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Floral Visits of *Cordyla murina* (Mycetophilidae) and Other Dipterans to *Asarum asaroides* (Aristolochiaceae) and the Possible Role of Mushroom-like Scents

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Abstract We assessed plausible linkages between floral variation and pollination-system diversity in the genus *Asarum* (sect. Heterotropa) by investigating the floral-visitor and floral-scent profiles of *Asarum asaroides* (C.Morren et Decne.) Makino, a large-flowered species endemic to western Japan. Using time-lapse photography and field collections of insects, we confirmed that multiple dipteran families visited *A. asaroides*. The fungus gnat *Cordyla murina* Winnertz, 1863 (Mycetophilidae), is likely the primary pollinator, since we observed repeated visits by this species at three spatially distinct sites. We also reassessed the previously posited primary pollinator of *A. tamaense* Makino and found that *C. murina* is in fact the likely primary pollinator of this species. The floral-scent profile of *A. asaroides* typically contains ethyl 3-methylcrotonate, 3-octanone, 3-octanol, safrole, and caryophyllene, and is strikingly different from that of *A. tamaense*, implying that different mechanisms underlie the attraction of *C. murina* to these closely related species.

Key words: brood-site mimicry, floral volatile compound, fungus gnat, mushroom mimicry, pollination.

Introduction

Floral variation is a prominent feature of angiosperm diversity and is hypothesized to be related to pollination-system diversity. Plant lineages that contain wide floral diversity are therefore useful for assessing linkages between floral diversity and pollination systems. The genus *Asarum* (sect. Heterotropa) is one such lineage, comprising 62 species with remarkably varied floral traits, including color, shape, and scent; it is endemic to a narrow geographic area in Japan and Taiwan (Okuyama *et al.*, 2020). Sect. Heterotropa is also noted for its presumed deceptive pollination strategy, which offers no reward to pollinators, and the varied traits observed within this taxon may be related to a diversity of deception strategies (Sinn *et al.*, 2015; Okuyama *et al.*, 2020). However, because visual observations of pollinator visits to concealed floral organs are logistically difficult and therefore rare in the literature, few sources have assessed the pollination biology of Heterotropa, excluding the relatively well-studied pollination system of *Asarum tamaense*, which is mediated by fungus gnats (Sugawara, 1988; Kakishima and Okuyama, 2020). We aimed to more fully elucidate the pollination systems and floral-scent profiles within

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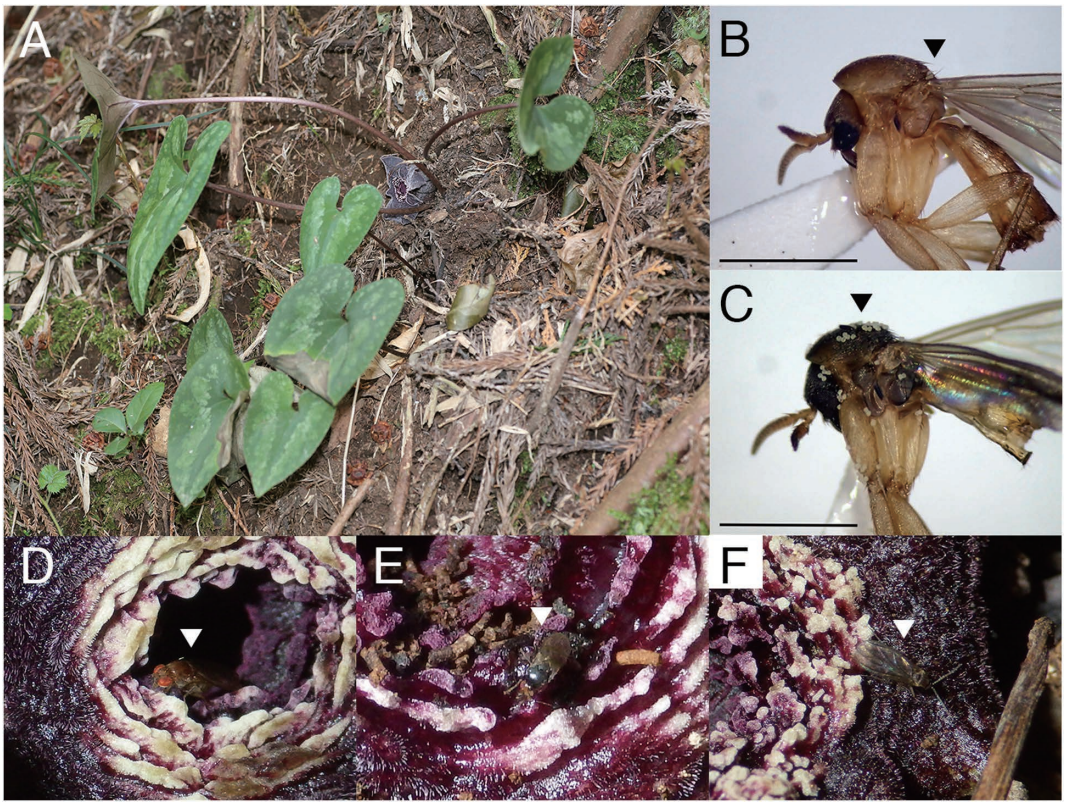

Fig. 1. Photographs of *Asarum asaroides* and associated flower visitors. A) A wild individual of *A. asaroides*. B and C) *Cordyla murina* collected from flowers at Mine (B) and Tsukuba (C) showing pollen grains on the body surface. D) Drosophilidae sp. E) Sphaeroceridae sp. F) Sciaridae sp.

this genus using *Asarum asaroides*, which is endemic to western Honshu and northern Kyushu, Japan. *A. asaroides* is a showy species with flowers up to 4 cm in diameter (Fig. 1) and a chamber-like space located above the column of the calyx tube. Given its unique floral structure, *A. asaroides* is an ideal species with which to assess pollinator attraction in the context of floral traits and pollination systems in Heterotropa.

Materials and Methods

Collection and time-lapse photography of floral visitors

Floral visitors to *A. asaroides* were collected from a native population located in Mine, Yamaguchi Prefecture, Japan (N34°10′, E131°12′), on April 22, 2018, and from potted plants located in the Tsukuba Botanical Garden in Ibaraki Prefecture (N36°06′, E140°07′) on April 22–23, 2021. The potted plants were grown in a greenhouse with open windows. We used an insect aspirator to collect insects that had entered the calyx. Specimens were stored dry to allow inspection for pollen on their bodies, and used for morphological and DNA barcoding identification, as described below.

Floral visitors were monitored using time-lapse photography (camera models WG-4 and WG-50, RICOH, Tokyo, Japan) at the Mine location on April 22–23, 2018, and at an additional population in Abu, Yamaguchi Prefecture (N34°30′, E131°34′) on April 23–24, 2018. Overall, 2 and 20 flowering individuals were monitored at Mine and Abu, respectively (Table 1). Cameras were placed at least 5 cm from each of

Table 1. The detailed information of the time-lapse photography

Plant ID	Location	Number of flowers	Date	Time	Interval (min)	Number of photos
1	Mine	2	22–23 Apr. 2018	16:25–18:31	2	784
2	Mine	1	22–23 Apr. 2018	16:17–18:37	2	791
3	Abu	1	23–24 Apr. 2018	10:03–2:43	1	1001
4	Abu	1	23–24 Apr. 2018	10:04–2:49	1	1006
5	Abu	1	23–24 Apr. 2018	10:17–0:36	1	860
6	Abu	1	23–24 Apr. 2018	10:12–1:31	1	920
7	Abu	1	23–24 Apr. 2018	10:23–3:02	1	1000
8	Abu	1	23–24 Apr. 2018	11:04–1:57	1	894
9	Abu	1	23–24 Apr. 2018	11:25–1:40	1	856
10	Abu	2	23–24 Apr. 2018	11:07–1:27	1	861
11	Abu	1	23–24 Apr. 2018	10:57–2:19	1	923
12	Abu	2	23–24 Apr. 2018	10:44–0:30	1	827
13	Abu	1	23–24 Apr. 2018	10:34–0:40	1	847
14	Abu	1	23 Apr. 2018	10:41–23:59	1	799
15	Abu	2	23 Apr. 2018	10:41–22:49	1	729
16	Abu	1	23–24 Apr. 2018	11:13–0:47	1	815
17	Abu	1	23–24 Apr. 2018	10:51–1:18	1	868
18	Abu	1	23 Apr. 2018	10:46–23:14	1	749
19	Abu	2	23–24 Apr. 2018	11:23–0:08	1	765
20	Abu	1	23 Apr. 2018	11:34–23:59	1	746
21	Abu	1	23–24 Apr. 2018	9:44–2:24	1	1001
22	Abu	2	23–24 Apr. 2018	10:11–2:51	1	1002
Total						19044

the target flowers and the time interval between photos was set to 2 min at Mine and 1 min at Abu. Any insect visitor that made contact with the upper surface of the calyx or entered it was considered a single visit and subsequent photos of the same individual were not counted. All target flowers were collected following the observation period and brought back to the laboratory to assess the presence of oviposited eggs inside the calyx tube.

Identification of floral visitors

Collected floral visitors were identified based on both morphology and DNA barcoding. First, insects were examined using a binocular microscope (SZ, Olympus, Tokyo, Japan). The genitalia of male fungus gnats were removed with sharpened tweezers, treated with a 10% potassium hydroxide solution at room temperature overnight, and then observed under a stereoscopic microscope in 99.5% ethanol. The genitalia were preserved in glycerin within plastic microvials pinned under the body remains. Then the legs of the specimens were removed and used for DNA extraction. The vouchers were deposited in National Museum of Nature and Science, Tsukuba.

Given the challenges of morphological identification of female fungus gnats, we used DNA barcoding based on mitochondrial cytochrome oxidase subunit I (COI) sequences using the primers LCO1490 and HCO2198 (Folmer *et al.*, 1994), with the nucleotide sequences of the morphologically identified male fungus gnats as the reference. DNA extraction and sequencing were performed as described in Kakishima and Okuyama (2018b) and Kakishima *et al.* (2020). Nucleotide sequences were deposited in the DNA Data Bank of Japan under accession numbers LC649054–LC649063. COI sequences of closely related species were obtained from GenBank or the Barcode of Life Data System (BOLD) (Ratnasingham and Hebert, 2007). We used a 4% uncorrected genetic distance as the threshold for differentiating species using DNA barcoding (Okuyama *et al.*, 2018). A phylogenetic analysis was conducted using RAxML version 8 (Stamatakis, 2014), using a maximum likelihood (ML)

method with a GTR + G likelihood model for nucleotide substitutions. The ML tree and bootstrap branch supports were obtained by running a rapid bootstrapping algorithm with 1,000 replicates, followed by a search for the ML tree. Finally, we assessed correspondence between the barcoding results and those obtained from morphological identification.

Floral-scent analyses

Floral-scent profiles of *A. asaroides* flowers were examined using 13 samples from six individuals. All individuals were assessed during the daytime. Three individuals (TBG160978, TBG160979, TBG160980) were sampled a second time during the day using different flowers, and two individuals (TBG160978, TBG160979) were sampled at night using different flowers. The scents from the cut leaves of two individuals (TBG160978, TBG160979) were also assessed to identify the volatile compounds unique to flowers. For sampling, one or two flowers (0–7 days after opening), or one leaf, were collected from each individual and placed in a 50- or 100-mL glass vial and sealed with aluminum foil. Volatile compounds were collected for 30 min using headspace-solid phase microextraction with 100-μm fibers of divinylbenzene/carboxen/polydimethylsiloxane (Supelco, Bellefonte, PA, USA). To distinguish the volatile compounds of flowers from those of the ambient air, volatiles from an empty vial were used as a control.

All samples were subjected to gas chromatography mass spectrometry with settings equivalent to those reported in Okamoto *et al.* (2015) and Kakishima and Okuyama (2018a). We used a GCMS-QP2010SE system (Shimadzu, Kyoto, Japan) equipped with 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 splitless mode for 1 min. Electron ionization mass spectra were obtained at a source temperature of 250°C. The oven temperature was programmed to the following sequence: 40°C for 5 min, an increase of 5°C/min to 210°C, an increase of 10°C/min to 280°C, and then holding at 280°C for 5 min. The relative peak area in the total ion chromatogram (TIC) was used as a rough estimate of the relative content of each compound in each sample.

For all volatile compounds, retention indices were calculated using n-alkane (C6–C20) standards (Wako, Tokyo, Japan). Tentative identification was made by comparing the mass spectra with those in libraries (NIST14 and NIST14s, National Institute of Standards and Technology, USA) using a cutoff of 94% similarity. The mass spectra, as well as the retention indices for the compounds, were compared with authentic standards. When authentic standards could not be obtained, the retention indices were compared with those reported in the National Institute of Standards and Technology Chemistry WebBook (Linstrom and Mallard, 2012).

Results

Floral visitors of A. asaroides

We collected two and seven dipteran flower visitors from *A. asaroides* flowers at Mine and the Tsukuba Botanical Garden, respectively. All individuals excluding one had 1–40 pollen grains on their bodies (Fig. 1B, C). All individuals were identified as *Cordyla* sp. (Mycetophilidae) based on morphology. Both individuals from Mine and five of the seven individuals from Tsukuba were male. We identified one male from each sampling location as *Cordyla murina* based on the genitalia. DNA barcoding results indicated that all collected individuals were of the same species, i.e., *Cordyla murina*. DNA barcoding also implied that the *Cordyla* sp. collected from *A. tamaense* in our previous study (Kakishima and Okuyama, 2020) was *C. murina* (Fig. 2). We therefore examined the genitalia of one male *Cordyla* sp. collected from *A. tamaense*, and confirmed the individual's identity as *C. murina*. We found no evidence of eggs inside any of the collected flowers of *A. asaroides*.

In total, 19,044 photographs were captured

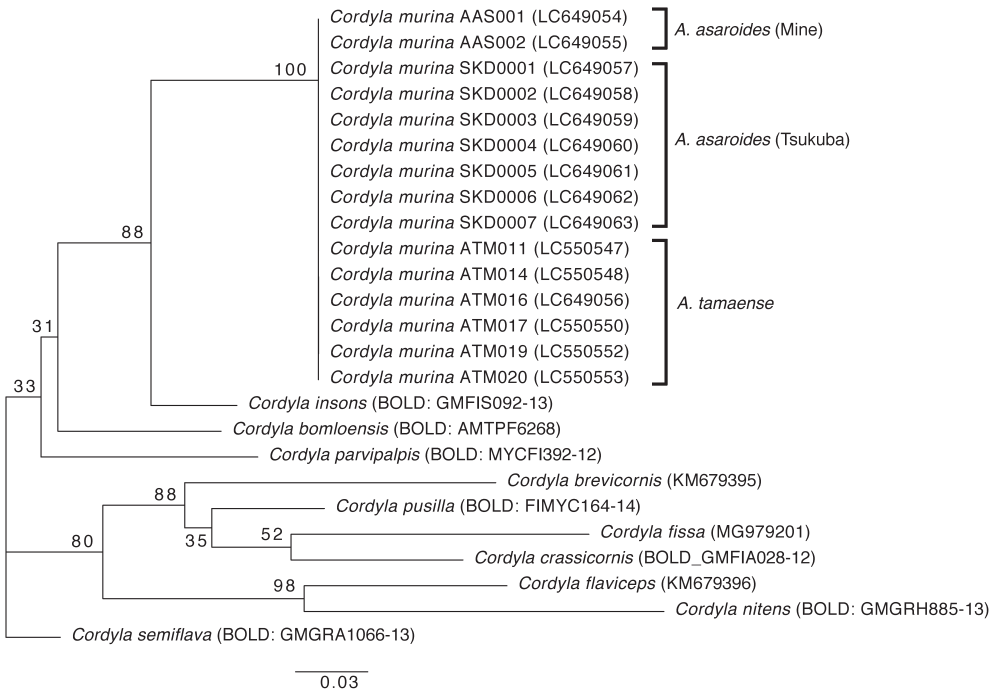

Fig. 2. A maximum-likelihood phylogenetic tree of *Cordyla* spp. based on a 617-base-pair sequence of the mitochondrial COI region. Bootstrap supports are shown for nodes above the species level. Each operational taxonomic unit (OTU) label represents the sample name followed by the database ID (BOLD or GenBank). For *C. murina*, specimen origins are indicated on the right.

Table 2. A list of the flower visitors photographed on each *A. asaroides* plant individual

Category of visitor		Plant ID																					Total
		Mine						Abu															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
Coleoptera	Carabidae	0	0	0	0	1	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	4
	Curculionidae	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
	Nitulidae*	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	1	0	4
	Staphilinidae	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2
Diptera	Drosophilidae	1	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	6
	Empididae	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
	Mycetophilidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Psychodidae	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	Sciaridae	0	0	0	1	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	4
	Sphaeroceridae	0	1	0	0	1	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
Hemiptera		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Hymenoptera	Formicidae	14	28	5	2	3	2	18	1	1	2	0	8	1	0	0	3	0	2	0	1	1	1	93
Orthoptera		0	0	0	1	0	1	0	1	1	0	1	1	1	0	0	2	0	0	0	0	1	0	10
Unidentified insects		0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	4
Amphipoda		0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Araneae		0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	4
Collembola		3	1	1	0	0	3	18	1	3	5	6	8	3	7	6	9	2	5	0	2	1	14	98
Isopoda		2	0	0	1	0	1	0	0	4	2	0	1	0	0	1	3	0	0	0	0	0	0	15
Myriapoda		0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	4
Opiliones		0	0	0	0	1	0	5	0	0	2	0	4	1	2	0	1	0	1	2	1	2	2	24
Total		21	35	7	8	6	10	44	5	9	12	11	24	6	10	9	21	2	8	4	4	6	21	283

* Tentative identification

over the observation period (Table 1). We counted 0–93 floral visitors to each individual and a total of 283 floral visitors across the observation period (Table 2). Springtails (Collembola) and ants (Formicidae) were the most frequently observed insects (Table 2), but there was little evidence that they entered the calyx tubes, indicating that they are not important pollinators of *A*. *asaroides*. Dipterans and coleopterans were the only winged insects recorded and at least some were photographed entering the calyx tube (Fig. 1D–F). Among dipterans, Drosophilidae, Empididae, Psychodidae, Sciaridae, and Sphaeroceridae were observed. No visitations by *Cordyla* spp. were photographed, but individuals were collected while visiting flowers at the Mine location.

Floral volatile compounds in A. asaroides

Overall, 81 floral volatile compounds were identified from the flowers of *A*. *asaroides*. We detected 14–48 volatile compounds in each individual headspace sample (Table 3). These compounds included 45 aliphatics, four benzenoids or phenylpropanoids, 13 monoterpenes, 16 sesquiterpenes, two nitrogen-containing compounds, and one sulfur-containing compound. The volatile compounds common among all flower samples were ethyl 3-methylcrotonate (ethyl 3-methyl-2-butenoate), 3-octanone, 3-octanol, safrole, and caryophyllene. Among these, safrole and caryophyllene were also detected in the leaf samples. The volatile composition, represented by the TIC peak area ratios, was variable among flower samples and was dominated by ethyl 3-methylcrotonate (38.65–93.84%), methyl 3,3-dimethacrylate (methyl 3-methyl-2-butenoate, 0.00–54.00%), 3-octanone (0.83–5.75%), and safrole (0.18–25.53%). We found no obvious difference in volatile compounds between samples taken during the day and at night.

Discussion

Our results represent a significant addition to the paucity of literature on the pollination systems of Heterotropa species, where mushroom mimicry has long been the posited pollinator attraction strategy (Vogel, 1978; Sugawara, 1988; Sinn *et al.*, 2015b). Congruent with the hypothesized mushroom mimicry, the flowers of *A*. *asaroides* attracted various potentially mycophagous dipterans (e.g., Drosophilidae and Mycetophilidae) and the scent profile consistently contained 3-octanone and 3-octanol, which are typical of mushroom scent profiles (Policha *et al.*, 2015; Kakishima *et al.*, 2019). The flowers of *A*. *asaroides* also attracted some coleopterans, which might act as pollinators with moderate frequency, although they all consisted of less-mobile, ground-dwelling species of Carabidae that were too large to enter the calyx tube, Curculionidae, Nitulidae, Staphylinidae, etc. Other floral visitors were primarily flightless and did not actively enter the calyx tube. Thus, we considered these non-dipteran species as reasonably unlikely to be major contributors to outcrossing in *A*. *asaroides*. The principal pollinators of *A*. *asaroides* are most likely dipterans, particularly Drosophilidae, Mycetophilidae, Sciaridae, and Sphaeroceridae, which were observed making repeated visits over the observation period.

Among these, the fungus gnat, *C*. *murina*, is likely a notable pollinator, given that we observed and collected this species making repeated floral visits in both wild and cultivated populations, although, probably by chance, it was not photographed. In addition, one of the authors (MS) reported *C*. *murina* to be a floral visitor of *A*. *asaroides* in a wild population in Hita, Oita Prefecture, Japan (Sueyoshi and Suetsugu, 2018). Therefore, *A*. *asaroides* appear to attract *C*. *murina*, with the attraction signal potentially involving an olfactory mechanism. Given that members of *Cordyla* pollinate the closely related *A*. *tamaense*, we reexamined specimens collected in Kakishima and Okuyama (2020) and identified these individuals as *C*. *murina*. However, we note that there are apparent differences in the pollination systems of *A*. *tamense* and *A*. *asaroides*. We found that *C*. *murina* attraction to *A*. *asaroides* appeared to be

Table 3. Relative amount (%) of volatile compounds detected from the flower of each *Asarum asaroides* individual

Name	Ret. Index	Identification[a]	Leaf	160978 (1st)	160978 (2nd)	160978 (3rd, night)	160979 (1st)	160979 (2nd)	160979 (3rd, night)	160980 (1st)	160980 (2nd)	163683	163684	SK18132	Average
Aliphatics															
Acetic acid	603	B	*	0.04	—	0.07	0.30	—	0.19	—	—	—	—	—	0.05
Ethyl acetate	608	B		—	—	—	1.57	—	—	0.22	1.88	1.94	1.53	—	0.55
Ethyl propionate	706	B		—	—	—	—	—	—	—	—	0.03	0.19	—	0.02
Propyl acetate	708	A		—	—	—	—	—	—	—	—	—	0.17	—	0.01
Acetoin	711	A		—	—	—	—	—	—	—	—	—	0.16	—	0.01
Aliphatics-1	778			—	—	—	—	—	—	—	—	—	0.04	—	0.00
Ethyl butyrate	800	A		—	—	—	—	—	—	—	0.05	0.14	0.40	—	0.05
Butyl acetate	813	A	*	—	—	—	0.12	—	—	—	—	—	—	—	0.01
1,3-Octadiene isomer	819	B		0.11	0.05	0.05	0.19	0.23	0.15	0.10	0.13	0.11	0.08	—	0.09
Methyl 3,3-dimethacrylate	838	B		—	0.03	0.18	—	9.27	54.00	0.07	0.06	0.05	—	0.04	4.90
Ethyl isovalerate	853	A		—	—	—	—	—	—	0.08	0.08	0.19	0.21	—	0.04
Aliphatics-2	856			—	—	—	—	—	—	—	—	—	0.11	—	0.01
Aliphatics-3	873			—	—	—	—	—	—	—	—	—	0.04	—	0.00
Isoamyl acetate	877	A		—	—	—	—	—	—	—	—	0.04	0.11	—	0.01
2-Acetoxy-3-butanone	892	A		—	—	—	—	—	—	—	—	—	0.06	—	0.00
Ethyl 3-methylcrotonate	924	A		87.25	90.47	93.15	38.74	75.77	27.53	93.84	80.74	69.19	42.09	93.13	60.92
Ethyl tiglate	940	A		—	—	—	—	—	—	—	—	—	0.09	—	0.01
Ethyl 3-hydroxy-3-methylbutanoate	952	B		0.04	0.04	—	0.10	—	—	0.07	0.22	0.28	0.07	0.07	0.07
Aliphatics-4	965			—	—	—	—	0.09	—	—	—	—	—	—	0.01
Aliphatics-5	966			—	—	—	—	—	—	—	—	—	—	0.06	0.00
1-Octen-3-ol	982	A		0.04	0.03	—	0.18	0.24	—	—	—	0.03	—	—	0.04
3-Octanone	987	A		4.48	7.37	2.28	4.53	5.79	2.22	2.52	0.83	1.20	1.93	2.76	2.76
3-Octanol	998	A		1.86	0.94	0.40	5.52	3.70	1.40	1.01	0.79	1.52	0.34	2.11	1.51
Ethyl hexanoate	999	B		—	0.08	—	—	—	—	—	—	—	0.19	—	0.02
Aliphatics-6	1006			—	0.08	—	—	—	—	—	—	—	—	—	0.01
Hexyl acetate	1013	A		—	—	—	0.09	—	—	—	—	0.06	0.03	—	0.01
Aliphatics-7	1022			0.25	0.27	—	—	0.15	—	0.10	0.03	0.11	—	0.07	0.08
Aliphatics-8	1034			—	0.04	0.07	—	—	0.11	—	—	0.05	—	—	0.02
Aliphatics-9	1046			0.06	0.04	—	0.15	0.22	0.10	0.06	0.03	0.04	—	—	0.05
trans-2-Octenal	1057	A		0.13	0.10	—	0.11	0.23	0.10	0.06	0.03	0.05	—	0.07	0.07
Aliphatics-10	1064			—	—	—	—	—	—	—	—	0.07	—	—	0.01
1-Octanol	1072	A	*	0.15	—	—	0.20	0.43	—	0.17	0.04	0.06	—	0.10	0.09
Aliphatics-11	1078			—	0.03	—	—	—	—	—	—	0.03	—	—	0.00
1-Octen-3-yl-acetate	1107	B		—	—	—	1.36	—	—	—	0.14	0.14	0.03	—	0.13
3-Octyl acetate	1120	A		—	—	—	9.09	—	—	0.18	1.57	2.67	0.28	—	1.06
2-Ethylhexyl acetate	1047	A		—	—	—	0.83	—	—	0.17	0.18	0.14	0.07	0.07	0.11
Aliphatics-12	1172		*	—	—	—	—	—	—	—	—	—	—	—	0.00
Aliphatics-13	1198			—	—	—	21.97	—	—	0.43	5.51	1.89	0.24	—	2.31
Octyl acetate	1210	B		—	—	—	4.21	—	—	0.64	1.32	1.27	0.07	—	0.58
Aliphatics-14	1291			—	—	—	—	—	—	—	0.05	0.05	—	—	0.01
Nonyl acetate	1309	B		—	—	—	—	—	—	—	0.04	—	—	—	0.00
Aliphatics-15	1345			—	—	—	0.13	—	—	—	—	—	—	—	0.01
3-Hydroxy-2,2,4-trimethylpentyl isobutyrate	1369	B	*	0.04	—	—	0.15	—	—	—	—	—	—	—	0.01
2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	1585	A		—	—	—	0.11	—	—	—	—	—	—	0.06	0.01
												0.00			

Table 3. Continued

Name	Ret. Index	Identification[a]	Leaf	160978 (1st)	160978 (2nd)	160978 (3rd, night)	160979 (1st)	160979 (2nd)	160979 (3rd, night)	160980 (1st)	160980 (2nd)	163683	163684	SK18132	Average
Benzenoids and Phenylpropanoids												0.00			
3,5-Dimethoxytoluene	1264	B		—	—	—	—	—	—	—	—	—	0.08	—	0.01
Safrole	1286	A	*	2.96	0.23	2.64	3.12	0.92	5.81	0.18	4.83	12.55	25.53	0.55	4.56
cis-Isosafrole	1337	A	*	—	—	—	—	—	—	—	—	—	0.08	—	0.01
trans-Isosafrole	1376	A	*	0.06	—	—	—	—	—	—	0.04	0.12	0.18	—	0.03
Monoterpenes															
α-Pinene	931	A	*	—	—	0.20	0.11	0.24	—	—	—	—	—	—	0.04
Camphene	947	A		—	—	—	—	—	—	—	—	—	0.10	—	0.01
Sabinene	971	A		—	—	—	—	0.11	—	—	—	—	—	—	0.01
β-Pinene	974	A	*	—	—	0.04	0.28	0.15	0.08	—	0.03	0.06	0.39	—	0.08
β-Myrcene	989	A	*	0.29	0.12	0.33	1.81	0.89	2.44	—	0.16	0.99	2.40	—	0.73
α-Phellandrene	1003	A		—	—	—	—	—	—	—	—	0.30	0.84	—	0.09
3-Carene	1005	A	*	—	—	—	0.65	0.26	0.69	0.02	0.09	—	—	0.07	0.14
Cymene isomer	1022	B		—	—	—	—	—	—	—	—	—	0.12	—	0.01
Monoterpene-1	1027			—	—	0.07	—	—	—	—	—	0.33	0.91	—	0.10
Limonene	1027	A	*	—	—	—	0.15	0.06	0.16	—	0.02	—	—	—	0.03
trans-β-Ocimene	1035	A	*	—	—	—	—	—	—	—	—	—	0.04	—	0.00
Terpinolene	1084	A		—	—	—	—	—	0.09	—	—	—	0.05	—	0.01
Monoterpene-2	1195			—	—	—	—	—	—	—	—	—	0.06	—	0.00
Sesquiterpenes															
β-Elemene	1388	B	*	0.25	—	0.07	—	—	0.10	—	0.07	0.40	0.34	—	0.09
Sesquiterpene-1	1387			—	—	—	0.23	0.13	—	—	—	—	—	—	0.03
Sesquiterpene-2	1401			—	—	—	—	—	—	—	—	0.05	—	—	0.00
Sesquiterpene-3	1412			—	—	—	—	—	—	—	—	0.04	—	—	0.00
Caryophyllene	1418	A	*	1.74	0.08	0.44	3.11	0.90	3.98	0.08	0.73	2.71	2.02	0.54	1.26
β-Gurjunene	1430	B		—	—	—	0.30	—	—	—	—	—	—	—	0.02
Sesquiterpene-4	1433			—	—	—	—	—	—	—	—	0.50	0.05	—	0.04
Sesquiterpene-5	1440			—	—	—	—	0.08	—	—	—	—	—	—	0.01
Sesquiterpene-6	1448			—	—	—	—	—	—	—	—	—	0.05	—	0.00
(*E*)-β-Farnesene	1452	A	*	0.24	—	—	0.43	0.09	0.67	—	0.30	—	1.99	0.26	0.31
Humulene	1454	A	*	—	—	—	0.11	—	0.17	—	—	0.18	—	—	0.04
γ-Curcumene	1477	B		—	—	—	—	—	—	—	—	0.08	0.18	—	0.02
α-Curcumene	1480	B	*	—	—	—	—	—	—	—	—	0.04	0.05	—	0.01
β-Selinene	1488	B		—	—	—	—	—	—	—	—	0.03	—	—	0.00
Bicyclogermacrene	1494	B		—	—	—	—	—	—	—	—	0.16	0.20	—	0.03
β-Curcumene	1508	B		—	—	—	—	—	—	—	—	0.04	0.07	—	0.01
												0.00			
Nitrogen-containing compounds												0.00			
Isovaleronitrile	721	A		—	—	—	—	—	—	—	—	—	0.06	—	0.00
Nitrogen-containing compound-1	905			—	—	—	—	—	—	—	—	—	15.66	—	1.20
												0.00			
Sulphur-containing compounds															
Dimethyl disulfide	730			—	—	—	—	0.04	—	—	—	0.06	—	0.06	0.01

a. Identification was based on A: mass spectrum and retention index of authentic compound, B: similarity of mass spectrum to those in the libraries and previously reported RI index in the NIST Chemistry WebBook. * Indicates those compounds were detected in a leaf sample.

male-biased (seven of nine individuals were male), whereas attraction to *A*. *tamaense* appeared to be female-biased (five of six individuals were female) and eggs were typically laid within the flowers of the latter. Although it is possible that these two species attract different sexes of *C*. *murina* using different signals, the sample sizes obtained in these two studies were too low to allow decisive conclusions on this point. However, we speculate that this could explain the substantial differences in the floral scent profiles of these two species; ethyl 3-methylcrotonate was the dominant floral volatile compound in *A*. *asaroides* (60.85% of the total on average) and was only marginally detected in *A*. *tamaense* (0.00–0.42% of the total; Kakishima and Okuyama, 2020), in which dimethyl disulfide was the dominant compound (42.1% of the total on average; Kakishima and Okuyama, 2020). Conversely, dimethyl disulfide was rare in *A*. *asaroides* (0.00–0.06% of the total).

The two dominant floral volatile compounds of *A*. *asaroides*, ethyl 3-methylcrotonate and methyl 3,3-dimethacrylate, have been reported from a type of yeast as well as from the flowers and fruits of other plant species (Lugemwa *et al.*, 1989, Dobson *et al.*, 1997; Mahidol *et al.*, 2005, Wijaya *et al.*, 2005, Grondin *et al.*, 2017, Wang *et al.*, 2017), although none of these are likely associated with *C*. *murina*. It is possible that *C*. *murina* may be attracted to fungal fruiting bodies that emit the same compounds, but such scent profiles have not yet been assessed. The genus *Cordyla* was recently highlighted for its role in pollination (e.g., Suetsugu and Sueyoshi, 2018a, b; Matsumoto *et al.*, 2021; Suetsugu *et al.*, 2021) and there is evidence that another Heterotropa member, *Asarum asperum*, is visited by *Cordyla pusilla* (Sasakawa and Ishizaki, 2003). Further surveys of the life-history strategies of *Cordyla* are crucial to understanding the role of these insects as plant pollinators and the mechanisms that may be used within Heterotropa to attract them.

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