with the exception of a few species (Arroyo and Dafni, 1995; Herrera, 1995) including *N. papyraceus* from this study (J. Arroyo, unpublished). Contributing to this paucity of records is that most *Narcissus* species bloom in early spring, when pollinators are few and abiotic factors (especially temperature) may be unfavorable to insect activity (Herrera, 1995; Barrett *et al.*, 1996). In addition, most pollination studies have been made during daytime hours, leaving the possibility that evening pollinators would be missed. Flower morphology, especially of the floral tube, has been used widely to predict the primary pollinators (Knuth, 1898; Vogel and Müller-Doblies, 1975). Based on both observed and predicted flower visitors, Loew (in Knuth, 1898) described the genus as being typically pollinated by bumble bees (i.e. large bees) and Lepidoptera (moths and butterflies), with individual species relying on either or both insect groups to differing extents. Examination of observation-based pollination data currently available confirm these trends and also suggest that some taxonomic groups are associated with certain pollinator types. Section *Pseudonarcissus* tends towards pollination mainly by bees, e.g. *N. bugei* (data here), *N. longispathus* (Herrera, 1995), and the hybrid *N. odorus* (Knuth, 1898); section *Jonquillae* appears to be visited by moths and butterflies, as well as bees (data here for three species); and section *Tazettae* is visited by butterflies and moths, bees, and also flies, e.g. *N. tazetta* (Dulberger, 1967; Arroyo and Dafni, 1995), *N. papyraceus* (data here), and *N. polyanthus* (Knuth, 1898).

The *Narcissus* species analyzed in the present study display two types of fragrance that correlate with whether or not their pollinators include Lepidoptera, especially moths. Species visited by Lepidoptera have an odor characterized by the presence of both esters (terpenoid and benzenoid) and indole, accompanied by *trans*- β -ocimene and linalool, whereas species visited by other insects to the exclusion of Lepidoptera (e.g. bees and flies) are united by their lack of this volatile mixture. The fragrances with the "Lepidoptera odor" show close similarities with the general scent chemistry reported to be typical of moth-pollinated flowers, namely the presence of acyclic terpene alcohols and hydrocarbons, benzenoid alcohols and esters, and nitrogen-containing compounds (Kaiser, 1993a; Knudsen and Tollsten, 1993). They also contained some of the mono-terpenes reported to be most common in such fragrances, namely *trans*- β -ocimene, myrcene, limonene, linalool and its furanoid oxides, and lilac compounds. Furthermore, the range in percent representation of the dominant isoprenoids (32–91%) and benzenoids (5–63%) in the moth-pollinated *Silene* species examined by Knudsen and Tollsten (1993) was similar to that found in our *Narcissus* samples. These data as a whole point to an evolutionary convergence in plants to certain fragrance-pollinator combinations, which appear to be particularly well-defined in plants pollinated by moths.

Close examination of the *Narcissus* species suggests that their fragrances form a continuum between the Lepidoptera and non-Lepidoptera odor that corresponds with the relative predominance of moths among their flower visitors. Coincidentally, this concept is similar to that set forth by Loew (in Knuth, 1898). Using flower morphology and the predicted and observed flower visitors, he divided the genus into five species groups that differed in the relative importance of bee vs. Lepidoptera pollinators (i.e. bumble bee flowers, intermediate stage between bumble bee and lepidopterid flowers, two types of lepidopterid flowers, and bumble bee and lepidopterid flowers). Our species with moth Lepidoptera visitors, namely *N. serotinus*, *N. papyraceus*, *N. jonquilla*, and *N.*

assoanus, fit the moth-pollination fragrance syndrome most closely (however, moths are not the exclusive insect visitors of these species, and their effectiveness in pollination as compared to other visitors remains to be determined). *Narcissus gaditanus*, although it has a Lepidoptera odor, occupies an intermediate position between the two pollinator-fragrance types, with its fragrance containing indole and abundant acyclic terpenes (e.g. *trans*- β -ocimene, linalool) but only few esters compared to the most-visited species. This may reflect its intermediate position with respect to insect visitors, since butterflies and bees but no moths have been recorded on the flowers to date.

The species visited by non-Lepidoptera, and mainly bees, are fewer and more heterogeneous in their fragrances. *Narcissus triandrus* and *N. bulbocodium* have fragrances characterized by their diversity of terpenoids, whereas *N. bugei*, shows intermediacy. Its fragrance shares many volatiles with the Lepidoptera-visited group, but differs in both the lack of indole and esters and the presence of a variety of benzenoid ethers. This may underlie the more diverse insect fauna recorded on flowers of *N. bugei* when compared to other non-Lepidoptera visited species. Flower visitor data are lacking for one species, *N. cuatrecasasi*, which coincidentally has a fragrance that is distinctly different from any other *Narcissus* species. Although its flower volatiles suggest non-Lepidopteran pollination, its position in the continuum is problematical. This species is both sympatric and morphologically similar to *N. assoanus*, and the strong contrast in fragrance between the two species may help maintain their reproductive isolation by minimizing pollinator sharing. More data on observed pollinators, taken over more hours of the day, for all *Narcissus* species would provide a stronger basis for establishing whether pollinators are influencing the fragrance chemistry patterns revealed here in *Narcissus* and would help clarify whether the suggested relationship between fragrance chemistry and relative importance of bee and Lepidoptera pollinators is justified.

Both Lepidoptera and non-Lepidoptera odors occur intraspecifically in *N. assoanus*, which suggests that individual plants differ in their relative dependence on Lepidopteran and bee pollination. The non-Lepidoptera odors (having no detectable indole) show varying degrees of similarity to the Lepidoptera odor as measured in their content of linalool and esters. This variation produces a checkerboard effect, showing no correlation with subspecific taxonomic boundaries or geographical areas. Fragrance chemotypes have been documented in several plant species, both within and between populations, where they have been shown to correlate clearly (Galen and Kevan, 1980; Pellmyr, 1986; Groth *et al.*, 1987) or only suggestively (Tollsten and Bergström, 1993; Tollsten and Øvstedal, 1994) with different pollinators. In *N. assoanus* the relationship between fragrance chemistry and pollinators is not clear, and more information on the pollination, as well as a larger number of fragrance samples (Harborne and Turner, 1984), are needed to clarify the meaning of these intraspecific variations.

Pollinator and floral biology data (see Barrett *et al.*, 1996) suggest that fragrance polymorphism might occur in other *Narcissus* species. Among the species we studied here, it is possible that fragrance chemotypes might have been missed due to our small number of samples and general restriction to single populations. Evidence pointing to the likelihood of fragrance chemotypes, however, is strongest in *N. tazetta*. This species has two distinct and geographically isolated "pollination ecotypes" (Dulberger, 1967; Arroyo and Dafni, 1995), where one ecotype is visited principally by hawk moths and the other by solitary bees and syrphid flies. These pollinator differences appear to be also associated with different expressions of style polymorphism, which could add another

dimension to the pollination-fragrance interaction if populations having one style condition differ in fragrance from populations having other style conditions. The general trend from monomorphic species with bowl-shaped or broadly tubular flowers to polymorphic species with well developed tubes suggests that stylar polymorphism evolved with a change from a relatively unspecialized to a more precise pollination (Barrett *et al.*, 1996). Thus, the evolution of stylar polymorphism could in turn be associated with selection for a more pollinator-specific floral chemistry.

Concluding Remarks

Our findings in *Narcissus* confirm that comparative studies of fragrance chemistry can provide insight into both the taxonomy and pollination biology of plants. Furthermore, grouping the fragrance compounds by biosynthetic pathways revealed more distinct differences among species than when the compounds were listed according to general chemical classes. For clarifying evolutionary relationships among taxa, we suggest that fragrances be described in terms of biosynthetic pathways whenever possible, since interspecific fragrance comparisons based on the expression of biosynthetic pathways are more informative than those based only on the identity of individual volatiles. The tentative patterns we observed between fragrance chemistry and pollinator-plant associations confirm earlier studies proposing the existence of fragrance syndromes that correlate with certain pollination syndromes (e.g. Knudsen and Tollsten, 1993) and point to the potential value of fragrances in predicting a plant's pollinators. However, to more firmly understand the evolutionary significance of flower fragrance chemistry, it is essential to place the chemical data of each species into a functional ecological context by conducting fragrance analyses in parallel with plant reproductive studies. Future field studies of *Narcissus* aimed at increasing our pollination data pool will establish the reliability of the fragrance-pollinator relationships that are suggested by our findings presented here, and will provide more clarification into the role of pollinators in driving the evolution of flower fragrances.

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