1 # Chemistry of floral rewards: intra- and interspecific variability of nectar and pollen
2 secondary metabolites across taxa

3 ## Authors

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21 # Abstract

22 Floral chemistry mediates plant interactions with pollinators, pathogens, and herbivores,
23 with major consequences for fitness of both plants and flower visitors. The outcome of such
24 interactions often depends on compound dose and chemical context. However, chemical
25 diversity and intraspecific variation of nectar and pollen secondary chemistry are known for very
26 few species, precluding general statements about their composition. We analyzed methanol
27 extracts of flowers, nectar, and pollen from 31 cultivated and wild plant species, including
28 multiple sites and cultivars, by liquid chromatography-mass spectrometry. To depict the
29 chemical niche of each tissue type, we analyzed differences in nectar and pollen chemical
30 richness, absolute and proportional concentrations, and intraspecific variability. We hypothesized
31 that pollen would have higher concentrations and more compounds than nectar, consistent with
32 Optimal Defense Theory and pollen's importance as a male gamete. To investigate chemical
33 correlations across and within tissues, which could reflect physiological constraints, we
34 quantified chemical overlap between conspecific nectar and pollen, and phenotypic integration of
35 individual compounds within tissue types.

36 Nectar and pollen were chemically differentiated both across and within species. Of 102
37 compounds identified, most occurred in only one species. Machine-learning algorithms assigned
38 samples to the correct species and tissue type with 98.6% accuracy. Consistent with our
39 hypothesis, pollen had 23.8- to 235-fold higher secondary chemical concentrations and 63%
40 higher chemical richness than nectar. The most common secondary compound classes were
41 flavonoids, alkaloids, terpenoids, and phenolics (primarily phenylpropanoids including
42 chlorogenic acid). The most common specific compound types were quercetin and kaempferol
43 glycosides, known to mediate biotic and abiotic effects. Pollens were distinguished from nectar

44 by high concentrations of hydroxycinnamoyl-spermidine conjugates, which affect plant
45 development, abiotic stress tolerance, and herbivore resistance.
46 Although chemistry was qualitatively consistent within species and tissue types,
47 concentrations varied across cultivars and sites, which could influence pollination, herbivory,
48 and disease in wild and agricultural plants. Analyses of multivariate trait space showed greater
49 overlap across sites and cultivars in nectar than pollen chemistry; this overlap reflected greater
50 within-site and within-cultivar variability of nectar. Our analyses suggest different ecological
51 roles of nectar and pollen mediated by chemical concentration, composition, and variability.
52

53 Key words
54 Floral chemistry, plant secondary metabolites, plant-pollinator interactions, plant-microbe
55 interactions, intraspecific variation, site variation, cultivar variation, floral rewards, n-
56 dimensional hypervolume, dynamic range boxes, phenotypic integration
57

# 58 Introduction

59 Floral reward chemistry is central to ecology, mediating interactions with pollinators,
60 flower-visiting antagonists, and microbes (Strauss and Whittall 2006, Irwin et al. 2010, Huang et
61 al. 2012, McArt et al. 2014, Good et al. 2014) that influence plant reproductive success.
62 Alkaloids, phenolics, terpenoids, and proteins have been found in nectar (Baker 1977, Adler
63 2000, Nicolson and Thornburg 2007, Heil 2011, Stevenson et al. 2017). Numerous secondary
64 metabolites, including phenolic compounds (De-Melo and Almeida-Muradian 2017), alkaloids
65 (Wink 1993, Dübecke et al. 2011), and terpenoids (Flamini et al. 2003) occur in pollen. Nectar
66 chemicals can deter nectar robbers (Barlow et al. 2017), preserve nectar from spoilage (Herrera

67 et al. 2010), or act as floral filters that conserve food rewards for effective pollinators (Tiedeken
68 et al. 2016), but could also occur as a pleiotropic consequence of plant defense against foliar
69 herbivory (Adler 2000, Heil 2011). Pollen secondary chemistry is also central to plant
70 reproduction, mediating interactions with pollinators, microbes and the abiotic environment
71 (Dobson and Bergstrom 2000, Murphy 2000, Pacini and Hesse 2005, Arnold et al. 2014).
72 Floral chemistry can have effects that are organism-, dose-, and context-dependent. First,
73 many floral compounds attract pollinators, but repel ants and other non-pollinating insects
74 (Stephenson 1982, Junker and Blüthgen 2010, Galen et al. 2011, Junker et al. 2011a) and inhibit
75 microbes (Dobson and Bergstrom 2000, Huang et al. 2012, Junker and Tholl 2013). In some
76 cases, however, nectar chemicals can deter consumption by pollinators (Hagler et al. 1990,
77 Hagler and Buchmann 1993, Kessler et al. 2008, Barlow et al. 2017), with negative as well as
78 positive effects on plant reproduction in different systems (Adler and Irwin 2005, 2012, Kessler
79 et al. 2008, Thomson et al. 2015). Second, the same compound can have different consequences
80 at different doses. For example, low concentrations of caffeine in nectar improved pollinator
81 memory and increased pollination services to artificial flowers (Wright et al. 2013, Thomson et
82 al. 2015), but high concentrations of caffeine and other compounds deterred pollinators
83 (Singaravelan et al. 2005, Wright et al. 2013). Third, compounds may have different effects in
84 the context of chemical mixtures. For example, individual floral volatiles may be attractive only
85 as components of a blend (Hebets and Papaj 2005).
86 Despite the importance of chemical concentration and context in floral ecology,
87 challenges associated with chemical analysis of nectar and pollen have limited the number of
88 species for which secondary chemistry has been fully and quantitatively described. Although
89 qualitative assays of particular compound classes date back many decades (Baker 1977, Dobson

90 1988), quantitative assessments are still limited to a handful of plant species, and often target
91 particular compounds. Within species, chemical composition of floral rewards can vary at the
92 scale of individual plants, patches, and populations (Kessler et al. 2012, Egan et al. 2016), and
93 this variation can influence plant-pollinator interactions (Kessler et al. 2012, Thomson et al. 2015,
94 Barlow et al. 2017). However, even in well-studied species, little is known about the extent of—
95 or contributors to— intraspecific variation in nectar and pollen chemistry.

96 The relative costs and benefits of attraction and defense may be different for pollen than
97 for nectar. Chemical defense of pollen makes intuitive sense because pollen is the male gamete,
98 and therefore requires chemicals for development (Grienenberger et al. 2009) and for protection
99 from insects, microbes, and abiotic stressors such as desiccation and UV light (Pacini and Hesse
100 2005), whereas the sole purpose of nectar is to reward mutualists. Optimal defense theory
101 predicts that defensive chemicals are preferentially allocated to a plant's most valuable tissues
102 (Zangerl and Rutledge 1996). Therefore, we might expect pollen to have higher concentrations
103 of defensive compounds than nectar (Cook et al. 2013). Indeed, in two *Delphinium* species,
104 anther alkaloid concentrations were 150- to 3,000-fold higher than nectar concentrations, and
105 comparable to levels in leaves, flowers, and fruits (Cook et al. 2013). However, in *Chelone*
106 *glabra,* iridoid glycoside concentrations were similar in nectar and pollen (Richardson et al.
107 2016), and in *Brugmansia aurea,* alkaloid concentrations were higher in nectar than pollen
108 (Detzel and Wink 1993). These examples emphasize the need to compare differences in chemical
109 concentrations of pollen and nectar in a wider range of plant species to make general statements
110 about relative amounts in nectar versus pollen.

111 Within a single species, the chemistry of nectar and pollen may be interdependent.
112 Studies on other plant parts reported chemical correlations between leaves and fruits (Wink 1988,

113 Agrawal et al. 2002), leaves and flowers (Kessler and Halitschke 2009, Kessler et al. 2011),
114 leaves and nectar (Adler et al. 2012), and flowers and nectar (Barlow et al. 2017). These
115 correlations suggest the hypothesis that secondary chemical concentrations in floral rewards may
116 reflect pleiotropic consequences of natural selection for greater defense of leaves or flowers
117 against herbivores (Adler 2000), or of artificial selection for lower secondary compound
118 concentrations in the edible parts of cultivated plants (Wink 1988). On the other hand, many
119 compounds are exclusive to either nectar, pollen, or leaves (Kessler and Baldwin 2007, Manson
120 et al. 2012, Marlin et al. 2014, Stevenson et al. 2017), which suggests that plants can selectively
121 allocate secondary compounds both quantitively and qualitatively. This selectivity could enable
122 plants to transcend ecological costs through maintenance of tissue-specific chemical composition
123 and consequent ecological function. For example, in *Nicotiana africana,* multiple insect-
124 deterrent alkaloids occur in leaves, but these compounds are absent from nectar; this selective
125 distribution may facilitate defense against herbivores without repellence of pollinators (Marlin et
126 al. 2014). A survey that assesses overlap between nectar and pollen chemical composition across
127 a range of species would help to elucidate the extent of interdependence between nectar and
128 pollen chemistry, and the degree to which chemistry of these two plant parts can evolve
129 independently.

130 Covariation among nectar and pollen compounds, termed "phenotypic integration"
131 (Pigliucci 2003), may mediate attractiveness to and repellency of specific chemical combinations
132 (Junker et al. 2017). In other words, covariation among compounds may modulate the effects of
133 individual chemicals and concentrations. For example, in many host-seeking
134 herbivore/pollinators, individual volatiles from host plants are less attractive than multi-
135 compound blends (Bruce and Pickett 2011). In pollinators, multiple integrated signals can help

136 floral visitors learn to associate food—or toxicity—with specific visual, olfactory, and gustatory
137 stimuli (Dobson 1988, Cook et al. 2005, Junker and Parachnowitsch 2015). This learning of
138 reward-associated signal patterns, which is facilitated by within-species consistency of multiple
139 floral traits, promotes efficient resource collection by pollinators and effective pollination of
140 plants (Heinrich 1975). In pollen specifically, integrated synthesis and degradation of different
141 metabolites may be critical to development and maturation of the pollen grain and surrounding
142 pollenkitt (Pacini and Hesse 2005, Blackmore et al. 2007), and therefore essential for plant
143 fecundity. However, to our knowledge, phenotypic integration of nectar and pollen has not been
144 investigated in any species (Dobson 1988, Cook et al. 2005, Junker and Parachnowitsch 2015).
145 Thorough characterizations of floral reward secondary chemistry in a diverse array of
146 species are needed to test ecological hypotheses related to tissue-specific differences in
147 composition, constraints between nectar and pollen chemistry of the same species, and the extent
148 of intraspecific variation across genotypes and environments. Therefore, we conducted a
149 comprehensive LC-MS-based characterization of nectar and pollen secondary chemistry from 31
150 cultivated and wild plant species in 21 angiosperm families to address the following questions:
151 1. What are the common classes of secondary compounds in nectar and pollen?
152 2. How diverse are secondary metabolites in nectar and pollen across species?
153 3. How do conspecific nectar and pollen differ quantitatively and qualitatively?
154 4. Within species, how does chemistry vary across cultivars and across sites?
155 5. Within a species and tissue type, what is the level of phenotypic integration, and is
156 integration of nectar correlated with integration of pollen?
157

158 # Materials and Methods

159 ## Study sites and sampling design

160 Nectar, pollen, and flower samples (hereafter referred to as "tissue types") were collected
161 from 31 phylogenetically diverse species of flowering plants from 21 families in Massachusetts,
162 Vermont, and California, United States, in 2013 and 2014 (Appendix S1, Table S1). To
163 characterize intraspecific variation in cultivated species, we collected up to 10 samples each of 3
164 cultivars; for wild species, we collected up to 10 samples from each of 3 sites (see
165 Supplementary Appendix S1: Table S1, Supplementary Data S1: file "Species_metadata.csv",
166 and Supplementary Data S1: data files "Sites.csv" and "Cultivars.csv" for all species names,
167 sample sizes, site locations, and cultivar codes). Samples were obtained from local farms, natural
168 areas or along roadsides (after obtaining permission where necessary), and in some cases plants
169 were purchased from nurseries (*Antirrhinum majus*, two cultivars of *Dicentra eximia*, *Digitalis*
170 *purpurea*, *Eupatorium perfoliatum*, *Lobelia siphilitica*, and *Penstemon digitalis*). We chose a
171 mix of native and introduced species, with an emphasis on common species that are bee-
172 pollinated or for which we had prior knowledge of floral secondary chemistry to facilitate
173 analyses. For crop plants, we focused on species whose yield is improved by pollination
174 (Delaplane et al. 2000).

175 ## Sample collection

176 Nectar was collected with microcapillary tubes from flowers bagged in mesh for 24 h to
177 allow nectar to accumulate. For most species, nectar was pooled across individual flowers and,
178 when necessary, across plants to obtain a sufficient volume for analysis. Care was taken to avoid
179 contamination of samples with pollen. Depending on the plant species, we collected nectar either
180 from the top or bottom of the corolla after removing the flower from the plant. Each nectar

181 sample contained at least 5 µL but typically 20 µL nectar, added to 80 µL EtOH to prevent
182 spoilage. Samples were kept on ice in the field, then stored at −20 °C until lyophilization.
183 Alcohol from *Thymus vulgaris* nectar samples was evaporated at room temperature. For
184 *Antirrhinum majus* and *Rhododendron prinophyllum,* nectar was initially too viscous to collect
185 with microcapillary tubes. We added 20 µL deionized water to each flower's nectary, and
186 collected the resulting liquid several hours later. Concentrations and composition of these
187 species' nectar should therefore be interpreted with caution.

188 Pollen was collected from plants with mature, undehisced or newly dehiscing anthers. For
189 17 species, we could only obtain sufficient quantities of pollen by collecting anthers, and, for
190 *Solidago canadensis*, whole flower tops. Anther samples consisted of pollen, the pollen sac, and
191 a small amount of filament. For simplicity, we refer to both anther and pollen samples as
192 "pollen". We aimed to collect at least 5 mg per sample. In most species, pollen was pooled
193 across flowers within plants, but not across plants. Samples were lyophilized and stored at
194 −20 °C until extraction. Flowers were also collected. These were mainly used to confirm
195 identification of compounds found in nectar and pollen, but full chemical profiles were analyzed
196 for 9 species. The flower sample consisted of the entire flower for 5 species, the flower without
197 anthers for 2 species, the flower without carpel for 1 species, and the flower without calyx for 1
198 species (see Table S1 in Appendix S1).

199 Sample processing and chemical analyses

200 Lyophilized nectar was redissolved in 50 µL methanol. Pollen samples were extracted in
201 methanol as previously described (Arnold et al. 2014, Palmer-Young et al. 2016). Dried,
202 unground pollen or flowers (5–50 mg) were sonicated for 10 min with 1 mL methanol in a 2 mL

203 microcentrifuge tube, then incubated without shaking for 24 h at room temperature. Samples
204 were centrifuged for 5 min at 13,000 rpm, and the supernatant transferred to a glass vial.
205 Extracts were analyzed by liquid chromatography (LC)-Electrospray Ionisation Mass
206 Spectroscopy (ESIMS) and UV spectroscopy using a Micromass ZQ LC-MS (Waters, Elstree,
207 Herts, United Kingdom). Aliquots of nectar or pollen extract (10 µL) were injected directly onto
208 a Phenomenex (Macclesfield, Cheshire, United Kingdom) Luna C18(2) column (150 × 3.0 mm
209 inner diameter, 5 µm particle size). Samples were eluted with solvents A = MeOH, B = $H_2O$, C
210 = 1% $HCO_2H$ in MeCN with the following program: A = 0%, B = 90% at t = 0 min; A = 90%, B
211 = 0% at t = 20 min; A = 90%, B = 0% at t = 30 min; A = 0%, B = 90% at t = 31 min; solvent C
212 was a constant 10% throughout the run. Column temperature was 30 °C and flow rate 0.5 mL
213 $min^{-1}$. To facilitate compound identification, High Resolution ESIMS data were recorded on a
214 subset of samples using a Thermo LTQ-Orbitrap XL mass spectrometer (Waltham, MA, USA)
215 coupled to a Thermo Accela LC system performing chromatographic separation of 5 µl
216 injections on a Phenomenex Luna C18(2) column (150 mm × 3.0 mm i.d., 3 µm particle size).
217 The Orbitrap used the same mobile phase gradient, column temperature, and flow rate as
218 described for the ZQ-LCMS. Spectra were recorded in positive and negative modes at high
219 resolution (30,000 FWHM (full width at half maximum)).
220 Compounds were identified by comparison with mass spectra in the NIST spectral
221 database version 2.0 (Kramida et al. 2013) and, when possible, spectral comparisons with
222 authentic standards in the library at Royal Botanic Gardens, Kew, UK. Compound quantities
223 were calculated from external standard curves based on mass spectra or UV absorbance of the
224 same compound; if the compound was not available, a standard curve for a compound with the
225 same chromophore was used instead. All concentrations are given in micromolar (µmol $L^{-1}$

226 original volume for nectar, μmol $kg^{-1}$ dry mass for flower and pollen). Nectar samples were
227 typically too small to obtain accurate dry masses, which obligated the use of fresh volume-based
228 concentrations, and pollen is generally partially dehydrated at maturity (Heslop-Harrison 1979,
229 Pacini et al. 2006), suggesting that dry- and fresh mass-based concentrations are reasonably
230 similar for pollen. Most amino acids eluted in the solvent front and could not be quantified;
231 therefore, we quantified only phenylalanine and tryptophan. "Alkaloids" as defined in the figures
232 include all nitrogen-containing compounds except amino acids, including spermidine derivatives,
233 and we note here that the boundaries of the alkaloid chemical class are not universally agreed
234 upon (Hesse 2002). "Chlorogenic acids" refer to all phenylpropenoid derivatives of quinic acid.

## 235 Statistical analyses

236 All analyses were conducted in R version 3.3 for Windows (R Core Team 2014).

### 237 ***Species accumulation curves***

238 To visualize chemical diversity across species, chemical species accumulation curves
239 were computed with the vegan package v2.5, function "specaccum" (Oksanen et al. 2017), and
240 graphed with ggplot2 v2.2 (Wickham 2009), cowplot v0.9 (Wilke 2016) and ggdendro v0.1
241 (Vries and Ripley 2016). Color palettes used in figures were recommended by P. Tol (Tol 2012).
242 Within- and cross-species accumulation curves were computed separately. We assessed
243 accumulation of new compounds as more samples of a given species were analyzed within
244 species, and as additional species were analyzed across species.

### 245 ***Random forest***

246 Distinctiveness of species and tissue types were assessed by random forest machine-
247 learning algorithm (Breiman 2001). This technique determined whether samples could be
248 reliably assigned to their correct species and tissue type based on proportional composition. and

249 has been used previously to distinguish between bacterial communities (Junker and Keller 2015),
250 and different blends of floral volatiles (Junker et al. 2011b). To convert absolute concentrations
251 to proportions, the absolute concentration of each compound (in μM) within each sample was
252 divided by the sample's total concentration of quantifiable compounds. The analysis was
253 implemented in R package "randomForest" v4.6 (Liaw and Wiener 2002) with 10,000 iterations
254 and 10 randomly sampled compounds used for each split in the tree (“mtry = 10”). The out-of-
255 basket rate indicated the proportion of incorrectly assigned samples.

256 ***Non-metric multidimensional scaling (NMDS)***

257 Clustering of sample chemical compositions by species and tissue type was visualized
258 with non-metric multi-dimensional scaling (NMDS) based on Bray-Curtis distances between
259 each sample's proportional concentrations with function "vegdist" (Oksanen et al. 2017). NMDS
260 of the distance matrix was performed with function “isoMDS” (Venables and Ripley 2002).
261 Within-species ordinations were produced with function "metaMDS", which applies a Wisconsin
262 double standardization and square-root transformation to the original data matrix, then computes
263 an ordination based on Bray-Curtis distances between samples (Oksanen et al. 2017). The
264 metaMDS ordination method was not used for the full cross-species data set because it resulted
265 in convergence errors, but was used for visualization of within-species variation because it
266 allows creation of convex hulls for each within-species group.

267 ***Differences in chemical composition across tissue types, cultivars, and sites***

268 Statistical differences between tissue types, sites, and cultivars were assessed with
269 permutational MANOVA function “adonis” in R package vegan (Oksanen et al. 2017). This
270 function conducts an analysis of variance based on distance matrices using a permutation test to
271 compute F-statistics and $R^2$ values. Model $R^2$ values are calculated as the sum of squares for

272 each factor divided by the total sum of squares for the model; they indicate the proportion of
273 variance explained by each factor in the model (Oksanen et al. 2017), and are henceforth referred
274 to as "percent of variance explained". Permutational MANOVA models were run separately
275 from the NMDS ordinations, which were used for visualization. When comparing across tissue
276 types, we used proportional chemical concentrations because nectar, pollen, and flower
277 concentrations were measured on different scales (by fresh volume for nectar, but by dry mass
278 for flower and pollen). However, we used absolute concentrations when comparing within a
279 species and tissue type. We elected to use absolute concentrations because we felt that they were
280 a more direct reflection of the collected data, possibly more ecologically meaningful for
281 interactions with mutualists and antagonists (Tiedeken et al. 2016, Barlow et al. 2017), and more
282 relevant to future bioassays that test activity of specific compounds. In addition, they are
283 statistically more appropriate for many analyses (Morton et al. 2017), and robust to different
284 levels of ability to quantify co-occurring compounds.

285 ***Comparisons of absolute concentrations and chemical species richness by tissue type***

286 We used general linear mixed models, fit with the lme4 package v1.1 (Bates et al. 2015),
287 to compare absolute chemical concentrations of each chemical class in nectar and pollen. Within
288 each sample, we calculated total concentration of each compound class by summation of the
289 micromolar concentrations of each constituent compound. Median species-level concentration
290 was then computed for each chemical class and tissue type. To conform to distributional
291 assumptions of the model, only non-zero (i.e., positive) values for median concentration were
292 used. Although this approach obscures within-species variation in concentrations—which were
293 pursued in detail in subsequent analyses—our aim in this analysis was to compare in general
294 terms the concentrations found in nectar and pollen. Models used a Gaussian error distribution

295 with species-level median ln (1 + μM) concentration within each chemical class as the response
296 variable, and tissue type (nectar or pollen) as the predictor variable. Plant species was used as a
297 random effect to account for possible non-independence of nectar and pollen concentrations in
298 samples from the same species. To compare chemical concentrations for species where we
299 collected anthers rather than pure pollen, a t-test was used to compare species-level median log-
300 transformed concentrations for chemical classes that were represented in at least six species of
301 each pollen type (alkaloids, amino acids, and flavonoids). To test for differences in chemical
302 species richness between nectar and pollen, we used a generalized linear mixed model with a
303 Poisson error distribution. Chemical richness (i.e., number of compounds found) was the
304 response variable, tissue type the predictor variable, and plant species the random effect. For this
305 and subsequent lme4 models, homogeneity of variance and distribution of residuals were
306 inspected with quantile-quantile and residuals vs. fitted-value plots to check for conformation to
307 model assumptions (Bolker et al. 2009).

308 ***Trait space overlap between nectar and pollen, and across cultivars and sites***

309 We used the dynamic range boxes package v0.10 (Junker et al. 2016) to assess
310 differences in volume and overlap of multivariate chemical trait spaces (niche hypervolumes)
311 across tissue types, and across cultivars (for cultivated species) or sites (for wild species) within
312 individual species. Independent analyses were performed for each species (for comparisons
313 across tissue types), or for each species and tissue type (for comparisons across cultivars or sites).
314 The "dynamic range box" is a multivariate measure of the chemical trait space occupied by a
315 tissue type, with each compound considered as a separate dimension of the $n$-dimensional trait
316 space. The size of the range box in each dimension corresponds to the variability in
317 concentration of each compound. Hence, a voluminous range box indicates a high variability in

318 chemical concentration of the compounds. For comparisons of trait space volume between nectar
319 and pollen, proportional (rather than absolute) concentrations were used to compute the sizes of
320 range boxes. We used proportional concentrations because nectar and pollen concentrations were
321 measured on different scales (fresh volume vs. dry mass), and because large differences in
322 absolute concentrations were already obvious based on visual inspection of the data. By using
323 proportional data, the composition of tissues with differences in absolute concentration can be
324 compared. Differences in trait space volume between tissue types were tested with Gaussian
325 family linear mixed-effects models using size of the *n*-dimensional hypervolume as the response
326 variable, tissue type as the predictor variable, and plant species as a random effect.
327 Proportional overlap between groups of samples was measured as the arithmetic mean of
328 overlap in chemical concentrations for each compound, i.e., in each dimension of trait space
329 (dynamic range boxes aggregation method "mean"). Proportional overlaps are, by construction,
330 asymmetric. This is because each group of samples occupies a different total volume of trait
331 space (Junker et al. 2016). Therefore, any shared trait space may represent a relatively small
332 proportion of total trait space for a group that occupies a large trait space, but a relatively large
333 proportion of total trait space for a group with that occupies a smaller trait space. In the case of
334 chemical trait space, asymmetric overlap indicates that one type of sample encompasses a larger
335 fraction of the number of compounds found in the other group, and/or spans a larger spectrum of
336 concentrations for compounds shared between the two groups. For example, if nectar contains 1
337 compound, and pollen contains the same compound, at the same concentrations, but also 3
338 additional compounds, then pollen will occupy a larger proportion of nectar trait space than
339 nectar does of pollen. As a result, we can expect pollen to perform many of the chemically
340 mediated functions performed by nectar in terms of, e.g., the number of microbe, herbivore, or

341 pollinator species that are attracted or repelled. Further examples can be found elsewhere
342 (Kuppler et al. 2017, Junker and Larue-Kontić 2018). Asymmetry in trait space overlap was
343 tested in Gaussian family general linear mixed models that used the proportional trait space
344 overlap (i.e., shared trait space divided by total trait space) as the response variable, tissue type
345 as the predictor variable, and plant species as a random effect.

346 Coefficients of variation (CV) were calculated as the ratio of standard deviation to mean
347 concentration for each compound within each species and tissue type. The coefficient of
348 variation was calculated at two levels of resolution: the "species level" (i.e., a CV calculated for
349 each compound within each species and tissue type, without consideration of sites and cultivars)
350 and the "within-species" level (i.e., a CV calculated for each compound within each combination
351 of species, tissue type, and site or cultivar). A Gaussian family linear mixed model was fit with
352 coefficient of variation as the response variable; tissue type, level of resolution, and their
353 interaction as predictors, and species as a random effect. Post hoc pairwise comparisons with
354 Tukey adjustment for multiple tests were made using R package lsmeans v2.27 (Lenth 2016).
355 We also tested for differences in CV for compounds from different chemical classes within each
356 tissue type. Square root-transformed CV was the response variable, chemical class and tissue
357 type were the predictor variables, and plant species was the random effect to account for non-
358 independence of CV for different compounds within the same species.

359 ***Phenotypic integration***

360 We assessed the extent of covariation among different compounds within each species or
361 tissue by calculating phenotypic integration (Pigliucci 2003). High phenotypic integration
362 indicates that compounds have consistent relative concentrations; low phenotypic integration
363 indicates variability in relative concentrations. Phenotypic integration was determined for each

364 species and tissue type with at least 8 samples following previously described approaches for
365 plant volatiles (Junker et al. 2017). Pearson's correlation coefficient *r* was computed for all
366 concentrations (in μM) of all pairs of compounds. Eigenvalues were calculated for the resulting
367 correlation matrix. Raw phenotypic integration index was measured as the variance of the
368 eigenvalues with a correction for sample size (Wagner 1984, Herrera et al. 2002, Junker et al.
369 2017). This index can be compared across species and tissue types with different numbers of
370 compounds and samples.

371 In addition to calculating the integration index using complete chemical profiles, we also
372 calculated within-module phenotypic integration (Junker et al. 2017). "Modules" are groups of
373 well-correlated compounds, defined by hierarchical cluster analysis of a dissimilarity matrix of
374 chemical concentrations (R function "hclust"). The optimal number of modules was determined
375 with the "silhouette" function (Maechler et al. 2005). The mixture was divided into the optimal
376 number of modules with the "cutree" function, and phenotypic integration was computed
377 separately for each module.

378 Differences in phenotypic integration between nectar and pollen were assessed with a
379 linear mixed-effects model that used integration index as the response variable (Gaussian
380 distribution), tissue type (flower, nectar, or pollen) as the predictor variable, and species as a
381 random effect. Post hoc pairwise comparisons with Tukey adjustment for multiple tests were
382 made using R package lsmeans (Lenth 2016).Correlation between phenotypic integration of
383 nectar and pollen was assessed with a Pearson correlation for all species with at least 8 samples
384 each for both nectar and pollen.

385 To assess the effects of shared biosynthetic pathways on correlation between
386 concentrations of compound pairs, we computed all pairwise correlation coefficients for species

387 and tissue types represented by at least 8 samples. Correlations were grouped as "within-class"
388 (i.e., both compounds belonged to the same chemical class) or "between-class" (i.e., the two
389 compounds belonged to different classes). We compared correlation strength (Pearson's $r$) for
390 within- versus between-class correlations in a general linear mixed model. The model used
391 Pearson's $r$ as the response variable (Gaussian distribution); tissue type, relationship between
392 compounds (within- vs. between-class), and their interaction as predictor variables; and plant
393 species as the random effect. Pairwise contrasts were computed with Tukey correction for
394 differences between tissue types. Additional comparisons were made for the effect of chemical
395 relationship within each tissue type. Whereas the phenotypic integration analysis treated each
396 species and tissue type as one observation, this analysis used each pair of compounds within a
397 species and tissue type as one observation. As a result, it had greater power to distinguish effects
398 of tissue type and shared biosynthetic pathway on covariation among compounds.

399 ***Phylogenetic signal***

400 We tested for phylogenetic signal in total concentrations of flavonoids, alkaloids and
401 spermidines, and terpenoids in nectar and pollen, and phenotypic integration index of nectar and
402 pollen. We used function "congeneric.merge" in the pez package v1.1 (Pearse et al. 2015) to
403 obtain a time-scaled, rooted tree by extraction of our species from an unparalleled molecular
404 phylogeny of flower plants (Zanne et al. 2014). Phylogenetic signal was assessed with the
405 function "phylosig" in R package phytools v0.6 (Revell 2012), which uses a permutation test
406 (10,000 iterations) to compute Bloomberg's K (Blomberg et al. 2003).

407 ***Data availability***

408 All raw data are available in the Supplementary Materials (Data_S1.zip). Please see
409 Metadata_S1_v1.docx for a complete guide to these data files.
410

411 # Results

412 ## Patterns of composition and diversity

413 Our survey identified 102 compounds across samples of flowers (9 species), nectar (26
414 species), and pollen (28 species). The most common secondary compound classes were
415 flavonoids, alkaloids including spermidine derivatives, terpenoids, and chlorogenic acids (Fig. 1).
416 Phenylpropanoids other than chlorogenic acids consisted of acylated sugars (feruloyl glucose in
417 *Fragaria* pollen and *Silene* nectar), rosmarinic acid (*Monarda* pollen and *Thymus* nectar), and a
418 lignin glycoside (*Penstemon* pollen). Also ubiquitous were the free amino acids phenylalanine
419 and tryptophan, which were recorded in 92% of nectars and 100% of pollens. The most
420 frequently recorded compounds were the flavonoids quercetin and kaempferol glycosides, which
421 were among the five most common compounds for all three tissue types (Table 1). Many pollens
422 (71% of species) contained hydroxycinnamoyl-spermidines, mainly triscoumaroyl and
423 trisferuloyl spermidines.

424 Aside from these common compounds, cross-species diversity of flower, nectar, and
425 pollen samples was high. Most compounds were found in only a single species (Fig. 2a), and
426 new compounds were discovered with each additional species sampled (Fig. 2b). Within species,
427 however, the qualitative composition of compounds was consistent (Fig. 2c). Because
428 lyophilization likely resulted in loss of the most volatile sample components, and we could not

429 simultaneously optimize our chromatographic methods for all possible compounds, the true
430 diversity of compounds in the samples is even greater than what is depicted here. We would
431 therefore encourage the analysis of fresh samples and the use of alternative methods of
432 separation and detection, such as GC-MS, to identify additional chemical components.

433 Differentiation across species and tissue types

434 Each species and tissue type exhibited characteristically unique phytochemistry, visible
435 using NMDS multivariate ordination based on proportional composition (Fig. 3). Species and
436 tissue type explained $R^2$ = 86.6% of the variation among samples. A random forest analysis
437 assigned compounds to the correct plant species and tissue type with 98.6% accuracy.

438 On an absolute scale, pollen had much higher concentrations of secondary metabolites
439 than did nectar. Non-zero median pollen concentrations were 23.8- (terpenoids) to 235-fold
440 (flavonoids) higher than those in nectar (Fig. 4; pairwise comparisons: alkaloids: $t = 6.76$, $P <$
441 $0.001$; amino acids: $t = 9.27$, $P < 0.001$; flavonoids: $t = 12.06$, $P < 0.001$; terpenoids: $t = 2.27$, $P =$
442 $0.025$). Pollen concentrations did not differ between species where we collected anthers rather
443 than pollen (t-test $P > 0.20$ for alkaloids, amino acids, and flavonoids).

444 Flowers, nectar, and pollen also had distinct proportional composition at the level of both
445 individual compounds (perMANOVA: $F_{2, 1482} = 65.9$, $P = 0.001$, $R^2 = 0.081$, Fig. 3) and
446 compound classes ($F_{2,58} = 4.18$, $P = 0.001$, $R^2 = 0.125$). Flowers had the highest proportion of
447 flavonoids (53% of documented chemical composition) and the lowest proportion of alkaloids
448 (9%) and free amino acids (4%, Fig. 5), nectar had the highest proportion of free amino acids
449 (23%) and terpenoids (19%, Fig. 5), and pollen had the highest proportion of alkaloids and
450 spermidines (42%) and the lowest proportion of terpenoids (1%, Fig. 5). Most samples not
451 covered by these chemical classes were dominated by chlorogenic acids, which comprised 85%

452 of composition of *Helianthus* flowers, 33% of *Dicentra* nectar, 62% of *Penstemon* nectar, and 60%
453 of *Rhododendron* nectar. Both nectar and pollen of *Geranium* were dominated by tannins.
454 Of the nectars with a high (>15% documented chemistry) proportion of alkaloids and
455 spermidines, *Citrus* contained only caffeine (42% of total concentration); *Dicentra* contained
456 aporphine-, aconitine-, and isoquinoloid-type alkaloids (total 17%); *Digitalis* (41%) and
457 *Helianthus* (71%) contained acylated spermidines; *Echium* contained several pyrrolizidine
458 alkaloids as echimidine derivatives (total 81%); and *Lobelia* contained two piperidyl and one
459 pyridyl alkaloid (total 51%).
460 Pollen also differed qualitatively and quantitatively from nectar (Fig. 6). Across all
461 species, nectar and pollen shared on average only 34% of compounds. Much of this overlap was
462 due to phenylalanine and tryptophan, which were common in both nectar and pollen (Fig. 1).
463 When amino acids were excluded, the qualitative contrast was even more stark (22% nectar only,
464 57% pollen only, 22 % shared). Pollen contained, on average, 63% more compounds than did
465 nectar ($9.3 \pm 0.67$ compounds SE in pollen vs $5.7 \pm 0.51$ compounds per species in nectar, $Z =$
466 $4.41$, $P < 0.001$).

467 Chemical trait space overlap between conspecific nectar and pollen

468 We used dynamic range boxes to obtain quantitative estimates of trait space overlap
469 between nectar and pollen of the same species. Despite the higher number of compounds in
470 pollen which allowed for variation in more chemical dimensions, nectar and pollen occupied
471 similar amounts of chemical trait space based on proportional composition (nectar and pollen
472 hypervolumes both had size $0.71 \pm 0.03$ SE). There was, accordingly, little asymmetry in trait
473 space overlap between the two tissue types, with median trait space overlap of 0.14 (Fig. 7). This
474 low overlap, which reflects both the proportion of shared compounds (Fig. 7) and their relative

475 concentrations (Fig. 5), adds further evidence of phytochemical differentiation between nectar
476 and pollen within a single species. When the same analysis was run on absolute concentrations
477 rather than proportional composition, trait space overlap was near zero (Appendix S1, Fig. S2),
478 reflecting higher absolute concentrations found in pollen (Fig. 4). On the absolute scale (Fig. S2),
479 trait space overlap between nectar and pollen was greatest in species that lacked unique
480 compounds in nectar (*Impatiens, Rhododendron,* and *Verbascum;* Fig. 6). In these cases, pollen
481 trait space overlapped more than half of nectar trait space (Fig. S2).

482 Intraspecific differences across cultivars and sites

483 Across cultivars of the same species, permutational MANOVA showed significant
484 variation in chemical concentrations for 11 of 15 comparisons (2/2 species for flowers, 4/5 for
485 nectar, 5/8 for pollen). These comparisons were chosen *a priori* to reflect species with high
486 levels of replication. Cultivar explained 32.5% of intraspecific variation across samples on
487 average (Table 2A). Across sites for wild species, we found significant variation in chemical
488 concentrations for 8 of 14 comparisons (0/1 for flower, 3/5 for nectar, 5/7 for pollen), and site
489 explained $R^2$ = 21.1% of intraspecific variation across samples on average (Table 2B).

490 We analyzed intraspecific trait space overlap across cultivars and sites with dynamic
491 range boxes (Fig. 8). Linear mixed model post-hoc comparisons indicated that for both cultivar-
492 and site-level comparisons, nectar trait spaces had significantly greater overlap across within-
493 species groups than did pollen trait spaces (Cultivars: $t = 2.1$, $P = 0.039$; Sites: $t = 3.74$, $P <$
494 $0.001$).

495 The greater overlap in nectar than pollen likely reflected higher intraspecific coefficients
496 of variation (CV) in nectar chemical concentrations than in pollen or flowers (Fig. 9). Nectar
497 concentrations had on average 90% higher CV than pollen; this difference was consistent

498 whether CV was calculated based on variation in concentrations at the species level ($t = 10.50$, $P$
499 $< 0.001$) or the within-species level (i.e., variation within sites and cultivars, $t = 12.77$, $P <$
500 $0.001$). Accounting for sites and cultivars significantly reduced CV by 14% relative to when
501 variation was calculated at the species level (Species-level CV = 0.82 ± 0.04 SE; Within-species
502 CV = 0.70 ± 0.04 SE, $t = -4.17$, $P < 0.001$). No significant effect of chemical class on CV was
503 found for flowers, nectar, or pollen (Class effect, $F_{4, 310} = 1.77$, $P = 0.13$; $P > 0.20$ for all Tukey-
504 corrected pairwise contrasts between classes within tissue types).

505 Domesticated apple (*Malus domestica*) exemplified chemical separation across tissue
506 types and cultivars within a single species (Fig. 10). Flowers, nectar, and pollen were completely
507 distinguished from one another, and tissue type explained $R^2 = 81\%$ of variation across samples
508 (MANOVA $F_{2, 84} = 207.4$, $P = 0.001$, Fig. 10A). Within nectar and within pollen, cultivars
509 exhibited almost complete separation in chemical trait space (nectar: $F_{2, 29} = 8.58$, $P = 0.001$, $R^2$
510 $= 0.39$; pollen: $F_{2, 29} = 13.93$, $P = 0.001$, $R^2 = 0.51$, Fig. 10B, C).

511 Phenotypic integration

512 Chemical mixtures were generally less integrated in flowers (least squares mean 9.91 ±
513 4.59 SE) than in nectar (21.30 ± 2.96 SE) and pollen (21.53 ± 3.17 SE), but these differences
514 were not statistically significant ($F_{2, 39.6} = 2.37$, $P = 0.10$, Fig. 11A). However, integration of
515 chemical modules varied significantly across tissue types ($F_{2,36.4} = 4.31$, $P = 0.021$). Within-
516 module integration was significantly higher in nectar (46.1 ± 4.30 SE) than in flowers (26.2 ±
517 6.26 SE, $t = 2.76$, $P = 0.024$, Fig. 11B). Within-module integration of pollen was intermediate
518 (35.33 ± 4.01 SE) and not significantly different from either nectar ($t = -1.98$, $P = 0.13$) or
519 flowers ($t = 1.26$, $P = 0.42$, Fig. 11B). Integration of nectar and pollen were not significantly
520 correlated ($t = -0.538$, $P = 0.60$, Fig. 11C).

521 Consideration of individual species showed that compounds tended to cluster by
522 biosynthetic relatedness. For example, in *Malus domestica* nectar (Fig. S3), there were seven
523 pairwise correlations with *r* values above 0.80. All were between pairs of flavonoids or a
524 flavonoid and chlorogenic acid (Fig. S3). Chlorogenic acid is an ester of quinic and caffeic acids.
525 Caffeic acid, like other flavonoids, is synthesized via the phenylpropanoid pathway (Rice-Evans
526 et al. 1996). These shared metabolic precursors may explain correlations between concentrations
527 of chlorogenic acid and flavonoids. Likewise, in *Digitalis purpurea* pollen, nine of the 10
528 strongest correlations (highest *r*-values) were between chemically similar spermidine derivatives
529 (Fig.'s S4, S5).
530 Analysis of all pairwise correlations between compounds indicated stronger positive
531 correlations for within-class (i.e., both compounds belonged to the same chemical class) than
532 between-class compound pairs ($F_{2,1238} = 12.35$, $P < 0.001$). Within each tissue type, the effect of
533 chemical relatedness was significant for both nectar ($t = 4.26$, $P < 0.001$) and for pollen ($t = 4.59$,
534 $P < 0.001$). The effect of chemical relatedness did not vary significantly across tissue types
535 (Relationship x Type interaction: $F_{2,1280} = 2.28$, $P = 0.10$), although the estimate for the effect of
536 chemical relatedness tended to be higher for nectar (0.21 ± 0.043 SE) than for pollen (0.13 ±
537 0.028 SE, Fig. S6). Across all compound pairs, correlation coefficients were higher in nectar
538 than in pollen (estimate of differences: 0.11 ± 0.030 SE, $t = 3.82$, $P < 0.001$), and marginally
539 higher in pollen than in flowers (estimate 0.075 ± 0.032 SE, $t = 2.36$, $P = 0.048$, Fig. S6).
540

541 Phylogenetic signal

542 No significant phylogenetic signal was found for median total concentrations of alkaloids,
543 amino acids, flavonoids, or terpenoids in nectar or pollen (Bloomberg's K randomization test, K

544 = 1.09, $P = 0.07$ for nectar terpenoids, $P > 0.25$ for all others), nor for number of compounds or
545 phenotypic integration of nectar or pollen (Bloomberg's K randomization test, $P > 0.45$ for all).
546

547 # Discussion

548 In the most comprehensive qualitative and quantitative cross-taxon description of nectar
549 and pollen chemistry to date, we found marked differentiation of nectar and pollen across species,
550 clear quantitative and qualitative distinction between nectar and pollen of the same species, and
551 intraspecific variation in both nectar and pollen chemistry across cultivars and sites. Pollen had
552 higher concentrations and more compounds than did nectar, consistent with Optimal Defense
553 Theory. These data provide a new level of insight into the secondary chemistry of nectar and
554 pollen, and provide a framework for future research on the heritability, ontogeny, and ecological
555 consequences of chemical variation in floral rewards.

556 ## Common compounds and potential functions

557 Most secondary chemicals were from a few common classes—flavonoids, alkaloids,
558 chlorogenic acids, and terpenoids. Flavonoids are widespread among plants and tissue types
559 (Taylor and Grotewold 2005). Flavonoids in our samples—mainly quercetin and kaempferol
560 glycosides—were among the most frequently recorded compounds in flowers, nectar, and pollen,
561 where they may mediate both biotic and abiotic interactions. First, flavonoids can serve primary
562 functions as plant growth regulators (Taylor and Grotewold 2005). For example, flavonoids can
563 govern pollen fertility (Mo et al. 1992). These growth-regulating properties could also contribute
564 to the allelopathic activity of flavonoids against microbes and insects (Taylor and Grotewold
565 2005), and inhibit germination of competing, heterospecific pollen (Murphy 2000). Second,

566 flavonoids can act as antioxidants, which could improve tolerance of pollen grains to abiotic
567 stressors that may reduce viability (Schoper et al. 1986). While hydroxycinnamic acids have
568 superior absorption of UVB irradiation, flavonoids also absorb wavelengths in the UV spectrum,
569 and accumulation is stimulated by both visible and UV light exposure, as well as by other abiotic
570 stressors that generate reactive oxygen species (Agati and Tattini 2010). The high flavonoid
571 concentrations in our pollen samples (median non-zero concentrations > 14,000 μM) are similar
572 to those reported for leaves grown in full sunlight (Agati and Tattini 2010), which suggests that
573 pollen has comparable abilities to withstand potentially damaging radiation. Third, flavonoids
574 can regulate biotic interactions with mutualists and antagonists. Flavonoids generally reduce
575 herbivory and infection (Karpinski et al. 2003, Cushnie and Lamb 2005). In multiple plant
576 species, high constitutive and inducible leaf flavonoid content has been correlated with insect
577 and pathogen resistance (Treutter 2005). Protection of nectar and pollen from microbial and
578 insect antagonists may help to preserve these resources for plant reproduction. Flavonoids may
579 also be an honest signal for insects with vision in the UV spectra; nectar with flavonoids
580 fluoresces under UV light (Thorp et al. 1975) and could visually guide pollinators to rewarding
581 flowers.
582 Alkaloids and spermidines in our samples were dominated by the spermidine conjugates
583 in pollen. Spermidines were generally esterified to one or more cinnamic acids, e.g.,
584 triscoumaroyl and trisferuloyl spermidines. These compounds likely play both developmental
585 and ecological roles. Found in all plants, hydroxycinnamoyl spermidines are thought to have
586 phytohormone-like roles in plant development and abiotic stress tolerance; synthesis is induced
587 by exposure to heat, UV, salinity, and dessication (Gill and Tuteja 2010) as well as by herbivory
588 (Bassard et al. 2010). In *N. attenuata,* foliar concentrations of 520 μM reduced herbivore growth

589 rates by 50%; the median nonzero alkaloid concentration in our pollen samples (23,000 μM) was
590 44-fold higher (Kaur et al. 2010).

591 Both developmental and ecological functions of spermidines are likely important for
592 pollen, which must endure abiotic stresses that can reduce viability (Schoper et al. 1986) before
593 it germinates to fertilize ovules. In *Arabidopsis*, deficiency of spermidine conjugates caused
594 pollen grains to become deformed, indicating the developmental role of these compounds
595 (Grienenberger et al. 2009). Prior to germination, pollen may be exposed to insects and
596 pathogens, which can be inhibited by spermidines (Walters et al. 2001), and UV irradiation,
597 which can be absorbed by spermidines (Gill and Tuteja 2010). In *Arabidopsis* pollen,
598 hydroxycinnamoyl spermidines are concentrated in the pollen coat, an ideal location to function
599 in UV absorption and inhibition of insects and pathogens (Grienenberger et al. 2009). Despite
600 their multi-functionality and developmental importance, nearly one-third of our tested pollens
601 lacked spermidines, suggesting that these compounds are dispensable for some species.

602 We recorded spermidine conjugates in nectar of *Helianthus annuus* and *Digitalis*
603 *purpurea*. Spermidines have not been previously reported in nectar, although they have been
604 found in xylem and phloem, and the enzymes that catalyze their synthesis have been found in
605 nectar (Friedman et al. 1986, Shah et al. 2016). In *H. annuus* and *D. purpurea*, nectar and pollen
606 contained the same spermidine conjugates, suggesting that spermidines in nectar could be a
607 result of contact with pollen. Regardless of their origin, the occurrence of spermidines in nectar
608 may still be ecologically relevant to organisms that interact with these species.

609 Overall, alkaloids comprised >15% of recorded metabolite concentrations in the nectar of
610 6 of 26 species. Nectar alkaloids included caffeine in *Citrus;* aconitine and isoquinoline alkaloids
611 in *Dicentra*, pyrrolizidine alkaloids in *Echium*, and piperidine and pyridyl alkaloids in *Lobelia*.

612 Alkaloids have antimicrobial and insect-deterrent properties (Wink 1993), which may defend
613 nectar against bacteria and non-pollinating insects that can deplete floral rewards (Good et al.
614 2014, Barlow et al. 2017). Whether nectar alkaloids are beneficial for pollination *per se* remains
615 a matter of debate. Effects may depend on ecological context. For example, alkaloids reduced
616 plant reproduction in *Gelsemium sempervirens* through deterrence of pollinators (Adler and
617 Irwin 2005), but increased outcrossing in *Nicotiana attenuata* by enforcement of modest
618 drinking behavior (Kessler et al. 2008), and had dose-dependent benefits for pollination of
619 artificial flowers (Thomson et al. 2015). Nectar alkaloids could benefit pollination when they are
620 preferred over alkaloid-free solutions by honey and bumble bees (Singaravelan et al. 2005,
621 Thomson et al. 2015); enhance pollinator memory and associative learning (Wright et al. 2013,
622 Baracchi et al. 2017); or deter nectar robbers, which preserves rewards for pollinators (Barlow et
623 al. 2017). For example, 10 µM caffeine in nectar of artificial flowers resulted in more pollination
624 from bumble bees than 100 µM or no caffeine (Thomson et al. 2015), and 129 µM caffeine at
625 artificial feeders increased recruitment of honey bees (Couvillon et al. 2015). The caffeine
626 concentrations in our *Citrus* nectar samples (median 25.6 µM, interquartile range 14.7-50.4 µM)
627 are within the concentration range that may benefit pollination by several of these mechanisms.

## 628 Differentiation across species

629 Across the species surveyed, each species and tissue type was chemically unique. Most
630 compounds were recorded only once, and new compounds were recorded with each additional
631 species sampled (Fig. 2). This is likely due, at least in part, to our phylogenetically diverse set of
632 species, which came from 21 plant families. Despite quantitative variation within species,
633 random forest (machine-learning) algorithms assigned samples to their correct taxon and tissue
634 type with over 98% accuracy. Each tissue type within a species was characterized by a unique

635 combination of chemicals not found in any other species, or even in other floral tissues of the
636 same plant. Nectar and pollen of the same species were chemically distinct in proportional
637 composition, absolute concentrations, and chemical identity, all of which suggest chemical
638 regulation to accomplish specific ecological functions. These results, which are consistent with
639 prior surveys that revealed high floral phytochemical diversity (Junker et al. 2011a, Courtois et al.
640 2016), suggest that nectar and pollen chemistry of the same plant can take independent
641 evolutionary trajectories. Prior studies of floral volatiles and nectar have shown lower levels of
642 insect-repellent compounds in species that benefit from animal pollination, which is thought to
643 reflect the high costs of pollinator deterrence for obligate outcrossers (Abel et al. 2009, Adler et
644 al. 2012). Future studies should test whether pollen exhibits the same chemical trends as these
645 other tissue types, with reduced levels of defensive chemicals in pollinator-dependent species.

646

647 Pollen and nectar of the same species had distinct phytochemistry

648 Differences between nectar and pollen are exemplified by alkaloids and spermidines,
649 where concentrations in nectar were orders of magnitude lower than those in pollen, consistent
650 with the lower concentrations of alkaloids in *Nicotiana* spp. nectar relative to leaves and flowers
651 (Adler et al. 2012). In our samples, caffeine concentrations in *Citrus* nectar were 2,900-fold
652 lower than those in pollen. In a variety of *Coffea* and *Citrus* spp., nectar caffeine concentrations
653 were always below the taste thresholds of honey bees, but were sufficient to enhance honey bee
654 memory for floral cues associated with a reward (Wright et al. 2013). Many alkaloids and
655 spermidines present in pollen were absent from nectar, which indicates that the presence of
656 alkaloids in nectar is not necessarily constrained by their presence in other tissues, at least in

657 pollen. This finding is consistent with previously documented lack of nectar alkaloids in
658 *Nicotiana africana* (Marlin et al. 2014), and nectar limonoids in *Citrus sinensis* (Stevenson et al.
659 2017). Generally, our results suggest selection for lower alkaloid levels in nectar to minimize
660 pollination-related costs (Adler et al. 2012), and are consistent with the disposability of nectar—
661 a dedicated floral reward—relative to the male gametes in pollen (Hargreaves et al. 2009).

662 We still have much to learn about mechanisms of nectar production, and the degree to
663 which nectar chemistry reflects secondary metabolism in other parts of the plant (Heil 2011,
664 Stevenson et al. 2017). Whereas pollen development, including the production of pollenkitt, have
665 been described in detail (Heslop-Harrison 1979, Pacini and Hesse 2005, Blackmore et al. 2007),
666 including at the molecular level (Grienenberger et al. 2009, Yonekura-Sakakibara et al. 2014),
667 the molecular basis of sugar transport in nectar was only elucidated recently (Lin et al. 2014).
668 Greater knowledge of nectar production would help to clarify physiological constraints on
669 chemical composition. Correlations between nectar and corolla chemistry (Cook et al. 2013,
670 Richardson et al. 2016, Barlow et al. 2017) may relate to the mode of nectar secretion. For
671 example, in Ranunculaceae, some species secrete nectar through cuticular microchannels,
672 whereas others release nectar by rupture of epidermal cells that line the nectary (Antoń and
673 Kamińska 2015). The latter mechanism releases the entire cytoplasmic contents into the nectary,
674 which could be a less selective process than secretion through microchannels (Antoń and
675 Kamińska 2015). Constraints between nectar and phloem chemistry may reflect sites of
676 secondary compound synthesis. For example, locally synthesized or adsorbed nectar chemicals
677 (Raguso 2004) might be less constrained by phloem chemistry relative to compounds that are
678 synthesized systemically and transported via xylem or phloem. For remotely synthesized
679 compounds, pleiotropic costs of foliar defenses could impose a lower limit on nectar

680 concentrations (Adler et al. 2012), whereas autotoxicity could impose an upper limit (Baldwin
681 and Callahan 1993). We also do not know to what extent nectar composition is environmentally
682 versus genetically determined (Mitchell 2004). Future study on regulation of nectar synthesis and
683 provisioning with phytochemicals in diverse species will indicate which phytochemicals are
684 constrained by versus independent from chemistry of other plant parts. Overall, our data suggest
685 strong independence of nectar and pollen secondary chemistry. They indicate that nectar
686 chemistry can evolve separately from that of pollen, both in terms of composition and
687 concentration.

688 Intraspecific variation across cultivars and sites

689 Across cultivars and sites, within-species nectar and pollen phytochemistry was
690 qualitatively conserved but quantitatively heterogeneous. Intraspecific differences were not only
691 statistically significant, but also of large magnitude. A median pair of cultivars or sites shared
692 less than two-thirds of chemical trait space for nectar and less than half for pollen, with possible
693 implications for disease resistance, herbivore resistance, and pollinator behavior, as discussed
694 below.

695 We found the clearest differentiation in chemistry across cultivars. This likely reflects
696 consequences of strong artificial selection, as well as the homogeneous age and genetic
697 background of cultivated plants relative to those in the wild, although we cannot exclude some
698 effects of environmental factors or maternal environment. In other work, nectar traits such as
699 volume and sugar composition had high heritability, but were generally measured in greenhouse
700 rather than field settings (Mitchell 2004). Genetic control over non-sugar nectar constituents has
701 not been explicitly addressed except with transformed plant lines (Kessler and Baldwin 2007),
702 and no other study to our knowledge has examined intraspecific variation in pollen composition.

703 Inter-cultivar variation in chemistry suggests a need for future study on how cultivars vary in
704 attractiveness to managed and wild pollinator communities, particularly in species where yields
705 are pollen-limited (Garibaldi et al. 2013). In addition, cultivar differences illustrate how
706 pleiotropic effects of selection on non-floral traits can alter nectar and pollen chemistry, which
707 may complicate theories of floral phytochemical evolution in wild species.

708 We found less consistent, but still statistically significant, variation across sites in
709 chemistry of wild species. These differences may reflect genetic or environmental effects, or
710 their interactions. Genetic differences across populations likely explain some differences
711 (Mitchell 2004). For example, deterministic effects of genetics on floral traits are demonstrated
712 by the within-species consistency of floral morphology (Heinrich 1975), the low inducibility of
713 floral chemical defenses relative to those of other tissues (Zangerl and Rutledge 1996), and the
714 qualitative consistency of conspecific nectar amino acid samples from widely separated sites
715 (Baker and Baker 1977). However, the environment can also have profound effects on floral
716 traits. These include scent emission (Dötterl et al. 2009, Kessler et al. 2011), floral color morph
717 (Baker and Baker 1977), diurnal rhythm of flowering (Kessler et al. 2010), and pollinator
718 attraction (Kessler et al. 2011). Nectar traits can also be influenced by the environment. For
719 example, nectar grayanotoxin concentrations were correlated with heat load across
720 *Rhododendron* populations (Egan et al. 2016), and nectar alkaloid levels were experimentally
721 modified by herbivory and nutrient addition (Adler et al. 2006). Each of these studies
722 demonstrates ways in which the environment can influence floral chemistry. Finally, genotype
723 by environment interactions have been found for nectar production rates (Boose 1997), and could
724 exist for nectar and pollen chemistry as well. Future experiments using plant genotypes grown
725 under different conditions could clarify the relative importance of genetics and environment to

726 nectar and pollen chemistry. Additional experiments could test the inducibility of secondary
727 chemical concentrations in response to environmental cues including fertilization, herbivory, and
728 pathogen challenge.

729 Chemical differences between sites have implications for both pollinator behavior and
730 plant evolution. Site-specific chemistry could alter pollinator foraging preferences, potentially
731 shaping inter- and intraspecific resource competition, nest site selection, and population
732 dynamics. Individual bumble bees, in particular, have a broad foraging range but consistent site-
733 and plant-specific preferences that are retained over multiple weeks (Heinrich 1976, Ohashi and
734 Thomson 2009). For plants, optimal chemistry of floral rewards may differ in response to abiotic
735 conditions; pollinator availability, effectiveness, and chemical sensitivity (Tiedeken et al. 2014);
736 and presence of non-pollinating insects and pathogens. Local selective pressures that act on pre-
737 existing variation could create chemical divergence across populations, as found in
738 *Rhododendron ponticum* (Egan et al. 2016), which could in turn shape flower-insect interaction
739 networks (Tiedeken et al. 2016). A related question is the scale at which pollinators make
740 foraging decisions. Nectar phytochemical concentrations can influence local interactions (Adler
741 and Irwin 2005, Kessler and Baldwin 2007), but can also vary by orders of magnitude among
742 flowers of a single inflorescence (Kessler et al. 2012). It is unknown whether pollinators can
743 detect inter-site differences against this background of within- and between-individual variation.
744 If they can, differences in chemical concentrations could be one driver of preferences for plant
745 species and foraging sites.

746 Phenotypic integration

747 Our results indicate that nectar (mean integration index = 21.5) and pollen (mean 21.3)
748 have levels of integration that are similar to those of leaf volatiles (mean 22.0) , which were

749 more integrated than flower volatiles (mean 10.8 (Junker et al. 2017)) and flower methanolic
750 extracts (mean 9.9 (this study)). The generally low levels of integration in flowers may reflect
751 several factors. First, flowers are physiologically complex, including include petals, corolla,
752 stigma, and anthers that differ in chemical composition (Flamini et al. 2002). This heterogeneity
753 may reduce the chemical integration of the pooled floral tissue. Second, flowers undergo rapid
754 chemical changes during maturation, bloom, and senescence that result in different chemical
755 ratios in samples that differ slightly in developmental stage (Schiestl et al. 1997). Third, flowers
756 may accomplish ecological functions with single compounds, which may lessen the need for
757 integration of the whole flower. For example, variation in the floral volatile 2-phenylethanol was
758 sufficient to alter both pollinator attraction and ant repellence in *Polemonium viscosum* (Galen et
759 al. 2011). Likewise, a single compound—the monoterpenoid linalool—was sufficient to alter
760 growth of some bacteria from *P. digitalis* flowers (Burdon et al. 2018).

761 In our study, correlations between different compounds were partly explained by
762 biosynthetic similarity. Overall, concentrations of compound pairs that belonged to the same
763 chemical class were more strongly correlated than were pairs that belonged to different chemical
764 classes (Fig. S6). For example, in *Malus domestica* nectar, the seven strongest correlations were
765 all between pairs of flavonoids or a flavonoid and chlorogenic acid (Fig. S3). All of these
766 compounds are synthesized via the phenylpropanoid pathway (Rice-Evans et al. 1996). Similarly,
767 in *Digitalis purpurea* pollen, 9 of the 10 strongest correlations were between spermidine
768 derivatives (Fig.'s S4, S5). These findings are consistent with prior analyses of phenotypic
769 integration in scent bouquets, where biosynthetic similarity between compounds was correlated
770 with strength of covariation (Junker et al. 2017).

771 On the other hand, both *Malus* and *Digitalis* (Fig.'s S3-S5)*,* as well as the entire dataset
772 (Fig. S6), showed numerous strong correlations between compounds from different classes.
773 These correlations could reflect similar solubilities or transport (in nectar), or selection for
774 specific chemical ratios or combinations that function in pollinator attraction, defense, or
775 development. Multimodal signals that combine scents with color can attract and condition
776 pollinators to rewards (Junker and Parachnowitsch 2015). For example, carbon dioxide, floral
777 volatiles, and leaf volatiles all functioned in concert with visual cues to attract adult *Manduca*
778 *sexta* to artificial flowers*;* in females, carbon dioxide was only attractive against a background of
779 host-plant leaf volatiles (Goyret et al. 2008). In nectar, which exhibited the highest within-
780 module integration (Fig. 11) and strongest average correlation between compound pairs (Fig. S6),
781 consistent secondary chemical ratios could promote pollinator constancy by allowing pollinators
782 to associate species-specific flavors with food rewards. This hypothesis has also been suggested
783 to explain the consistency of amino acid composition of conspecific nectars (Baker and Baker
784 1977) and the morphological similarity of conspecific flowers (Heinrich 1975). Further research
785 is needed to determine the primary and secondary significance of correlations between secondary
786 compounds in nectar and pollen, and how covariation is differentially regulated in the two tissue
787 types. Manipulative studies are necessary to determine whether damage by herbivores reduces
788 the level of integration in nectar and pollen, as found for leaf volatiles (Junker et al. 2017).
789 There was no significant correlation between the integration of a species' nectar and the
790 integration of its pollen. This is an important result, because it indicates that forces acting on
791 phenotypic integration of nectar may be different from those acting on phenotypic integration of
792 pollen, and that integration of these two tissues may be independently regulated. For example,
793 *Malus domestica* had the second highest integration of all species for nectar (PI = 49.4), but the

794 ninth lowest integration for pollen (PI = 12.7). Likewise, *Catalpa speciosa* had second highest
795 integration for pollen (47.3), but below average integration for nectar (10.0). Together with the
796 low levels of chemical overlap between nectar and pollen, this finding emphasizes that secondary
797 chemistry of conspecific nectar and pollen can chemically diverge from one another. This
798 divergence may reflect the unique selective pressures exerted on their different ecological roles.

799 This description of nectar and pollen secondary chemistry complements an expanding
800 knowledge of scent- and morphology-mediated interactions between flowers, insects, and
801 microbes (Junker and Blüthgen 2010, Junker et al. 2011a, Junker and Parachnowitsch 2015).
802 Nectar and pollen secondary chemistry mediates interaction with pollinators, floral antagonists,
803 and pathogens, and thereby influences the ecology and evolution of many plant communities.
804 Our analyses summarize the variety of chemical strategies used in floral food rewards of diverse
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806

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1159

1160 # Tables

1161 **Table 1.** Most common compounds by tissue type.

| Type | Compound | Presences | Prevalence (%) |
|---|---|---|---|
| **A. Flower (9 spp)** | | | |
| | Quercetin-O-glycoside | 8 | 88.9 |
| | Chlorogenic acid | 6 | 66.7 |
| | Kaempferol-O-glycoside | 6 | 66.7 |
| | Tryptophan | 5 | 55.6 |
| | Acylated sugar | 4 | 44.4 |
| **B. Nectar (26 spp)** | | | |
| | Phenylalanine | 24 | 92.3 |
| | Tryptophan | 17 | 65.4 |
| | Quercetin-O-glycoside | 9 | 34.6 |
| | Chlorogenic acid | 6 | 23.1 |
| | Kaempferol-O-glycoside | 5 | 19.2 |
| **C. Pollen (28 spp)** | | | |
| | Phenylalanine | 27 | 100 |
| | Tryptophan | 25 | 92.3 |
| | Kaempferol-O-glycoside | 19 | 67.9 |
| | Quercetin-O-glycoside | 14 | 50.0 |
| | Triscoumaroyl spermidine | 11 | 39.3 |

1162

1163 **Table 2.** Results of permutational MANOVA tests for intraspecific variation in chemistry across
1164 cultivars and sites. Bold print indicates P < 0.05. N: number of samples. Df(n): numerator
1165 degrees of freedom. Df(d): denominator degrees of freedom.

| **A. Cultivars** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Species** | **Type** | **N** | **Cultivars** | **F** | **Df(n)** | **Df(d)** | **P** | **$R^2$** |
| ***Helianthus annuus*** | **Flower** | **40** | **4** | **2.44** | **3** | **36** | **0.023** | **0.17** |
| ***Malus domestica*** | **Flower** | **29** | **3** | **11.29** | **2** | **26** | **0.001** | **0.46** |
| ***Citrus sinensis*** | **Nectar** | **23** | **2** | **13.09** | **1** | **21** | **0.001** | **0.38** |
| *Cucurbita pepo* | Nectar | 45 | 3 | 1.77 | 2 | 42 | 0.062 | 0.08 |
| ***Digitalis purpurea*** | **Nectar** | **30** | **3** | **1.96** | **2** | **27** | **0.02** | **0.13** |
| ***Helianthus annuus*** | **Nectar** | **20** | **4** | **5.99** | **3** | **16** | **0.001** | **0.53** |
| ***Malus domestica*** | **Nectar** | **30** | **3** | **8.58** | **2** | **27** | **0.001** | **0.39** |
| ***Citrus sinensis*** | **Pollen** | **23** | **2** | **19.84** | **1** | **21** | **0.001** | **0.49** |
| *Cucurbita pepo* | Pollen | 32 | 3 | 1.77 | 2 | 29 | 0.138 | 0.11 |
| *Digitalis purpurea* | Pollen | 17 | 3 | 0.57 | 2 | 14 | 0.913 | 0.08 |
| ***Fragaria ananassa*** | **Pollen** | **30** | **3** | **7.78** | **2** | **27** | **0.001** | **0.37** |
| *Helianthus annuus* | Pollen | 30 | 3 | 0.91 | 2 | 27 | 0.406 | 0.06 |
| ***Malus domestica*** | **Pollen** | **30** | **3** | **13.93** | **2** | **27** | **0.001** | **0.51** |
| ***Persea americana*** | **Pollen** | **30** | **3** | **86.00** | **2** | **27** | **0.001** | **0.86** |
| ***Prunus dulcis*** | **Pollen** | **30** | **3** | **4.88** | **2** | **27** | **0.007** | **0.27** |
| | | | | | | | | |
| **B. Sites** | | | | | | | | |
| **Species** | **Type** | **N** | **Sites** | **F** | **Df(n)** | **Df(d)** | **P** | **$R^2$** |
| *Geranium maculatum* | Flower | 21 | 3 | 2.03 | 2 | 18 | 0.1 | 0.18 |
| *Geranium maculatum* | Nectar | 19 | 2 | 0.72 | 1 | 17 | 0.508 | 0.04 |
| ***Impatiens capensis*** | **Nectar** | **31** | **3** | **2.55** | **2** | **28** | **0.036** | **0.15** |
| **Kalmia latifolia** | **Nectar** | **20** | **3** | **4.16** | **2** | **17** | **0.004** | **0.33** |
| **Linaria vulgaris** | **Nectar** | **31** | **4** | **1.85** | **3** | **27** | **0.031** | **0.17** |
| *Lythrum salicaria* | Nectar | 33 | 3 | 0.96 | 2 | 30 | 0.444 | 0.06 |
| *Verbascum thapsus* | Nectar | 27 | 2 | 2.14 | 1 | 25 | 0.101 | 0.08 |
| ***Geranium maculatum*** | **Pollen** | **30** | **4** | **4.70** | **3** | **26** | **0.001** | **0.35** |
| ***Impatiens capensis*** | **Pollen** | **24** | **3** | **12.14** | **2** | **21** | **0.001** | **0.54** |
| ***Kalmia latifolia*** | **Pollen** | **15** | **3** | **2.97** | **2** | **12** | **0.033** | **0.33** |
| ***Linaria vulgaris*** | **Pollen** | **32** | **5** | **2.24** | **4** | **27** | **0.046** | **0.25** |
| *Solanum carolinense* | Pollen | 28 | 3 | 2.18 | 2 | 25 | 0.07 | 0.15 |
| ***Solidago canadensis*** | **Pollen** | **25** | **3** | **3.41** | **2** | **22** | **0.014** | **0.24** |
| *Verbascum thapsus* | Pollen | 29 | 2 | 2.70 | 1 | 27 | 0.091 | 0.09 |

1166 # Figure captions

1167 **Fig. 1.** Prevalence of major compound classes in flowers (9 species), nectar (26 species), and
1168 pollen (28 species). Alkaloids include all nitrogen-containing compounds except the amino acids,
1169 including spermidine derivatives. Chlorogenic acids refer to all phenylpropenoid derivatives of
1170 quinic acid.

1171 **Fig. 2.** Chemical diversity in nectar, pollen, and floral samples. (a) Most compounds were found
1172 in only a single species. Flower samples: solid yellow line. Nectar samples: dotted red line.
1173 Pollen samples: dashed blue line. (b) Chemical species accumulation curves indicated that new
1174 compounds were found for each additional species sampled. Neither nectar nor pollen
1175 accumulation curves approached saturation. Lines and shaded bands show mean ± standard
1176 deviation. (c) Within-species chemical species accumulation curves. All compounds within each
1177 species were found after analysis of the first few samples for both nectar (solid red lines) and
1178 pollen (dashed blue lines).

1179 **Fig. 3.** Non-metric multidimensional scaling-based ordination of Bray-Curtis distances between
1180 flower (circles), nectar (triangles), and pollen (squares) samples. Samples clustered strongly by
1181 species and tissue type, with significant differences between tissue types ($F_{2, 1482} = 65.9$, $P =$
1182 $0.001$). Random forest discriminant analysis showed that 98.6% of samples could be assigned to
1183 the correct species- tissue type combination. Ellipses show 95% confidence bands for flower
1184 (solid line), nectar (dotted line), and pollen (dashed line). Colors indicate different species.
1185 Ordination is based on proportional chemical composition.

1186 **Fig. 4.** Absolute ln (μM + 1) concentrations of all compound classes were 23.8- to 235-fold
1187 lower in nectar (red circles) than in pollen (blue triangles). Vertical lines show median non-zero
1188 concentrations in nectar (solid red line) and pollen (dashed blue line). Points and error bars show
1189 means and 95% confidence intervals. Where no error bars are visible, either all concentrations
1190 are zero or error bars are smaller than symbols for points. Concentrations are in μmol $L^{-1}$ for
1191 nectar and μmol $kg^{-1}$ dry mass for pollen. Alkaloids include all nitrogen-containing compounds
1192 except the amino acids, including spermidine derivatives.

1193 **Fig. 5.** Median proportional compositions of flower, nectar, and pollen samples by chemical
1194 class. Bar chart in (a) shows median proportions across all species (b). Tissue types differed
1195 significantly in class-wise proportional composition (permutational MANOVA on median
1196 proportional composition for each species and tissue type, $F_{2,58} = 4.18$, $P = 0.001$). Tissue type
1197 explained 12.5% of variance in proportional composition across species. Alkaloids include all
1198 nitrogen-containing compounds except the amino acids, including spermidine derivatives.

1199 **Fig. 6.** Number of quantifiable compounds detected in nectar, pollen and both nectar and pollen.
1200 (a) Pie chart indicates totals aggregated across all species. (b) Individual species. Pollen
1201 contained on average 63% more compounds than did nectar (9.3 ± 0.67 compounds SE vs 5.7 ±
1202 0.51 compounds per species, $\chi^2 = 19.5$, Df = 1, P < 0.001).

1203 **Fig. 7.** Nectar and pollen exhibited similar levels of variability in proportional composition, with
1204 no significant asymmetry in trait space overlap of one tissue type by the other. Graphs show
1205 dynamic range boxes-based trait space volume of nectar (red bars) and pollen (blue bars), and
1206 overlap between the two types. (a) Median hypervolume size and (b) proportional hypervolume
1207 overlap, aggregated across species. (c) Hypervolume size and (d) proportional overlap for each

1208 individual species. The hypervolume size indicates the variability of proportional concentrations.
1209 Trait space overlap indicates how much the nectar trait space covers the pollen trait space
1210 ("Nectar over Pollen") and *vice versa*. Calculations are based on proportional composition.
1211 *Vaccinium corymbosum* samples are separated into samples from cultivated ("cult") and wild
1212 taxa. P-values in (a) and (b) are for generalized linear mixed model pairwise comparisons
1213 between nectar and pollen volume size (a) and asymmetry in overlap between nectar and pollen
1214 in (b). See Appendix S1, Fig. S2 for trait space volumes and proportional overlap based on
1215 absolute concentrations.

1216 **Fig. 8.** Intraspecific variation in nectar and pollen composition across cultivars (cultivated
1217 species: a, c) and sites (wild species: b, d). Horizontal axis shows median proportional overlap of
1218 trait space (n-dimensional hypervolume) for all pairs of sites and cultivars, as quantified by
1219 dynamic range boxes. Median proportional hypervolume overlap in (a) and (b) are pooled across
1220 species. The trait space overlap indicates how much trait space is shared between a typical pair
1221 of cultivars or sites. Analyses are based on proportional composition. P-values in (a) and (b) are
1222 for generalized linear mixed model pairwise comparisons between nectar and pollen site- or
1223 cultivar-wise overlap. Nectar chemistry overlapped more across both sites and cultivars than did
1224 pollen chemistry (cultivars: $t = -2.1$, $P = 0.039$; sites: $t = -3.74$, $P = 0.0002$).

1225 **Fig. 9.** Nectar chemical concentrations were relatively more variable than either flower or pollen
1226 concentrations, whether variation was calculated at the level of species (left panel) or the level of
1227 cultivars (for cultivated species) and sites (for wild species, right panel). Coefficients of variation
1228 were calculated as the ratio of the standard deviation to the mean for each compound within each
1229 species and tissue type (“Species level”), or for each compound within species, tissue type, and

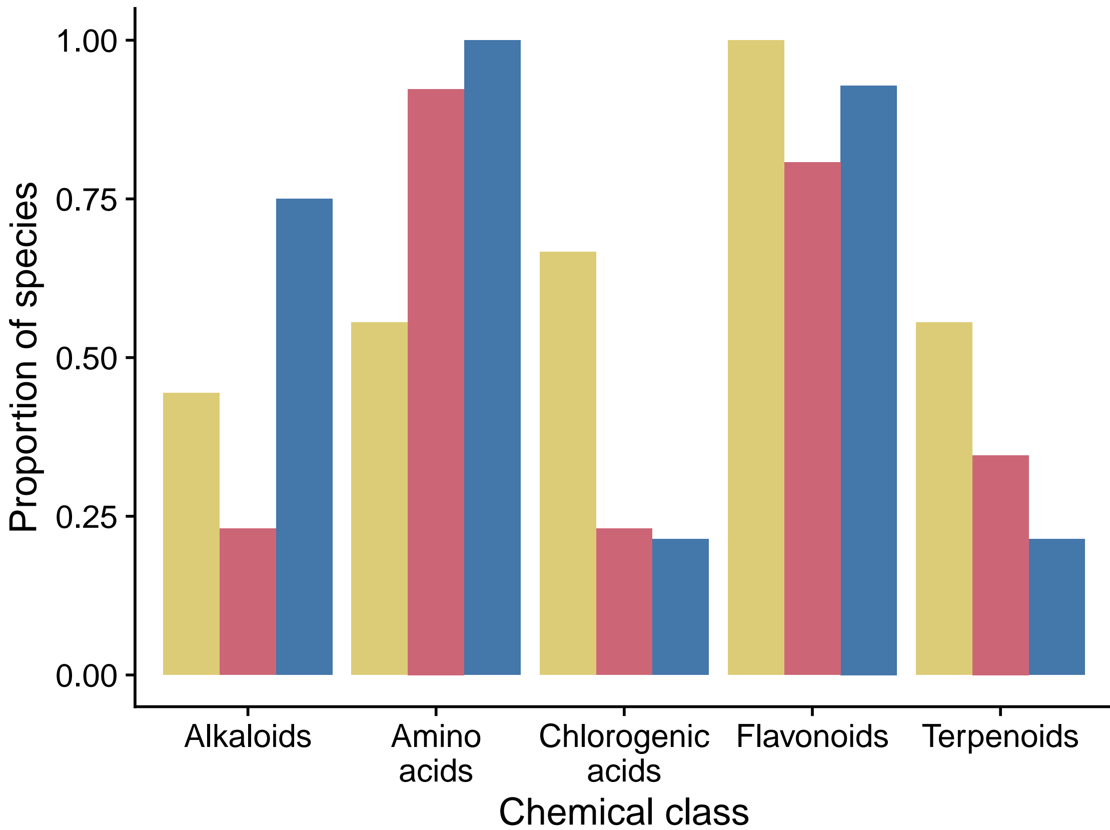
1230 site or cultivar (“Within species”). Different lower-case letters indicate significant differences ($P$
1231 $< 0.05$) between tissue types within each level of resolution in linear mixed model post hoc
1232 comparisons.

1233 **Fig. 10.** Example of distinct chemical compositions of flower, nectar, and pollen (a) and
1234 intraspecific variation in nectar and pollen composition across cultivars (b, c) in *Malus domestica*.
1235 Graphs show ordinations based on Bray-Curtis distances after Wisconsin double standardization
1236 of μM concentrations. Permutational MANOVA showed that tissue type ($F_{2, 84} = 207$, $P = 0.001$)
1237 explained $R^2 = 81\%$ of variation across samples in (a). Differences between cultivars were
1238 significant for both nectar ($F_{2, 27} = 8.58$, $P = 0.001$, panel b) and pollen ($F_{2, 27} = 13.93$, $P = 0.001$,
1239 panel c). Cultivar abbreviations: Fuji: Fuji-Autumn Red. Mac: Macintosh. See Table 2 for full
1240 results of cultivar-wise permutational MANOVA.

1241 **Fig. 11.** Median species-wise phenotypic integration of flower, nectar, and pollen samples. (a)
1242 Integration of the full chemical mixture was generally higher in nectar and pollen, but did not
1243 differ significantly across tissue types (linear mixed model $F_{2, 2.42} = 39.6$, $P = 0.11$). (b)
1244 Integration within modules of compounds within each mixture (defined by hierarchical
1245 clustering) indicated significant differences across tissue types ($F_{2,36.4} = 4.31$, $P = 0.021$). Nectar
1246 had higher within-module integration than did flowers ($t = 2.76$, $P = 0.024$). (c) No significant
1247 correlation was found between species-level nectar integration and pollen integration.

1248

1249


Type
Flower
Nectar
Pollen
Proportion of species
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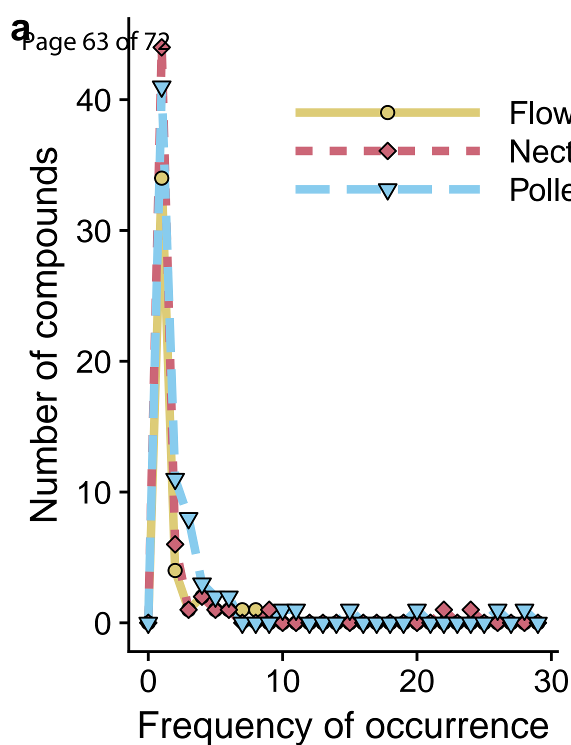
0.50
0.25
0.00
Alkaloids
Amino acids
Chlorogenic acids
Flavonoids
Terpenoids
Chemical class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Number of compounds
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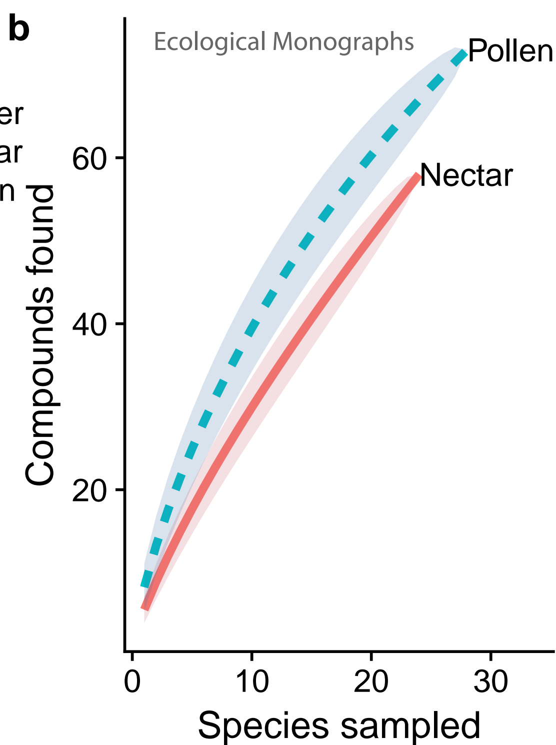
10
20
30
40
Frequency of occurrence
0
10
20
30
Flower
Nectar
Pollen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40
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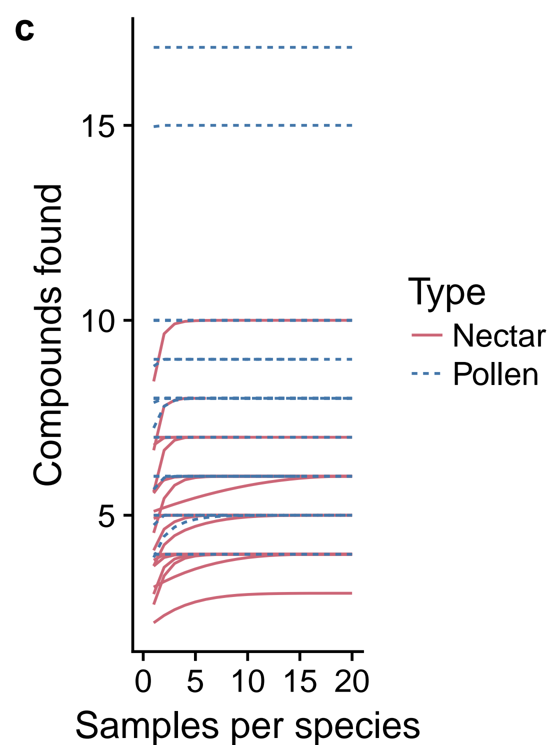
20
30
Pollen
Nectar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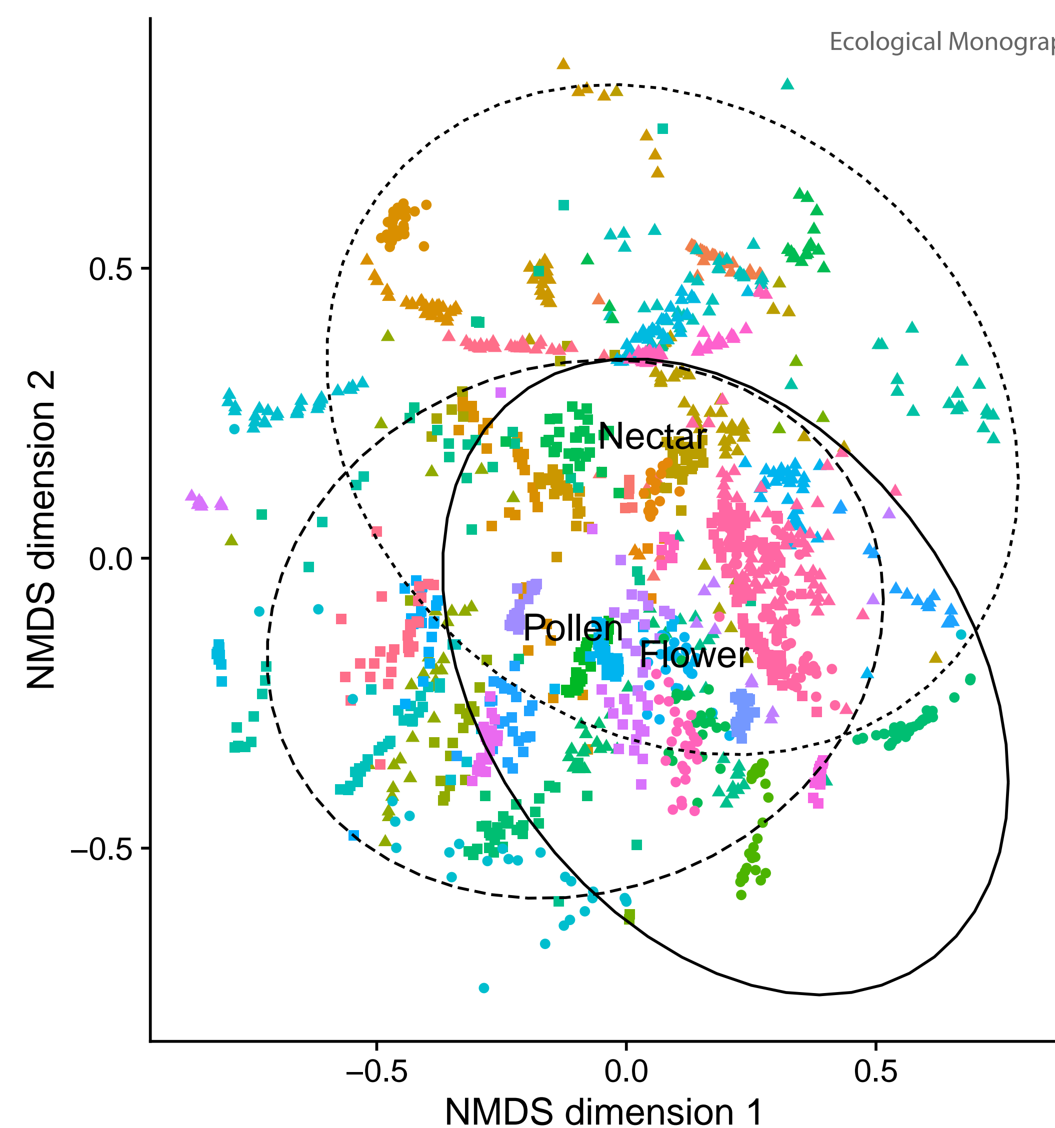
5
10
15
20
Type
Nectar
Pollen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NMDS dimension 1
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0.0
−0.5
−0.5
0.0
0.5
Nectar
Pollen
Flower
Type
Flower
Nectar
Pollen
Flower
Nectar
Pollen
Species
Aesculus_carnea
Antirrhinum_majus
Brassica_napus
Catalpa_speciosa
Citrus_sinensis
Cucurbita_pepo
Dicentra_eximia
Digitalis_purpurea
Echium_vulgare
Eupatorium_perfoliatum
Fragaria_ananassa
Geranium_maculatum
Helianthus_annuus
Impatiens_capensis
Kalmia_latifolia
Linaria_vulgaris
Lobelia_siphilitica
Lythrum_salicaria
Malus_domestica
Monarda_didyma
Penstemon_digitalis
Persea_americana
Prunus_dulcis
Rhododendron_prinophyllum
Silene_vulgaris
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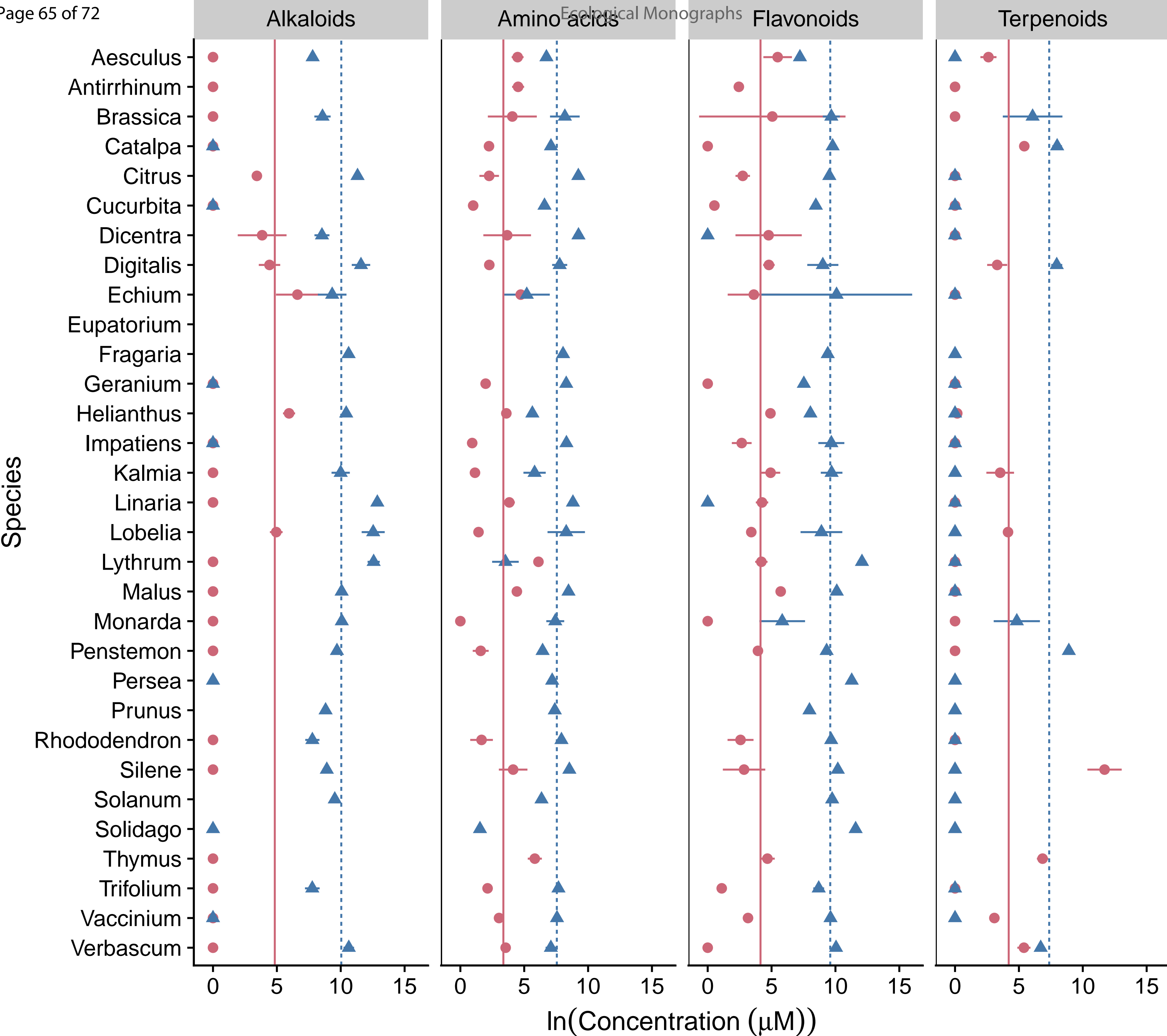
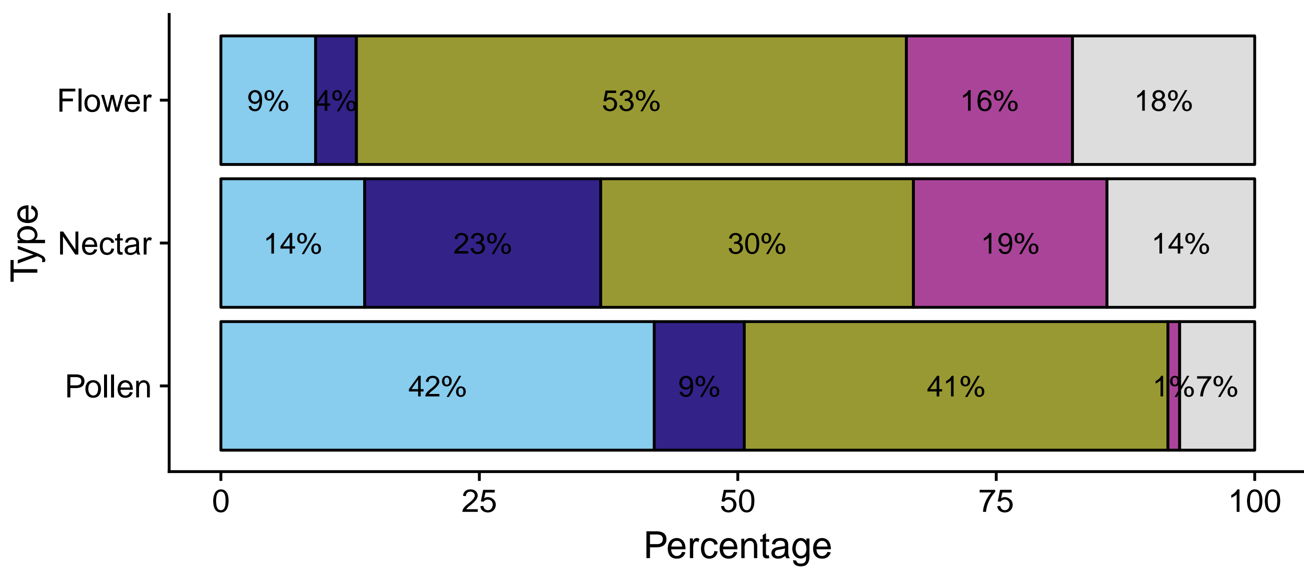
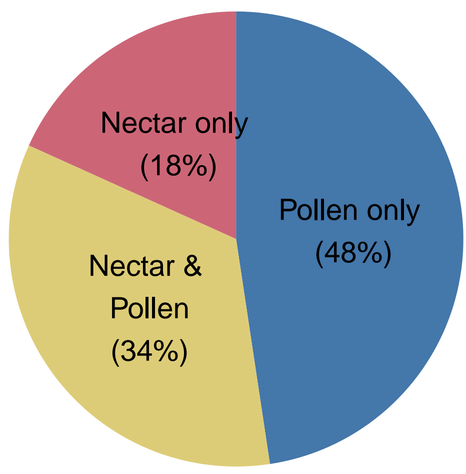
Trifolium_pratense
Vaccinium_corymbosum
Verbascum_thapsus

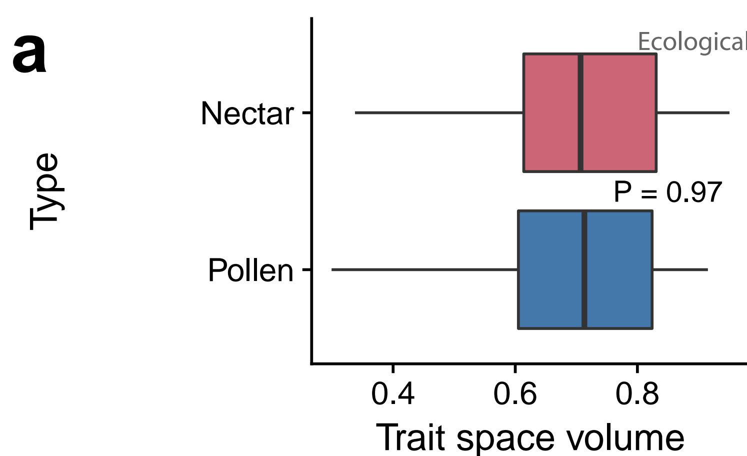


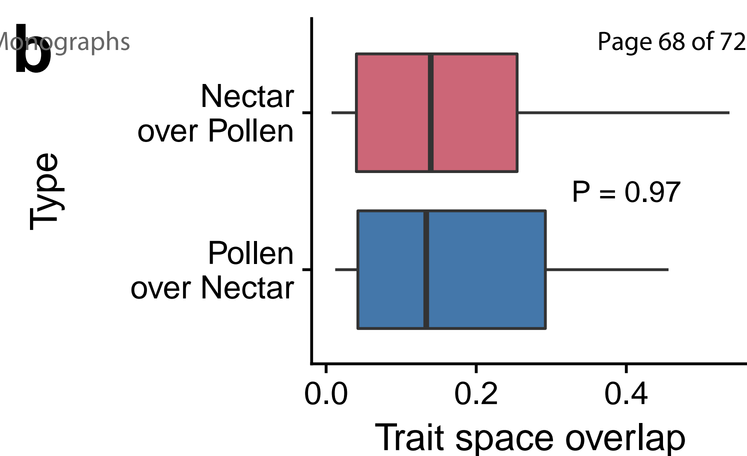
Alkaloids
Amino acids
Flavonoids
Terpenoids
Species
Aesculus
Antirrhinum
Brassica
Catalpa
Citrus
Cucurbita
Dicentra
Digitalis
Echium
Eupatorium
Fragaria
Geranium
Helianthus
Impatiens
Kalmia
Linaria
Lobelia
Lythrum
Malus
Monarda
Penstemon
Persea
Prunus
Rhododendron
Silene
Solanum
Solidago
Thymus
Trifolium
Vaccinium
Verbascum
0
5
10
15
ln(Concentration (μM))
Type
Nectar
Pollen

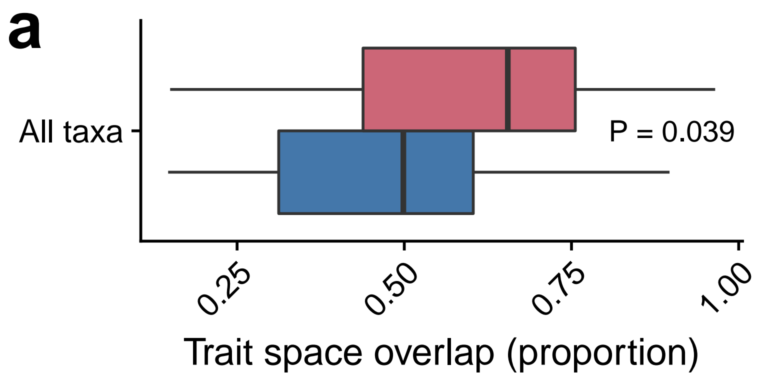

a
Alkaloids
Amino acids
Flavonoids
Terpenoids
Other
Type
Flower
Nectar
Pollen
9%
4%
53%
16%
18%
14%
23%
30%
19%
14%
42%
9%
41%
1%
7%
0
25
50
75
100
Percentage

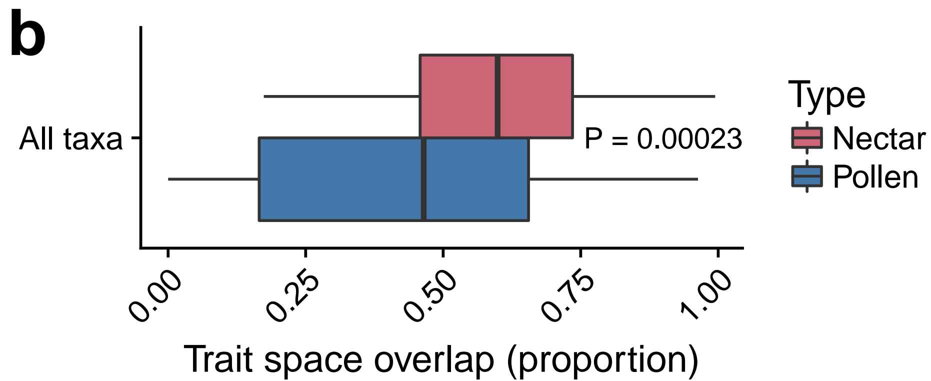
b
Flower
Nectar
Pollen
Species
Aesculus
Antirrhinum
Brassica
Catalpa
Citrus
Cucurbita
Dicentra
Digitalis
Echium
Eupatorium
Fragaria
Geranium
Helianthus
Impatiens
Kalmia
Linaria
Lobelia
Lythrum
Malus
Monarda
Penstemon
Persea
Prunus
Rhododendron
Silene
Solanum
Solidago
Thymus
Trifolium
Vaccinium
Verbascum
0
25
50
75
100
Percentage

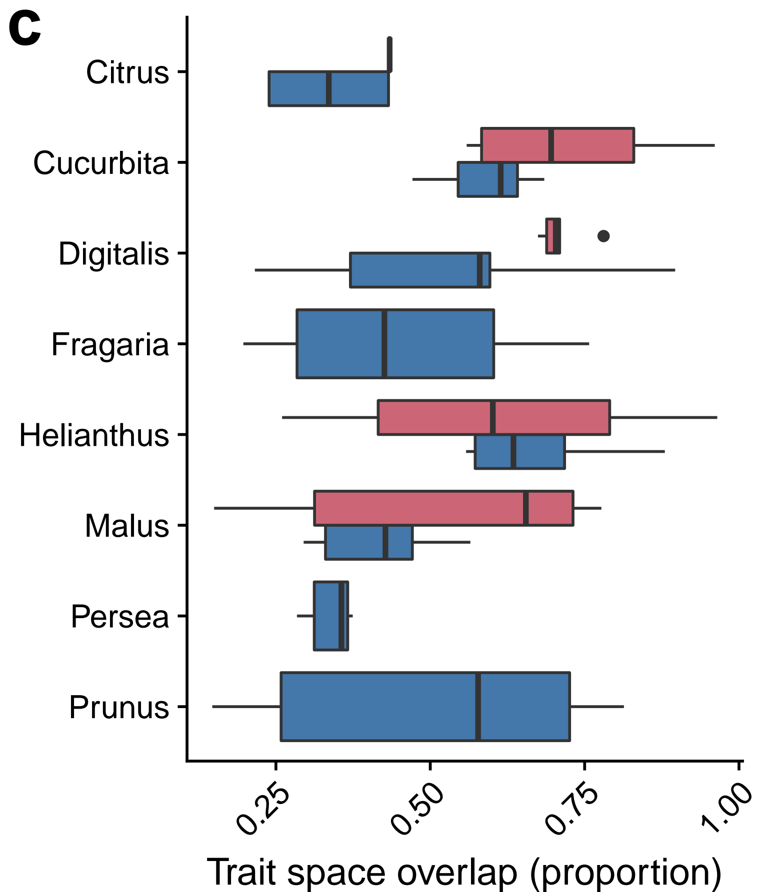

a
Nectar only
(18%)
Pollen only
(48%)
Nectar &
Pollen
(34%)
b
Aesculus
Brassica
Catalpa
Citrus
Cucurbita
Dicentra
Digitalis
Echium
Geranium
Helianthus
Impatiens
Kalmia
Linaria
Lobelia
Lythrum
Malus
Monarda
Penstemon
Rhododendron
Silene
Trifolium
Vaccinium
Verbascum
Species
0
3
6
9
12
Number of Compounds
Nectar only
Nectar & Pollen
Pollen only

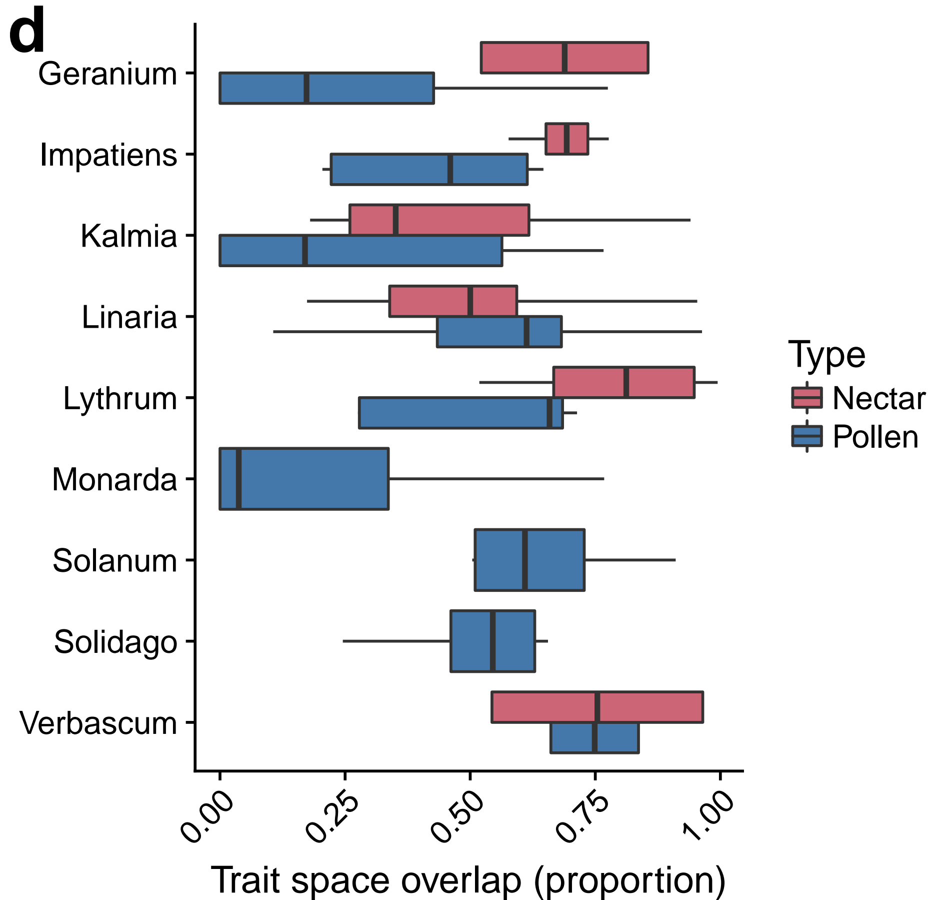

a
Type
Nectar
Pollen
P = 0.97
0.4
0.6
0.8
Trait space volume

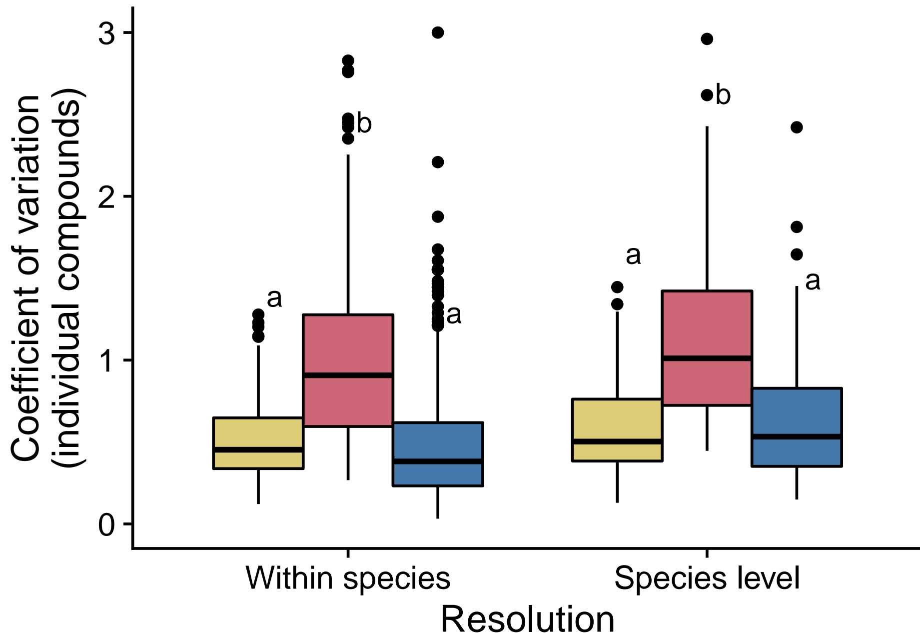

b
Type
Nectar over Pollen
Pollen over Nectar
P = 0.97
0.0
0.2
0.4
Trait space overlap
c
Nectar space
Pollen space
Species
Aesculus
Brassica
Catalpa
Citrus
Cucurbita
Dicentra
Digitalis
Echium
Geranium
Helianthus
Impatiens
Kalmia
Linaria
Lobelia
Lythrum
Malus
Penstemon
Rhododendron
Silene
Trifolium
Vaccinium_cult
Vaccinium_wild
Verbascum
0.00
0.25
0.50
0.75
Trait space volume
d
Nectar over Pollen
Pollen over Nectar
Species
Aesculus
Brassica
Catalpa
Citrus
Cucurbita
Dicentra
Digitalis
Echium
Geranium
Helianthus
Impatiens
Kalmia
Linaria
Lobelia
Lythrum
Malus
Penstemon
Rhododendron
Silene
Trifolium
Vaccinium_cult
Vaccinium_wild
Verbascum
0.0
0.2
0.4
Trait space overlap

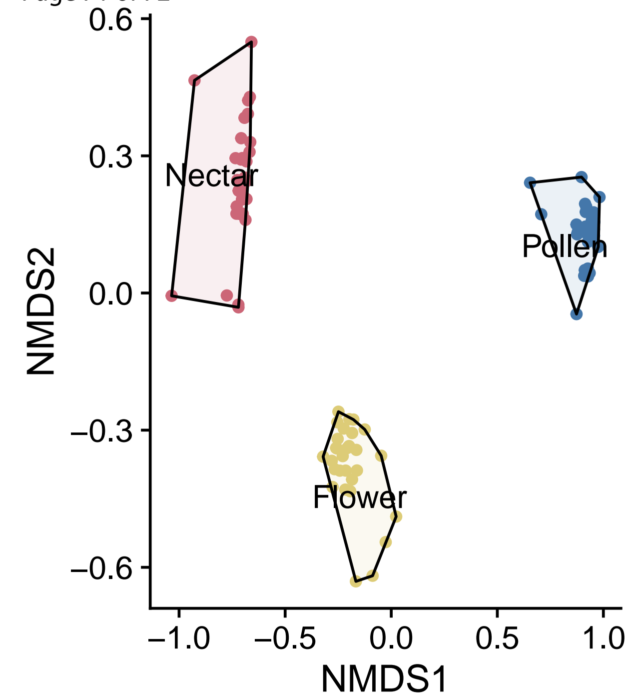

Cultivars
a
All taxa
P = 0.039
Type
Nectar
Pollen
0.25
0.50
0.75
1.00
Trait space overlap (proportion)

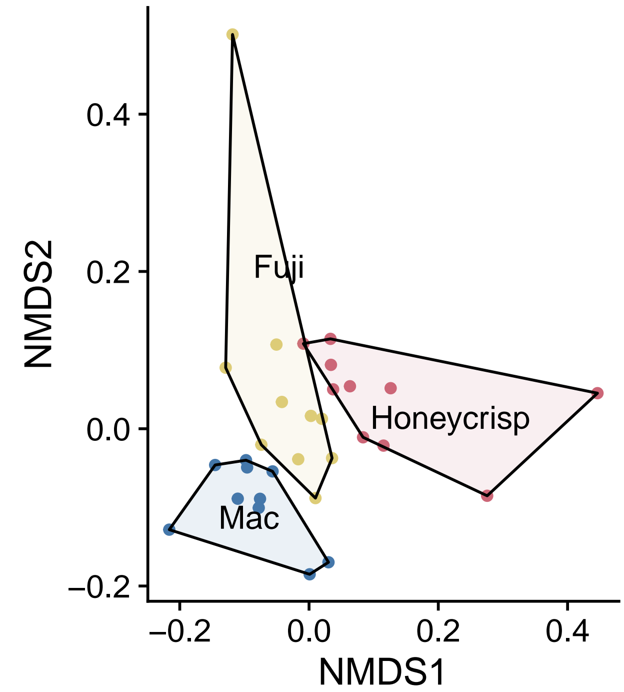

Sites
b
All taxa
P = 0.00023
Type
Nectar
Pollen
0.00
0.25
0.50
0.75
1.00
Trait space overlap (proportion)

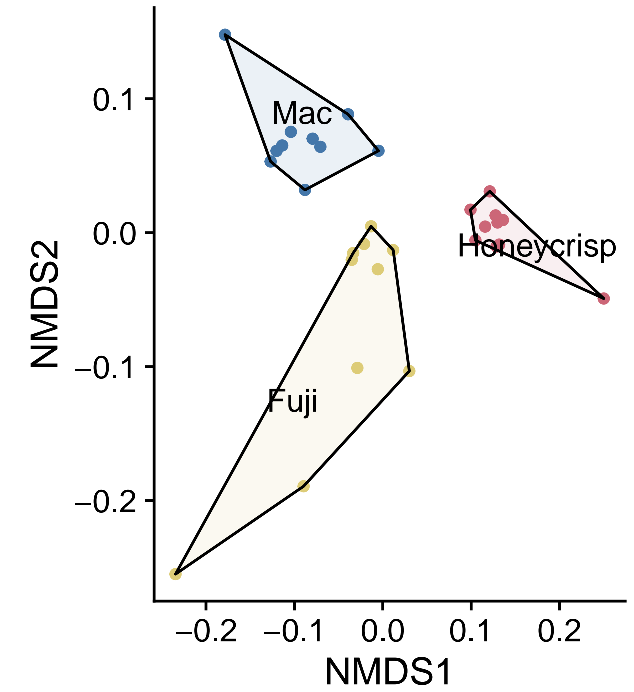

c
Citrus
Cucurbita
Digitalis
Fragaria
Helianthus
Malus
Persea
Prunus
Type
Nectar
Pollen
0.25
0.50
0.75
1.00
Trait space overlap (proportion)

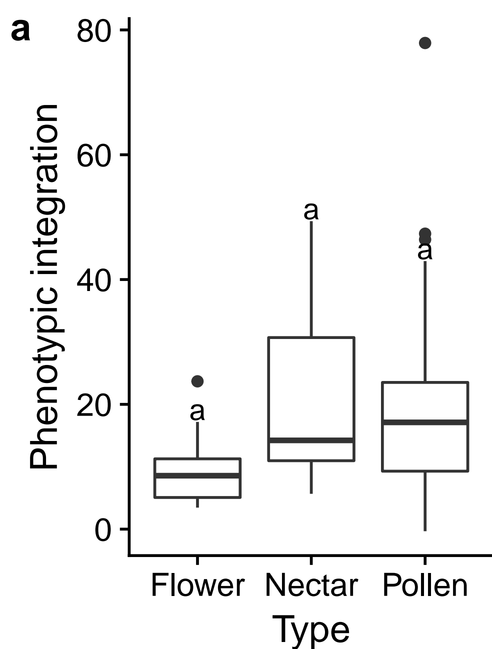

d
Geranium
Impatiens
Kalmia
Linaria
Lythrum
Monarda
Solanum
Solidago
Verbascum
Type
Nectar
Pollen
0.00
0.25
0.50
0.75
1.00
Trait space overlap (proportion)

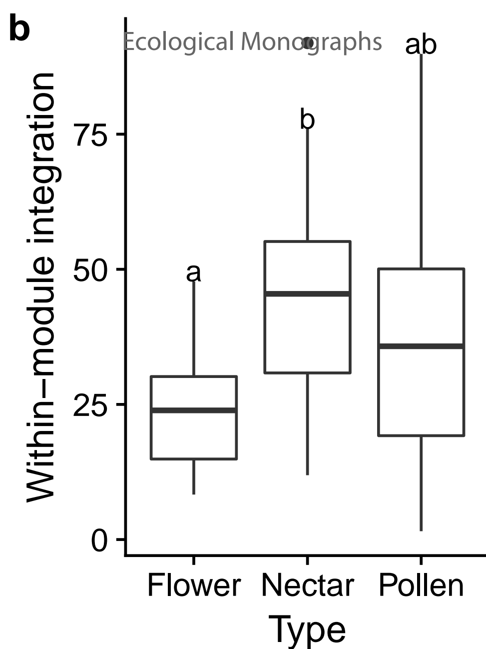

Type
Flower
Nectar
Pollen
Coefficient of variation
(individual compounds)
3
2
1
0
a
b
a
a
b
a
Within species
Species level
Resolution

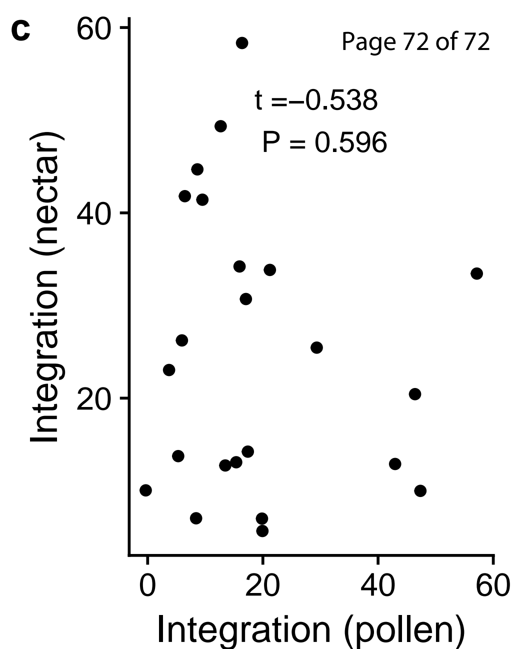

a
All cultivars
Nectar
Pollen
Flower
NMDS1
NMDS2
0.6
0.3
0.0
−0.3
−0.6
−1.0
−0.5
0.0
0.5
1.0


b
Nectar
Fuji
Honeycrisp
Mac
NMDS1
NMDS2
0.4
0.2
0.0
−0.2
−0.2
0.0
0.2
0.4


c
Pollen
Mac
Honeycrisp
Fuji
NMDS1
NMDS2
0.1
0.0
−0.1
−0.2
−0.2
−0.1
0.0
0.1
0.2


a
Phenotypic integration
0
20
40
60
80
a
a
a
Flower
Nectar
Pollen
Type


b
Within−module integration
0
25
50
75
a
b
ab
Flower
Nectar
Pollen
Type


c
Integration (nectar)
20
40
60
t =−0.538
P = 0.596
0
20
40
60
Integration (pollen)