American Journal of Botany

RESEARCH ARTICLE

Pollen and vegetative secondary chemistry of three pollen-rewarding lupines

Jacob M. Heiling[1,2,4], Daniel Cook[3], Stephen T. Lee[3], and Rebecca E. Irwin[1,2]

Manuscript received 14 December 2018; revision accepted 19 March 2019.

[1] Department of Applied Ecology, North Carolina State University, Raleigh, NC, USA

[2] Rocky Mountain Biological Laboratory, Gothic, CO, USA

[3] USDA ARS Poisonous Plant Research Laboratory, Logan, UT, USA

[4] Author for correspondence (e-mail: jacob.michael.heiling@gmail.com)

Citation: Heiling, J. M., D. Cook, S. T. Lee, and R. E. Irwin. 2019. Pollen and vegetative secondary chemistry of three pollen-rewarding lupines. *American Journal of Botany* 106(5): 643–655.

doi:10.1002/ajb2.1283

PREMISE: Optimal defense theory predicts that selection should drive plants to disproportionally allocate resources for herbivore defense to tissues with high fitness values. Because pollen's primary role is the transport of gametes, plants may be expected to defend it from herbivory. However, for many animal-pollinated plants, pollen serves a secondary role as a pollinator reward. These dual roles may present a conflict between selection to defend pollen from herbivores and selection to reward pollinators. Here, we investigate whether pollen secondary chemistry in three pollen-rewarding *Lupinus* species better reflects the need to defend pollen or reward pollinators.

METHODS: *Lupinus* (Fabaceae) species are nectarless, pollen-rewarding, and produce defensive quinolizidine and/or piperidine alkaloids throughout their tissues. We used gas chromatography to identify and quantitate the alkaloids in four aboveground tissues (pollen, flower, leaf, stem) of three western North American lupines, *L. argenteus*, *L. bakeri*, and *L. sulphureus*, and compared alkaloid concentrations and composition among tissues within individuals.

RESULTS: In *L. argenteus* and *L. sulphureus*, pollen alkaloid concentrations were 11–35% of those found in other tissues. We detected no alkaloids in *L. bakeri* pollen, though they were present in other tissues. Alkaloid concentrations were not strongly correlated among tissues within individuals. We detected fewer alkaloids in pollen compared to other tissues, and pollen contained no unique alkaloids.

CONCLUSIONS: Our results are consistent with the hypothesis that, in these pollen-rewarding species, pollen secondary chemistry may reflect the need to attract and reward pollinators more than the need to defend pollen from herbivory.

KEY WORDS *Bombus*; defense-attraction; Fabaecae; floral reward; *Lupinus*; optimal defense theory; pollen chemistry; pollen foraging; pollination.

Plant secondary compounds mediate interactions between plants and a diverse array of other organisms. Distasteful, repellant, and toxic secondary compounds are characteristic of plant defense against herbivory (Ehrlich and Raven, 1964). While individual secondary compounds often target certain species or guilds of interaction partners (Ehrlich and Raven, 1964; Agrawal and Weber, 2015), the expression of plant secondary compounds may also affect plant interactions with nontarget species (Strauss and Armbruster, 1997; Strauss and Irwin, 2004; Theis et al., 2007; Kessler and Halitschke, 2009). For example, some floral volatiles that deter ants that steal nectar from flowers and damage the gynoecium without pollinating can also deter pollinators (Galen et al., 2011). Secondary compounds occur not only in leaves, stems, and roots, but also in seeds, fruit, flowers, and floral rewards, including nectar and pollen (McKey, 1974; Cipollini and Levey, 1997; Adler, 2000). Optimal defense theory (ODT, McKey, 1974; Rhoades, 1979) predicts that reproductive structures and tissues should be among the most well-defended plant parts due to their high fitness values. Indeed, studies comparing secondary compound allocation between vegetative and reproductive tissues in rockcress (*Boechera stricta*; Keith and Mitchell-Olds, 2017), wild parsnip (*Pastinaca sativa*; Zangerl and Rutledge, 1996), and lungwort (*Lobaria scrobiculata*; Asplund et al., 2010) have found that secondary compounds are disproportionally allocated to reproductive tissues. Further, reproductive tissues may exhibit not only higher constitutive defenses, but also a greater ability to induce defense following damage compared to

vegetative tissues (Zangerl and Rutledge, 1996; Keith and Mitchell-Olds, 2017), resulting in reduced herbivore damage relative to other tissues (Zangerl and Rutledge, 1996; Asplund et al., 2010).

Most studies that have measured the secondary chemistry of floral rewards have focused on nectar, finding that nectar secondary compounds tend to either mirror or represent a subset of the secondary compounds found in vegetative and floral tissues, but are typically present at considerably lower concentrations (Manson et al., 2012; Cook et al., 2013). Because nectar usually serves solely as a reward, it is not surprising that concentrations of defense related compounds are lower in nectar than in other plant tissues. Pollen serves two very different functions in many plants—as carriers of gametes and as a pollinator reward. In plants that offer both pollen and nectar as rewards, defending pollen from nonpollinating pollen consumers should be advantageous, and selection for well-defended pollen follows from the ODT (McKey, 1974; Rhoades, 1979). Consistent with this hypothesis, in some plants that offer nectar as a reward, the concentrations of secondary compounds in pollen far exceed those in nectar and may be comparable to those found in vegetative tissues (Palmer-Young et al., 2018). However, approximately 20,000 nectarless plant species offer pollen as the sole reward to pollinators (Willmer, 2011), and little is known about the pollen secondary chemistry in these pollen rewarding species or about the variation in pollen chemistry among pollen-rewarding species (Parachnowitsch and Manson, 2015). Hereafter, we refer to plants that produce no nectar and only offer pollen as a reward to pollinators as pollen-rewarding plants. While potentially advantageous in defense against nonpollinating pollen consumers, chemically defended pollen may also come with the ecological cost of deterring pollinators in search of pollen rewards, presenting a conflict between the gamete and reward roles of pollen for pollen-rewarding species. How pollen-rewarding plants resolve the conflict between the pressure to defend pollen vs. enticing and rewarding pollinators has received little empirical attention within and across plant genera.

Lupines (*Lupinus* spp., Fabaceae) are nectarless, pollen-rewarding, and primarily bee-pollinated (though some may be capable of autogamous self-pollination) (Dunn, 1956; Gori, 1989), and thus represent ideal plants to assess patterns of secondary compound expression within pollen relative to a whole-plant context. Lupines produce quinolizidine and/or piperidine alkaloids, secondary compounds which are deterrent and toxic to some invertebrate herbivores and which may be toxic and/or teratogenic to ungulates (Dolinger et al., 1973; Panter et al., 1998; Lee et al., 2007; Kozłowski et al., 2017). Only two studies, to our knowledge, have measured lupine pollen secondary chemistry, both finding that alkaloid concentrations in seeds, leaves, and/or flowers exceeded those in pollen (Detzel and Wink, 1993; Arnold et al., 2014). Detzel and Wink (1993) compared alkaloid concentrations from a composite sample within and between tissue types in *Lupinus polyphyllus*, and Arnold et al. (2014) only reported a single value of pollen alkaloid concentration from a composite sample of *Lupinus mutabilis* and did not report concentrations in other tissues. Detzel and Wink (1993) found lower alkaloid concentrations in pollen than in other tissues tested, approximately 20 and 3.5% of the concentrations reported in flowers and leaves, respectively.

As alkaloids are broadly toxic to invertebrates, many lupine alkaloids may be toxic or deterrent to bees in sufficient concentrations, though to date only a few lupine alkaloids have been assessed for toxicity in bees (Detzel and Wink, 1993). Because lupines use pollen as their sole pollinator reward and are entirely reliant on bee pollinators for reproduction, they may experience selection against the use of alkaloids to defend their pollen from nonpollinating pollen consumers such as beetles and lepidopteran larvae. Alternatively, alkaloid-rich pollen could be selected for if selection to defend pollen from nonpollinating pollen consumers overwhelms selection to attract and reward pollinators or is important to reduce over-collection of pollen by bee pollinators (i.e., pollen that is lost in corbicular pollen loads and not available for pollination).

To determine whether the lower concentrations of secondary compounds in pollen relative to other plant tissues is common among species in the pollen-rewarding genus *Lupinus*, we compared the alkaloid profiles of pollen to other aboveground tissues in three western North American *Lupinus* species: *Lupinus argenteus* Pursh, *Lupinus bakeri* Greene subsp. *amplus* Fleak and Dunn, and *Lupinus sulphureus* Douglas ex. Hook var. *sulphureus*. We hypothesized that the aboveground tissues, including pollen, would contain quinolizidine and/or piperidine alkaloids, based on prior studies in other *Lupinus* species (Detzel and Wink, 1993; Arnold et al., 2014), but that concentrations would be lower in the pollen relative to other tissues. We also hypothesized that the alkaloid profiles of pollen would be qualitatively similar to other aboveground tissues. This study provides much needed empirical insight into the secondary chemistry of pollen and tests predictions about its evolutionary ecology in pollen-rewarding plants.

MATERIALS AND METHODS

Study system

The ranges of the three species included in our study span a broad swath of western North America from British Columbia to Washington State and Oregon (*L. sulphureus*), to Colorado and New Mexico (*L. bakeri*) and across all of the western United States and into northern Mexico (*L. argenteus*). This trio of species was ideal for our study because they cover a broad geographic range but share a common reward strategy (nectarless and pollen-rewarding) (Dunn, 1956), are pollinated by similar assemblages of insects (medium to large bodied bees including *Bombus* spp., *Andrena* spp., *Megachile* spp., and *Osmia* spp.) (Dunn, 1956; J. M. Heiling, personal observation), and share similar herbivores (primarily lycaenid butterflies and grazing mammals) (Dolinger et al., 1973; Severns, 2003).

Plant material

We collected *L. argenteus* and *L. bakeri* samples from four and two sites, respectively, in and around the Rocky Mountain Biological Laboratory (RMBL), Gothic, Colorado, United States, and *L. sulphureus* from one site near Pendleton, Oregon, United States (Table 1). For all species, we collected single flowering stems from 6 to 7 plants at each site. Following collection, samples were separated into leaf, stem, flower, and pollen. For *L. argenteus* and *L. bakeri*, we included only the corolla and gynoecium in flower samples, excluding the androecium. Due to minor differences in sampling protocols, *L. sulphureus* flower samples included some material from the androecium. For all three species, pollen samples consisted of granular pollen, no anther material was included. At each site, we collected and pressed a voucher specimen (Table 1). The *L. argenteus*

TABLE 1. GPS coordinates, elevation, and voucher identification numbers for *Lupinus* sites sampled. *Lupinus argenteus* and *L. bakeri* vouchers are housed in the herbarium of the Rocky Mountain Biological Laboratory (global unique identifier: dcc3c0d4-53b3-4be4-9de7-a6c1d63736c5).

Species	Site	Latitude	Longitude	Elevation (m a.s.l.)	Voucher ID
Lupinus argenteus	401	39.008381	–107.02717	3306	RMBL0011502
	Gothic (GTH)	38.957441	–106.9891	2872	NA
	Baldy (BDY)	38.977309	–107.04126	3521	RMBL0011548
	Elko (ELK)	39.008124	–107.05431	3278	RMBL0011503
Lupinus bakeri	Upper Loop (UPL)	38.88303	–107.95976	2814	RMBL0011499
	Kapuchion (KAP)	38.90362	–107.01328	2745	NA
Lupinus sulphureus	Pendelton (PEN)	45.593617	–118.53918	1127	UTC247072

Note: L. sulphureus vouchers are housed in the Intermountain Herbarium, Utah State University.

and *L. bakeri* specimens are retained at the RMBL Herbarium, and the *L. sulphureus* specimen is retained at the Intermountain Herbarium (UTC) at Utah State University. Geographical information for sites and voucher numbers can be found in Table 1.

Chemicals and reagents

We purchased ammonium hydroxide, sodium sulfate, and chloroform from Fisher Scientific (Pittsburgh, PA, USA), Baker (Phillipsburg, NJ, USA), and Mallinckrodt Baker (Paris, KY, USA) respectively; caffeine and sparteine from Sigma-Aldrich (St. Louis, MO and Milwaukee, WI, USA); lupanine from Biomedical Research Co. (Los Angeles, CA, USA); and D-α-isolupanine perchlorate from Koch-Light Laboratories Ltd. (Colnbrook, Bucks, UK).

Alkaloid extraction

All plant material was air-dried and ground to pass through a 2 mm screen. Once samples had dried, we weighed a measured quantity into a 16 mL screw-top glass test tube for extraction. In brief, the plant material was extracted by mechanical rotation using the Rugged Rotator (Glas-Col LLC, Terre Haute, IN, USA) with a mixture of 1 N HCl (4.0 mL) and $CHCl_3$ (4.0 mL) for 15 min (as reported by Lee et al., 2007). Following extraction, we centrifuged the samples (5 min) and removed the aqueous layer. To each test tube containing plant material and $CHCl_3$, we added an additional 2.0 mL of 1 N HCl, then repeated the extraction by mechanical rotation (15 min), centrifugation, and removal of the aqueous layer. The aqueous portions were combined in clean test tubes. We adjusted their pH to 9.0–9.5 with concentrated NH_4OH and then extracted the basic solution twice with $CHCl_3$, first with 4.0 mL and then with 2.0 mL. These solutions were combined and filtered through anhydrous Na_2SO_4 into clean test tubes, and the solvent was evaporated under N_2 at 60°C. After reconstituting the extracted alkaloid fraction with a measured volume of methanol containing 1.3 µg/mL caffeine (an internal standard), we transferred ~1 mL to 1.5 mL GC autosample vials for GC/FID or GC/MS analysis.

GC/FID analysis

All samples were analyzed by GC/FID using a Shimadzu GC-2010 gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a Shimadzu AOC-20i autosampler, J&W DB-5 column (30 m × 0.32 mm, 0.25 µm film thickness; J&W Scientific, Folsom, CA, USA) and a flame ionization detector (FID). Samples were injected (1.0 µL) splitless at 250°C using helium as the carrier gas at a constant flow rate of 2.0 mL/min. The column oven temperature program started at 100°C for 1 min; increased to 200°C at 50°C/min; then to 260°C at 5°C/min; then to 320°C at 50°C/min; and held at 320°C for 8.8 min for a total run time of 25 min. To calculate alkaloid concentrations, we used an eight-point sparteine standard curve (4–500 µg/ml). Alkaloid concentrations are expressed as relative micrograms of sparteine because standards were not available for all alkaloids.

GC/MS analysis

We performed GC/MS analysis as previously reported by Lee et al. (2007). In brief, we analyzed a minimum of one representative sample from each locality from the representative taxa by GC/MS using a Finnigan MAT GCQ (Finnigan MAT, San Jose, CA, USA) equipped with a split/splitless injector and a DB-5MS (30 m × 0.25 mm; J&W Scientific) column. The injection port temperature was 250°C and was operated in the splitless mode. The split vent flow rate was 50 mL/min and purged after 0.80 min. The oven temperature was 100°C for 1 min; 100–200°C at 40°C/min; 200–275°C at 5°C/min; and then held at 275°C for 1.5 min. We used electron impact ionization (EI) at 70 eV with an ion source temperature of 200°C.

Alkaloid identification

Alkaloid identification was performed as previously reported by Lee et al. (2007). In brief, we identified six individual alkaloids from authenticated (MS, NMR) samples of ammodendrine, anagyrine, and thermopsine from the alkaloid collection of the Poisonous Plants Research Laboratory and from commercially obtained standards (sparteine, lupanine, and D-α-isolupanine). We determined the yet to be identified alkaloids from correlation of measured retention times (RT) to retention indices (RI) calculated by linear extrapolation from RI values generated from known standards and assigned RI numbers from the literature and their electron ionization (EI) mass spectra (Wink et al., 1995). In addition, alkaloid identification was further supported by correlation of measured relative retention times (RR_t) to lupanine RT and EI mass spectra reported in the literature (Kinghorn and Balandrin, 1984).

Statistical analyses

Total alkaloid concentrations by tissue and site—We conducted all analyses in R version 3.5.1 (R Core Team, 2018). To compare total alkaloid concentrations across tissue types in each species, we used linear mixed effects models (LMM) with tissue type (leaf, flower,

TABLE 2. Alkaloids identified in *Lupinus argenteus*, *L. bakeri*, and *L. sulphureus* tissues in samples from populations near Gothic, Colorado (*L. argenteus* and *L. bakeri*) and Pendleton, Oregon (*L. sulphureus*). Columns are arranged by species, variety, and site, respectively. Letters denote presence of a given alkaloid in samples of corresponding tissue (pollen: P, flower: F, leaf: L, stem: S). – indicates the alkaloid was not detected; † indicates the alkaloid was detected in <75% of samples.

	Lupinus argenteus				*L. bakeri*		*L. sulphureus*
Alkaloid	**401**	**GTH**	**BDY**	**ELK**	**KAP**	**UPL**	**PEN**
Ammodendrine	–	–	–	–	F L S	F L S	–
Anagyrine	–	P F L S	P F L S	–	–	–	P F L S
Aphyllidine	–	F L S	–	–	–	–	–
Argyrolobine	–	P† F L S	–	–	–	–	–
5,6-Dehydrolupanine	–	F L S	P F L S	–	–	F† L†	F L S
11,12 Dehydrolupanine	F S†	–	–	P F† L S	–	–	–
5,6-Dehydro-α-isolupanine	F L S	–	–	P† F L S	–	–	–
Dihydroxyaphyllidine	–	F L S	–	–	–	–	–
3-Hydroxy-lupanine	–	–	P† F† L† S†	–	–	–	–
α-Isolupanine	P F L S	–	–	P F L S	–	–	–
α-Isosparteine	F L S†	–	–	F† L S†	–	–	–
β-Isosparteine	–	L	F† L S†	–	–	–	–
Lupanine	–	F L S	P F L S	–	–	F† L†	P F L S
Sparteine	–	F L S†	F L S†	–	–	–	–
Thermopsine	P F L S	–	–	P F L S	–	–	–
Unknown	–	–	–	–	F† L† S†	F† L† S†	–

stem, and pollen) and site as fixed effects (for *L. sulphureus*, which came from a single site, tissue was the only fixed effect), an interaction between fixed effects of site and tissue, and individual as a random effect. The secondary chemistry profiles of lupine are known to vary systematically between sites (Carey and Wink, 1994; Muzquiz et al., 1994; Adler and Kittelson, 2004; Cook et al., 2018). While the primary goal of our study was to compare alkaloid patterns between tissues within individuals, we were still interested in capturing any between site variation in the populations that we studied. Hence, we included site as a fixed (rather than a random) effect. We used the lme4 package (Bates et al., 2014) to construct all LMMs, and fit these models by restricted maximum likelihood (REML) with Kenward–Roger approximations for degrees of freedom. We used the lmerTest package (Kuznetsova et al., 2015) to perform inference tests (ANOVA) on LMMs. For post-hoc significance tests on the main effect of tissue type, we performed Tukey contrasts using the emmeans package (Lenth, 2018).

Between-tissue correlations of total alkaloid concentrations—To evaluate whether total alkaloid concentrations were correlated across tissue types within plants, we performed correlation analyses for each site-species combination. We used the function rcorr() in the Hmisc package (Harrell and Harrell, 2018) to construct correlation matrices with Spearman's ρ rank correlation coefficients. Although it resulted in smaller sample sizes, we were unable to pool data across sites because of significant tissue × site interactions in our LMMs (see Results).

Alkaloid compositional analysis—To characterize the tissue-specific alkaloid profiles for each species, we performed nonmetric multidimensional scaling (NMDS) on the alkaloid composition and concentration matrices (Bray–Curtis dissimilarity index) using the metaMDS() function in the vegan package (Cornille et al., 2012; Oksanen et al., 2013). *Lupinus argenteus* populations exhibit a variety of chemotypes (Wink and Carey, 1994). Pooled data from all four *L. argenteus* sites suggested two distinct subgroups (Table 2). Accordingly, we divided the *L. argenteus* data into two separate NMDS analyses, one including data from the Gothic and Baldy sites representing one chemotype and another including data from the Elko and 401 sites representing another chemotype.

RESULTS

Alkaloid identification

We identified 15 alkaloids and found one unknown alkaloid in our samples across all tissue types (Table 2 and Appendices S1–S3), with 15 in *L. argenteus*, four (including the unknown) in *L. bakeri*, and three in *L. sulphureus*. All taxa yielded quinolizidine alkaloids (typical of lupines), and *L. bakeri* yielded one piperidine alkaloid (ammodendrine). *Lupinus argenteus* and *L. sulphureus* pollen contained only a subset of the alkaloids identified in other aboveground tissues, and no alkaloids were unique to pollen (Table 2).

Total alkaloid concentrations by tissue and site

The tissue × site interaction term was significant in the initial models for *L. argenteus* and *L. bakeri* using pooled data from all of the sites for each species ($F_{9, 66} = 5.46$, $P < 0.0001$ and $F_{3, 36} = 3.29$, $P = 0.032$, respectively). Since we were interested in the patterns of alkaloid concentrations within individuals, not between sites, we ran separate models for each site.

Total alkaloid concentrations differed significantly by tissue type for all *L. argenteus* sites (Baldy: $F_{3, 15} = 8.77$, $P = 0.0013$; Elko: $F_{3, 18} = 11.74$, $P = 0.0002$; 401: $F_{3, 18} = 35.44$, $P < 0.0001$; Gothic: $F_{3, 15} = 59.31$, $P < 0.0001$), for all *L. bakeri* sites (Upper Loop: $F_{3, 18} = 31.12$, $P < 0.0001$; Kapuchion: $F_{3,18} = 27.52$, $P < 0.0001$), and for *L. sulphureus* ($F_{3,18} = 17.21$, $P < 0.0001$). Mean pollen alkaloid concentrations were 17–35% of those in other tissues in *L. argenteus* (Fig. 1A–D), and 11–28% of those in other tissues in *L. sulphureus* (Fig. 1G). We did not detect any alkaloids in *L. bakeri* pollen (Fig. 1E, F; Table 2). Tukey contrasts for tissue-by-tissue concentration comparisons are reported in Table 3.

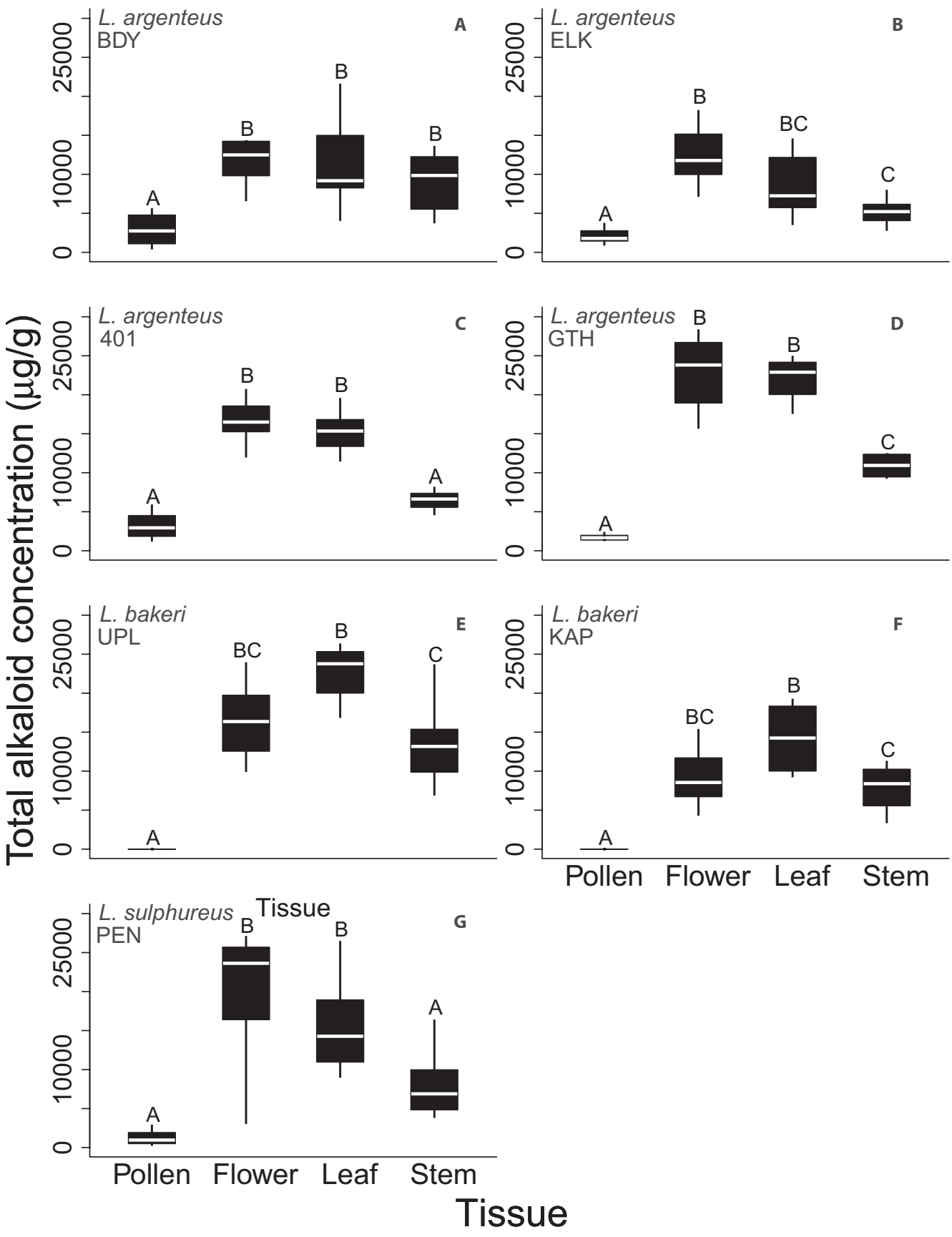
L. argenteus
BDY
A
L. argenteus
ELK
B
L. argenteus
401
C
L. argenteus
GTH
D
L. bakeri
UPL
E
L. bakeri
KAP
F
L. sulphureus
PEN
G
Total alkaloid concentration (µg/g)
0
10000
25000
Pollen
Flower
Leaf
Stem
Tissue

FIGURE 1. Total alkaloid concentrations (μg/g) in tissues of *Lupinus argenteus* from (A) Baldy, (B) Elko, (C) 401, and (D) Gothic, *Lupinus bakeri* from (E) Upper Loop and (F) Kapuchion, and (G) *Lupinus sulphureus* from Pendleton. Bars are box plots; lower and upper ends of boxes depict the lower and upper quartiles, respectively. Solid bands indicate medians. Whiskers extend across the data range. Different uppercase letters above boxes indicate significant differences in alkaloid concentrations at $P \leq 0.05$ based on Tukey contrasts.

Between-tissue correlations of total alkaloid concentrations

Correlations in total alkaloid concentrations among tissue types ranged from −0.93 to 0.93 across all sites and species, but few of the correlations were statistically significant (Fig. 2). For *L. argenteus*, flower alkaloid concentrations were significantly negatively correlated with stem alkaloids at 401 ($\rho = -0.93$, $P = 0.0025$, Fig. 2B lower) while pollen and flower alkaloid concentrations were positively correlated at Baldy but negatively correlated at Elko ($\rho = 0.89$, $P = 0.0068$; $\rho = -0.82$, $P = 0.0234$; Fig. 2A upper and lower, respectively). In *L. bakeri*, the only significant correlation was between leaf and stem alkaloid concentrations at Kapuchion ($\rho = 0.86$, $P < 0.05$, Fig. 2C upper). Likewise, in *L. sulphureus*, the only significant correlation was between pollen and stem alkaloid concentrations ($\rho = 0.93$, $P < 0.01$; Fig. 2D). There were eight other tissue–tissue correlations with a $|\rho| > 0.5$, but none of these were statistically significant.

Alkaloid compositional analysis

The ordination analyses suggest differentiation between pollen alkaloid profiles and those of the other tissues (Fig. 3). Pollen alkaloid profiles were distinct from both flower and leaf profiles for all three species (Figs. 3–5; note that pollen is not included in the *L. bakeri* plot in Fig. 3 because there were no detectable alkaloids in any pollen samples in this species). Flower, leaf, and stem tissues also formed distinct clusters from one another in *L. argenteus* and *L. sulphureus* but were not clearly differentiable in *L. bakeri* (Fig. 3). The apparent lack of separation in *L. bakeri* may be driven by an extreme flower profile observation (Fig. 3 upper right of panel C), but a Grubbs test (using the R package *outliers*; Komsta, 2011) did not identify this value as an outlier ($G = 2.684$; $P = 0.158$).

DISCUSSION

Pollen is critical to reproduction in most flowering plants, and pollen secondary chemistry should shape and be shaped by interactions with pollen foragers. However, surprisingly few studies have measured pollen secondary chemistry in a whole-plant context (reviewed by Irwin et al., 2014) and even fewer in pollen-rewarding plant species (but see Detzel and Wink, 1993; Arnold et al., 2014). We predicted that alkaloid concentrations would be lower in pollen than other tissue types but that the composition (identity and concentration) of alkaloids in pollen would be qualitatively similar to those of other tissues in the three pollen-rewarding *Lupinus* species that we studied. Our results generally supported these predictions. We found that alkaloid concentrations were significantly lower in pollen relative to other tissues (Fig. 1) and that alkaloid concentrations were not strongly correlated among tissues within individuals (Fig. 2). The distinct clustering of pollen observed in Fig. 3 is likely primarily explained by differences in concentration relative to other tissues (Figs. 1, 4, and 5). While pollen contained only a subset of alkaloids observed in the other aboveground tissues, no alkaloids were unique to pollen (Table 2), and no alkaloids were present in higher concentrations in pollen than in other tissues (Appendices S1 and S3). Instead, alkaloids that tended to be lowest in concentration in other aboveground tissues were absent in pollen.

Floral parts are closely tied to plant reproductive success, and thus fitness (Strauss, 1997; Adler et al., 2001; Irwin et al., 2004; Kessler and Halitschke, 2009). The ODT predicts that, of the tissues we tested, flowers and pollen should be the most well-defended because they are most closely tied to reproductive success (McKey, 1974; Rhoades, 1979; Kessler and Halitschke, 2009). Consistent with the predictions of ODT, total alkaloid concentrations in flower tissue were consistently as high as or higher than those of leaves or stems. The universally high alkaloid concentration in leaves relative to other tissues is also consistent with the predictions of ODT because the growing season for these species is brief (only 2–3 months at higher elevations), which may select for high investment in defense of leaf tissue. However, our results for pollen were not consistent with ODT but rather with the hypothesis that, due to its dual roles as gametophyte and pollinator reward, pollen should have lower concentrations of secondary compounds relative to other tissues in pollen rewarding plants.

While nonpollinating pollen consumers should select for defended pollen, Muth et al. (2016) found that *Bombus impatiens* allowed to forage on artificial flowers containing *Prunus avium* pollen treated with either sucrose, cellulose (control), or quinine (a defensive alkaloid) made fewer visits to and spent less time collecting pollen from quinine treated artificial flowers, and were more likely to switch to a novel artificial flower type than

TABLE 3. Tukey contrasts (*p*-values) from tissue-by-tissue alkaloid concentration comparisons. NS = not significant, NA = no contrast performed due to lack of detectible alkaloids in pollen.

Species	Site	Pollen–Flower	Pollen–Leaf	Pollen–Stem	Flower–Stem	Flower–Leaf	Leaf–Stem
L. argenteus	BDY	0.0011	0.0067	0.0445	NS	NS	NS
	ELK	0.0002	0.0152	NS	0.003	NS	NS
	401	<0.0001	<0.0001	NS	<0.0001	NS	0.001
	GTH	<0.0001	<0.0001	0.0002	0.0002	NS	0.005
L. bakeri	UPL	NA	NA	NA	NS	NS	0.0066
	KAP	NA	NA	NA	NS	0.0302	0.0174
L. sulphureus	PEN	<0.0001	0.0005	NS	0.0018	NS	0.0476

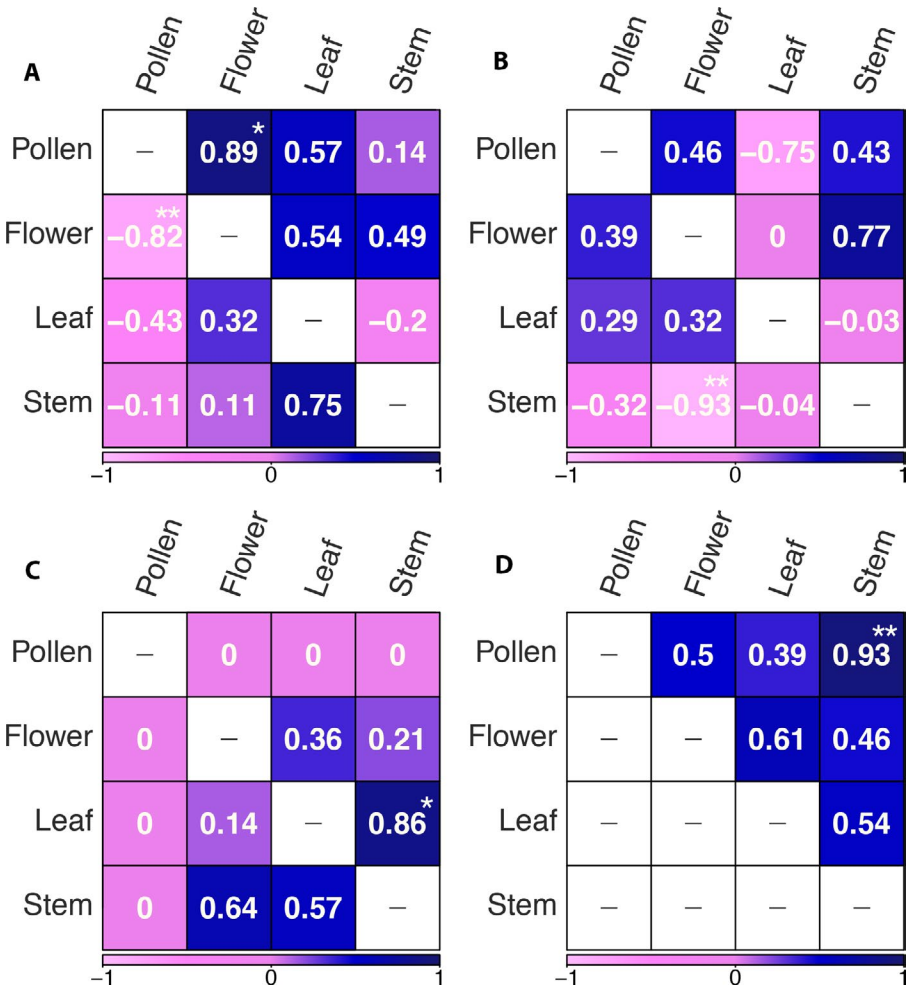

FIGURE 2. Correlograms of total alkaloid concentrations showing correlations among tissue types (pollen, leaf, flower, and stem) for (A, B) *Lupinus argenteus*, (C) *L. bakeri*, and (D) *L. sulphureus*. In panels A–C, two sites are shown per panel, A: Baldy (upper) and Elko (lower); B: Gothic (upper), 401 (lower); C: Kapuchion (upper), Upper Loop (lower). Correlation coefficients are Spearman's ρ rank. * $P \leq 0.05$, ** $P \leq 0.01$.

bees in the other two groups, indicating that the preferences of pollen-foraging pollinators may select against chemically defended pollen. While these experimental results are compelling, we know little about the secondary chemistry of pollen in pollen-rewarding plants.

In particular, many of the studies that have assessed pollen secondary chemistry have been limited to single species and single or few tissues, limiting the comparative value of these data (Kessler and Halitschke, 2009). Further, the majority of this work has focused on species that also provide other rewards, most commonly nectar (e.g., London-Shafir et al., 2003; Cook et al., 2013; Egan et al., 2018). Such species are not strictly pollen rewarding and so may be expected to chemically defend their pollen. Arnold et al. (2014) reported negative effects of the lupine alkaloid D-lupanine on production of male bumble bees (*Bombus terrestris*) in microcolonies fed exclusively on pollen containing 0.02–0.2% D-lupanine (syn. lupanine), while Detzel and Wink (1993) found that concentrations of the lupine alkaloid sparteine were deterrent (ED_{50}) and toxic (LD_{50}) to honey bees when fed in sugar solutions at concentrations of 0.03 and 0.05%, respectively. Beyond these two alkaloids, we do not know whether or to what degree bees are sensitive to other lupine alkaloids, and this should be the subject of further study. The different animal taxa that plants interact with often vary in their sensitivities to a given secondary compound (e.g., Saunders et al., 1992; Galen et al., 2011). Pollinators, such as *Bombus*, may be more sensitive to defensive secondary compounds than the herbivores that have driven the evolution of the defenses (Roslin and Salminen, 2008; Kelly and Bowers, 2016; but see Bernays et al., 2004). For example, while bees are sensitive to lupanine and sparteine at relatively low concentrations (Detzel and Wink, 1993; Arnold et al., 2014), Johnson and Bentley (1988) found that much higher concentrations of these same alkaloids were required to reduce growth and survivorship of herbivorous army worm (*Spodoptera eridania*) larvae. We suspect that many of the other alkaloids that we describe here may also have toxic or deterrent effects on bees in high enough concentrations.

The total alkaloid concentrations in pollen were variable among individual plants in our study (from 0 to 1.1% dry mass), but they were very low overall (mean in *L. argenteus* and *L. sulphureus*: 0.27% ± 0.05 SE dry mass) relative to other tissues (Fig. 1, Appendices S1–3). We determined that mean pollen alkaloid concentrations were within the ranges shown by Arnold et al. (2014) and Detzel and Wink (1993) to negatively affect bees fed exclusively on food (pollen and sugar, respectively) with these alkaloid concentrations. However, it seems unlikely that natural lupine pollen alkaloids concentrations would deter or intoxicate pollen-foraging polylectic bees such as the *Bombus* spp., which are the primary pollinators of North American lupines (Dunn, 1956) because the concentrations that such bees are effectively exposed to will be lower than the population mean due to diet mixing. Because bees collect pollen from several individual plants and polylectic bees collect pollen from several species, the high variability in alkaloid concentration and composition among individual plants may limit the exposure of a colony to any one alkaloid. This limited exposure should be contrasted with the more focused exposure to a given set of lupine alkaloids that an herbivore, which spends its entire juvenile stage on an individual plant (e.g., lepidopteran larvae), would experience. Further, as within-population variation in pollen alkaloid concentrations was fairly high (1–2 orders of magnitude), and bees have been demonstrated to use taste to selectively forage on pollen with lower alkaloid concentrations when given the choice (Muth et al., 2016), bees may limit their exposure to alkaloids by avoiding individuals that express higher alkaloids concentrations in their pollen. However, the oligolectic species that pollinate *Lupinus* species may experience much higher effective doses of lupine pollen alkaloids, suggesting an exciting avenue for future work. Interestingly, in sites near Gothic, Colorado, the Fabaceae specialist *Osmia iridis* does not collect lupine pollen, though the flowers

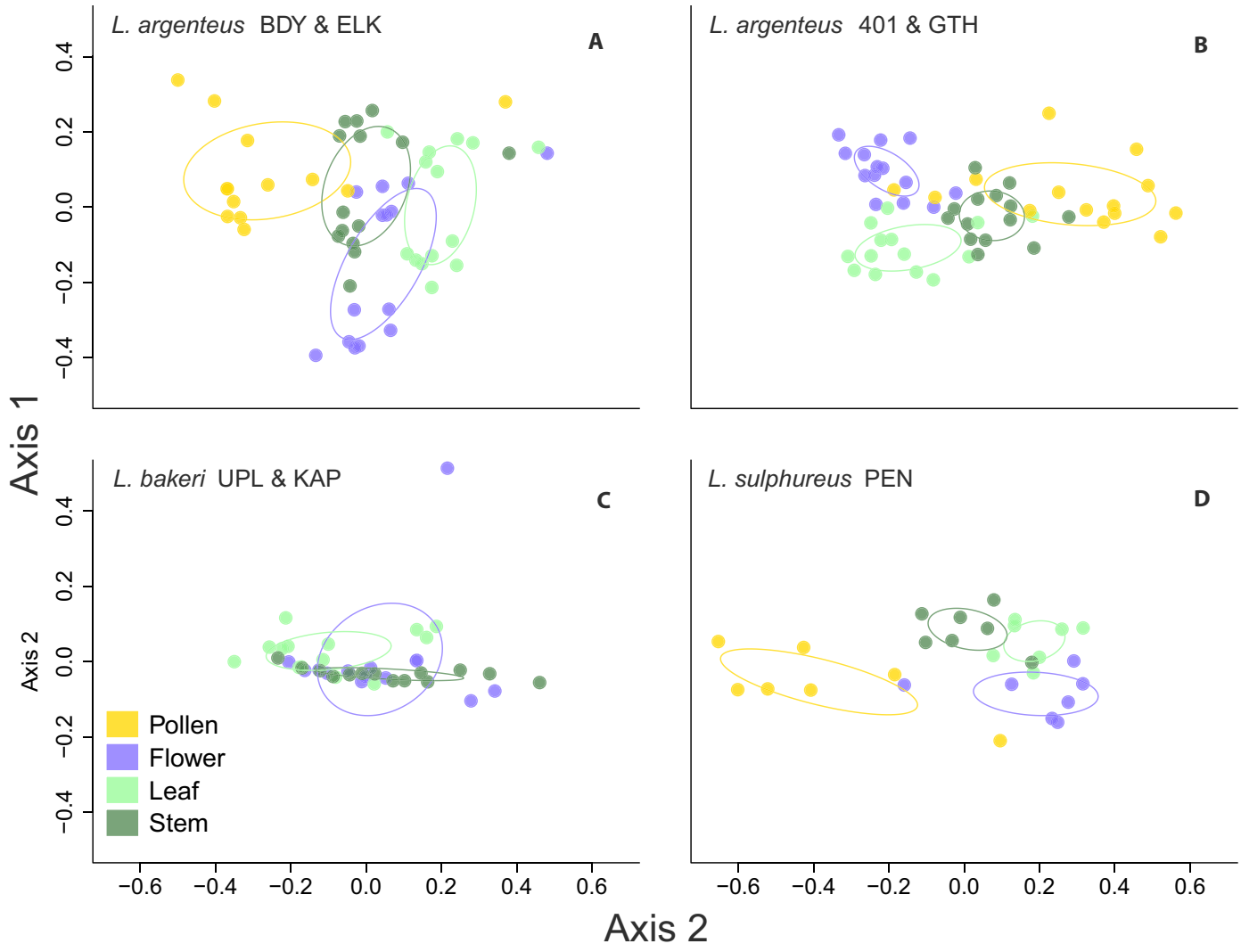

FIGURE 3. Nonmetric multidimensional scaling ordination plots of alkaloid profiles across four tissue types (pollen, leaf, flower, and stem) of (A, B) *Lupinus argenteus* (stress = 0.158 and 0.092), (C) *L. bakeri* (stress = 0.041), and (D) *L. sulphureus* (stress = 0.049). Data for *Lupinus argenteus* are divided into two plots: (A) Gothic and Baldy sites and (B) Elko and 401 sites. Pollen is not included in the plot for *L. bakeri* because we did not detect any alkaloids in the pollen samples from this species. Ellipses are SD.

are locally abundant in areas where they forage (Spear et al., 2016; Forrest and Chisholm, 2017). It is possible that, while low relative to other tissues, the alkaloids present in the pollen of *L. argenteus* are at high enough concentrations to deter these oligolectic and relatively small-bodied bees.

The overall lack of significant correlations between tissue alkaloid concentrations across all three species is, on the surface, surprising. While our knowledge of within-individual variation in secondary chemistry lags behind our understanding of variation among individuals, some studies have found correlations in secondary compound profiles across tissues within individuals (Adler et al., 2006, 2012). However, for all species and populations of *Lupinus* in our study, we found that, at most, only two tissue types were ever correlated. Further, there were no consistent trends in the tissue alkaloid correlations within or across species. Two factors may limit the strength of these results. First, sample sizes were small ($N = 6$ or 7 individuals per site), which may mean that the type II error was high and that we were unable to detect some true correlations, if they existed. Second, while we collected samples at roughly the same phenological stage at each site, we did not fully control for phenology. In general, relative chemical concentrations across tissues vary temporally as secondary compounds produced in one tissue are transported to other tissues (Lee et al., 2006), and as secondary compounds degrade with time (Wink and Witte, 1984). With these limitations in mind, a cautious interpretation of our results is that selection may act on the alkaloid concentrations of several or all of these tissues independently, consistent with the hypothesis that pollen foraging by pollinators could select for lower concentrations of defensive secondary compounds in pollen. Similar results were described by Kessler et al. (2009), for the pollen-rewarding wild tomato (*Solanum peruvianum*), and echo patterns described in some studies of nectar secondary chemistry (Manson et al., 2012). These results suggest that the uncoupling of defensive secondary chemistry between rewards and other tissues may be common.

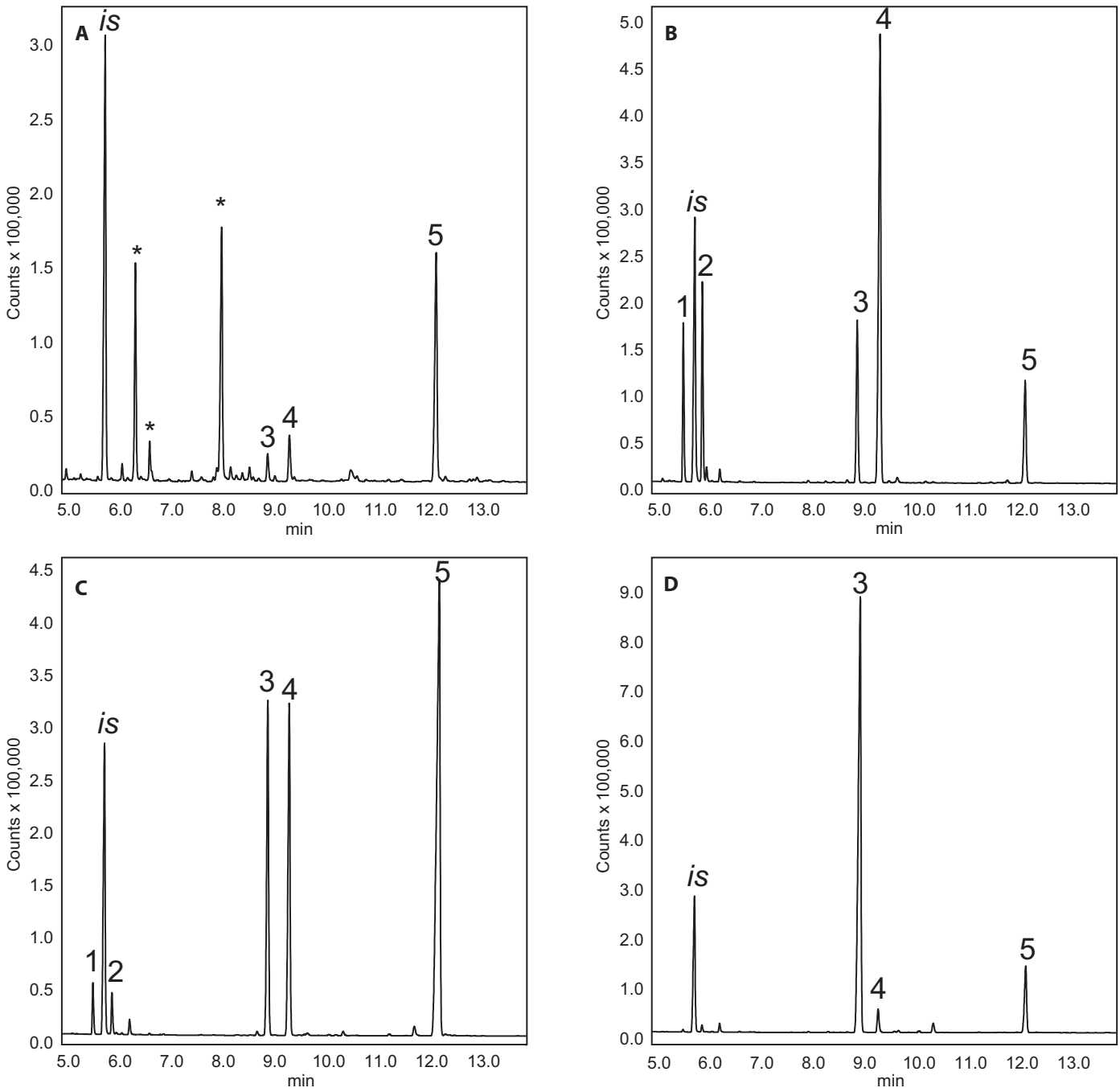

FIGURE 4. GC-FID chromatogram representative of *Lupinus argenteus* collected near Gothic, Colorado (BDY). (A) Pollen, (B) leaves, (C) flowers, and (D) stems. Peak 1: sparteine; 2: β-isosparteine; 3: 5,6-dehydrolupanine; 4: lupanine; 5: anagyrine; *is*: internal standard (caffeine); * unidentified pollen-specific analytes, found in all pollen samples.

While alkaloid profiles of *Lupinus* species, as described here and by previous authors (e.g., Dolinger et al., 1973; Wink and Carey, 1994; Wink et al., 1995; Cook et al., 2009), are characteristically variable both within and among species and populations, two features were common to all populations and species in our study. First, pollen alkaloid concentrations were consistently and significantly lower than in other tissues, and second, pollen alkaloid composition represented only a subset of other aboveground tissues (Table 2; Appendices S1, S3). The simplified composition of alkaloids in pollen relative to other tissues may reflect either the lack of expression of these alkaloids in pollen or proportionally lower expression in pollen such that that the concentrations fall below the detection threshold of our analytical methods. Interestingly, of the two lupine alkaloids found in our samples and known to be toxic and deterrent to bees (Detzel and Wink, 1993; Arnold et al., 2014), lupanine was only present in pollen from two populations

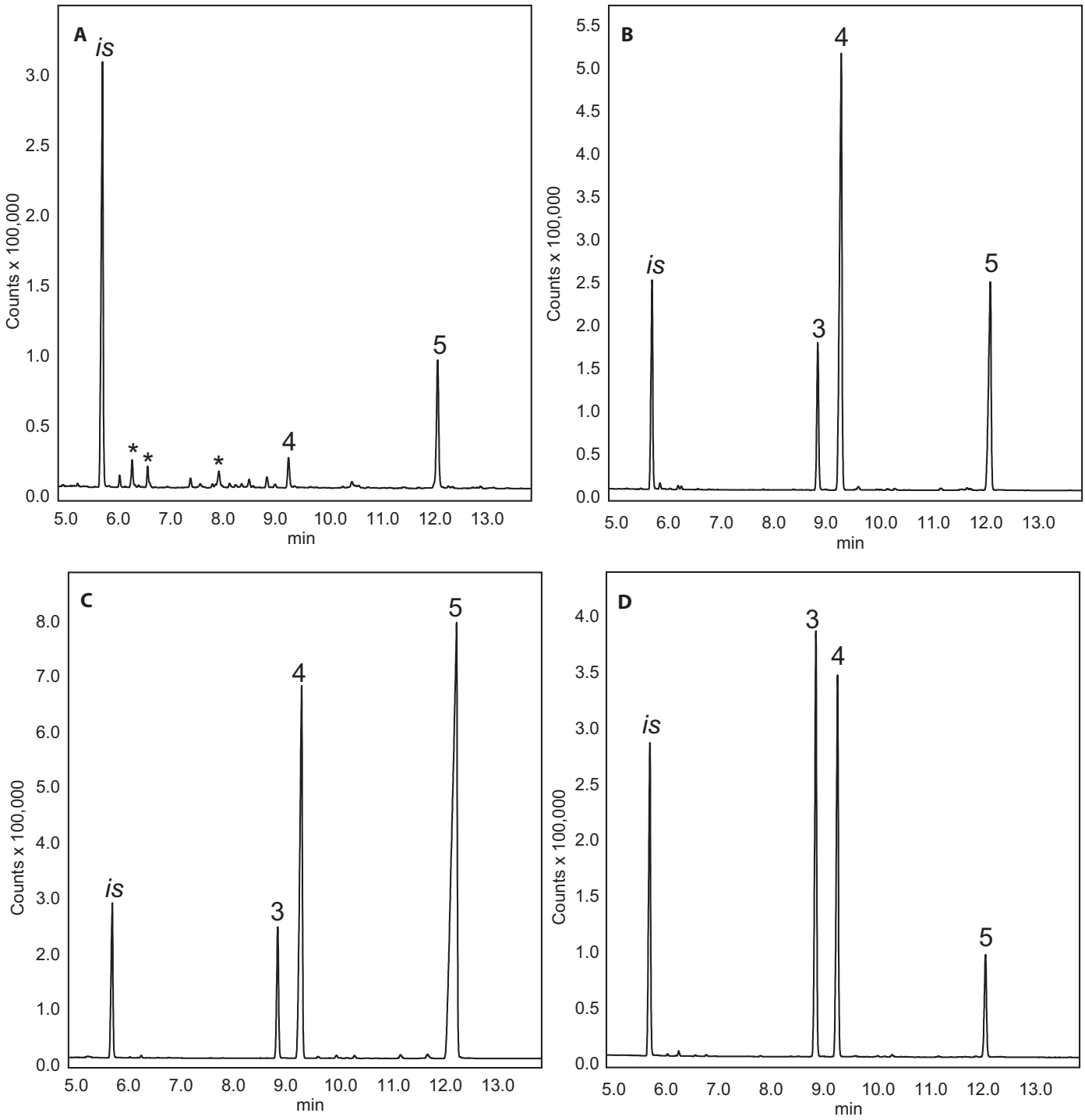

FIGURE 5. GC-FID chromatogram representative of *Lupinus sulphureus* collected near Pendleton, Oregon. (A) Pollen, (B) leaves, (C) flowers, and (D) stems. Peak 3: 5,6 dehydrolupanine; 4: lupanine; 5: anagyrine; *is*: internal standard (caffeine); * unidentified pollen-specific analytes, found in all pollen samples.

(Appendices S1, S3), and we did not detect sparteine or its stereoisomers (α-isosparteine and β-isosparteine), in the pollen of any of the species in our study (Table 2; Appendices S1–S3). Whether *Bombus* are as sensitive to the alkaloids that are more abundant in the *Lupinus* we sampled remains unclear. The absence of these compounds in the pollen that we sampled may reflect selection against their inclusion in pollen, possibly because of deterrent or toxic effects on pollinators. However, the apparent absence of individual alkaloids in our samples could be the result of small sample sizes, spatial or temporal variation in alkaloid expression (either due to phenotypic plasticity or population-genetic variation), or a combination of these factors.

CONCLUSIONS

Pollen-rewarding plant species may be engaged in a defense–attraction tug of war. The relative importance of selection on pollen chemistry via pollinator attraction vs. plant defense will depend on the magnitude of pollen limitation of plant reproduction as well as levels of pollen herbivory and their effect on plant fitness. We cannot yet rule out the possibility that pollen alkaloids, or lack thereof, may reflect pleiotropic effects of the production and transport of alkaloids across other tissues (Adler, 2000), selection via pollen's role as a gamete, or to reduce over-collection by pollen-harvesting bees (Parachnowitsch and Manson, 2015). However, the low concentration of alkaloids that we observed in the pollen of these three *Lupinus* species is consistent with the hypothesis that these species may experience selection against the use of alkaloids to defend their pollen from nonpollinating pollen consumers. Studies assessing whether pollen secondary chemistry serves an adaptive function in these and other species are now needed. In light of these results, future studies should ask: (1) What is the relative importance of pollinator attraction vs. plant defense with respect to pollen loss and how might this vary across species and communities, (2) how might this differ for male and female components of plant fitness, and (3) is there evidence that selection for chemically defended pollen undermines the pollen-rewarding strategy? Answering these questions is an important next step in understanding the patterns and implications of pollen secondary chemistry and the pollen-rewarding character state, which is so widespread among the angiosperms.

ACKNOWLEDGEMENTS

The authors thank K. Brennan, J. Park, and C. Hailes for field and lab assistance; M. Stemkovski, G. Calabrese, G. Pardee, and L. Harmon for statistical and programming advice; and J. Forrest for information regarding non-*Bombus* visitors of *Lupinus argenteus* and *L. bakeri*. We also thank N. Waser, M. Price, P. CaraDonna, S. Rivest, N. Sletvold, and two anonymous reviewers for insightful comments on the manuscript. Access to field sites was provided by RMBL and the United States Forest Service as well as the Kapuchion family. We acknowledge that we performed our collections of *L. argenteus* and *L. bakeri* on traditional Parianuches (Ute) territory. This work was funded by graduate student research awards to J.M.H. from the Botanical Society of America, Colorado Mountain Club (Al Ossinger Fellowship), the Rocky Mountain Biological Laboratory, and North Carolina State University, and a grant from the National Science Foundation (DEB-1641243) to R.E.I. Any opinions, findings and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

AUTHOR CONTRIBUTIONS

J.H., D.C., and R.I. designed the study. J.H. and D.C. carried out fieldwork. D.C. and S.L. carried out lab work. J.H. analyzed the data with the input of D.C. and R.I. J.H., D.C., S.L., and R.I. wrote the manuscript.

DATA ACCESSIBILITY

Data are archived with and available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.586f011 (Heiling et al., 2019).

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Concentrations of alkaloid species in *Lupinus argenteus* tissues.

APPENDIX S2. Concentrations of alkaloid species in *Lupinus bakeri* tissues.

APPENDIX S3. Concentrations of alkaloid species in *Lupinus sulphureus* tissues.

LITERATURE CITED

Adler, L. S. 2000. The ecological significance of toxic nectar. *Oikos* 91: 409–420.

Adler, L. S., R. Karban, and S. Y. Strauss. 2001. Direct and indirect effects of alkaloids on plant fitness via herbivory and pollination. *Ecology* 82: 2032–2044.

Adler, L. S., and P. M. Kittelson. 2004. Variation in *Lupinus arboreus* alkaloid profiles and relationships with multiple herbivores. *Biochemical Systematics and Ecology* 32: 371–390.

Adler, L. S., M. Wink, M. Distl, and A. J. Lentz. 2006. Leaf herbivory and nutrients increase nectar alkaloids. *Ecology Letters* 9: 960–967.

Adler, L. S., M. G. Seifert, M. Wink, and G. E. Morse. 2012. Reliance on pollinators predicts defensive chemistry across tobacco species. *Ecology Letters* 15: 1140–1148.

Agrawal, A. A., and M. G. Weber. 2015. On the study of plant defense and herbivory using comparative approaches: how important are secondary plant compounds. *Ecology Letters* 18: 985–991.

Arnold, S. E. J., M. E. P. Idrovo, L. J. L. Arias, S. R. Belmain, and P. C. Stevenson. 2014. Herbivore defense compounds occur in pollen and reduce bumblebee colony fitness. *Journal of Chemical Ecology* 40: 878–881.

Asplund, J., K. A. Solhaug, and Y. Gauslaa. 2010. Optimal defense: snails avoid reproductive parts of the lichen *Lobaria scrobiculata* due to internal defense allocation. *Ecology* 91: 3100–3105.

Bates, D., M. Maechler, B. Bolker, S. Walker, R. H. B. Christensen, H. Singmann, B. Dai, and G. Grothendieck. 2014. Package lme4. R Foundation for Statistical Computing, Vienna, Austria. Website: https://cran.r-project.org/web/packages/lme4/index.html.

Bernays, E. A., T. Hartmann, and R. F. Chapman. 2004. Gustatory responsiveness to pyrrolizidine alkaloids in the Senecio specialist, Tyria jacobaeae (Lepidoptera, Arctiidae). *Physiological Entomology* 29: 67–72.

Carey, D. B., and M. Wink. 1994. Elevational variation of quinolizidine alkaloid contents in a lupine (*Lupinus argenteus*) of the Rocky Mountains. *Journal of Chemical Ecology* 20: 849–857.

Cipollini, M. L., and D. J. Levey. 1997. Why are some fruits toxic? Glycoalkaloids in *Solanum* and fruit choice by vertebrates. *Ecology* 78: 782–798.

Cook, D., S. T. Lee, D. R. Gardner, J. A. Pfister, K. D. Welch, B. T. Green, T. Z. Davis, and K. E. Panter. 2009. The alkaloid profiles of *Lupinus sulphureus*. *Journal of Agricultural and Food Chemistry* 57: 1646–1653.

Cook, D., J. S. Manson, D. R. Gardner, K. D. Welch, and R. E. Irwin. 2013. Norditerpene alkaloid concentrations in tissues and floral rewards of larkspurs and impacts on pollinators. *Biochemical Systematics and Ecology* 48: 123–131.

Cook, D., I. W. Mott, S. R. Larson, S. T. Lee, R. Johnson, and C. A. Stonecipher. 2018. Genetic relationships among different chemotypes of *Lupinus sulphureus*. *Journal of Agricultural and Food Chemistry* 66: 1773–1783.

Cornille, A., J. Underhill, A. Cruaud, M. Hossaert-McKey, S. Johnson, K. Tolley, F. Kjellberg, et al. 2012. Floral volatiles, pollinator sharing and diversification in the fig–wasp mutualism: insights from *Ficus natalensis*, and its two wasp pollinators (South Africa). *Proceedings of the Royal Society, B, Biological Sciences* 279: 1731–1739.

Detzel, A., and M. Wink. 1993. Attraction, deterrence or intoxication of bees (*Apis mellifera*) by plant allelochemicals. *Chemoecology* 4: 8–18.

Dolinger, P. M., P. R. Ehrlich, W. L. Fitch, and D. E. Breedlove. 1973. Alkaloid and predation patterns in Colorado lupine populations. *Oecologia* 13: 191–204.

Dunn, D. B. 1956. The breeding systems of *Lupinus*, group *Micranthi*. *American Midland Naturalist* 55: 443–472.

Egan, P. A., L. S. Adler, R. E. Irwin, I. W. Farrell, E. C. Palmer-Young, and P. C. Stevenson. 2018. Crop domestication alters floral reward chemistry with potential consequences for pollinator Health. *Frontiers in Plant Science* 9: 1357.

Ehrlich, P. R., and P. H. Raven. 1964. Butterflies and plants: a study in coevolution. *Evolution* 18: 586–608.

Forrest, J. R., and S. P. Chisholm. 2017. Direct benefits and indirect costs of warm temperatures for high-elevation populations of a solitary bee. *Ecology* 98: 359–369.

Galen, C., R. Kaczorowski, S. L. Todd, J. Geib, and R. A. Raguso. 2011. Dosage-dependent impacts of a floral volatile compound on pollinators, larcenists, and the potential for floral evolution in the alpine skypilot *Polemonium viscosum*. *American Naturalist* 177: 258–272.

Gori, D. F. 1989. Floral color change in *Lupinus argenteus* (Fabaceae): Why should plants advertise the location of unrewarding flowers to pollinators? *Evolution* 43: 870–881.

Harrell, F. E. Jr., and M. F. E. Harrell Jr. 2018. Package Hmisc. R Foundation for Statistical Computing, Vienna, Austria. Website: https://cran.r-project.org/web/packages/Hmisc/index.html.

Heiling, J. M., D. Cook, S. T. Lee, and R. E. Irwin 2019. Data from: Pollen and vegetative secondary chemistry of three pollen-rewarding lupines. Dryad Digital Repository. https://doi.org/10.5061/dryad.586f011.

Irwin, R. E., L. S. Adler, and A. K. Brody. 2004. The dual role of floral traits: pollinator attraction and plant defense. *Ecology* 85: 1503–1511.

Irwin, R. E., D. Cook, L. L. Richardson, J. S. Manson, and D. R. Gardner. 2014. Secondary compounds in floral rewards of toxic rangeland plants: impacts on pollinators. *Journal of Agricultural and Food Chemistry* 62: 7335–7344.

Johnson, N. D., and B. L. Bentley. 1988. Effects of dietary protein and lupine alkaloids on growth and survivorship of *Spodoptera eridania*. *Journal of Chemical Ecology* 14: 1391–1403.

Keith, R. A., and T. Mitchell-Olds. 2017. Testing the optimal defense hypothesis in nature: Variation for glucosinolate profiles within plants. *PloS One* 12: e0180971.

Kelly, C. A., and M. D. Bowers. 2016. Preference and performance of generalist and specialist herbivores on chemically defended host plants. *Ecological Entomology* 41: 308–316.

Kessler, A., and R. Halitschke. 2009. Testing the potential for conflicting selection on floral chemical traits by pollinators and herbivores: predictions and case study. *Functional Ecology* 23: 901–912.

Kinghorn, A. D., and M. F. Balandrin. 1984. Quinolizidine alkaloids of the Leguminosae: structural types, analysis, chemotaxonomy, and biological activities. *Alkaloids: Chemical and Biological Perspectives* 2: 105–148.

Komsta, L. 2011. Outliers: tests for outliers. R package version 014. R Foundation for Statistical Computing, Vienna, Austria. Website: https://cran.r-project.org/web/packages/outliers/index.html.

Kozłowski, J., M. Jaskulska, and M. Kozłowska. 2017. The role of alkaloids in the feeding behaviour of slugs (Gastropoda: Stylommatophora) as pests of narrow-leafed lupin plants. *Acta Agriculturae Scandinavica B, Soil & Plant Science* 67: 263–269.

Kuznetsova, A., P. B. Brockhoff, and R. H. B. Christensen. 2015. Package lmerTest. R package version 2. R Foundation for Statistical Computing, Vienna, Austria. Website: https://cran.r-project.org/web/packages/lmerTest/index.html.

Lee, M. J., J. S. Pate, D. J. Harris, and C. A. Atkins. 2006. Synthesis, transport and accumulation of quinolizidine alkaloids in *Lupinus albus* L. and *L. angustifolius* L. *Journal of Experimental Botany* 58: 935–946.

Lee, S. T., D. Cook, K. E. Panter, D. R. Gardner, M. H. Ralphs, E. S. Motteram, J. A. Pfister, and C. C. Gay. 2007. Lupine induced "crooked calf disease" in Washington and Oregon: identification of the alkaloid profiles in *Lupinus sulfureus*, *Lupinus leucophyllus*, and *Lupinus sericeus*. *Journal of Agricultural and Food Chemistry* 55: 10649–10655.

Lenth, R. 2018. Emmeans: estimated marginal means, aka least-squares means. R package version 1.1. R Foundation for Statistical Computing, Vienna, Austria. Website: https://cran.r-project.org/web/packages/emmeans/index.html.

London-Shafir, I., S. Shafir, and D. Eisikowitch. 2003. Amygdalin in almond nectar and pollen – facts and possible roles. *Plant Systematics and Evolution* 238: 87–95.

Manson, J. S., S. Rasmann, R. Halitschke, J. D. Thomson, and A. A. Agrawal. 2012. Cardenolides in nectar may be more than a consequence of allocation to other plant parts: a phylogenetic study of *Asclepias*. *Functional Ecology* 26: 1100–1110.

McKey, D. 1974. Adaptive patterns in alkaloid physiology. *American Naturalist* 108: 305–320.

Muth, F., J. S. Francis, and A. S. Leonard. 2016. Bees use the taste of pollen to determine which flowers to visit. *Biology Letters* 12: 20160356.

Muzquiz, M., C. Cuadrado, G. Ayet, C. de la Cuadra, C. Burbano, and A. Osagie. 1994. Variation of alkaloid components of lupin seeds in 49 genotypes of *Lupinus albus* from different countries and locations. *Journal of Agricultural and Food Chemistry* 42: 1447–1450.

Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. O'Hara, G. L. Simpson, et al. 2013. Package vegan. Community ecology package version 2. R Foundation for Statistical Computing, Vienna, Austria. Website: https://cran.r-project.org/web/packages/vegan/index.html.

Palmer-Young, E. C., I. W. Farrell, L. S. Adler, N. J. Milano, P. A. Egan, R. R. Junker, R. E. Irwin, and P. C. Stevenson. 2018. Chemistry of floral rewards: intra-and interspecific variability of nectar and pollen secondary metabolites across taxa. *Ecological Monographs* 89: e01335. https://doi.org/10.1002/ecm.1335.

Panter, K., D. Gardner, and R. Molyneux. 1998. Teratogenic and fetotoxic effects of two piperidine alkaloid-containing lupines (*L. formosus* and *L. arbustus*) in cows. *Journal of Natural Toxins* 7: 131–140.

Parachnowitsch, A. L., and J. S. Manson. 2015. The chemical ecology of plant–pollinator interactions: recent advances and future directions. *Current Opinion in Insect Science* 8: 41–46.

R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Website http://www.R-project.org/.

Rhoades, D. F. 1979. Evolution of plant chemical defense against herbivores. *In* G. A. Rosenthal and M. R. Berenbaum [eds.], Herbivores: their interactions with secondary plant metabolites, vol. 2, 3–54. Academic Press, NY, NY, USA.

Roslin, T., and J. P. Salminen. 2008. Specialization pays off: contrasting effects of two types of tannins on oak specialist and generalist moth species. *Oikos* 117: 1560–1568.

Saunders, J. A., N. R. O'neill, and J. T. Romeo. 1992. Alkaloid chemistry and feeding specificity of insect herbivores. *In* Alkaloids: chemical and biological perspectives, 151–196. Springer, New York, NY, USA.

Severns, P. 2003. Inbreeding and small population size reduce seed set in a threatened and fragmented plant species, *Lupinus sulphureus* ssp. *kincaidii* (Fabaceae). *Biological Conservation* 110: 221–229.

Spear, D. M., S. Silverman, and J. R. Forrest. 2016. Asteraceae pollen provisions protect *Osmia* mason bees (Hymenoptera: Megachilidae) from brood parasitism. *American Naturalist* 187: 797–803.

Strauss, S. Y., and W. S. Armbruster. 1997. Linking herbivory and pollination: new perspectives on plant and animal ecology and evolution. *Ecology* 78: 1617–1618.

Strauss, S. Y., and R. E. Irwin. 2004. Ecological and evolutionary consequences of multispecies plant–animal interactions. *Annual Review of Ecology, Evolution, and Systematics* 35: 435–466.

Theis, N., M. Lerdau, and R. A. Raguso. 2007. The challenge of attracting pollinators while evading floral herbivores: patterns of fragrance emission in *Cirsium arvense* and *Cirsium repandum* (Asteraceae). *International Journal of Plant Sciences* 168: 587–601.

Willmer, P. 2011. Pollination and floral ecology. Princeton University Press, Princeton, NJ, USA.

Wink, M., and D. B. Carey. 1994. Variability of quinolizidine alkaloid profiles of *Lupinus argentous* (Fabaceae) from North America. *Biochemical Systematics and Ecology* 22: 663–669.

Wink, M., and L. Witte. 1984. Turnover and transport of quinolizidine alkaloids. Diurnal fluctuations of lupanine in the phloem sap, leaves and fruits of *Lupinus albus* L. *Planta* 161: 519–524.

Wink, M., C. Meißner, and L. Witte. 1995. Patterns of quinolizidine alkaloids in 56 species of the genus *Lupinus*. *Phytochemistry* 38: 139–153.

Zangerl, A. R., and C. E. Rutledge. 1996. The probability of attack and patterns of constitutive and induced defense: a test of optimal defense theory. *The American Naturalist* 147: 599–608.