

1 Class I. Data set descriptors

2 Title

3 Secondary metabolites extracted in methanol from nectar and pollen: a resource for ecological and
4 evolutionary studies

5 Data set identification code: Nectar_pollen_chemistry_20180919_v1

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19 Abstract

20 Floral chemistry mediates plant interactions with herbivores, pathogens, and pollinators. The
21 chemistry of floral nectar and pollen—the primary food rewards for pollinators—can affect both plant
22 reproduction and pollinator health. Although the existence and functional significance of nectar and
23 pollen secondary metabolites has long been known, comprehensive quantitative characterizations of
24 secondary chemistry exist for only a few species. Moreover, little is known about intraspecific variation
25 in nectar and pollen chemical profiles. Because the ecological effects of secondary chemicals are dose-
26 dependent, heterogeneity across genotypes and populations could influence floral trait evolution and
27 pollinator foraging ecology. To better understand within- and across-species heterogeneity in nectar and
28 pollen secondary chemistry, we undertook exhaustive LC-MS and LC-UV-based chemical
29 characterizations of nectar and pollen methanol extracts from 31 cultivated and wild plant species.

30 Nectar and pollen were collected from farms and natural areas in Massachusetts, Vermont, and
31 California, USA, in 2013 and 2014. For wild species, we aimed to collect 10 samples from each of 3 sites.
32 For agricultural and horticultural species, we aimed for 10 samples from each of 3 cultivars. Our dataset
33 (1535 samples, 102 identified compounds) identifies and quantifies each compound recorded in
34 methanolic extracts, and includes chemical metadata that describe the molecular mass, retention time,
35 and chemical classification of each compound. A reference phylogeny is included for comparative
36 analyses.

37 We found that each species possessed a distinct chemical profile; moreover, within species, few
38 compounds were found in both nectar and pollen. The most common secondary chemical classes were
39 flavonoids, terpenoids, alkaloids and amines, and chlorogenic acids. The most common compounds

40 were quercetin and kaempferol glycosides. Pollens contained high concentrations of hydroxycinnamoyl-
41 spermidine conjugates, mainly triscoumaroyl and trisferuloyl spermidine, found in 71% of species. When
42 present, pollen alkaloids and spermidines had median nonzero concentrations of 23,000 μM (median
43 52% of recorded micromolar composition). Although secondary chemistry was qualitatively consistent
44 within each species and sample type, we found significant quantitative heterogeneity across cultivars
45 and sites. These data provide a standard reference for future ecological and evolutionary research on
46 nectar and pollen secondary chemistry, including its role in pollinator health and plant reproduction.

47 Key words

48 Floral chemistry, plant secondary metabolites, allelopathy, plant-pollinator interactions, plant-microbe
49 interactions, diversity, intraspecific variation, site variation, cultivar variation, floral rewards, liquid
50 chromatography-mass spectrometry, mutualisms

51

52

53 Introduction

54 Floral nectar and pollen provide rewards for the services of pollinators. However, these rewards
55 face multiple and sometimes conflicting selective pressures to not only attract pollinators, but also to
56 defend against exploitation by folivores, nectar robbers, and microbes that can cause nutrient
57 degradation and plant disease (Dobson and Bergstrom 2000, Heil 2011, McArt et al. 2014). The
58 composition and concentration of plant secondary metabolites in floral food rewards can influence
59 interactions with mutualists and antagonists (Adler and Irwin 2005, Kessler et al. 2008, Galen et al. 2011,
60 Barlow et al. 2017), and are therefore important to plant ecology and evolution.

61 Previous studies of secondary metabolites in floral rewards have typically focused on one or
62 several metabolites in one or a few plant species, such as aconitine alkaloids in *Aconitum* spp. (Barlow et
63 al. 2017), cardenolides in *Asclepias* spp. (Manson et al. 2012), iridoid glycosides in *Chelone glabra*
64 (Richardson et al. 2016), grayanotoxins in *Rhododendron ponticum* (Egan et al. 2016), gelsemine in
65 *Gelsemium sempervirens* (Adler and Irwin 2012), or nicotine in *Nicotiana* spp. (Adler et al. 2006, 2012).
66 Although a few earlier studies encompassed a wide variety of species and chemical classes (Baker 1977,
67 Dobson 1988), the techniques available to these authors provided only non-specific identification of
68 nectar and pollen compounds, and semi-quantitative estimates of chemical concentrations. Aside from
69 taxonomic and chemical breadth, within-species variation in floral reward chemistry can shape
70 pollinator behavior and plant reproduction, but has seldom been explored (Kessler et al. 2012, Egan et
71 al. 2016). Finally, the raw data from many of these earlier studies are not readily available, which
72 hinders their reuse and value to new experiments and syntheses.

73

74 To fill some of these knowledge gaps, we present data on methanol-soluble nectar and pollen
75 secondary metabolites from 31 wild, horticultural, and crop species. This dataset is unique in its
76 combination of diverse plant taxa, specific and exhaustive identification and quantification of methanol-
77 soluble secondary compounds, and explicit consideration of intraspecific variation in chemical
78 composition. Compounds were separated by liquid chromatography, identified by UV and mass spectra,
79 and quantified using standard curves. Intraspecific variation was accounted for by sampling with
80 replication from multiple sites (for wild species), and varieties and cultivars (for horticultural and crop
81 species). We predict that these data will be a useful reference in future investigations of (i) the
82 chemistry of individual species, (ii) the bioactivity of specific compounds and mixtures, and (iii) in
83 phylogenetic comparisons across taxa, and thereby further the understanding of the ecological and
84 evolutionary pressures that shape the chemistry of floral rewards.

85

86 Metadata

87 Class II. Research origin descriptors

88 A. Project description

89 1. **Identity:** Secondary metabolites extracted in methanol from nectar and pollen: a resource
90 for ecological and evolutionary studies

91 2. **Originators:**

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101 3. **Period of study:** Samples were collected in 2013 and 2014 and analyzed in 2015 and 2016.

102 4. **Objectives:** To characterize the nectar and pollen secondary chemistry of a wide range of
103 cultivated and wild plant species, to better understand the role of secondary chemistry in
104 interactions with pollinators and other organisms. Specifically, we determined intra and
105 inter-species variation in chemistry and how nectar and pollen chemistry varied within a
106 species and across space. These data can be used as background information or preliminary
107 data to support future ecological and evolutionary research.

108 5. **Abstract:** See above.

109 6. **Sources of funding:** This research was funded by the United States Department of
110 Agriculture and the United States National Science Foundation (NSF). Please see
111 acknowledgments for grant information.

112

113 B. Methods

114 1. **Study sites**

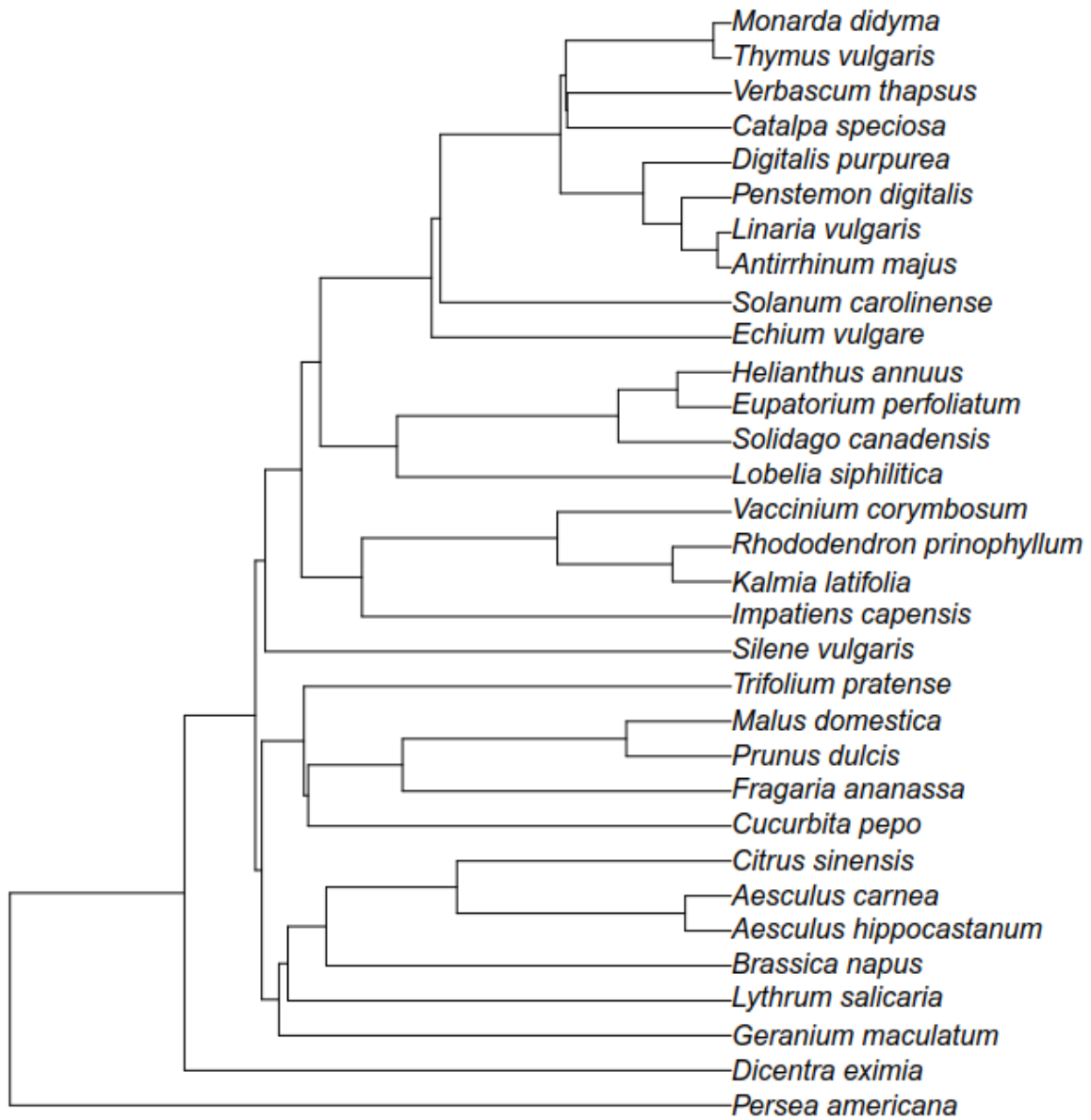
115 Nectar, flower, and pollen samples were collected from 32 species of flowering plants in
116 Massachusetts, Vermont, and California, United States, in 2013 and 2014. Massachusetts
117 and Vermont have a temperate continental climate. Sites in California (Santa Ana, CA for

118 *Persea americana* and *Citrus sinensis*; Sacramento, CA for *Prunus dulcis*) have a hot-summer
119 Mediterranean climate. We chose a mix of native and introduced species, with an emphasis
120 on those that are bee-pollinated and either common or those for which we had prior
121 knowledge of floral secondary chemistry to facilitate analyses. For crop plants, we also
122 focused on species whose yield is improved by pollination (Delaplane et al. 2000). A
123 phylogeny of the sampled species is shown in Figure 1.

124

125 **2. Sampling design**

126 To characterize intraspecific variation, we collected 10 samples each of 3 cultivars (for
127 cultivated plants), or 10 samples from each of 3 sites (for wild species). Within each cultivar
128 or site, plants were selected based on availability of flowers. Unless otherwise specified in
129 data column "Pooled.p", each pollen sample came from a separate plant. In contrast, nectar
130 was often pooled across flowers from multiple plants to obtain sufficient volume for
131 chemical analysis; however, any given plant was never used for multiple samples. Samples
132 were obtained from plants grown at local farms, or found in natural areas or along
133 roadsides. *Antirrhinum majus*, two cultivars of *Dicentra eximia*, *Digitalis purpurea*,
134 *Eupatorium perfoliatum*, *Lobelia siphilitica*, and *Penstemon digitalis* were purchased from
135 local nurseries. No special permits were required for the sample collection. Where
136 necessary, permission was obtained from local farms, parks, and landowners. Sample sizes
137 are given in Table 1. This information is also given in "Species_metadata.csv". Site locations
138 and cultivar codes are given in data files "Sites.csv" and "Cultivars.csv", respectively.



139

140 **Figure 1.** Phylogeny of sampled species.

141

142 **Table 1.** Overview of species, sample sizes, number of cultivars and sites, and collection notes. “Type” columns give details of sample collection.
 143 “Class” abbreviations: C = Crop, H = Horticultural, W = Wild.

Species	Family	Class	Flower N	Flower Ncult	Flower Nsite	Nectar N	Nectar Ncult	Nectar Nsite	Pollen N	Pollen Ncult	Pollen Nsite	Nectar type	Pollen type ¹	Flower type ²	Notes
<i>Aesculus carnea</i> Zeyher	Sapindaceae	H	NA	NA	NA	5	1	1	5	1	1	Nectar	Anther	None	NA
<i>Antirrhinum majus</i> L.	Plantaginaceae	H	NA	NA	NA	29	3	1	NA	NA	NA	Water added	Pollen	None	30 µL water per flower added prior to nectar sampling; purchased from greenhouse
<i>Brassica napus</i> L.	Brassicaceae	C	15	1	1	2	1	1	3	1	1	Nectar	Pollen	Whole	Collection difficult; very small sample masses
<i>Catalpa speciosa</i> Warder ex. Engelmann	Bignoniaceae	W	28	1	7	30	1	7	29	1	7	Nectar	Pollen	No carpel	NA
<i>Citrus sinensis</i> Osbeck	Rutaceae	C	NA	NA	NA	23	2	1	23	2	1	Nectar	Anther	None	NA
<i>Cucurbita pepo</i> L.	Cucurbitaceae	C	NA	NA	NA	46	3	3	32	3	3	Nectar	Pollen	None	NA
<i>Dicentra eximia</i> Torrey	Papaveraceae	H	NA	NA	NA	6	1	1	8	1	3	Nectar	Pollen	None	Purchased from greenhouse
<i>Digitalis purpurea</i> L.	Plantaginaceae	H	NA	NA	NA	30	3	2	17	3	2	Nectar	Pollen	None	Purchased from greenhouse
<i>Echium vulgare</i> L.	Boraginaceae	W	NA	NA	NA	3	1	1	2	1	1	Nectar	Anther	None	NA
<i>Eupatorium perfoliatum</i> L.	Asteraceae	W/H	27	1	1	1	1	1	NA	NA	NA	Nectar	None	Whole	Single nectar sample, no quantifiable peaks; purchased from greenhouse
<i>Fragaria ananassa</i> Duchesne	Rosaceae	C	NA	NA	NA	NA	NA	NA	30	3	1	Nectar	Anther	None	NA
<i>Geranium maculatum</i> L.	Geraniaceae	W	21	1	3	19	1	2	30	1	4	Nectar	Anther	No anther	Few flowers per plant; flower samples taken after pollen collection

<i>Helianthus annuus</i> L.	Asteraceae	C	40	4	3	20	4	1	30	3	2	Nectar	Pollen	Whole	Some plants: damaged leaves
<i>Impatiens capensis</i> Meerburgh	Balsaminaceae	W	NA	NA	NA	31	1	3	24	1	3	Nectar	Pollen	None	NA
<i>Kalmia latifolia</i> L.	Ericaceae	W	NA	NA	NA	20	1	3	15	1	3	Nectar	Anther or pollen	None	7 anther samples, 4 of which >1mg
<i>Linaria vulgaris</i> Miller	Plantaginaceae	W	NA	NA	NA	31	1	4	32	1	5	Nectar	Anther	None	NA
<i>Lobelia siphilitica</i> L.	Campanulaceae	W/H	29	1	1	30	1	1	3	1	1	Nectar	Pollen	Whole	Pollen: n=3 >1mg; purchased from greenhouse
<i>Lythrum salicaria</i> L.	Lythraceae	W	NA	NA	NA	33	1	3	9	1	3	Nectar	Anther	None	NA
<i>Malus domestica</i> Miller	Rosaceae	C	30	3	1	30	3	1	30	3	1	Nectar	Anther	No anther	11 anther samples, 3 of which >1mg
<i>Monarda didyma</i> L.	Lamiaceae	W	NA	NA	NA	31	1	4	21	1	4	Nectar	Anther or pollen	None	NA
<i>Penstemon digitalis</i> Nuttall ex Sims	Scrophulariaceae	W/H	15	1	1	15	1	1	22	1	1	Nectar	Anther	No anther	Flowers partially analyzed; purchased from greenhouse
<i>Persea americana</i> Miller	Lauraceae	C	NA	NA	NA	NA	NA	NA	30	3	1	None	Pollen	None	NA
<i>Prunus dulcis</i> Webb	Rosaceae	C	NA	NA	NA	NA	NA	NA	30	3	1	None	Pollen	None	NA
<i>Rhododendron prino-phyllum</i> Millais	Ericaceae	W	NA	NA	NA	11	1	2	15	1	4	Water added	Anther	None	30 µL water added to flowers on day of collection (Pelham samples 8,9,10) or day before collection (all others)
<i>Silene vulgaris</i> Garcke	Caryophyllaceae	W	NA	NA	NA	10	1	1	19	1	1	Nectar	Anther	None	NA
<i>Solanum carolinense</i> L.	Solanaceae	W	NA	NA	NA	NA	NA	NA	28	1	3	None	Pollen	None	NA

<i>Solidago canadensis</i> L.	Asteraceae	W	NA	NA	NA	NA	NA	NA	25	1	3	None	Flower tops	Whole	NA
<i>Thymus vulgaris</i> L.	Lamiaceae	H	NA	NA	NA	12	2	1	NA	NA	NA	Nectar	None	None	NA
<i>Trifolium pretense</i> L.	Fabaceae	W	29	1	3	30	1	3	7	1	2	Nectar	Anther and filament	No calyx	Aphids on flowers
<i>Vaccinium corymbosum</i> L. (cult)	Ericaceae	C	29	6	1	55	8	4	54	8	4	Nectar	Anther	Whole	NA
<i>Vaccinium corymbosum</i> L. (wild)	Ericaceae	W	30	1	3	30	1	3	30	1	3	Nectar	Anther	Whole	NA
<i>Verbascum Thapsus</i> L.	Scrophulariaceae	W	NA	NA	NA	27	1	2	29	1	2	Nectar	Anther	None	NA

144 ¹ Pollen types: “Anther” refers to the pollen-containing anther and a small amount of filament, removed from the rest of the stamen with
145 forceps. “Pollen” indicates that pollen grains were removed from the anther with paintbrushes or the vibrating wand of an electric toothbrush.
146 For *Solidago canadensis*, “flower tops” refers to clippings from the distal end of the inflorescence, above the involucre bracts.
147 ² Flower types: For Asteraceae, “flower” refers to inflorescences rather than individual florets

148

149

150 3. Sample collection

151 Nectar was collected with microcapillary tubes from flowers that had been bagged in
152 mesh for 24 h to exclude pollinating insects and allow nectar to accumulate. For samples in
153 Asteraceae, whole inflorescences were bagged. Because nectar typically occurs in flowers at
154 very low volume, each sample generally included nectar from multiple individual flowers
155 and, when necessary, multiple plants to obtain a sufficient volume (~20 μL) for analysis. Care
156 was taken to avoid contamination of nectar samples with pollen. Because nectar
157 concentrations can vary substantially due to evaporative concentration and condensation,
158 we did not collect samples on rainy days. When plants were visibly wet, we checked nectar
159 sugar concentrations with a refractometer and, if nectar sugar concentrations were <5%,
160 postponed our sampling.

161 Depending on the plant species, we collected nectar either from the top or bottom of
162 the corolla; in the latter case, the flower was removed from the plant and probed with
163 microcapillary tubes from below. Each nectar sample contained at least 5 μL but typically 20
164 μL nectar, added to 80 μL ethanol (Palmer-Young et al. 2016, Egan et al. 2018). Ethanol was
165 used to kill any microorganisms and denature enzymes in the nectar that might
166 subsequently degrade secondary chemicals before the nectar was lyophilized. Samples
167 were kept on ice in the field, then stored at $-20\text{ }^{\circ}\text{C}$ until lyophilization. Alcohol from *Thymus*
168 *vulgaris* nectar samples was evaporated at room temperature. For *Antirrhinum majus* and
169 *Rhododendron prinophyllum*, nectar was initially too viscous to collect with microcapillary
170 tubes. We therefore added 30 μL deionized water to each flower's nectary, and collected
171 the resulting liquid several hours later. Concentrations and composition determined for
172 nectar of these species may include chemicals not normally present in nectar (e.g.,

173 compounds dissolved from adjacent tissue) , and chemical concentrations in the diluted
174 nectar may be different from those in the nectar produced by the plants.

175 Pollen was collected from plants with mature, undehisced or newly dehiscing anthers.
176 We initially attempted to collect pollen with paintbrushes and electric toothbrushes.
177 However, for 17 species, it was only feasible to collect sufficient pollen for analysis in the
178 form of anthers, and, for *Solidago canadensis*, whole flower tops (obtained by clipping the
179 inflorescence above the involucre bracts; Table 1). Pollen samples were collected using
180 clean forceps by pinching off anthers, avoiding as much filament as possible. We aimed to
181 collect at least 5 mg per sample, consisting of pollen, the pollen sac, and a small amount of
182 filament. In most species, pollen was pooled across flowers within plants, but not across
183 plants. Samples were stored at -20°C until extraction. Flowers were also collected (whole
184 flowers for 5 species, flowers without anthers for 2 species, the flower without carpel for 1
185 species, and flowers without calyces for 1 species; see Table 1. In the case of Asteraceae
186 species, 'whole flowers' refers to inflorescences rather than individual florets. These flower
187 samples were mainly used for confirmation of compound identities, but full chemical
188 profiles were analyzed for 9 species.

189

190 **4. Sample processing**

191 Lyophilized nectar (original volume $\sim 10\ \mu\text{L}$) was extracted in $50\ \mu\text{L}$ methanol. Pollen
192 samples were extracted in methanol following previously published methods (Arnold et al.
193 2014, Palmer-Young et al. 2016). Unground pollen or flowers (5–50 mg) were sonicated for
194 10 min with 1 mL methanol in a 2 mL microcentrifuge tube, then incubated without shaking
195 for 24 h at room temperature. Samples were centrifuged for 5 min at 12,000 rpm, and the
196 supernatant transferred to a glass vial. We chose methanol as the extraction solvent due to

197 its ability to extract a wide range of secondary metabolites known to occur in nectar and
198 pollen, as well as in plants more generally. These include sesquiterpenes (Green et al. 2017),
199 diterpenoids (Tiedeken et al. 2014), acylated triterpenoids (Stevenson et al. 2016), saponins
200 (Stevenson et al. 2009), iridoid glycosides (Stevenson et al. 2002), flavonoids (Serra Bonvehi
201 et al. 2001) and phenolics (Ainsworth and Gillespie 2007). Microscopic examination of
202 extracted pollen samples indicated that the methanol completely penetrated the pollenkitt
203 after 24 h of extraction, and in preliminary tests we found no differences between the
204 chemical profiles of ground vs. unground pollen samples (PCS, unpublished data).

205 206 **5. Chemical analyses**

207 All extracts were analyzed by liquid chromatography (LC) using Electrospray Ionisation
208 Mass Spectroscopy (ESIMS) and UV spectroscopy. Aliquots (10 μ L) were injected directly
209 onto an LC-MS system with a Micromass ZQ LC-MS detector (Waters, Elstree, Herts, United
210 Kingdom) on a Phenomenex (Macclesfield, Cheshire, United Kingdom) Luna C18(2) column
211 (150 \times 3.0 mm inner diameter, 5 μ m particle size). Samples were eluted with solvents A =
212 MeOH, B = H₂O, C = 1% HCO₂H in MeCN with the following program: A = 0%, B = 90% at t = 0
213 min; A = 90%, B = 0% at t = 20 min; A = 90%, B = 0% at t = 30 min; A = 0%, B = 90% at t = 31
214 min; solvent C was maintained at 10% throughout the run. Column temperature was 30 °C
215 and flow rate 0.5 mL min⁻¹. To facilitate compound identification, HRESIMS data were
216 recorded on a subset of samples using a Thermo (Waltham, MA, USA) LTQ-Orbitrap XL mass
217 spectrometer coupled to a Thermo Accela LC system performing chromatographic
218 separation of 5 μ l injections on a Phenomenex Luna C18(2) column (150 mm \times 3.0 mm i.d., 3
219 μ m particle size). The Orbitrap used the same mobile phase gradient, column temperature,

220 and flow rate as described for the ZQ-LCMS. Spectra were recorded in positive modes at
221 high resolution (30,000 FWHM (full width at half maximum)).

222 Compounds were identified by comparison with mass spectra in the NIST spectral
223 database version 2.0 (Kramida et al. 2013) and, when possible, spectral comparisons with
224 authentic standards in the library at Royal Botanic Gardens, Kew, UK. Quantifications were
225 made based on external standard curves of the same compound, or, for UV-based
226 quantifications, a compound with the same chromophore. All concentrations are given in
227 micromolar ($\mu\text{mol L}^{-1}$ original volume for nectar, $\mu\text{mol kg}^{-1}$ dry mass for pollen). Most amino
228 acids are not retained on the solid phase and elute together at the beginning of the run,
229 thus only phenylalanine and tryptophan were quantitated.

230 Each compound was further classified according to its chemical structure, as described
231 in **[Chemicals.txt]**. The most common chemical groups were amino acids (only
232 phenylalanine and tryptophan quantified), flavonoids, alkaloids and amines (includes
233 spermidine derivatives), terpenoids, and chlorogenic acids (includes 3-, 4-, and 5-
234 caffeoylquinic acids and derivatives). Total concentrations by chemical groups are given in
235 **[Major_class_totals_uM.txt]** and **[Major_class_totals_ppm.txt]**.

236

237 6. Extraction of reference phylogeny

238 We used function “congeneric.merge” in the pez package (Pearse et al. 2015) of R v3.3
239 (R Core Team 2014) to obtain a time-scaled, rooted tree by extraction of our species
240 from an unparalleled molecular phylogeny of flower plants (Zanne et al. 2014). This
241 phylogeny (Figure 1 and **[Npchem_phylogeny.txt]**) can be used in comparative analyses
242 to test or correct for phylogenetic non-independence of chemical traits.

243

244 7. **Permits and authorizations:** No special permits were required for the sample collection.

245 Where necessary, permission was obtained from local farms, parks, and landowners.

246

247 8. **Project personnel:** Undergraduate project managers responsible for sample collection are

248 listed in the acknowledgements.

249 Class III. Data set status and accessibility

250 A. Status

251 1. **Latest update:** Data were last modified in November 2017.

252 2. **Latest archive date:** All data were archived in September 2018.

253 3. **Metadata status:** All metadata are up to date and were uploaded with the original data.

254 4. **Data verification:**

255 **Sample collection:** Plant species identities were verified by reference to field guides and

256 dichotomous keys (Peterson and McKenny 1968, Clemants and Gracie 2006) and, when

257 necessary, by comparison with reference specimens in the University of Massachusetts

258 Amherst herbarium. However, many of the species were obtained from nurseries, or locally

259 common and distinct from co-occurring species, and hence not difficult to identify.

260 Given the abundant and widespread nature of most of the species sampled, we did not

261 collect or deposit voucher specimens. However, remaining plant material, extracts, and

262 chromatograms are available from PCS upon request.

263 **Chemical analyses:** Sample codes were cross-checked with field assistants at University of

264 Massachusetts upon arrival at Royal Botanic Gardens Kew. Quality of chemical extraction

265 data was assessed by searching for the two resolvable amino acids, phenylalanine and

266 tryptophan, which were present in nearly all species and sample types. Compounds were
267 identified by comparison with spectral databases and, when possible, authentic standards in
268 the compound library at Royal Botanic Gardens Kew. Sample metadata, compound
269 identifications, and quantifications were checked by ECPY and IWF during analysis and by
270 exploratory visualizations in R.

271 **B. Accessibility**

272 1. **Storage location and medium:** All data will be electronically archived in *Ecological Archives*.
273 Local copies are maintained at the University of Massachusetts by LSA and ECPY. Original
274 chromatograms are archived at Royal Botanical Gardens, Kew, and available on request.

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285 3. **Copyright restrictions:** Data are published under a Creative Commons Attribution License
286 (CC BY 3.0 US).

287 **4. Proprietary restrictions:**

288 a. **Proprietary restrictions on use:** Data may be freely used if properly cited.

289 b. **Related citations:**

- 290 1. Palmer-Young, E., I. W. Farrell, L. S. Adler, N. J. Milano, P. Egan, R. Juncker, R. E.
291 Irwin, and P. Stevenson. 2018. Chemistry of floral rewards: intra- and
292 interspecific variability of nectar and pollen secondary metabolites across taxa.
293 Ecological Monographs (in press).
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295 Stevenson. 2018. Crop domestication alters floral reward Chemistry with
296 potential consequences for pollinator health. *Frontiers in Plant Science* (in
297 press). doi: 10.3389/fpls.2018.01357
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299 Irwin. (2018). Disease where you dine: Plant species and floral traits associated
300 with pathogen transmission in bumble bees. *Ecology* (in press).
- 301 4. Palmer-Young EC, Sadd BM, Stevenson PC, Irwin RE, Adler LS. Bumble bee
302 parasite strains vary in resistance to phytochemicals. *Sci Rep.* 2016;6: 37087.
303 doi:10.1038/srep37087

304 5. Disclaimers:

305 **Sample Collection:** For 2 species, *Antirrhinum majus* and *Rhododendron*
306 *prinophyllum*, distilled water had to be added to reconstitute nectar that had
307 congealed (as described in II.B.3: [Sample collection](#)). In addition, the presence of
308 acyl-spermidines in nectars of *Digitalis purpurea* and *Helianthus annuus* most likely
309 reflects contamination from pollen, which were found in pollen of both *D. purpurea*
310 and *H. annuus*, but are not known to occur in nectar. However, spermidine synthase
311 has been found in extrafloral nectar from *Ricinus communis* (Shah et al. 2016), and
312 spermidines have been found in the phloem exudates of *H. annuus* (Friedman et al.
313 1986); therefore we cannot rule out that spermidine may be present in nectar

314 independent of contact with pollen. Pollen samples included anthers when it was
315 not feasible to isolate sufficient quantities of pure pollen for analysis. Taxon-specific
316 notes are listed in Table 1.

317

318 **Chemical analyses:** No single chemical analysis can extract and quantify all
319 chemicals found in a plant material. Because we lyophilized samples to avoid
320 spoilage during shipment, and used liquid chromatography rather than gas
321 chromatography, we were not able to characterize nectar and pollen volatiles. In
322 addition, because most amino acids eluted together at the beginning of the
323 chromatographic run, it was only possible to quantify phenylalanine and
324 tryptophan. The absence of quantifications of volatiles and amino acids in our data
325 does not imply that they are absent from nectar, pollen, or flowers of the sampled
326 taxa.

327 6. **Costs of acquisition:** None

328 Class IV. Data structural descriptors

329 A. Data set files

330 All files are provided in .txt format

- 331 1. **[Species_metadata.txt]** (4 KB) Site locations and cultivar codes are given in data files
332 **[Sites.txt]** and **[Cultivars.txt]**.

333 **Description:** Species names, plant families, sample sizes, and sampling notes of sampled
334 plant taxa.

335 **Variables:**

336 Species: Plant species

337 Family: Plant family
338 Flower_N: Number of flower samples
339 Flower_Ncult: Number of flower cultivars
340 Flower_Nsite: Number of flower sites
341 Nectar_N: Number of nectar samples
342 Nectar_Ncult: Number of nectar cultivars
343 Nectar_Nsite: Number of nectar sites
344 Pollen_N: Number of pollen samples
345 Pollen_Ncult: Number of pollen cultivars
346 Pollen_Nsite: Number of pollen sites
347 Nectar.note: "Nectar" indicates that nectar was sampled. "Water.added" indicates when
348 water was added prior to sampling, to reduce viscosity.
349 Pollen.type: Whether anthers, pollen, or floral tops (for *Solidago canadensis*) were collected.
350 Flower.type: Which floral structures were included in the flower samples (analyzed for 9
351 species, NA for remaining species).
352 Notes: Miscellaneous comments

353

354 2. **[Sites.txt]** (9 KB)

355 **Description:** Explanation of site codes with GPS coordinates.

356 **Variables:**

357 Species: Plant species

358 Site: Site abbreviation

359 Location: Description of site

360 GPS: Site coordinates

361 *Catalpa_sample*: For *Catalpa speciosa*, we sampled individual trees that were dispersed
362 across three different towns. Therefore, for this species only, we give GPS coordinates for
363 each sample within each town-level site.

364 Cultivars: For agricultural and horticultural species, which cultivars were sampled at the site.

365

366 3. **[Cultivars.txt]** (2 KB)

367 **Description:** Explanation of cultivar codes.

368 **Variables:**

369 Species: Plant species

370 Cultivar: Cultivar abbreviation

371 Name: Cultivar description

372

373 4. **[Chemicals.txt]** (122 KB)

374 **Description:** List of chemicals identified and measured in each species and sample type.

375 Includes information on compound molecular mass, retention time, and chemical class.

376 **Variables:**

377 Species: Plant species

378 Type: Sample type (flower, nectar, or pollen)

379 Retention_time_min: Elution time in minutes

380 m_z_negative: Characteristic m/z in negative ion mode

381 m_z_positive: Characteristic m/z in positive ion mode

382 UV_nm: Peak UV absorbance (nm)

383 Peak_quantified: Trace used for quantification

384 Molecular_weight: Molecular weight

385 Compound: Name of compound

386 MF: Molecular formula

387 Class: Chemical class

388 Subclass_1 through Subclass_6: Additional chemical classification

389 5. **[Concentrations_long.txt]** (16,246 KB)

390 **Description:** Compilation of concentration measurements, with one row per sample and
391 compound.

392 **Variables:**

393 Species: Plant species

394 Type: Sample type (flower, nectar, or pollen)

395 Cultivar: Cultivar abbreviation. Please note that both wild and cultivated *Vaccinium*
396 *corymbosum* were sampled. The wild plants are assigned cultivar "W", for "Wild".

397 Site: Site abbreviation

398 Number: Sample number

399 Date: Date of collection

400 Mass: Sample mass (dry mass in mg for flower and pollen, fresh nectar volume in μL for
401 nectar)

402 Pool: For nectar samples, "Y" indicates that nectar was pooled from multiple plant
403 individuals.

404 Pooled.p: For pollen samples, "Y" indicates that pollen was pooled from multiple plant
405 individuals

406 Pollen.type: Whether anthers, pollen, or floral tops (for *Solidago canadensis*) were collected.

407 Compound: Name of compound

408 Concentration: Concentration in $\mu\text{mol kg}^{-1}$ dry mass (flower and pollen) or μM (for nectar)

409 Conc_ppm: Concentration in mg kg⁻¹ dry mass (flower and pollen) or mg L⁻¹ (for nectar).

410

411 6. **[Concentrations_wide.txt]** (565 KB)

412 **Description:** Compilation of concentration measurements, with one row per sample.

413 **Variables:**

414 The first 10 columns are identical to the sample identifiers in **[Concentrations_long.txt]**. The
415 subsequent columns include concentrations of each compound (in μmol kg⁻¹ dry mass for
416 flower and pollen, or μM for nectar).

417

418 7. **[Major_class_totals_uM.txt]** (272 KB)

419 **Description:** Total concentrations for each chemical class, with one row per sample. Classes
420 correspond to “Class” in file **[Chemicals.txt]**

421 **Variables:**

422 The first 10 columns are identical to the sample identifiers in **[Concentrations_long.txt]**. The
423 subsequent columns include concentrations of each chemical class (in μmol kg⁻¹ dry mass for
424 flower and pollen, or μM for nectar). “Alkaloids” column includes both alkaloids and amines.

425

426 8. **[Major_class_totals_ppm.txt]** (278 KB)

427 **Description:** Total concentrations for each chemical class, with one row per sample. Classes
428 correspond to “Class” in file **[Chemicals.txt]**

429 **Variables:**

430 The first 10 columns are identical to the sample identifiers in **[Concentrations_long.txt]**. The
431 subsequent columns include concentrations of each chemical class (in mg kg⁻¹ dry mass

432 (flower and pollen) or mg L^{-1} (for nectar)). “Alkaloids” column includes both alkaloids and
433 amines.

434

435 9. **[Concentration_summary.txt]** (70 KB)

436 **Description:** Summary statistics for concentration measurements, with one row per species,
437 sample type, and compound.

438 **Variables:**

439 Species: Plant species

440 Type: Sample type (flower, nectar, or pollen)

441 Compound: Name of compound

442 N: Number of samples

443 Mean: Mean concentration (in $\mu\text{mol kg}^{-1}$ dry mass for flower and pollen, or μM for nectar).

444 SD: Standard deviation of concentration

445 CV: Coefficient of variation

446 Median: Median concentration

447 First.quartile: First quartile of concentrations

448 Third.quartile: Third quartile of concentrations

449

450 10. **[Npchem_phylogeny.txt]** (2 KB)

451 **Description:** Phylogeny of sampled species, in Newick format.

452

453

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