Class I. Data set descriptors

Title

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

Data set identification code: Nectar_pollen_chemistry_20180919_v1

Authors

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Abstract

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

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

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

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

Key words

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

Introduction

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

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

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

Metadata

Class II. Research origin descriptors

A. Project description

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

2. **Originators:**

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3. **Period of study:** Samples were collected in 2013 and 2014 and analyzed in 2015 and 2016.
4. **Objectives:** To characterize the nectar and pollen secondary chemistry of a wide range of cultivated and wild plant species, to better understand the role of secondary chemistry in interactions with pollinators and other organisms. Specifically, we determined intra and inter-species variation in chemistry and how nectar and pollen chemistry varied within a species and across space. These data can be used as background information or preliminary data to support future ecological and evolutionary research.
5. **Abstract:** See above.
6. **Sources of funding:** This research was funded by the United States Department of Agriculture and the United States National Science Foundation (NSF). Please see acknowledgments for grant information.

B. Methods

1. Study sites

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

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

2. Sampling design

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

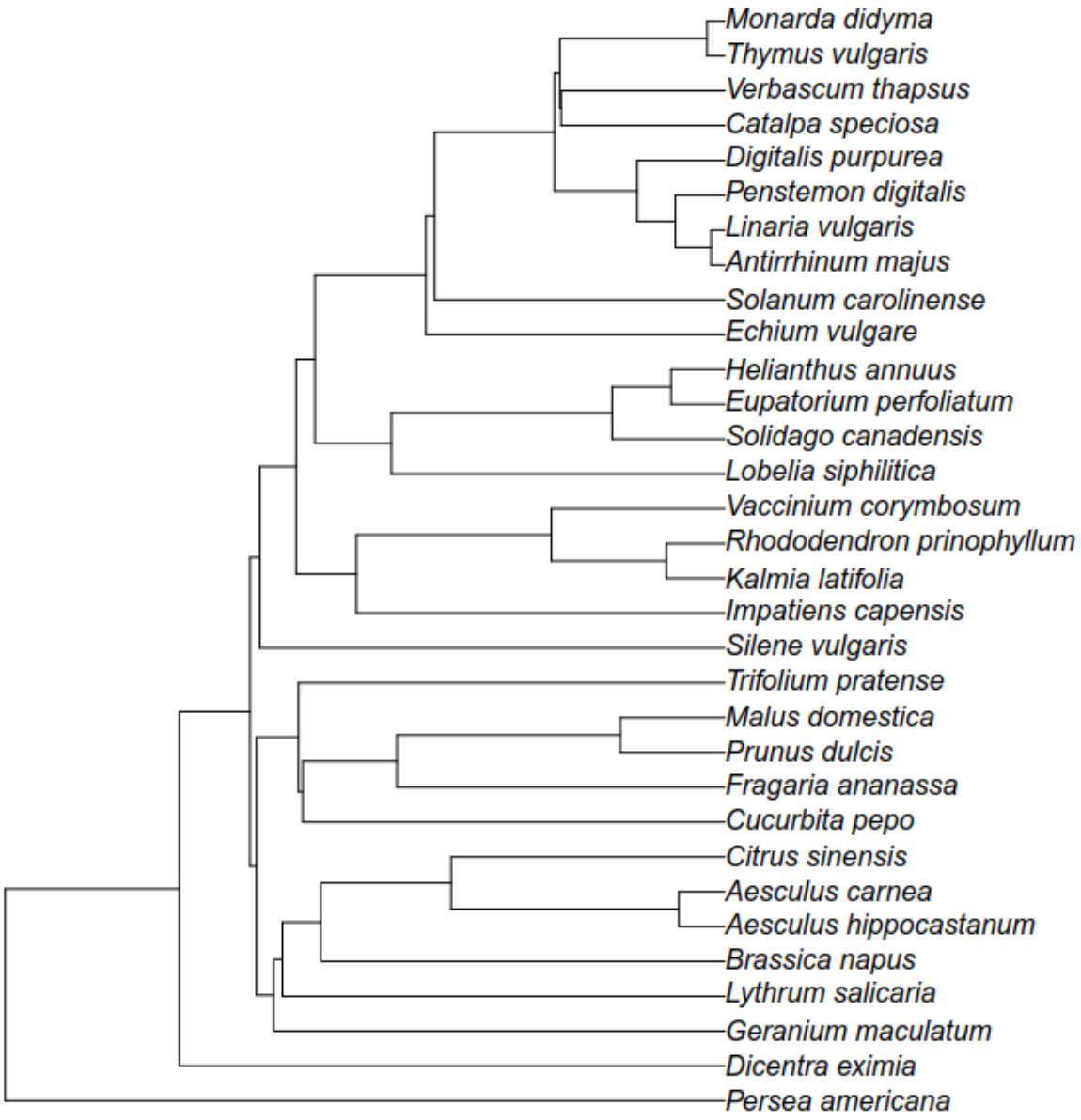

Figure 1. Phylogeny of sampled species.

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
Aesculus carnea Zeyher	Sapind-aceae	H	NA	NA	NA	5	1	1	5	1	1	Nectar	Anther	None	NA
Antirrhinum majus L.	Plantagin-aceae	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
Brassica napus L.	Brassic-aceae	C	15	1	1	2	1	1	3	1	1	Nectar	Pollen	Whole	Collection difficult; very small sample masses
Catalpa speciosa Warder ex. Engelmann	Bignoni-aceae	W	28	1	7	30	1	7	29	1	7	Nectar	Pollen	No carpel	NA
Citrus sinensis Osbeck	Rutaceae	C	NA	NA	NA	23	2	1	23	2	1	Nectar	Anther	None	NA
Cucurbita pepo L.	Cucurbit-aceae	C	NA	NA	NA	46	3	3	32	3	3	Nectar	Pollen	None	NA
Dicentra eximia Torrey	Papaver-aceae	H	NA	NA	NA	6	1	1	8	1	3	Nectar	Pollen	None	Purchased from greenhouse
Digitalis purpurea L.	Plantagin-aceae	H	NA	NA	NA	30	3	2	17	3	2	Nectar	Pollen	None	Purchased from greenhouse
Echium vulgare L.	Boragin-aceae	W	NA	NA	NA	3	1	1	2	1	1	Nectar	Anther	None	NA
Eupatorium perfoliatum 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
Fragaria ananassa Duchesne	Rosaceae	C	NA	NA	NA	NA	NA	NA	30	3	1	Nectar	Anther	None	NA
Geranium maculatum L.	Gerani-aceae	W	21	1	3	19	1	2	30	1	4	Nectar	Anther	No anther	Few flowers per plant; flower samples taken after pollen collection

Helianthus annuus L.	Asteraceae	C	40	4	3	20	4	1	30	3	2	Nectar	Pollen	Whole	Some plants: damaged leaves
Impatiens capensis Meerburgh	Balsamin-aceae	W	NA	NA	NA	31	1	3	24	1	3	Nectar	Pollen	None	NA
Kalmia latifolia L.	Ericaceae	W	NA	NA	NA	20	1	3	15	1	3	Nectar	Anther or pollen	None	7 anther samples, 4 of which >1mg
Linaria vulgaris Miller	Plantagin-aceae	W	NA	NA	NA	31	1	4	32	1	5	Nectar	Anther	None	NA
Lobelia siphilitica L.	Campanu-laceae	W/H	29	1	1	30	1	1	3	1	1	Nectar	Pollen	Whole	Pollen: n=3 >1mg; purchased from greenhouse
Lythrum salicaria L.	Lythraceae	W	NA	NA	NA	33	1	3	9	1	3	Nectar	Anther	None	NA
Malus domestica Miller	Rosaceae	C	30	3	1	30	3	1	30	3	1	Nectar	Anther	No anther	11 anther samples, 3 of which >1mg
Monarda didyma L.	Lamiaceae	W	NA	NA	NA	31	1	4	21	1	4	Nectar	Anther or pollen	None	NA
Penstemon digitalis Nuttall ex Sims	Scrophulari-aceae	W/H	15	1	1	15	1	1	22	1	1	Nectar	Anther	No anther	Flowers partially analyzed; purchased from greenhouse
Persea americana Miller	Lauraceae	C	NA	NA	NA	NA	NA	NA	30	3	1	None	Pollen	None	NA
Prunus dulcis Webb	Rosaceae	C	NA	NA	NA	NA	NA	NA	30	3	1	None	Pollen	None	NA
Rhodo-dendron prino-phyllum Millais	Ericaeae	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)
Silene vulgaris Garcke	Caryophyll-aceae	W	NA	NA	NA	10	1	1	19	1	1	Nectar	Anther	None	NA
Solanum carolinense L.	Solanaceae	W	NA	NA	NA	NA	NA	NA	28	1	3	None	Pollen	None	NA

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

[1] Pollen types: “Anther” refers to the pollen-containing anther and a small amount of filament, removed from the rest of the stamen with forceps. “Pollen” indicates that pollen grains were removed from the anther with paintbrushes or the vibrating wand of an electric toothbrush. For *Solidagao canadensis,* “flower tops” refers to clippings from the distal end of the inflorescence, above the involucral bracts.

[2] Flower types: For Asteraceae, “flower” refers to inflorescences rather than individual florets

3. Sample collection

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

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

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

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

4. Sample processing

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

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

5. Chemical analyses

All extracts were analyzed by liquid chromatography (LC) using Electrospray Ionisation Mass Spectroscopy (ESIMS) and UV spectroscopy. Aliquots (10 µL) were injected directly onto an LC-MS system with a Micromass ZQ LC-MS detector (Waters, Elstree, Herts, United Kingdom) on a Phenomenex (Macclesfield, Cheshire, United Kingdom) Luna C18(2) column (150 × 3.0 mm inner diameter, 5 µm particle size). Samples were eluted with solvents A = MeOH, B = H_2O, C = 1% HCO_2H in MeCN with the following program: A = 0%, B = 90% at t = 0 min; A = 90%, B = 0% at t = 20 min; A = 90%, B = 0% at t = 30 min; A = 0%, B = 90% at t = 31 min; solvent C was maintained at 10% throughout the run. Column temperature was 30 °C and flow rate 0.5 mL min^{-1}. To facilitate compound identification, HRESIMS data were recorded on a subset of samples using a Thermo (Waltham, MA, USA) LTQ-Orbitrap XL mass spectrometer coupled to a Thermo Accela LC system performing chromatographic separation of 5 µl injections on a Phenomenex Luna C18(2) column (150 mm × 3.0 mm i.d., 3 µm particle size). The Orbitrap used the same mobile phase gradient, column temperature,

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

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

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

6. **Extraction of reference phylogeny**

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

7. **Permits and authorizations:** No special permits were required for the sample collection. Where necessary, permission was obtained from local farms, parks, and landowners.

8. **Project personnel:** Undergraduate project managers responsible for sample collection are listed in the acknowledgements.

Class III. Data set status and accessibility

A. Status

1. **Latest update:** Data were last modified in November 2017.
2. **Latest archive date:** All data were archived in September 2018.
3. **Metadata status:** All metadata are up to date and were uploaded with the original data.
4. **Data verification:**

Sample collection: Plant species identities were verified by reference to field guides and dichotomous keys (Peterson and McKenny 1968, Clemants and Gracie 2006) and, when necessary, by comparison with reference specimens in the University of Massachusetts Amherst herbarium. However, many of the species were obtained from nurseries, or locally common and and distinct from co-occurring species, and hence not difficult to identify. Given the abundant and widespread nature of most of the species sampled, we did not collect or deposit voucher specimens. However, remaining plant material, extracts, and chromatograms are available from PCS upon request.

Chemical analyses: Sample codes were cross-checked with field assistants at University of Massachusetts upon arrival at Royal Botanic Gardens Kew. Quality of chemical extraction data was assessed by searching for the two resolvable amino acids, phenylalanine and

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

B. Accessibility

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

2. Contact persons:

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

4. Proprietary restrictions:

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

b. Related citations:

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

5. Disclaimers:

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

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

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

6. **Costs of acquisition:** None

Class IV. Data structural descriptors

A. Data set files

All files are provided in .txt format

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

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

   Variables:

   Species: Plant species

Family: Plant family

Flower_N: Number of flower samples

Flower_Ncult: Number of flower cultivars

Flower_Nsite: Number of flower sites

Nectar_N: Number of nectar samples

Nectar_Ncult: Number of nectar cultivars

Nectar_Nsite: Number of nectar sites

Pollen_N: Number of pollen samples

Pollen_Ncult: Number of pollen cultivars

Pollen_Nsite: Number of pollen sites

Nectar.note: "Nectar" indicates that nectar was sampled. "Water.added" indicates when water was added prior to sampling, to reduce viscosity.

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

Flower.type: Which floral structures were included in the flower samples (analyzed for 9 species, NA for remaining species).

Notes: Miscellaneous comments

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

Description: Explanation of site codes with GPS coordinates.

Variables:

Species: Plant species

Site: Site abbreviation

Location: Description of site

GPS: Site coordinates

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

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

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

   Description: Explanation of cultivar codes.

   Variables:

   Species: Plant species

   Cultivar: Cultivar abbreviation

   Name: Cultivar description

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

   Description: List of chemicals identified and measured in each species and sample type. Includes information on compound molecular mass, retention time, and chemical class.

   Variables:

   Species: Plant species

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

   Retention_time_min: Elution time in minutes

   m_z_negative: Characteristic m/z in negative ion mode

   m_z_positive: Characteristic m/z in positive ion mode

   UV_nm: Peak UV absorbance (nm)

   Peak_quantified: Trace used for quantification

   Molecular_weight: Molecular weight

Compound: Name of compound

MF: Molecular formula

Class: Chemical class

Subclass_1 through Subclass_6: Additional chemical classification

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

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

Variables:

Species: Plant species

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

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

Site: Site abbreviation

Number: Sample number

Date: Date of collection

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

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

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

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

Compound: Name of compound

Concentration: Concentration in µmol kg^{-1} dry mass (flower and pollen) or µM (for nectar)

Conc_ppm: Concentration in mg kg^{-1} dry mass (flower and pollen) or mg L^{-1} (for nectar).

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

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

   Variables:

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

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

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

   Variables:

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

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

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

   Variables:

   The first 10 columns are identical to the sample identifiers in [Concentrations_long.txt]. The subsequent columns include concentrations of each chemical class (in mg kg^{-1} dry mass

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

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

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

   Variables:

   Species: Plant species

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

   Compound: Name of compound

   N: Number of samples

   Mean: Mean concentration (in µmol kg^{-1} dry mass for flower and pollen, or µM for nectar).

   SD: Standard deviation of concentration

   CV: Coefficient of variation

   Median: Median concentration

   First.quartile: First quartile of concentrations

   Third.quartile: Third quartile of concentrations

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

    Description: Phylogeny of sampled species, in Newick format.

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