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Interspecific variation of scent characteristics in the *Cyclamen* genus and the utility of the variation

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Abstract

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All the currently available interspecific scented cyclamen were bred by crossing *Cyclamen persicum* with only a wild species, *C.purpurascens.* To develop cyclamen with a wider variety of fragrances, we clarified the diversity of volatile compounds emitted from the flowers of 17 wild cyclamen species. We found that 14 of the wild species emitted fragrant compounds. In particular, *C. pseudibericum*, *C. cyprium*, *C. libanoticum*, *C. purpurascens*, *C. cilicium* and *C. alpinum* emitted floral compounds, and *C. mirabile* emitted fruity compounds. We produced interspecific hybrids between two *C. persicum* cultivars and *C. purpurascens* (which emitted the greatest number of volatile compounds) and analysed the scent characteristics of the resulting hybrids. We found that the hybrids varied in scent characteristics, even when the same parents were crossed; for example, we obtained hybrids with various proportions of citronellol, nerol and geraniol and various ratios of floral-scented and fruity-scented compounds.

Keywords: cyclamen; odour; wild species; interspecific hybrids; volatile compound

Colour, shape and scent are the main breeding objectives for flowers. Cyclamen with blue flowers (Murayama et al. 2011) and yellow flowers (Takamura et al. 1993) have already been bred, in addition to varieties with red, white, pink and purple flowers. The flowers of the cultivated varieties are larger than those of the wild species, and varieties with double flowers, have recently been developed by means of mutation or transgenic technology (Tanaka et al. 2013). Among the 23 species of the genus *Cyclamen*, only *C. persicum* has been subject to intensive breeding and selection, and this species is the source of all the currently available cyclamen cultivars (Mathew, Clennett 2013). Although wild *C. persicum* are scented, not many cultivated cyclamen varieties are scented, and the development of such varieties would be highly desirable. Flower breeders have attempted to regain lost fragrance by crossing cultivated species with scented wild species. For example, Kishimoto et al. (2013) produced interspecific hybrids between carnations and wild *Dianthus* species and compared the volatiles of the hybrids with those of the parents. These investigators reported that both the variation and amounts of fragrant compounds tended to be greater in the hybrids than in the parental carnations. Ishizaka and Uematsu (1995) have bred scented interspecific cyclamen hybrids, but in this case, only the wild species *C. purpurascens* was used as the breeding parent, and therefore only a few varieties were produced. Several wild species in *Cyclamen* are known to have fragrance (Clery et al. 2013). It may be possible to introduce new fragrances into cyclamen by using

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different wild species as breeding materials. Clery et al. (2013) analysed the volatile compounds in six wild species (*C. purpurascens*, *C. hederifolium*, *C. mirabile*, *C. rohlfsianum*, *C. cilicium* and *C. intaminatum*) and evaluated the fragrant characteristics of these species. Producing a wide range of fragrant interspecific hybrids will require evaluation and effective use of the fragrant characteristics of a greater number of wild species. In this study, we analysed the volatile compounds in 17 wild species. Furthermore, we produced interspecific hybrids between two cyclamen cultivars and the wild species *C. purpurascens* (which contained the largest number of volatile compounds), and we evaluated how the scent characteristics of the hybrids varied depending on the combination of parents.

MATERIAL AND METHODS

Analysis of floral volatiles of wild cyclamen species. We obtained bulbs of 17 wild cyclamen species from Tile Barn Nursery (Benenden, Kent, UK) and investigated the fragrance of the flowers. The flowers of *C. repandum*, *C. creticum*, *C. alpinum*, *C. cilicium*, *C. libanoticum*, *C. cyprium*, *C. pseudibericum*, *C. purpurascens* and *C. persicum* were strongly scented; whereas the flowers of *C. mirabile*, *C. hederifolium*, *C. africanum*, *C. graecum*, *C. rohlfsianum*, *C. coum*, *C. parviflorum* and *C. balearicum* were weakly scented.

To collect the volatiles from the flowers of each species, we covered the corollas of a few potted plants with a Tedlar® bag (GL Sciences, Tokyo, Japan), and the upper part of the bag was equipped with a glass column containing Tenax® TA (400 mg, GL Sciences, Tokyo, Japan) as the volatile trap. A constant stream of air was pumped through the bag for 24 h at a flow rate of 1,000 ml/min.

The volatiles adsorbed on the column were either removed by solvent extraction (for strongly scented species) or thermally desorbed by means of a dynamic headspace method (for weakly scented species), as proposed by Nohara et al. (1991). Specifically, the volatile compounds from *C. repandum*, *C. creticum*, *C. cilicium*, *C. alpinum*, *C. libanoticum*, *C. cyprium*, *C. pseudibericum*, *C. purpurascens* and *C. persicum* were removed by solvent extraction, whereas the volatile compounds from *C. mirabile*, *C. hederifolium*, *C. africanum*, *C. graecum*, *C. rohlfsianum*, *C. coum*, *C. parviflorum* and *C. balearicum* were removed by thermal desorption. For solvent extraction, the volatiles were removed from the column with 3 ml of diethyl ether, and the ether was evaporated by means of a 45°C bath. For thermal desorption, the glass column (GC) connected with a transfer line to a GC held at 250°C for 3 min under helium gas stream. Desorbed volatile compounds were introduced into a GC inlet through the transfer line on a splitless mode. They were focused on a part of GC capillary column in GC oven at −80°C cooled by a dry ice-acetone bath for 6 min, then they were released according to GC oven temperature.

Samples were analysed by means of gas chromatography–mass spectrometry (GC-MS) (GC, HP-5790; MS, Hitachi M-80B). Ionization was performed in electron-impact mode at 20 V at an ion source temperature of 200°C. We used a BC-WAX column (0.25 mm id. × 50 m× 0.15 µm, GL Sciences; temperature program, 70–220°C at 4°C/min). We also carried out GC on an instrument equipped with a flame-ionization detector (GC, HP-5890 Series II; column, Hewlett Packard HP-20M, 20 m × 0.20 mm × 0.10 µm; temperature program, 55–215°C at 4°C/min).

We also classified the volatile compounds on the basis of odour type according to the database of Takasago International Corporation Corporate Research and Development Division and evaluated scent characteristics of interspecific hybrids generated from two cyclamen cultivars ('Akebono' and 'Schubert') and *C. purpurascens*.

Production of cyclamen hybrids from two *C. persicum* cultivars and *C. purpurascens* and analysis of the floral volatiles of the hybrids. The floral volatiles emitted by 18 cyclamen cultivars and eight *C. purpurascens* were analysed by solvent extraction, and intraspecific variation of the volatile compounds in each individual was confirmed. As 18 cyclamen cultivars, we used two 'Akebono', two 'Beethoven', two 'Bryan pink', one 'Candy mix', one 'Honoka', one 'Lips orange', two 'Lips pink', one 'Pavilion pretty', four 'Schubert', one 'Shining red' and one 'Victoria', and as eight *C. purpurascens*, used '39-3', '39-5', '39-8', '40-1', '40-2', '40-3', '40-7' and '40-8'. Cyclamen cultivars were emasculated about two days before anthesis. Crossings of cyclamen cultivars and *C. purpurascens* were performed three days after flowering of cyclamen cultivars. Ovules with placenta were isolated from the ovary 28 days after pollination and used as explants for

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ovule culture. Initially these explants were cultured on MS media supplemented with 60 g/l sucrose, adjusted to pH 5.8. Cultures were maintained at 20°C in the dark. Plantlets obtained by the culture were transplanted to flasks with MS medium containing 30g/l sucrose and flasks were placed in a incubator at 20°C with 16hr light period. Among these cultivars, we used 'Akebono' and 'Schubert' because they were more popular cultivars, and we used '40-2' and '40-3' as crossing parents because they could produce relatively a lot of hybrids.

An individual plant of the cyclamen diploid cultivar 'Akebono' ($2n = 48$) was pollinated with pollen from an individual *C. purpurascens* '40-3' plant ($2n = 34$), the hybrid ovules obtained were cultured, and six interspecific hybrids ($2n = 41$) were produced. Likewise, a cyclamen diploid cultivar 'Schubert' ($2n = 48$) individual was pollinated with pollen from a *C. purpurascens* '40-2' individual, and five interspecific hybrids were produced by ovule culture. The volatile compounds from the individuals were obtained by the solvent extraction method and analysed by means of GC-MS.

RESULTS AND DISCUSSION

Volatile compounds in the wild cyclamen species

There are more than 1,000 identified fragrant compounds in flowers, and most of the compounds are biosynthesized by one of three pathways (Muhlemann et al. 2014). On the basis of these pathways, we classified the compounds analysed in this study as terpenoids, benzenoids or fatty acid derivatives. Table 1 shows the percentages of each of the volatile compounds obtained from wild cyclamen species by means of the solvent extraction method. The only compound that was found in all of the wild species was geranylacetone. *C. repandum* had a fresh floral green note and contained 35 compounds, with geranylacetone as the major compound (33.55%). *C. creticum* had a muguet-like floral green, earthy note and contained 20 compounds; heptadecan-2-one (8.01%) and β-bisabolene (7.35%) predominated. *C. cilicium* had a hyacinth-like aldehydic note and contained 20 compounds, the major compound being phenylacetaldehyde (38.02%). *C. alpinum* had a hyacinth-like aldehydic note and contained 32 compounds; the main components were phenylacetaldehyde (32.12%) and 2-acetyl-*N*-methylanilline (31.33%). *C. libanoticum* had a geranylacetone note and contained 17 compounds, the major one being geranylacetone (56.36%). *C. cyprium* had a lemon muguet note and contained 29 compounds; the major compounds were geranylacetone (42.0%) and geraniol (31.12%). *C. pseudibericum* had a lemon muguet note and contained 21 compounds; the major compounds were geranylacetone (42.81%), geraniol (22.83%) and nerol (12.86%). *C. purpurascens* had a floral fruity note and contained 62 compounds, the highest number among all the wild species evaluated in this study. The major compounds were citronellol (39.34%) and (*E*)-2,3-dihydrofarnesol (11.7%). Other components were detected, such as geraniol (4.6%), linalool (1.58%) and nerol (3.71%) (which were the sources of a floral note) and methyl citronellate (0.96%), (*E*)-2,3-dihydrofarnesal (0.28%) and (*Z*)-2,3-dihydrofarnesal (1.57%). These latter three compounds have a strong floral scent, even in extremely low quantities, because of their high olfactive capacity. *C. persicum* had a rose-like floral note and contained 24 compounds; the major compounds were nerol (29.30%) and geraniol (29.26%), as well as (*E*)-2,3-dihydrofarnesol (2.29%).

Table 2 shows the percentages of volatile compounds obtained from wild cyclamen by means of the thermal desorption method. *C. mirabile* had a fruity-coconutty note and contained 30 compounds, with butyl acetate (4.02%) and calamenene (4.0%) predominating. *C. mirabile* contained a lactone (octan-1,4-olide, 1.83%) that brings fruity-coconutty odour. *C. hederifolium* had a green aldehydic note and contained 27 compounds; the major compounds were geranylacetone (7.21%) and anisole (6.84%). *C. africanum* had a modern rose note and contained 31 compounds; the major compounds were anisole (12.40%) and 6-methyl-5-heptan-2-one (8.20%). (*E*)-linalool-3,6-oxide (2.37%), citronellol (3.92%), calamenene (3.20%) and geraniol (1.53%) were also detected. *C. graecum* had an orange-like aldehydic note and contained 22 compounds, and the major component being undecan-2-one (5.80%). *C. rohlfsianum* had an aldehydic note and contained 23 compounds; the major compounds were decane (5.78%), butyl acetate (5.26%) and nonanal (4.03%). The fragrances of *C. coum*, *C. parviflorum* and *C. balearicum* were very weak. None of the volatile compounds in *C. coum*, *C. parviflorum* and *C. balearicum* were present in large enough amounts to be analysed, so

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Table 1. Volatile compounds from flowers of wild cyclamen species, obtained by solvent extraction

Compounds	Content (%)[a]								
	C. rep	*C. cre*	*C. cil*	*C. alp*	*C. lib*	*C. cyp*	*C. pse*	*C. pur*	*C. per*
Terpenoids									
Monoterpenes									
Esters									
Linalyl acetate								0.16	
Methyl citronellate								0.96	
Methyl geranate								0.21	
Aldehydes									
Citronellal	0.35							3.40	
Geranial	0.40				0.33	1.50	1.54	2.00	3.57
Lilac aldehyde				0.34					
Neral	0.30					0.15	0.68	1.85	2.58
Alcohols									
Citronellol	1.41							39.34	4.58
Geraniol	0.04					31.12	22.83	4.60	29.26
Linalool			0.06	1.66		1.00	0.03	1.58	0.54
Menthol		0.22							
Nerol	0.48					0.18	12.86	3.71	29.30
α-Terpineol	0.43								1.31
Ethers									
1,8-Cineole	0.34								
(*E*)-Linalool-3,6-oxide				0.37			0.07		0.03
(*Z*)-Linalool-3,6-oxide				0.12					
(*E*)-Linalool-3,7-oxide				0.14					
(*Z*)-Linalool-3,7-oxide				0.11					
Hydrocarbons									
p-Cymene		0.86	0.27	0.55	0.51	0.25			0.13
Limonene	0.67	1.70	0.21			0.07		0.05	0.13
Myrcene	2.77					0.01		0.11	
Sabinene	0.58								
Sesquiterpenes									
Esters									
(*E*,*E*)-Farnesyl acetate									0.06
Ketones									
Geranylacetone	33.55	0.81	0.38	0.13	56.36	42.00	42.81	0.63	0.35
Nerylacetone						0.45	0.31		
Aldehydes									
(*E*)-2,3-dihydrofarnesal								0.28	
(*Z*)-2,3-dihydrofarnesal								1.57	
(*Z*,*E*)-farnesal								0.01	
(*Z*,*Z*)-farnesal								0.01	
(*E*,*Z*)-farnesal						0.04		2.37	
(*E*,*E*)-farnesal						0.33		2.40	

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Table 1. to be continued

Compounds	Content (%)[a]								
	C. rep	*C. cre*	*C. cil*	*C. alp*	*C. lib*	*C. cyp*	*C. pse*	*C. pur*	*C. per*
Alcohols									
(*E*)-2,3-dihydrofarnesol	1.60							11.70	2.29
Elemol							2.16	0.97	
β-Eudesmol								0.73	
γ-10-epi-Eudesmol								0.67	
α-Eudesmol								0.01	
(*E*,*Z*)-farnesol						0.68		0.94	0.13
(*E*,*E*)-farnesol						5.05		1.90	1.88
(*Z*,*Z*)-farnesol								0.01	
(*Z*,*E*)-farnesol								0.01	
Nerolidol	0.84	3.00			5.34	0.46		0.11	
Ethers									
Caryophyllene oxide	0.20								

[a]content (%) of the individual compounds were calculated based on the peak area of the gas chromatogram; *C. rep* – *C. repandum*; *C. cre* – *C. creticum*; *C. cil* – *C. cilicium*; *C. alp* – *C. alpinum*; *C. lib* – *C. libanoticum*; *C. cyp* – *C. cyprium*; *C. pse* – *C. pseudibericum*; *C. pur* – *C. purpurascens*; *C. per* – *C. persicum*

data for these compounds are not included in Table 2. In all three of these species, the major compounds were hydrocarbons. Small amounts of butyl acetate, benzaldehyde, undecan-2-one, acetophenone and geranylacetone were detected in *C. coum*. Small amounts of butyl acetate, isoamyl acetate, benzaldehyde, linalool, acetophenone and benzyl acetate were detected in *C. parviflorum*. Small amounts of butyl acetate, benzaldehyde, acetophenone, citronellol, geranylacetone and (*E*)-2,3-dihydrofarnesol were detected in *C. balearicum*.

There have been a number of studies of the diversity of fragrant compounds in species related to each other. Most of these studies dealt with species divergence or with the relationships between plants and pollinators (Dobson et al. 1997; Knudsen et al. 2001; Jurgens et al. 2003, 2010; Feulner et al. 2009, 2011; Nowak 2005), and in some studies, the results of analysis of volatile components were used to identify promising breeding materials (Takatsu et al. 2002; Kishimoto et al. 2011; Sun et al. 2015). *C. purpurascens*, which is used as a parent for breeding scented cyclamen, is useful because it contains a large variety of fragrant compounds (Ishizaka et al. 2002). In this study, we also investigated some other wild cyclamen species that contain fragrant compounds, with the aim of identifying breeding materials that can be used to develop cultivars with new fragrances because of their diversity of scent characteristics. Specifically, because geranylacetone was the main compound in *C. repandum*, *C. cyprium*, *C. pseudibericum* and *C. libanoticum*, we expect that these species could be used to introduce a geranium-like scent. Because *C. cyprium* and *C. pseudibericum* contained a lot of geraniol, they could be used to introduce a rose-like scent. Phenylacetaldehyde was the main compound in *C. cilicium* and *C. alpinum*, so they could be used to introduce a hyacinth-like scent. *C. alpinum* contained 2-acetyl-*N*-methlaniline, and also could be used to introduce a lilac-like scent. These compounds were floral odour type. *C. mirabile,* which had a relatively large amount of lactone, as well as butyl acetate, could be used to introduce a fruity scent. Because floral fragrances and fruity fragrances are the most important for introducing pleasing scents into flowers, *C. pseudibericum*, *C. cyprium*, *C. libanoticum*, *C. purpurascens*, *C. cilicium* and *C. alpinum* can be expected to be useful breeding parents because they contain a lot of floral compounds. Furthermore, *C. mirabile* is also potentially useful because they contain a lot of fruity compounds. In addition, small amounts of waxy compounds sometimes give flowers a unique fragrance. The waxy-scented compound anisole was a major compound of *C. africanum* and *C. hederi-*

https://doi: 10.17221/111/2017-HORTSCI

Table 2. Volatile compounds from wild cyclamen species, obtained by thermal desorption

Compounds	Content(%)[a]				
	C. mir	*C. hed*	*C. afr*	*C. gra*	*C. roh*
Terpenoids					
Monoterpenes					
Alcohols					
Citronellol		3.00	3.92		
Geraniol		0.68	1.53		
Linalool			0.73		
Nerol		0.65			
Ethers					
(*E*)-Linalool-3,6-oxide			2.37		
(Z)-Linalool-3,6-oxide			0.31		
Rose oxide			1.14		
Hydrocarbons					
Camphene	0.20				
p-Cymene	1.48		2.12	1.74	2.46
Limonene	1.12	2.40	0.96	0.70	2.94
p-Mentha-1,3,8-triene			0.29		
Myrcene		0.98	0.01		
Sesquiterpenes					
Ketones					
Geranylacetone	0.55	7.21	0.76		0.53
Nerylacetone		0.87			
Hydrocarbons					
Calamenene	4.00	1.07	3.20	4.05	2.75
β-Elemene		2.35			
(*E*)-β-Farnesene		0.83			
Total of Terpenoids	7.35	20.04	17.34	6.49	8.68
Benzenoids					
Ketones					
Acetophenone	2.59	0.89	1.51	1.24	1.71
Aldehydes					
p-Anisaldehyde	0.48				
Benzaldehyde	1.63	0.65	1.36	0.66	1.06
Alcohols					
Phenoxyethanol	1.25				
Other					
Anisole	0.83	6.84	12.40		2.66
1,2-Dimethoxybenzene			0.55		
1,4-Dimethoxybenzene			1.93		
Total of Benzenoids	6.78	8.38	17.75	1.90	5.43
Fatty acid derivatives					
Esters					
Butyl acetate	4.02	1.40	0.52	1.33	5.26

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Table 2. to be continued

Compounds	Content(%)[a]				
	C. mir	*C. hed*	*C. afr*	*C. gra*	*C. roh*
Isoamyl acetate		0.45			
Isobutyl acetate			1.15		
(*Z*)-3-Hexenyl acetate		1.35	0.86		
Ketones					
2,3-Butanedione			0.58		
3-Heptanone	0.22	0.09			0.86
6-Methyl-5-hepten-2-one	0.92		8.20	1.40	1.30
Undecan-2-one				5.80	1.62
Aldehydes					
Decanal	0.68	0.50	0.79	0.31	1.44
Dodecanal	1.10				
Furfural	0.70				0.58
Hexanal	0.60		0.34	0.50	
Isopentanal	2.00			1.00	
3-Methyl-2-butenal			0.02	0.12	
Nonanal		2.52	0.90	0.67	4.03
Octanal	0.44		0.49	0.50	
Undecanal				1.07	
Alcohols					
Heptanol		0.01			
Octanol	0.70				
Acids					
Acetic acid			0.50		0.57
2-Ethylhexanoic acid					1.07
Lactones					
Octan-1,4-olide	1.83				
Hydrocarbons					
Decane	3.00	5.30			5.78
Dodecane	1.95	1.90		1.03	
Hexadecane	1.13	0.58			
Nonane	0.80	2.40	1.28	1.52	1.39
Octane	1.20		0.48	1.50	3.51
Pentadecane	1.32			0.77	0.55
Tetradecane	1.31	1.77		1.50	1.82
Tridecane	1.13			0.89	4.30
Undecane	1.78	2.90	1.48	1.30	1.33
Total of fatty acids	26.83	21.17	17.59	21.21	35.41
Total of compounds	40.96	49.59	52.68	29.60	49.52

[a] content (%) of the individual compounds were calculated based on the peak area of the gas chromatogram; *C. mir – C. mirabile; C. hed – C. hederifolium; C. afr – C. africanum; C. gra – C. graecum; C. roh – C. rohlfsianum*

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Table 3. Percentage of individuals containing volatile compounds and relative amounts of volatile compounds in *C. purpurascens* and cyclamen cultivars

Compounds	*C. purpurascens* (*n* = 8)		Cultivar (*n* = 18)	
	individual rate (%)	content (%)[a]	individual rate (%)	content (%)[a]
Floral odour compounds				
Citronellal	100	1.79 ± 0.37	5.6	0.04
Citronellol	100	27.94 ± 3.96	11.1	4.66 ± 0.03
Geranial	100	2.03 ± 0.40	16.7	1.95 ± 0.79
Geraniol	100	3.81 ± 0.93	16.7	19.69 ± 9.65
Linalool	100	18.35 ± 16.95	77.8	1.54 ± 1.27
Neral	87.5	1.82 ± 0.53	16.7	1.85 ± 0.44
Nerol	100	3.72 ± 1.05	22.2	20.08 ± 11.61
Methyl citronellate	100	1.28 ± 0.18		
(*E*,*Z*)-farnesal	100	2.27 ± 0.66	11.1	0.07 ± 0.01
(*E*,*E*)-farnesal	100	2.49 ± 0.63	11.1	0.1
(*E*,*Z*)-farnesol	100	1.83 ± 0.28	11.1	0.16 ± 0.06
(*E*,*E*)-farnesol	100	4.22 ± 1.11	11.1	2.06 ± 0.06
(*E*)-2,3-dihydrofarnesal	100	2.03 ± 0.26	5.6	0.1
(*E*)-2,3-dihydrofarnesol	100	9.12 ± 1.62	11.1	2.50 ± 0.29
Nerolidol	62.5	0.27 ± 0.06	5.6	0.0
Benzyl banzoate	100	1.36 ± 0.50		
Benzyl acetate			11.1	0.28 ± 0.13
Methyl *p*-anisate	62.5	0.20 ± 0.07		
Benzyl alcohol	50	0.15 ± 0.08		
Geranylacetone	100	1.09 ± 0.14	66.7	0.43 ± 0.10
Fruity odour compounds				
Methyl benzoate	62.5	0.23 ± 0.07		
Benzaldehyde	50	0.23 ± 0.06	44.4	0.42 ± 0.16
Decan-1,4-olide	25	0.36 ± 0.02		
Butyl acetate	12.5	0.1	11.1	0.46 ± 0.44
Methyl caprate	100	1.45 ± 0.25		
Methyl caprylate	62.5	0.30 ± 0.08		
Waxy odour compounds				
α-Terpineol			72.2	1.25 ± 0.35
Acetophenone			27.8	0.78 ± 0.50
6-Methyl-5-hepten-2-one	100	0.95 ± 0.18	55.6	1.13 ± 0.23
Undecan-2-one			100	4.71 ± 0.84

[a]content(average%±SE) of the individual compounds were calculated based on the peak area of the gas chromatogram

forium, so these species are promising as breeding materials for new cultivars with unique fragrances.

C. persicum, which is the breeding parent of all the currently available cyclamen cultivars, and *C. purpurascens*, which is the parent we used in this study to generate scented interspecific hybrids. By using wild species, it should be possible to produce hybrids with different types of scents from the existing hybrids produced with *C. purpurascens*. The results of our study clearly indicate that wild cyclamen species have a diversity of scents and are thus valuable as breeding materials.

Scent characteristics of interspecific hybrids generated from two cyclamen cultivars ('Akebono' and

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Table 4. Volatile compounds of interspecific hybrids of two cyclamen cultivars and *C. purpurascens*

Compounds	Content (%)[a]							
	seed parent	pollen parent	hybrids					
	cultivar 'Akebono'	*C. purpurascens* '40–3'	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
Floral odour compounds								
Citronellal		0.93	0.32	0.25	0.24			
Citronellol		12.04	17.71	18.09	11.53	6.64	9.12	6.74
Geranial		2.96	1.42	1.62	1.20	1.53	1.40	1.56
Geraniol	0.44	8.37	10.34	5.62	13.90	4.20	7.02	9.70
Linalool	0.06	1.37	0.98	2.64	0.89	0.58	0.89	1.44
Neral	0.97	2.12	1.05	1.93	0.90	0.89	0.78	0.78
Nerol	0.04	4.49	7.03	23.21	12.28	2.17	4.70	5.82
Methyl citronellate		1.41	0.57	0.40	0.28			
(*E*,*Z*)-farnesal		1.10				0.92	1.32	1.01
(*E*,*E*)-farnesal		1.64		0.36	0.20		0.54	
(*E*,*Z*)-farnesol		2.24	1.26	0.85	1.54	0.38	0.50	0.67
(*E*,*E*)-farnesol		8.81	6.18	2.58	6.00	1.72	2.02	2.78
(*E*)-2,3-dihydrofarnesal		0.58	0.51	0.84	0.52			0.65
(*E*)-2,3-dihydrofarnesol		2.26	7.66	15.37	5.54	2.09	2.79	2.99
Nerolidol		0.15		0.37				1.27
Benzyl banzoate		3.95		0.14	0.23		0.18	0.21
Benzyl acetate						0.92	0.36	0.29
Methyl *p*-anisate		0.35						
Benzyl alcohol		0.10	0.22	0.36	0.30			
Geranylacetone	1.19	0.90	0.91	0.43	0.50	0.59	0.97	0.83
F**ruity odour compounds**								
Methyl benzoate		0.50						
Benzaldehyde		0.42	0.24	0.23	0.14	0.71	0.59	0.40
Decan-1,4-olide		0.38	0.11		0.07			0.28
Butyl acetate		0.05	0.11	0.05	0.07	0.92	0.36	0.29
Methyl caprate		2.60		0.11			0.36	
Methyl caprylate		0.35						
Waxy odour compounds								
α-Terpineol	0.28		0.06	4.12	9.34	2.27	2.52	7.84
Acetophenone	0.19		0.29		0.17	1.68	0.93	0.57
6-Methyl-5-hepten-2-one	1.56	0.20	1.52		0.18	0.47	2.47	1.54
Undecan-2-one	7.43		1.91	0.77	2.09	1.26	1.52	2.04

[a]content (%) of the individual compounds were calculated based on the peak area of the gas chromatogram

'Schubert') and *C. purpurascens.*Variation of volatile compounds in *C. purpurascens* and cyclamen cultivars used as breeding parents.

Table 3 shows the percentages of individuals containing each volatile compound and the relative amounts of volatile compounds from *C. purpurascens* and cyclamen cultivars. We observed the following results with regard to floral compounds, fruity compounds and waxy compounds:

(1) Among the floral compounds citronellal, citronellol, geranial, geraniol, linalool, nerol, methyl citronellate, (*E,Z*)-farnesal, (*E,E*)-farnesal, (*E,Z*)-farnesol, (*E,E*)-farnesol, (*E*)-2,3-dihydrofarnesal, (*E*)-2,3-dihydrofarnesol, benzyl benzoate and gera-

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nylacetone were present in all of the *C. purpurascens*. More than half of the *C. purpurascens* contained neral, nerolidol, methyl *p*-anisate and benzyl alcohol. The amount of citronellol was higher than the amounts of nerol and geraniol. Among the cyclamen cultivars, 77.8% of the individuals contained linalool and 66.7% of the individuals contained geranylacetone, but relatively few individuals contained the other floral compounds. Methyl citronellate, which imparts a strong fragrance, was not detected in any of the cultivars. Few of the cultivars contained (*E,Z*)-farnesal, (*E,E*)-farnesal, (*E,Z*)-farnesol, (*E,E*)-farnesol, (*E*)-2,3-dihydrofarnesal or (*E*)-2,3-dihydrofarnesol. Benzyl benzoate, methyl *p*-anisate and benzyl alcohol, which are benzenoids, were not detected in any of the cultivars. The amounts of nerol and geraniol, which impart a rosy odour, tended to be high, and the amount of citronellol, which also imparts a rosy odour, tended to be lower than the amounts of nerol and geraniol.

(2) Among the fruity compounds, methyl benzoate, benzaldehyde, decan-1,4-olide, butyl acetate, methyl caprate and methyl caprylate were detected in *C. purpurascens*; for all but decan-1,4-olide and butyl acetate, the percentages were ≥ 50%. Few of the cultivar individuals contained fruity compounds; benzaldehyde and butyl acetate were the only fruity compounds we detected.

(3) Among the waxy compounds, α-terpineol, acetophenone and undecan-2-one were not detected in *C. purpurascens*. Many of the cyclamen cultivars contained α-terpineol, 6-methyl-5-hepten-2-one and undecan-2-one.

Variation of volatile compounds in interspecific hybrids

Table 4 shows the proportions of various volatile compounds in the flowers of the interspecific hybrids and the parent materials. The flowers of both cyclamen cultivars, ('Akebono' and 'Schubert') contained the waxy compounds α-terpineol and undecan-2-one, and small amounts of the floral compounds geraniol and nerol were detected in 'Akebono'. When we used 'Akebono' as a breeding parent, the resulting interspecific hybrids showed variation in the proportions of citronellol, nerol and geraniol. For example, citronellol predominated in hybrid 1, nerol and citronellol predominated in hybrid 2, and citronellol, geraniol and nerol were present in almost equal amounts in hybrid 3. Nerol and geraniol were not detected in 'Schubert', and when we used 'Schubert' as the breeding parent, citronellol predominated in most of the resulting hybrids, and nerol or geraniol were present only in small amounts. There were exceptions, such as hybrid 11, in which geraniol was the main component.

Among the other floral compounds, methyl citronellate was detected in three of the six hybrids obtained from 'Akebono' and in three of the five hybrids obtained from 'Schubert'. (*E,Z*)-farnesol, (*E,E*)-farnesol and (*E*)-2,3-dihydrofarnesol were detected in all 11 hybrids. Hybrid 2 had a particularly high proportion of (*E*)-2,3-dihydrofarnesol. In the case of crossing with 'Akebono', benzyl acetate, which was not detected in any of the parents, was detected in three of the 'Akebono' hybrids; and in the case of crossing with 'Schubert', benzyl alcohol, which was also not detected in the parents, was detected two of the 'Schubert' hybrids.

Among the fruity compounds, butyl acetate (which has a strong fruity odour) was detected in all the 'Akebono' hybrids and in two of the 'Schubert' hybrids. In the 'Akebono' hybrids, the butyl acetate was derived from the *C. purpurascens*, whereas in the 'Schubert' hybrids, it was derived from the 'Schubert'. Methyl benzoate and methyl caprylate were not detected in any of the hybrids, whereas other fruity compounds, including benzaldehyde, decan-1,4-olide, butyl acetate and methyl caprate, were detected in some of the hybrids. α-Terpineol, which has an unpleasant smell, was not detected in three of the 'Schubert' hybrids.

The results of our analysis of the volatile compounds in hybrids between *C. purpurascens* and the two cultivars show that when we used a cultivar containing even a small amount of nerol or geraniol, we could produce interspecific hybrids with various proportions of citronellol, nerol and geraniol. Yomogida (1992, 2015) reported that in roses, variation in the relative amounts of citronellol, geraniol and nerol affects the scent impression, which suggests that by producing interspecific hybrids with various proportions of these three compounds, we could breed individuals with various scent impressions. Methyl citronellate, which was present only in *C. purpurascens*, imparts a strong fragrance even when present in only small amounts, and its introduction into flowers is highly desirable. In this study, we were able to produce

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hybrids containing methyl citronellate. In addition, we could introduce floral odour compounds such as farnesol and dihydrofarnesol. Among the fruity odour compounds, methyl benzoate has a strong fruity fragrance, so introducing it can be expected to lead to scent improvement. However, methyl benzoate was not observed in any of the hybrids we produced. Ishizaka et al. (2002) obtained a methyl-benzoate-containing interspecific hybrid from *C. purpurascens*, which suggests that we might be able to introduce this compound by using a different *C. purpurascens* individual. α-Terpineol imparts an unpleasant smell to cyclamen cultivars, and this compound was present in most, but not all, of the hybrids we produced. That is, it was possible to breed hybrids that did not contain this compound. Our results indicate that interspecific hybrids with various scent characteristics can be produced from crosses between the same parents.

We found that benzyl acetate and benzyl alcohol, which were not present in any of the parents, were present in some of the hybrids. It has been reported that in *Dianthus* (Kishimoto et al. 2013) and *Ipomopsis* (Bischof et al. 2014), scent compounds do not show simple Mendelian inheritance patterns. Hybrids that emit larger amounts of certain volatile compounds than their parents are sometimes produced. Kishimoto et al. suggested that this phenomenon might be due to heterosis. In *Freesia,* Fu et al. (2007) found that the compounds in the flowers of the parent was not found in F1 hybrids and, conversely, the other compounds in F1 hybrids was not found in the parents. Because the inheritance of fragrant compounds is not simple, the use of wild species with unique biosynthesis pathways or new enzymes not found in cultivars may permit breeding of unexpected new types of scented cyclamen. Further investigation is necessary.

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