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 disproportionately 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; Kozlowski 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 polypyllus, 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 circumbellular 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
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 Mallinkrodt 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 Rotor (Glas-Col LLC, Terre Haute, IN, USA) at 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 NH₄OH and then extracted 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.

### 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 (RRt) 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, etc.) as fixed effects and site as a random effect.
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.

<table>
<thead>
<tr>
<th>Alkaloid</th>
<th><strong>Lupinus argenteus</strong></th>
<th><strong>L. bakeri</strong></th>
<th><strong>L. sulphureus</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>401</td>
<td>GTH</td>
<td>BDY</td>
</tr>
<tr>
<td>Ammodendrine</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Anagyrine</td>
<td>—</td>
<td>P</td>
<td>FLS</td>
</tr>
<tr>
<td>Aphyllidine</td>
<td>—</td>
<td>FLS</td>
<td>—</td>
</tr>
<tr>
<td>Argyroline</td>
<td>—</td>
<td>—</td>
<td>FLS</td>
</tr>
<tr>
<td>5,6-Dehydrolupanine</td>
<td>—</td>
<td>—</td>
<td>FLS</td>
</tr>
<tr>
<td>11,12-Dehydrolupanine</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>5,6-Dehydro-α-isolupanine</td>
<td>—</td>
<td>FLS</td>
<td>—</td>
</tr>
<tr>
<td>Dihydroxyaphyllidine</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3-Hydroxy-lupanine</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>α-Isoleucine</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>β-Isoleucine</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Lupanine</td>
<td>—</td>
<td>—</td>
<td>FLS</td>
</tr>
<tr>
<td>Sparteine</td>
<td>—</td>
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<tr>
<td>Thermopsine</td>
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<td>—</td>
</tr>
<tr>
<td>Unknown</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

**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*<sub>3,66</sub> = 5.46, *P* < 0.0001 and *F*<sub>3,36</sub> = 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*<sub>3,15</sub> = 8.77, *P* = 0.0013; Elko: *F*<sub>3,14</sub> = 11.74, *P* = 0.0002; 401: *F*<sub>3,18</sub> = 35.44, *P* < 0.0001; Gothic: *F*<sub>3,14</sub> = 59.31, *P* < 0.0001), for all *L. bakeri* sites (Upper Loop: *F*<sub>3,18</sub> = 31.12, *P* < 0.0001; Kapuchion: *F*<sub>3,18</sub> = 27.52, *P* < 0.0001), and for *L. sulphureus* (*F*<sub>3,15</sub> = 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.
Total alkaloid concentration (µg/g)
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 (ρ = −0.93, P = 0.0025, Fig. 2B lower) while pollen and flower alkaloid concentrations were positively correlated at Baldy but negatively correlated at Elko (ρ = 0.89, P = 0.0068; ρ = −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 (ρ = 0.86, P < 0.05, Fig. 2C upper). Likewise, in L. sulphureus, the only significant correlation was between pollen and stem alkaloid concentrations (ρ = 0.93, P < 0.01; Fig. 2D). There were eight other tissue–tissue correlations with a |ρ| > 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 profiles were distinctive from both flower and leaf profiles for all three 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 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>
<thead>
<tr>
<th>Species</th>
<th>Site</th>
<th>Pollen–Flower</th>
<th>Pollen–Leaf</th>
<th>Pollen–Stem</th>
<th>Flower–Stem</th>
<th>Flower–Leaf</th>
<th>Leaf–Stem</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. argenteus</td>
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<td>0.0445</td>
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<tr>
<td></td>
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<td></td>
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<td>&lt;0.0001</td>
<td>NS</td>
<td>&lt;0.0001</td>
<td>NS</td>
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</tr>
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<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0002</td>
<td>0.0002</td>
<td>NS</td>
<td>0.005</td>
</tr>
<tr>
<td>L. bakeri</td>
<td>UPL</td>
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<td>NA</td>
<td>NS</td>
<td>NS</td>
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</tr>
<tr>
<td></td>
<td>KAP</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NS</td>
<td>0.0302</td>
<td>0.0174</td>
</tr>
<tr>
<td></td>
<td>PEN</td>
<td>&lt;0.0001</td>
<td>0.0005</td>
<td>NS</td>
<td>0.0018</td>
<td>NS</td>
<td>0.0476</td>
</tr>
<tr>
<td>L. sulphureus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.
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 pollen. While these experimental results are compelling, we know little about the secondary chemistry of pollen in pollen-rewarding plants.

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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 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 ≤ 0.05, ** P ≤ 0.01.
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 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.
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 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.
(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.

**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); * unidentifed pollen-specific analytes, found in all pollen samples.
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.

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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.586f911 (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


