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Compartmentalized Floral Scent Emission in Two Species of *Mitella* (Saxifragaceae)

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Abstract As an initial step to clarify the role of individual floral scent chemicals in *Mitella*-fungus gnats pollination systems, I examined which part of the flower the volatiles were emitted from in *M. pauciflora* and *M. furusei* var. *subramosa*. Using solid-phase microextraction (SPME) method with flower samples separated for petals, anthers, and the rest, I found that the emission patterns of volatile compounds were different among samples. In *M. pauciflora*, *β*-caryophyllene was emitted mainly from petals, while linalool was emitted mainly from the flower parts other than petals. In *M. furusei*, the principal volatile compounds, lilac aldehydes and lilac alcohols were emitted from the flower parts other than petals. Because these patterns probably reflect the floral adaptation to optimize the way they interact with animals, the present finding will give some insights with the roles of individual volatile compounds for the *Mitella*-fungus gnat pollination systems.

Key words: flower, petal, pollination, SPME, volatile.

Floral scents are the mixture of volatile compounds that are considered one of the key signals to influence flower-visiting animals in the way they interact with the flower. Specifically, floral scents are known to have dual functions, i.e., attractant to obligate flower visitors that mostly act as pollinators, while repellent to facultative flower visitors that often act as antagonists (Junker and Blüthgen, 2010). Although the functions of individual floral scent compounds are largely unknown, not all compounds emitted by a flower necessarily contribute to pollinator attraction (Friberg *et al.*, 2013), and the responses of the animals to flowers are dependent on the quality and quantity of the floral scents (e.g., Terry *et al.*, 2007). Therefore, as an essential reproductive organ, it is reasonable that a flower emits differentiated compositions of scents in different part of flowers to optimize the behaviors of flower-visiting animals. Nevertheless, we still have a limited knowledge on how floral scents are emitted differently among specific floral structures (but see Dötterl and Jürgens, 2005 and Friberg *et al.*, 2013).

The genus *Mitella* section *Asimitellaria* is a plant lineage that has specific interactions with pollinating fungus gnats (Okuyama *et al.*, 2008). In *Asimitellaria*, floral scents are strongly associated with their interactions with different species of pollinators, i.e., long-tongued fungus gnats (*Gnoriste mikado*) or shot-tongued fungus gnats (*Boletina* spp. and *Coelosia* spp.) (Okamoto *et al.*, 2015). Among the floral scent compounds found in *Asimitellaria*, a stereoisomeric set of lilac aldehydes is likely to determine the pollinator specificity by eliciting nectaring behavior of *G. mikado* while repulsing short-tongued fungus gnats, whereas the functions of other compounds are less understood (Okamoto *et al.*, 2015).

Toward a better understanding of the role of individual floral scent compounds, here I examine if there is a tissue-specific pattern of floral

Table 1. The plant individuals used in the present study

	Plant ID	Population of origin
M. pauciflora	MP-NSS1404	Saruhachi, Sado city, Niigata pref., Japan
	MP-STW1501	Tsuwano town, Shimane pref., Japan
	MP-STW1502	Tsuwano town, Shimane pref., Japan
	MP-STI1502	Takairi fall, Yasugi city, Shimane Pref., Japan
M. furusei	MSU-KO1507	Koryo-cho, Izumo city, Shimane pref., Japan
	MSU-CH1501	Chomonkyo, Yamaguchi city, Yamaguchi pref., Japan

scent emission in *M. pauciflora and M. furusei* var. *subramosa*, a pair of closely related species of *Asimitellaria* with contrasting floral scent profiles associated with the differentiated pollination systems.

Materials and Methods

Four individuals of *M. pauciflora* and two individuals of *M. furusei* var. *subramosa*, cultivated in Tsukuba Botanical Garden of the National Museum of Nature and Science, Japan, were used (Table 1). Floral tissue, peduncles, petals, and anthers, were collected from 3–7 flowers (0–2 days after blooming) and placed in a 1.5 mL glass vial with silicone-septa (Shimadzu, Kyoto, Japan). Volatile compounds were collected for 30 min using head space-solid phase microextraction (SPME) with fibers of 100 μm Polydimethylsiloxane (PDMS) or Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS). All the plants used in this study are cultivated in Tsukuba Botanical Garden of the National Museum of Nature and Science, Japan. The samples were subjected to the GC/MS with the equivalent settings to those reported previously (Okamoto *et al.*, 2015). Specifically, we used an Rtx-5SilMS capillary column (30 m × 0.25 mm; film thickness, 250 μm; Restek, Bellefonte, PA, USA). Helium was used as the carrier gas at a velocity of 48.1 cm s^{-1}, and the injector temperature was 250°C. The injector was operated in the splitless mode for 1 min. Electron ionisation mass spectra were obtained at a source temperature of 200°C. The oven temperature was programmed to the following sequence: 40°C for 5 min, followed by an increase of 5°C/min to 210°C and then 10°C/min to 280°C, at which the oven was held for 5 min.

For every volatile compounds, retention indices were calculated with *n*-alkane (C9–C20) standards. Then identification was made by comparing the mass spectra and the retention indices with those reported in the NIST Chemistry WebBook (Linstrom and Mallard, 2012), Boachon *et al.* (2015), and Elsharif *et al.* (2015).

To distinguish volatile compounds of flowers from those of the damaged or vegetative tissues, the volatiles from the peduncle samples in *M. pauciflora* and *M. furusei* var. *subramosa* were used as the control. The volatile compounds only detected in the flower samples were regarded as flower-specific volatile compounds.

Results

Evaluation of SPME fiber for sensitivity to floral volatiles

Compared to PDMS, the use of DVB/CAR/PDMS for SPME fiber could retrieve more floral scent compounds. Specifically, PDMS failed to detect 2-hexenoic acid methyl ester in a flower sample of *M. furusei* var. *subramosa* (Fig. 1A and B). Therefore, hereafter I analyzed the tissue-specific floral scent profiles for all the samples using SPME with DVB/CAR/PDMS.

Compartmentalized patterns of floral scent emission in Mitella

In this study, I detected 9 and 12 flower-specific volatile compounds in *M. pauciflora* and *M. furusei*, respectively, where all but one (2-Hexenoic acid methyl ester) were terpenoids (Table 2). In *M. pauciflora*, the dominant compounds of floral

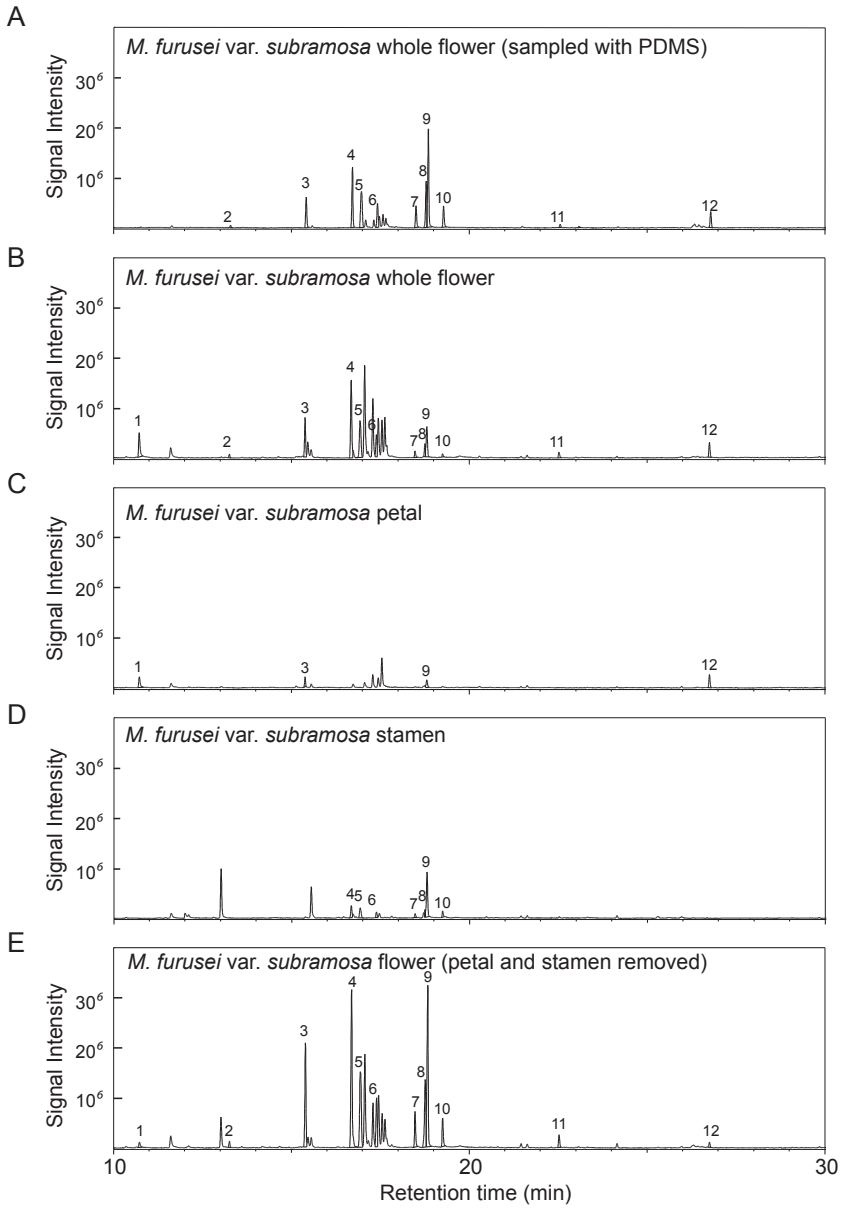

Fig. 1. Chromatograms of the volatile samples collected from different parts of flowers of *M. furusei* individual MSU-KO1507 under the same tuning conditions. The peak marked with numbers are the flower-specific volatile compounds. 1: 2-Hexenoic acid methyl ester. 2: (*E*)-*β*-ocimene. 3: linalool. 4: lilac aldehyde isomer 1. 5: lilac aldehyde isomer 2. 6: lilac aldehyde isomer 3. 7: lilac alcohol isomer 1. 8: lilac alcohol isomer 2. 9: lilac alcohol isomer 3. 10: lilac alcohol isomer 4. 11: 8-oxolinalool. 12: *α*-farnesene. A: Whole flowers (using PDMS for SPME fiber). B: Whole flowers. C: Petals. D: Stamens. E: Petal- and stamen-removed flowers. For B–E, DVB/CAR/PDMS was used for SPME fiber.

scents were linalool and *β*-caryophyllene, while in *M. furusei*, those were lilac aldehydes and lilac alcohols. Although there was substantial variation of volatile composition among individuals (Table 2), these observations were congruent with the previous study (Okamoto *et al.*, 2015).

Table 2. Floral scent compounds of the studied samples expressed as relative amounts (%)

Compound	RI[a]	*M. pauciflora*												*M. furusei* var. *subramosa*						
		MP-NSS1404			MP-STW1501				MP-STW1502			MP-STI1502		MSU-KO1507				MSU-CH1501		
		Whole flower	Flower (petal removed)	Petal	Whole flower	Flower (petal and stamen removed)	Stamen	Petal	Flower (petal and stamen removed)	Stamen	Petal	Flower (petal removed)	Petal	Whole flower	Flower (petal and stamen removed)	Stamen	Petal	Whole flower	Flower (petal removed)	Petal
Terpenoid																				
α-Pinene[b]	930	—	—	—	—	—	—	—	0.68	—	3.26	—	—	—	—	—	—	—	—	—
β-Myrcene[b]	989	—	—	—	—	—	—	—	1.00	—	—	—	—	—	—	—	—	—	—	—
Eucalyptol[b]	1029	0.89	—	—	—	—	—	—	9.29	—	54.23	0.34	51.14	—	—	—	—	—	—	—
(*E*)-*β*-ocimene[b]	1036	—	—	—	—	—	—	—	—	—	—	—	—	1.12	0.77	—	—	—	—	—
Linalool[b]	1098	3.67	14.35	—	83.54	97.08	—	—	87.25	91.73	21.47	97.72	48.86	12.80	13.36	—	20.49	15.68	9.54	78.36
Lilac aldehyde isomer 1[c]	1137	—	—	—	—	—	—	—	—	—	—	—	—	26.50	21.39	13.00	—	44.28	38.44	—
Lilac aldehyde isomer 2[c]	1145	—	—	—	—	—	—	—	—	—	—	—	—	18.34	14.52	14.91	—	13.50	24.50	—
Lilac aldehyde isomer 3[c]	1160	—	—	—	—	—	—	—	—	—	—	—	—	7.11	6.20	5.66	—	5.60	9.18	—
α-Terpineol[b]	1192				—	—	—	—	1.78	8.27	21.04	—	—	—	—	—	—	—	—	—
Lilac alcohol isomer 1[c]	1196	—	—	—	—	—	—	—	—	—	—	—	—	2.22	4.51	4.24	—	1.53	2.25	—
Lilac alcohol isomer 2[c]	1206	—	—	—	—	—	—	—	—	—	—	—	—	3.87	9.81	6.39	—	1.92	—	—
Lilac alcohol isomer 3[c]	1208	—	—	—	—	—	—	—	—	—	—	—	—	10.65	22.77	49.57	19.60	11.82	12.10	16.39
Lilac alcohol isomer 4[c]	1223	—	—	—	—	—	—	—	—	—	—	—	—	0.99	3.67	6.23	—	—	—	—
8-oxolinalool[d]	1339	—	—	—	—	—	—	—	—	—	—	—	—	2.03	1.61	—	—	2.34	3.99	
8-hydroxylinalool[c]	1360	—	—	—	—	—	—	—	—	—	—	—	—					3.33	—	—
β-Caryophyllene[b]	1421	92.00	80.71	94.16	15.98	2.92	—	100.00	—	—	—	—	—	—	—	—	—	—	—	—
10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0]undecane[b]	1426	0.72	2.51	2.67	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Humulene[b]	1456	2.72	2.43	3.17	0.48	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Germacrene D[c]	1478	—	—	—	—	—	—	—	—	—	—	1.95	—	—	—	—	—	—	—	—
α-farnesene[b]	1502	—	—	—	—	—	—	—	—	—	—	—	—	4.81	0.63	—	29.49	—	—	5.25
Aliphatics																				
2-Hexenoic acid methyl ester[b]	963	—	—	—	—	—	—	—	—	—	—	—	—	9.56	0.76	—	30.42	—	—	—

[a] Retention indices relative to *n*-alkanes on the Rtx-5Sil column.

[b] Identified by similarity (>94%) of mass spectrum to those in the libraries and previously reported RI index in the NIST Chemistry WebBook.

[c] Identified by similarity (>90%) of mass spectrum to those in the libraries and previously reported RI index in the NIST Chemistry WebBook.

[d] Reference mass spectrum was from Boachon *et al.* (2015).

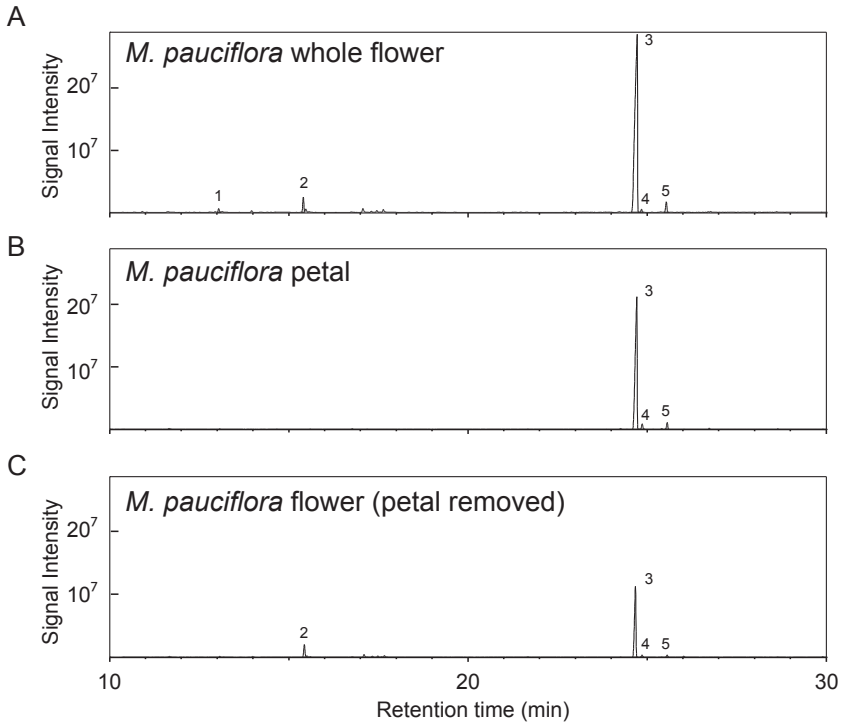

Fig. 2. Chromatograms of the volatile samples collected from different parts of flowers of *M. pauciflora* individual MP-NSS1404 under the same tuning conditions. The peaks marked with numbers are the flower-specific volatile compounds. 1: eucalyptol. 2: linalool. 3: *β*-caryophyllene. 4: 10,10-dimethyl-2,6-dimethylenebicyclo[7.2.0]undecane. 5: humulene. A: Whole flowers. B: Petals. C: Petal-removed flowers.

A subsequent comparison among the floral parts revealed that there is a clear pattern of tissue-specific floral scent emission. In *M. pauciflora*, *β*-caryophyllene, if present, was emitted mostly from petals, while linalool was not (Fig. 2B). As the result, the petal-removed flowers emitted much less *β*-caryophyllene, compared to the intact flowers (Fig. 2A and C). This pattern was common between two individuals that emit *β*-caryophyllene. The stamens emitted little or no volatiles (Table 2). In *M. furusei*, volatile emission was very weak in the petals, and only four flower-specific volatiles, linalool, lilac alcohol isomer 3, *α*-farnesene, and 2-hexenoic acid methyl ester were detected in small amount (Fig. 1C). The stamens emitted lilac alcohols and lilac aldehydes in small amount (Fig. 1D). Accordingly, the petal- and/or stamen-removed flowers had similar compositions of volatiles with the intact ones (Fig. 1E, Table 2).

Discussion

In this study, I revealed the patterns of compartmentalized volatile emission in flowers of two species of *Mitella*. Although similar patterns have also been reported in *Lithophragma*, a closely-related genus of *Mitella*, there is an apparent difference between the two genera. In *Lithophragma*, the tissue-specific patterns of volatile emission were observed mainly between the benzenoids and terpenoids (Friberg *et al.*, 2013), each of which are biosynthesized by different metabolic pathways. In *Mitella*, however, such the patterns were found in the same class of compounds, terpenoids.

Specifically, in *M. pauciflora*, *β*-caryophyllene was emitted mostly from petals, although there

was a clear pattern of polymorphism for presence or absence of the compounds even in a single population (Table 2). In the previous study, a significant association between *β*-caryophyllene emission and pollination by short-tongued fungus gnats has been detected (Okamoto *et al.*, 2015), suggesting that the compound has some roles for pollination by these insects. By contrast, there was no volatile compound specifically emitted from the petals of *M. furusei*. The petal of the genus *Mitella* is characteristic in its pinnately-cleft, linear morphology, and its role as the pollinator's footing has been suggested although not demonstrated experimentally (Okuyama *et al.* 2004). Field experiments with manipulation of the petals and other flower parts will elucidate the functions of *β*-caryophyllene in the pollination system.

It is also noteworthy that lilac aldehydes, a set of key volatile compounds associated with pollination by *Gnoriste mikado*, were emitted mainly from the flower parts other than petals. Because the behavioral bioassay have revealed that the lilac aldehydes stimulate nectaring behavior of *G. mikado*, the present finding suggests that the compounds function not only as the attractant but also as the fine guide for the long proboscis of *G. mikado* for successful pollen transport. Using SEM, a cluster of bumps at the base of pistils is visible in the flowers of *Mitella* (Y. Okuyama unpublished data). Whether or not it corresponds to the osmophore, i.e., a glandular floral tissue from which volatile compounds evaporate, where the lilac aldehydes are emitted would be of interest for the future study.

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References

Boachon, B., Junker, R. R., Miesch, L., Bassard, J. E., Höfer, R., Caillieaudeaux, R., Seidel, D. E., Lesot, A., Heinrich C., Ginglinger, J.-F., Allouche, L., Vincent, B., Wahyuni, D. S. C., Paetz, C., Beran, F., Miesch, M., Schneider, B., Leiss, K. and Werck-Reichhart, D. 2015. CYP76C1 (cytochrome P450)-mediated linalool metabolism and the formation of volatile and soluble linalool oxides in *Arabidopsis* flowers: A strategy for defense against floral antagonists. The Plant Cell 27: 2972–2990.

Dötterl, S. and Jürgens, A. 2005. Spatial fragrance patterns in flowers of *Silene latifolia*: Lilac compounds as olfactory nectar guides? Plant Systematics and Evolution 255: 99–109.

Elsharif, S. A., Banerjee, A. and Buettner, A. 2015. Structure-odor relationships of linalool, linalyl acetate and their corresponding oxygenated derivatives. Frontiers in Chemistry 3: 57. DOI: 10.3389/fchem.2015.00057.

Friberg, M., Schwind, C., Raguso, R. A. and Thompson, J. N. 2013. Extreme divergence in floral scent among woodland star species (*Lithophragma* spp.) pollinated by floral parasites. Annals of Botany 111: 539–550.

Junker, R. R. and Blüthgen, N. 2010. Floral scents repel facultative flower visitors, but attract obligate ones. Annals of Botany 105: 777–782.

Linstrom, P. J. and Mallard, W. G. 2012. NIST Chemistry WebBook, NIST Standard Reference Database Number 69. Gaithersburg, MD: National Institute of Standards and Technology, 20899. http://webbook.nist.gov.

Okamoto, T., Okuyama, Y., Goto, R., Tokoro, M. and Kato, M. 2015. Parallel chemical switches underlying pollinator isolation in Asian *Mitella*. Journal of Evolutionary Biology 28: 590–600.

Okuyama, Y., Murakami, N. and Kato, M. 2004. Pollination by fungus gnats in four species of the genus *Mitella* (Saxifragaceae). Botanical Journal of the Linnean Society 144: 449–460.

Okuyama, Y., Pellmyr, O. and Kato, M. 2008. Parallel floral adaptations to pollination by fungus gnats within the genus *Mitella* (Saxifragaceae). Molecular Phylogenetics and Evolution 46: 560–575.

Terry, I., Walter, G. H., Moore, C., Roemer, R. and Hull, C. 2007. Odor-mediated push-pull pollination in cycads. Science 318: 70.