

The Effects of Mine Disturbance and Contamination on Pollination of Subalpine Wildflowers

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Abstract

Metals-rich soils can occur naturally or through human activity, such as mining. Plants growing in metal-rich soils often incorporate metals into their tissues; the concentration of these metals can vary among tissues within a plant, among plants of the same species, and among species. Accumulation of metals in plant tissues has mainly been studied in the context of phytoremediation. The effects of metals on plant pollination in these metal-contaminated sites is less well studied. I examined the effects mine spoils on natural systems in Gunnison County, Colorado. I examined concentrations of metals and other elements in plant tissue as well as conducting plant diversity surveys at 6 pairs of mine and control sites. I measured plant traits and reproductive success and observed pollinator visits on *Draba aurea*, *Thlaspi montanum*, and *Lupinus argenteus* at three site pairs. To examine within-site variation, I used GIS mapping techniques. I found no differences in the metal concentration of either soils or plant tissue from mine sites. However, plants growing on mines were generally shorter and had fewer flowers compared to those from control sites, including *D. aurea*, *L. argenteus*, and *T. montanum*. For *D. aurea*, pollination rates were lower at mine sites, which could be a result of changes in plant or pollinator community composition or of the shorter, less showy plants. In addition, plant communities growing on control sites were more diverse than those growing on mine sites, which could suggest that such sites were less attractive to pollinators. Although there was no evidence that these differences were driven by current soil metals concentrations, this study suggests that mining has long-lasting effects on native plant communities and the pollination services they receive; more work should be done to identify which aspects of mining disturbance are causing the changes.

Introduction

One of the fundamental interests of ecology is how variation in abiotic factors affects the survival, performance, and abundance of plants and animals. An example of such an abiotic factor is metals. Metals occur in trace amounts in all soils, and in some cases may occur in high concentrations naturally, for example in serpentine soils (Brady et al. 2005). In other cases, high metal concentrations may be human-induced. There are 23,000 abandoned mines in the state of Colorado alone (“Abandoned Mines” 2008), encompassing hundreds of acres of land contaminated by waste mining material. While acid mine drainage and its effects on aquatic life and food webs are well-studied (Dills and Rogers 1974, Verb and Vis 2000), metal contamination in terrestrial systems, particularly pollination webs, is less well studied.

Mine tailings (rocks and particles remaining from the ore milling process) and their surrounding soil have elevated levels of many metals (Fey and Church 1999). Their small particle size and high surface area to volume ratio make them very reactive so that metals can easily enter the soil-water system (Nash 2002); water flowing from the bottoms of tailings piles has been shown to have far higher concentrations of metals than water entering these areas (Manning et al. 2008). Previous studies of terrestrial systems have focused on invertebrates (Peterson et al. 2003) as well as vertebrates such as prairie dogs (Quinn et al. 2007) and birds (Custer et al. 2008, Larison et al. 2000). The goal of this study was to assess whether metal contamination in abandoned mine sites affected native plant size, diversity, pollination, and plant reproductive success.

In general, metal uptake by plants occurs in three ways: by roots from the soil and its pore water, through leaves from the atmosphere, or onto leaves from wet and dry

deposition (Witte et al. 2004). The natural rate of each of these processes may increase with contamination when more metals are present in soil and air.

For some plants, added soil metals are toxic. Others can grow in metal-rich soils, but growth may be reduced compared to normal soils, and only a few species thrive (Foy et al. 1978). Plants rely on soil water for hydration, nutrients, and minerals (Brady and Weil 2002). For plants growing in metal-rich soils, metal tolerance can be accomplished either by excluding metals when soil water is taken up by roots, or by taking them up with the water and accumulating them in the plant tissue (Chaney 1994, Boyd and Martens 1998). The ability to take up metals at different levels can be constitutive (always present in a species) or facultative (present to varying degrees depending on the environment) depending on species, or even for different metals within the same species (Remon et al. 2007). Plants incorporating high concentrations of metals into their tissues are termed hyperaccumulators (Baker and Brooks 1989).

Because some plants can better tolerate metals in the soil, whether by exclusion or assimilation, differences in plant diversity are common between contaminated and non-contaminated sites. Differences in plant community composition have been noted on metal-rich serpentine soils in Tuscany since the 19th century (reviewed Chiarucci 2003). In Spain, plant diversity was significantly lower around a barium mine than in an uncontaminated pasture (Hernandez and Pastor 2008). Similarly, grassland community composition changes over a gradient of increasing soil copper concentration (Strandberg et al. 2005).

Not only can soil metals affect plant community composition, but they can affect the morphology and success of plants at contaminated sites. Plants growing in extreme

environments, which include those contaminated with heavy metals, may partition resources away from floral growth towards traits associated with plant survival (Grundt et al. 1999). For instance, plants growing in high-copper environments are often shorter than those in uncontaminated environments (Strandberg et al. 2005). Monkeyflower (*Mimulus guttatus*) growing in metal-rich soils often flower and set seed earlier than their counterparts in uncontaminated soils and have different floral sizes which attract different pollinators (Gardner and McNair 2000). In addition, monkeyflower with higher metal tolerance have been found to have lower pollen and seed output per flower, but larger seeds (Harper et al. 1997).

These changes in morphology may affect pollinator behavior and plant reproductive success, which could lead to reproductive isolation of these populations (Brady et al. 2005). While floral characteristics are under selection pressure from many factors, including predation and resource partitioning, pollination can also be an important selective agent (Waser and Price 1981, Cariveau et al. 2004). Plants with more flowers are often more successful at attracting pollinators, which can confer reproductive benefits if plants are pollinator and pollen limited for seed set (Brody and Mitchell, 1997, Ashman et al. 2004). Flower morphology, including shape and size, also has an important role in attracting pollinators (Mitchell 1994, Galen and Cuba 2001). Changes in these characteristics due to resource partitioning could lead to changes in potential pollinator visitation rates to individual plants. Gardner and MacNair (2000) postulated that differences in floral size in mine populations created a niche separate from nearby populations which are unaffected by soil metals, since these plants would be at a disadvantage in direct competition for pollinators. At the community level, increased

diversity and density of plants which share pollinators can lead to higher pollination rates (Moeller 2004, although this is not always the case; see also Elliott and Irwin 2009), so the decreased plant diversity at metal-rich sites found by Chiarrucci (2003), Hernandez and Pastor (2008), and Strandberg et al. (2005) could lead to fewer pollinator visits to even the most attractive plants at such sites. Seed set is often lower on metal-rich soils (Grundt et al. 1999), which could be due to reduced pollinator visitation, to partitioning of resources away from reproduction and towards survival, or to reduced availability of nutrients at mine sites. In either case, reduced seed set and presumably plant recruitment indicates that the next generation of plants on a site will probably not fare significantly better at attracting pollinators.

Besides altering the structure and frequency of plant-pollinator interactions, metal-accumulating plants can provide a mechanism for metals to enter food webs in contaminated areas. For instance, ptarmigan (*Lagopus leucurus*) feeding on willow in contaminated environments accumulate high levels of cadmium over their lifetimes (Larison et al. 2000). Arthropods feeding on the leaves of nickel hyperaccumulators have also been shown to have elevated body loads of nickel (Boyd et al. 2006). However, no studies to my knowledge have tested whether metals accumulate in pollination webs. For example, no studies have tested pollen from plants growing on mine soils for metal content, although pollen in areas with high levels of air pollution has been shown to have elevated metal levels (Kalbande et al. 2008). Due to some functional and structural similarities between herbivory and pollination (e.g., Williams 2003), it is reasonable to hypothesize that the consumption of pollen and nectar by pollinators could mobilize metals into pollination webs just as herbivory mobilizes metals into more general food

webs. Bodies of honeybees (*Apis mellifera*) foraging on hyperaccumulating plants have been shown to have elevated levels of nickel (Boyd et al. 2006), supporting this hypothesis. In addition, metal concentrations below hyperaccumulator levels – for instance 1,000 µg/g dry weight Co, Cu, Cr, Pb, and Ni, or 10,000 µg/g dry weight Mn and Zn (Baker and Brooks 1989) – have been shown to reduce herbivore performance (Coleman et al. 2005). Thus even tissue metal concentrations below levels deemed hyperaccumulation could affect herbivores and pollinators.

I investigated the effects of abandoned mine sites on plants and plant-pollinator interactions in the Rocky Mountains of Colorado. I focused on Colorado mine sites because of the significant number of mines in Colorado and because the area is known for highly attractive meadows of flowering plants, many of which require pollinator vectors to transfer pollen. I investigated five research questions related to the effects of soil metals on pollination systems. (1) Do metals from the soil accumulate in leaves and flowers of native plants? (2) Do plants growing on mine sites express different plant or floral traits and reproductive success than their counterparts on control soils? (3) Is there spatial autocorrelation in these plant and reproductive traits within a site, indicating spatial variation in soil quality? (4) Does pollinator behavior or visitation rates differ between abandoned mine and control sites? (5) Are there differences in plant diversity and plant and floral density between abandoned mine and control sites?

Methods and Materials

Study sites

This study was conducted in the Elk Mountains of Crested Butte, Colorado, near the Rocky Mountain Biological Laboratory (RMBL: Gothic, Gunnison County, Colorado, USA, elev: 2900 m), and specifically in the East River Valley, the Slate River Valley, Paradise Basin, Virginia Basin, and Elk Basin on Mount Emmons. These areas were affected by a mining boom which came to the Elk Mountains in the early 1880s. Mining predominantly involved coal, although silver was also a major product; the coal lines closed in 1952, but some mining continues today, and a new molybdenum mine is being proposed (Warren 1916, Hallowell 2008, “Directory of Colorado State Register Properties” 2008). The area features ample alpine wildflower species, and the Elk Mountains are marketed as the “Wildflower Capital of Colorado.”

Based on personal observation, soil parent material and substrate were not strikingly different among sites in the area; differences in vegetation on the sites should not have been driven by this factor. Study sites were selected as matched pairs, with one contaminated (“mine site”) and one uncontaminated (“control site”) per pair. Due to lack of easy access to laboratory facilities and a short timeframe for fieldwork, I was unable to test soil samples from the sites for metal concentration prior to beginning fieldwork. The control sites were selected based on visual analysis (i.e., soil color appeared normal), proximity to known mine sites, and proximity to drainage of known mine sites. Three site pairs were used for all aspects of the study, and several additional site pairs were used for only the metal analysis and plant diversity portions of the study (described below). In

total, I studied nine matched pairs across four mine sites. Locations of the sites are mapped in Figure 1, and their pairing and elevations are summarized in Table 1.

Two mine sites were located at the Peanut Mine in the Slate River Valley. The Peanut Mine was a coal mine with on-site hard-rock ore processing from 1904 to the 1970s (USEPA 2005) and then housed a silver mill in the 1970s (Renner 2006). It was acquired in 2001 by the Crested Butte Land Trust and, with an EPA grant, reclamation was completed in 2005 (Crested Butte Land Trust 2008). Tailings and refuse were excavated and covered with a layer of topsoil, which was seeded with native grasses and forbs (Renner 2006). Although remediation is deemed complete, there are small areas of the mine which were never remediated and remain contaminated; I selected two study sites at different parts of the mine. The control sites for the Peanut Mine site were across the narrow valley on Slate River Road, where the hillsides have similar vegetation patterns but no mining has occurred.

A third pair of study sites was located above the Elk Creek Trail off of Kebler Pass, Colorado. The mine site was located in the upper basin of the Standard Mine. The Standard Mine was an active silver mine from approx. 1874 to 1974, and was one of the three largest silver-producing mines in the area. The Colorado Geological Survey deemed it one of the most environmentally degraded mines in the district, and it was designated a Superfund site in 2005. The site has arsenic, barium, lead, zinc, cadmium, copper, and chromium contamination in the surface and ground water as well as the soil (USEPA 2008). The control site was located three quarters of a mile below the mine on the Elk Creek Trail; while the creek itself may have carried residue from the mine, the soil was assumed to be uncontaminated since it was in an unmined area.

Additional site pairs were located in other parts of the Standard Mine, with control sites at different locations along the Elk Creek Trail; in Paradise Basin with control sites in Elko Park; and at the Virginia Mine with control sites in areas below the mine on the Copper Creek Trail.

Metal content of plant tissue and soil

Tissue samples were collected from plants growing in each of the site pairs. Samples included leaf and floral tissue. I attempted to collect each tissue type from each species at each site, but not every species was present at every site, and sometimes there were no flowering individuals, so replicates were not perfectly balanced.

There are no known hyperaccumulators in the Rocky Mountains of Colorado (E. Pilon-Smits, personal communication), but there is evidence of accumulation of metals in close relatives of native species in the Colorado Rockies. *Draba japonica*, a congener of *D. aurea* (Brassicaceae), accumulates nickel (Horie et al, 2000), as does *Thlaspi caerulescens*, a congener of *T. montanum* (Brassicaceae) (Seregin and Kozhevnikova, 2003). *Salix* species (Salicaceae), of which there are several in the Rockies, accumulate cadmium (Larison et al 2003), as does *Fragaria anassa*, a congener of *F. virginiana* (Rosaceae) (Cieslinski et al 1996). *Lupinus albus*, a congener of *L. argenteus* (Fabaceae), accumulates zinc (Pastor et al 2003). Samples were taken from the native congeners of these accumulator species: *T. montanum*, *D. aurea*, *Salix* species, *L. argenteus*, and *F. virginiana* (Rosaceae). Samples were also collected from *Potentilla pulcherrima* (Rosaceae), one of the most common species present across all sites. Colorado species are hereafter referred to by genus.

A total of 116 tissue samples were collected, of which 56 were from mine sites and 60 from control sites. Samples included 10 *Lupinus*, 16 *Fragaria*, 17 *Draba*, 18 *Salix*, 28 *Thlaspi*, and 33 *Potentilla*, of both leaf and floral tissue (Table 2). In addition, 19 soil samples were collected from the sites, in most cases a single sample from each site.

All samples and their polypropylene collection vials were handled with gloves and separated from other field materials to avoid any possible metal contamination; these efforts were continued throughout processing. Tissue samples were placed in polypropylene Eppendorf tubes with the tops open and air-dried for several days. Soil samples were placed in paper bags, dried in an oven at 60° C, and then sieved using a 2 mm aluminum sieve. Samples were digested using approx. 1 mL of trace elements-grade nitric acid in acid-washed screw-top teflon vials which could sustain heat and pressure buildup. The vials were placed on a hot-plate overnight to aid digestion. Samples were then removed from the vials, diluted with filtered, deionized water to ~10 mL, and centrifuged to remove any remaining solids.

Samples were processed using Inductively Coupled Plasma-Atomic Emission Spectra (ICP-AES: Perkins Elmer Optima 3000) at the Colorado School of Mines in Golden, Colorado. The ICP-AES was calibrated with five standards before runs, as well as with metal and non-metal standards before and after each sample run, and at an interval of every 15 samples during the run to ensure that the instrument remained calibrated. Each sample was measured three times and the average and standard deviation were calculated to determine accuracy. The ICP-AES detected concentrations of 27 metals, metalloids, and other elements: Silver (Ag), Aluminum (Al), Arsenic (As), Boron

(B), Barium (Ba), Beryllium (Be), Calcium (Ca), Cadmium (Cd), Chromium (Cr), Cobalt (Co), Copper (Cu), Iron (Fe), Potassium (K), Lithium (Li), Magnesium (Mg), Manganese (Mn), Molybdenum (Mo), Sodium (Na), Nickel (Ni), Phosphorus (P), Lead (Pb), Sulfur (S), Antimony (Sb) Selenium (Se), Silicon (Si), Tin (Sn), Strontium (Sr), Vanadium (V), Zinc (Zn), and Uranium (U). Using the dry mass (measured to 0.0001 grams) of each tissue sample, solution concentrations were converted to tissue concentrations. For some samples, element concentrations were below detection limits.

Statistical analyses. All statistics were performed using JMP 7.0.2 statistical software. Soil samples were first analyzed for normality and sample size. Elements were dropped from subsequent analysis for the soil samples if detected in four or fewer samples: this group consisted of silver, arsenic, beryllium, cobalt, chromium, lithium, lead, selenium, tin, vanadium, and uranium. The other distributions were log-transformed. The distributions were analyzed using contingency tables to determine the effects of run number, sample mass, and site type on probability of detection. I was unable to use the matched-pair t-test to analyze differences between concentrations of elements at mine and control sites because some cells were empty due to lack of detection. Instead, an unequal sample size ANOVA was used to compare means between mine and control sites; if variances were unequal, I used a Welch ANOVA.

As with soil samples, the first step in the analysis of tissue samples was to examine which factors affected probability of detection of an element in a sample. Because lab ware and processing materials and were different between runs (the first in July and the second in August), contingency tables were constructed for each element to determine whether run had an effect on the probability of detection. I used the likelihood

ratio with an α value of 0.05 to determine statistical significance of differences in probability of detection. Likewise, I examined whether sample dry mass had an effect on detection using logistic regression, predicting that smaller samples would be less likely to be detected for most elements. Finally, I used logistic regression to determine whether site type (mine vs. control), the main factor of interest, had an effect on probability of detection.

I analyzed the distribution of each element for normality and outliers, checking outliers against known levels in other studies if possible. All distributions were natural log-transformed, except for the calcium and iron data, which were discarded because many values were too high compared to background literature (Larison et al. 2000, Custer et al. 2008).

Site of collection was treated as the experimental unit to avoid pseudoreplication. The original experimental design was a nested, fully crossed ANOVA. Site was nested within site type, and tissue type was nested within species; these two sets of factors were then crossed. However, because not all tissue types and species were found at all sites and because some values were below detection limits and therefore missing, I did not end up with a balanced design. With a small number of replicates for each treatment and some empty cells, I could not accurately analyze the nested factors or the matched pairs using the JMP software. Instead, I analyzed the effect of each factor individually, using ANOVA's. This resulted in a large number of statistical tests being performed; I did not use the Bonferroni correction and instead reported all values to be interpreted (Moran 2003).

For site type, if variances were equal between site types, I used simple ANOVA to determine whether means were different between site types. If variances were unequal, I used a Welch ANOVA.

For species, I used one-way ANOVA to determine whether overall means were different between species. Due to the fact that different species adapt to soil metals by either excluding or accumulating metals, I wanted to compare the native species and identify accumulators and, potentially, hyperaccumulators. If results of the ANOVA were significant, I used the Tukey-Honestly Significant Difference (HSD) test to determine which species means were different. For distributions with unequal variances between species, I used the Welch ANOVA.

For tissue type, I used simple ANOVA to determine whether means were different between flower and leaf tissue. I hypothesized that metal concentrations would be higher in floral tissue, based on Optimal Defense Theory, which states that plants should most strongly defend the structures which are most essential to fitness, such as reproductive structures (Nitao and Zangeri 1987). I hypothesized that metal accumulation could serve as a defense. If variances were unequal between floral and leaf samples, I used a Welch ANOVA.

Plant and floral traits and reproductive success

Ten plants of each of three focal species (*Draba*, *Lupinus*, and *Thlaspi*) were selected haphazardly at each site where they were present: *Draba* and *Lupinus* at the Peanut Mine site pairs, and *Thlaspi* at the Standard Mine site pair. For approximately five consecutive days during the peak of flowering season (June 23-30 for *Draba*, June 27-

July 4 for *Lupinus*, July 11-17 for *Thlaspi*), floral display size (number of open flowers) and plant height (to nearest 0.5 cm) were recorded for each plant. These data were averaged across all observation days (described below) for each plant. All of the fruits from each focal plant were collected when they matured, and seeds were counted in the lab. For *Lupinus*, seeds from all the fruits were counted. However, *Draba* and *Thlaspi* have very small seeds which are difficult to count, so a subset of fruits were examined and the average number of seeds in those fruits was multiplied by the total number of fruits. From these data, I calculated seed set per fruit.

Statistics. I tested the effect of site type on two plant growth traits which might attract pollinators (plant height and floral display size) and two estimates of plant reproduction (total fruits and seed set per fruit). These variables were analyzed separately for each plant species because I wanted to test for species-specific responses to site type. Within each species, means for each variable were compared between sites using the Tukey – HSD test. Because I acknowledged that I was not controlling for all factors within sites and wanted to include variation within each site, I treated samples as replicates rather than treating sites as replicates, and did not adjust for pseudoreplication.

For each variable, a hypothesized model explaining variation was constructed. I predicted that plant height would be explained by site type and that floral display would be explained by site type and plant height. For reproductive success, I predicted that both total fruit set and seed set per fruit would be predicted by site type, plant height, floral display, and estimates of pollinator visitation rate (described below). The models were tested using ANCOVA, and each factor was analyzed for significance using effect tests. I checked explanatory variables for multicollinearity using variance inflation factors (VIF),

and left out factors which exhibited high multicollinearity ($VIF > 9$) (Petraitis et al. 1996).

Pollinator and nonpollinator visitation rate

I observed pollinator visitation rates to the 10 tagged *Draba* and *Thlaspi* plants from the previous part of the study. I observed each focal plant 10 min each day for three to five days, alternating the time of day when each site was visited to control for temperature and time-of-day effects (morning vs. afternoon). Since not all flower visitors are pollinators (Inouye 1980), I only observed in detail those visitors which came into contact with the flower reproductive parts. I also noted the number of nonpollinating insects present on each plant each day. Because non-pollinating insects usually stayed on a single plant throughout the 10 min observation session, these counts were transformed into an average number of nonpollinators per plant per session. Pollinators were identified on the wing to functional group: solitary bee, bumblebee (to species), fly, butterfly (including butterflies and moths), or unknown.

Pollinators were followed throughout a visit, and the time spent on each flower, the number of flowers visited, and the time spent on the plant were recorded (Kearns and Inouye 1993). Data were standardized by the total amount of time the plant was observed. From these data, I calculated percent of time that a plant had visitors (hereafter referred to as pollinator visitation rate). I also calculated time per plant visit (to multiple flowers on the plant as a whole) and time per flower visit as estimates of the quality of pollination services rendered. Net effects for pollination mutualisms often increase with pollinator density until a point of saturation (Holland et al. 2002), and data on visitation frequency

could help identify this relationship in the specific study system. Pollinator visitation rate, time per plant visit, and time per flower visit were natural log-transformed.

Statistics. I tested the effect of site type on nonpollinator visitation rate and on three estimates of pollination services (pollinator visitation rate, time per plant visit, and time per flower visit). For each variable, a hypothesized model explaining variation was constructed. I predicted that nonpollinator density, pollinator visitation rate, time per plant visit and time per flower would be predicted by site type, plant height, and floral display. The models were tested using ANCOVA, and each factor was analyzed for significance using effect tests. I checked explanatory variables for multicollinearity using VIF's as described above.

Spatial Variation

Although it is possible to measure multiple factors which could affect plant growth and reproduction, as well as plant-pollinator interactions, it is impossible to account for all possible sources of variation and any model will have a large amount of unexplained variance. GIS analysis has been used in many ecological applications, perhaps most notably towards identification of habitat and species diversity (Scott et al. 1993). However, GIS can be used to map spatial patterns on scales both small and large, for example in insect ecology (Liebhold et al. 1993) and agricultural soil science (White and Zasoski 1999). Mapping spatial variation could serve two important purposes in examining the effects of mining on plant-pollinator interactions. First, if spatial patterns are present within sites, they could indicate variation in soil micronutrients, contaminants, and other factors at very small distances, which could account for some of the

unexplained variation in non-spatial models. Secondly, spatial variation in plant traits could affect pollinator behavior because, for example, clusters of tall, showy plants could more strongly attract pollinators than if height and floral display were evenly or randomly distributed.

The location of each of the *Draba* and *Thlaspi* study plants at the three main site pairs were mapped using a Trimble GeoXT handheld GPS unit. These data were used to interpolate the spatial variation in plant traits and reproduction traits at each site. Files were viewed using the Trimble Pathfinder program. Using ArcGIS software, I created a file geodatabase for each species at each site, which joined study plant locations to field data (plant height, floral display, fruit count, seed count, and seeds per fruit).

Because each dataset contained only ten sample points, I used jackknifing to create subsamples for interpolation. For each dataset, each point was removed once to create 10 subsamples of nine points each. Jackknifing is an accepted technique and has been used previously in spatial analysis to create subsamples for interpolation (Bowen and Revenaugh 2003).

I interpolated the values of plant height, flower number, fruit set, seed set, and seeds per fruit for each of the subsamples using the natural neighbor technique, creating 50 raster grids for each dataset. The natural neighbor technique was chosen because it is an exact interpolator (preserving sample values), functions smoothly at a local scale, and did not appear to either omit or create major features in the data.

For each variable within each dataset, two averages were created from the raster grids. The first included all ten interpolations. This produced a grid only in the areas covered by all of the subsamples, mainly the centers of each dataset. The second was an

average of the interpolations which included all of the corners in the dataset; depending on the shape of the dataset, this could be as few as three or as many as six interpolations. The first average highlighted the areas most reliably modeled by the second average, which showed the interpolated spatial variation of the variables at each site. The interpolation was classified using six classes and the Jenks natural breaks system.

The shape of the points selected at each site presented a challenge for interpolation. As a result, I left out one site pair of *Lupinus*, since there was only a small area which could be interpolated. I was left with maps for two site pairs (four sites) of *Draba*, and one site pair (two sites) per species for *Lupinus* and *Thlaspi*.

Plant diversity and density

Two 20 m transects were set up at the two Peanut Mine site pairs, the first Standard Mine site pair, both Paradise Basin site pairs, the Virginia Mine site pair. The two transects were 3-5 meters apart running parallel to the areas of highest visual soil contamination, based on texture and color. Plant diversity and density, including forbs and grasses, were measured in 1 m² quadrats every 5 meters on each transect. I sampled every two weeks throughout the flowering season (late June to mid-August) at the three main site pairs and once in August at the additional site pairs as a snapshot at the time of plant tissue collection. Numbers of flowering and non-flowering plants of each species were counted in each quadrat, as well as the number of flowers on flowering species. All plant individuals were identified to species except for grasses, due to the difficulty of identifying grasses to the species level in the field; grasses were pooled together and

counted as one species. The percent ground cover was visually estimated for each species.

Species richness was calculated by counting all the species present (flowering and non-flowering) in the two transects combined on a given day, and averaged across all days for each site. Richness was calculated for total species number, as well as for flowering plants separately. Plant density was estimated by averaging the percent cover of bare ground in all quadrats over the two transects. The density of flowering plants was calculated by averaging the percent cover per quadrat of any species which was flowering in any quadrat of the transect. Floral density was calculated for each site by dividing the total number of open flowers in all quadrats of a site by the area of the quadrats. I compared all these measures of diversity and density using matched-pairs t-tests.

Results

Metal content of plant tissue and soil

No significant differences were detected in log-transformed mean concentrations of any elements in soil samples between mine and control sites. In terms of detection, run had no significant effect for any elements ($P > 0.07$ in all cases). I found marginal effects of sample dry mass and site type on probability of detection. Sample dry mass had a significant effect on detection of boron ($\chi^2_1 = 7.835$, $P = 0.0051$), but this was based on a single sample for which boron was not detected. Site type had a significant effect on the detection of copper ($\chi^2_1 = 5.117$, $P = 0.0237$) and silicon ($\chi^2_1 = 7.049$, $P = 0.0079$), with detection in a higher proportion of samples at mine sites in each case. However, overall,

the soil samples tested seemed to indicate that there was no difference in metal concentrations between mine and control sites.

Site type did not have a significant effect on the detection of any elements (Table 3). I found marginal effects of sample run and sample dry mass on the probability of detection of metals in plant tissue. Antimony and Tin were left out of subsequent analyses because so few samples had detectable concentrations. After testing the distributions of 25 elements, run number only had a significant effect on detection of cadmium ($\chi^2_1 = 3.991$, $P = 0.0458$). The Chi-square test showed that sample dry mass had a significant effect on detection of three of the 25 elements. For cobalt ($\chi^2_1 = 5.361$, $P = 0.0206$) and lithium ($\chi^2_1 = 9.388$, $P = 0.0022$), a greater sample mass was associated with a lower probability of detection. However, for silicon ($\chi^2_1 = 6.297$, $P = 0.0121$), a greater sample mass was associated with a higher probability of detection. Most r-square values were extremely small ($r^2 = 0.134$ for Manganese, $r^2 < 0.07$ for all other cases), suggesting that sample dry mass explained only a small amount of the variation in detection probability.

For most elements, I found that the log-transformed mean concentration of metals in plant tissue at control sites was higher than that at mine sites across all other factors (Figure 2). This ran counter to my hypotheses about metal accumulation at mine sites, but since the soil samples did not indicate higher soil metal concentrations at mine sites, it was less surprising. Means were significantly higher at control sites for boron ($F_{1,21} = 9.635$, $P = 0.0024$), potassium ($F_{1,21} = 3.954$, $P = 0.0492$), and strontium ($F_{1,21} = 4.121$, $P = 0.0447$). For all other elements, means were not significantly different between mine and control sites ($P > 0.13$ in all cases).

Comparing metal concentrations among plant tissue from different species, at least one log-transformed mean concentration was significantly different for boron ($F_{5,50} = 3.840$, $P = 0.0031$), barium ($F_{5,51} = 2.946$, $P = 0.0156$), potassium ($F_{5,51} = 4.774$, $P = 0.0005$), sodium ($F_{5,51} = 3.342$, $P = 0.0077$), nickel ($F_{5,46} = 2.363$, $P = 0.0466$), phosphorus ($F_{5,51} = 2.506$, $P = 0.0344$), and sulfur ($F_{5,51} = 2.577$, $P = 0.0303$). For each of these elements, the Tukey HSD test showed that *Thlaspi* had the highest log-transformed mean concentration (Table 4).

Across all plant species, for all elements except uranium, log-transformed mean concentrations were higher in floral tissue than in leaf tissue (Figure 3). This relationship was significant for boron ($F_{1,40} = 7.942$, $P = 0.0057$), potassium ($F_{1,41} = 9.715$, $P = 0.0023$), lithium ($F_{1,22} = 4.690$, $P = 0.0379$), magnesium ($F_{1,39} = 10.539$, $P = 0.0016$), nickel ($F_{1,39} = 7.709$, $P = 0.0067$), phosphorus ($F_{1,41} = 13.482$, $P = 0.0004$), sulfur ($F_{1,41} = 5.688$, $P = 0.0187$) and strontium ($F_{1,41} = 5.688$, $P = 0.0417$).

Plant and floral traits and reproductive success

Across most plant and floral traits and estimates of plant reproductive success, analyses revealed differences between mine and control sites for *Draba* and *Lupinus* (Tables 5 and 6). Response variables did not tend to be significantly different between sites for *Thlaspi*, even though the confidence intervals were much smaller than those for the other species. This suggested that there was less variation both within and between sites in this species.

Plant height was significantly greater at mine sites for two of the three species (Figure 4). For *Draba*, mean plant height differed significantly between site types ($F_{1,38} =$

28.475, $P < 0.001$). Plants were 68% taller at control than at mine sites. *Lupinus* plants were 90% taller at control sites than at mine sites ($F_{1,38} = 10.146$, $p < 0.001$). Mean plant height for *Thlaspi* was not significantly different between mine and control sites ($F_{1,18} = 2.922$, $P < 0.15$)

For all species, I predicted that site type and plant height would explain variation in floral display size, with a potential interaction between the two factors. *Draba* plants had 220% larger floral displays at control sites, and the model was significant ($F_{3, 39} = 6.618$, $P = 0.0011$). However, a positive effect of plant height explained most of the variation ($F_{1, 36} = 7.849$, $P = 0.0081$) and site type was not a significant explanatory variable ($F_{1, 36} = 5.830$, $P = 0.583$). *Lupinus* plants had a 554% larger floral display size at control sites, and the model was once again significant ($F_{3, 39} = 22.113$, $P < 0.0001$). There was a significant site type by plant height interaction for floral display ($F_{1, 36} = 6.371$, $P < 0.025$), in which increasing plant height corresponded to comparatively greater increases in floral display size at control sites than at mine sites (Figure 5). *Thlaspi* plants showed the opposite trend: they had 59% larger floral displays at mine sites. The overall model was significant ($F_{3, 19} = 7.727$, $P = 0.0027$), with both site type ($F_{1, 16} = 16.311$, $P = 0.0010$) and plant height ($F_{1, 16} = 7.793$, $P < 0.025$) having significant factor effects; taller plants had larger floral displays. The interaction between the two variables was not significant.

To predict total fruit set, I ran a full factorial model including site type, plant height, floral display size, pollinator visitation rate, time per flower visit, and time per plant visit. After adjusting for multicollinearity, two of the three resulting models were significant, but not all of the factors in each model were important (Table 7). For *Draba*,

control site plants produced 120% more fruits than those at mine sites. In the model, all interactions were discarded except for the plant height by site type interaction due to multicollinearity. The resulting model was significant ($F_{7, 27} = 2.7155$, $P = 0.0377$), however the only significant factor effect was that of plant height ($F_{1, 20} = 5.8316$, $P = 0.0254$), which was positively associated with total fruit set. For the *Lupinus* model, there were problems with multicollinearity for all interactions except the plant height by site type and floral display by site type interactions. With these two included, the model was significant ($F_{5, 39} = 8.0349$, $P < 0.0001$). While site type and floral display size were both significant factor effects, their main effects were complicated by a significant interaction ($F_{1, 34} = 5.1057$, $P = 0.0304$): floral display size had a stronger positive effect on total fruit set at mine sites than it did at control sites (Figure 6). For *Thlaspi*, after removing factors with VIF's > 9 , the resulting model included site type, plant height, floral display size, time per plant visit, and time per flower visit, and was not significant ($F_{5, 10} = 0.7236$, $P = 0.6344$).

I had hypothesized that the same suite of plant traits and pollinator visitation estimates that I had used to predict fruit set would have an effect on seed set per fruit. Once again, many factors, had high VIF's and had to be discarded (Table 8). For *Draba* ($F_{1, 38} = 16.871$, $P < 0.001$), seed set was 67% higher at control sites than at mine sites. However, the model including all of the explanatory factors and the interaction of each by site type was not significant ($F_{11, 27} = 1.1771$, $P = 0.3727$). As shown in Table 6, there were few significant differences between sites for seed set per fruit for *Lupinus*. Therefore it was not entirely surprising that the model, which included site type, plant height, floral display size, the site type by plant height interaction, and the site type by

floral display size interaction, was not significant ($F_{5,33} = 1.6247$, $P = 0.1861$). Likewise, the *Thlaspi* model containing site type, plant height, floral display size, time per flower visit, and the plant height by site type interaction was not significant ($F_{5,10} = 2.862$, $P = 0.1366$).

Pollinator and nonpollinator visitation rate

Pollinator visitation rate, time per plant visit, and time per flower visit sometimes differed between mine and control sites (Table 7). In almost all datasets, means did not vary between sites of the same type. For each of these factors, as well as for nonpollinator visitation rate, I hypothesized that variation would be explained by a factorial model including site type, plant height, and floral display size, as well as all of the appropriate interactions. However, in the first three models, problems with multicollinearity led me to discard floral display size as an explanatory variable (its VIF > 9), and use only site type, plant height, and their interaction.

In analyzing pollinator visitation rate for *Draba*, the overall model was significant ($F_{3,27} = 6.0951$, $P = 0.0031$). Site type was the only significant factor effect ($F_{1,24} = 7.8417$, $P = 0.0099$); *Draba* plants growing on control sites had a 345% higher visitation rate than those growing on mine sites. The interaction between site type and plant height was not significant ($F_{1,24} = 3.085$, $P = 0.0918$), but trends showed that plant height had a positive effect at mine sites and a negative effect at control sites (Figure 7). For *Thlaspi*, floral display size was once again discarded due to multicollinearity, but in this case the model was not significant for explaining variation in visitation rate ($F_{3,12} = 2.6351$, $P = 0.1136$).

The hypothesized model explained significant variation in time per plant visit to *Draba* ($F_{3, 27} = 6.1550$, $P = 0.0030$). Both site type ($F_{1, 24} = 3.5338$, $P = 0.0723$) and plant height ($F_{1, 24} = 3.2128$, $P = 0.0857$) had marginally significant factor effects: pollinators spent more time per plant on *Draba* growing at control sites, and also on taller plants. The interaction between the two variables was not significant. For *Thlaspi*, the model was not significant ($F_{3, 10} = 1.1853$, $P = 0.3821$).

Time per flower visit was significantly explained by the model for *Draba* ($F_{3, 27} = 5.5714$, $P = 0.0048$). In this case, site type did not have a significant effect, and the only marginally significant factor effect came from plant height ($F_{1, 24} = 3.0583$, $P = 0.0931$). Across site types, pollinators spent more time on flowers belonging to tall plants than to those belonging to short plants. Once again, the model was not significant for *Thlaspi*, ($F_{3, 7} = 0.1873$, $P = 0.9018$).

For nonpollinator visitation rate to *Draba*, the full factorial model of site type, plant height, and floral display size was significant ($F_{7, 39} = 6.1161$, $P < 0.0001$). However, none of the interactions were significant, and the only significant factor effect was site type ($F_{1, 32} = 14.3546$, $P = 0.0006$); average number of visits per plant per observation session increased from 0.1 at mine sites to 1.9 at control sites. The model was also significant for *Lupinus* ($F_{5, 39} = 7.6838$, $P < 0.0001$). The interaction between site type and plant height was a significant factor effect ($F_{1, 34} = 5.9750$, $P = 0.0198$); plant height had a positive effect on nonpollinator visitation rate at control sites, but no effect at mine sites, partially because the rates were so close to zero at those sites. No model was significant for *Thlaspi* ($F_{3, 19} = 0.6888$, $P = 0.5720$).

Spatial Variation

As shown in the previous analyses, there was variation in plant height, floral display size, and plant reproductive success within sites. For example, height in *Draba* varied by a factor of four at most sites, and seed production varied by up to three orders of magnitude. Fruit and seed production varied by at least an order of magnitude in *Lupinus*. Similar variation was present within sites for *Thlaspi*.

At some sites and for some variables, a portion of this variation was due to spatial autocorrelation (Appendix I). In the *Draba* dataset from Peanut Mine 1, the spatial patterns of variation for plant height, floral display size, and fruit set were highly similar (Figure 8). At Peanut Mine 2, the spatial patterns in *Draba* were highly similar for floral display size and total fruit set, while a separate spatial pattern was common between plant height and seed set per fruit (Figure 9). However, spatial patterns were not as similar between *Draba* variables at the control sites; the only similarities were between floral display size and total fruit set (Figures 10 and 11). This was also true in the *Lupinus* Datasets from Peanut Mine 1 (Figure 12), while at Slate River 2, the pattern was also somewhat similar to the plant height distribution (Figure 13). For the *Thlaspi* datasets, none of the patterns of spatial variation were similar between variables (Figures 14 and 15).

Plant diversity and density

A total of 130 species, excluding grasses, were observed across all sites. Of those, 97 were present in more surveys of control sites than those of mine sites. Some families with species much less common at mine sites included Asteraceae (*Achillea lanulosa*,

Erigeron flagellaris, *Heliomeris multiflora*, *Senecio cana*), Fabaceae (*Lathyrus leucanthus*, *Vicia americana*), Violaceae (*Viola adunca*, *Viola praemorsa*), Ranunculaceae (*Delphinium nuttalianum*, *Ranunculus inaemoeus*), Chenopodiaceae (*Chenopodium album*), Onagraceae (*Epilobium brachycarpum*), Rubiaceae (*Galium boreale*), and Polygonaceae (*Polygonum douglasii*). Conversely, 36 species were present in more mine site surveys than control site surveys. The most prominent of this group was the invasive chamomile *Matricaria perforata* (Asteraceae).

Overall species richness was 43% greater at control sites than mine sites, corresponding to a mean difference of 7.97 species ($t_5 = 4.736$, $P = 0.0026$). Richness of flowering species was 118% greater at control sites than at mine sites, corresponding to a mean difference of 5.57 species ($t_5 = 5.8088$, $P < 0.0001$). In terms of density, bare ground was 101% more prevalent at mine sites than at control sites, an increase of 25 % of the ground in each quadrat ($t_5 = -4.706$, $P = 0.0002$).

Density of flowering plants was 66% greater at control sites than at mine sites ($t_5 = 3.0074$, $P = 0.0050$). Floral density itself was only marginally greater at control sites than at mine sites ($t_5 = -1.494$, $P = 0.0795$).

Discussion

Although I did not find significant differences in metal concentration in either soil or in plant tissue between mine and control sites, site type significantly affected many of the measures of the plant-pollinator community in this study. Species richness, plant density, and floral density were all lower at mine sites than at control sites. Focal plants of three species were generally shorter, had smaller floral display size, and produced

fewer fruits at mine sites than at control sites. Pollinators visited study plants of one species much less frequently at mine sites than at control sites. In short, the structure of plant communities was different between site types, which corresponded to significant differences in plant-pollinator interactions. This suggests that some non-metal aspect of mine disturbance or contamination has a strong effect on these native communities.

Due to time constraints, this project was limited to three main site pairs; site replication was very low. Individual plants were used as replicates in the plant trait, reproductive success, and pollinator visitation portions of the study. Soil conditions can vary over extremely small areas (White and Zasoski 1999), and spatial analysis showed spatial trends of response variables that might indicate varying conditions within sites. However, definitively determining whether mine sites affect plants and pollination would require working in more sites so that site type could be used as the experimental unit. This was beyond the scope of this study, but could be assessed in future research.

Metal content did not differ significantly in soil samples from mine and control sites. It is possible that the mine sites chosen due to visual contamination had actually been effectively remediated; on the other hand, it is possible, though less probable, that the control sites chosen due to lack of visual contamination had not been effectively remediated, or had naturally high levels of soil metals. However, sample size was small and power was low, even compared to other parts of the study; variation in soil metals may have existed, but not been detected. Due to the possibility of soil variation over very small areas, it would have been idea to take a soil sample from the base of each plant collected for ICP-AES or used for plant and pollinator observations. However, the cost of such analysis was far beyond the scope of the project.

Metal content in plant tissue, for the most part, did not vary between mine and control sites. If all the populations used the same uptake mechanisms for nutrients and metals and there was no difference in soil metal concentrations, plant tissue concentrations would be indistinguishable between mine and control sites. Another possible explanation is that even if there were differences in soil metal concentration which were not detected due to small sample size and low power, plants adaptively excluded metals from being taken up by roots. It is interesting to note that despite a lack of significant results, in all cases concentrations tended to have lower concentrations at mine sites; while this is counterintuitive for metals, it may be important for nutrients, if the trends continued towards significance. Lower nutrient levels would strongly effect plant growth and reproduction.

Even though there were few significant differences between tissue concentrations at mine and control sites, I found differences in concentrations due to other factors, such as species and tissue type. For example, *Thlaspi montanum* accumulated significantly more of several elements in its tissue than the other species studied. These accumulated elements included boron, barium, phosphorus, potassium, sodium, sulfur, and, marginally, nickel. These findings are similar to a previous study which reported that one variant of *T. montanum* accumulated nickel, calcium, potassium, and phosphorus in its leaves (Heath et al. 1997). While much attention has been paid to this plant's hyperaccumulating congener *Thlaspi caerulescens* for the purpose of phytoremediation, *T. montanum* may be a useful tool for mine clean-up in the American Rockies, since as a native its presence would likely not upset community dynamics. Scientists search for

plants which would be appropriate for phytoremediation, but native communities may already be providing these services to some extent.

In all cases, metal concentration, as well as that of non-metal elements, was higher in floral tissue than in leaf tissue. This difference was significant for boron, potassium, lithium, magnesium, nickel, phosphorus, sulfur, and strontium. One hypothesis for why this might be is that the metals are accumulated for defense against florivory (Adler 2000). Optimal defense theory predicts that the tissues most closely tied to plant fitness – among which are floral parts, without which sexual reproduction is impossible – should be most heavily defended, and defensive chemicals, whether they are a single element or a compound, are often present in higher quantities in floral tissue than in other parts of the plant (Nitao and Zangeri 1987). Cadmium hyperaccumulation in *T. caerulescens* leaves has been shown to deter herbivores (Jiang et al. 2005), so accumulation of similar elements in reproductive parts could deter floral herbivory. Whether the accumulation was evolved as a defense mechanism or simply because plants needed a place to store metals, this brings up an interesting trade-off: accumulating metals in floral tissue could defend against herbivores, but it could also be detrimental to pollinators (Irwin et al. 2004).

My initially proposed mechanism for the effect of mining on plant-pollinator interactions was that soil metals affect plant growth, which leads to fewer pollinator visits and thus declining reproductive success. There was no evidence that soil metals were higher at mine sites or that metals were accumulated in plant tissue at higher rates at those sites. However, the data supported other parts of the hypothesized mechanism. Floral traits were generally weaker at mine sites: plants were shorter and had smaller

floral displays. While this may not have been due to soil metals, which may have been effectively remediated, the disturbance to the soil cannot be remediated so easily. The mine sites are likely poor places to grow: they potentially lack organic material, are depleted of soil nutrients, and have disrupted soil structures and water storage and transport capacities. Even if mining did not cause the decline in plant traits at the sites due to soil metals, it is likely still the culprit through other pathways.

Pollinator visitation was also lower at mine sites than at control sites, and the plant traits explained a significant amount of this variation. As suggested for another species in this region by Brody and Mitchell (1997), shorter plants with fewer flowers led to lower pollination rates at mine sites. This was especially true for *Draba*. Another possible reason for the decrease in visitation is that the contaminated sites provide less desirable habitat for pollinators, and the decrease in visitation rate is due to the presence of fewer individuals. Unfortunately, an assessment of pollinator density was outside of the scope of this study. This outcome would be interesting in itself, and the question should be revisited in future studies.

In many cases, fruit set and seed set per fruit were lower at mine sites than at control sites, as I had initially predicted. However, pollination rates did not significantly explain these results. This implies that the plants studied may not be pollen-limited for fruit or seed set. *Thlaspi caerulescens* is known to be capable of self-pollination (Pollard and Baker 1996), so it is likely that *T. montanum* has this capacity as well.

Broadening the view past only individuals, site type affected plants' abilities to attract pollinators on a population or community level. Changes in plant and floral density within a population can significantly affect pollinator behavior and visitation

choice (Smithson and MacNair, 1997) as well as fitness, due to the quality of pollen donations (Bosch and Waser 1999). Meanwhile, the diversity of a plant community, or of congeners within that community, can attract more or fewer pollinators (Moeller, 2004). Thus the disappearance of some species within a family from control to mine sites could lead to fewer pollinator visits. For example, seven *Senecio* (Asteraceae) species were observed across the sites; however, the diversity of *Senecio* species and density of plants in each population may affect pollinator visits to individual plants within a site.

Spatial variation within a site could also affect pollinator behavior. Some distributions showed a relatively simple gradient in the plant trait or reproductive response variable from one side of the plot to another (for example, many maps of *Draba* at Peanut Mine 1), or from the edges to the center (for example, many maps of *Draba* at Peanut Mine 2). In addition, the maps showed that in many cases, spatial patterns of variation in one factor matched spatial patterns of variation in another factor. In these cases, one area of a site may have many more tall, showy plants, and pollinators may be more likely to visit this dense concentration of attractive plants than they would be to visit a single attractive plant on the other side of the plot. While not all sites showed strong spatial patterning of variables, the fact that some did suggests that the effects of mining are not homogeneous across a site, and that spatial variation in soil characteristics – whether this means metal concentrations, water storage, organic matter content, or something else – may be responsible for variation in plant traits. Without drawing multiple soil samples from each site, this may be the best way to identify underlying spatial variation in soil characteristics. This technique should be considered for use in remediation efforts.

Finally, these results underscore the complexity of plant-pollinator interactions. The same factors did not significantly explain variation in pollination estimates across species, or even across different measures for the same species. In some cases, models using multiple factors were significant, but none of the factors themselves were significant. Adding the variable of site type only added to this complexity, as interactions between site type and other factors were common. Ecological systems are not simple, and the ways in which they are perturbed by human activity such as mining are not easy to predict, nor will they be easy to remedy. This study touched on many ways in which mining disturbance affects plant-pollinator interactions, all of which would benefit from being studied in more detail.

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Table 1. Mining type and elevation for site pairs. Italicized pairs were used only for metal concentration and plant diversity sections of the study.

Site Pair	Mine Site	Mining Type	Elevation	Control Site	Elevation
1	Peanut Mine 1	coal, silver	2700 m	Slate River Road 1	2750 m
2	Peanut Mine 2	coal, silver	2700 m	Slate River Road 2	2750 m
3	Standard Mine 1	silver	3375 m	Elk Creek Trail 1	3300 m
4	<i>Standard Mine 2</i>	<i>silver</i>	<i>3375 m</i>	<i>Elk Creek Trail 2</i>	<i>3300 m</i>
5	<i>Standard Mine 3</i>	<i>silver</i>	<i>3375 m</i>	<i>Elk Creek Trail 3</i>	<i>3300 m</i>
6	<i>Standard Mine 4</i>	<i>silver</i>	<i>3375 m</i>	<i>Elk Creek Trail 4</i>	<i>3300 m</i>
7	<i>Virginia Mine</i>	<i>silver</i>	<i>3400 m</i>	<i>Copper Creek Trail</i>	<i>3050 m</i>
8	<i>Paradise Basin 1</i>	<i>gold or silver</i>	<i>3400 m</i>	<i>Elko Park 2</i>	<i>3200 m</i>
9	<i>Paradise Basin 2</i>	<i>gold or silver</i>	<i>3400 m</i>	<i>Elko Park 2</i>	<i>3200 m</i>

Table 2. Sample sizes of plant tissue taken for analysis with ICP-AES.

		control	mine
Draba	<i>flower</i>	5	3
	<i>leaf</i>	5	4
Fragaria	<i>flower</i>	4	2
	<i>leaf</i>	5	3
Lupine	<i>flower</i>	2	2
	<i>leaf</i>	3	3
Potentilla	<i>flower</i>	8	7
	<i>leaf</i>	10	8
Salix	<i>flower</i>	0	5
	<i>leaf</i>	3	7
Thlaspi	<i>flower</i>	6	7
	<i>leaf</i>	9	6

Table 3. A. Proportion of plant tissue samples (n=116) above detection limit for each element. **B.** Contingency analysis by site type (n_{control}=60, n_{mine}=56), with likelihood ratio χ^2 . If element was detected in all samples, statistics were not calculated. For all elements, $P > \chi^2$ was greater than 0.15 (d.f. = 1).

		A. Total	B. By Site Type		
element	detection limit (µg/g)	proportion detected	proportion detected at control sites	proportion detected at mine sites	χ^2
Ag	0.0024	0.17	0.22	0.13	1.732
Al	0.0180	0.99	1	0.98	1.466
As	0.0320	0.11	0.08	0.14	1.037
B	0.0080	0.96	0.97	0.95	0.289
Ba	0.0003	0.99	1	0.98	1.466
Be	0.0003	0.19	0.15	0.23	1.275
Ca	0.0100	1	1.00	1.00	
Cd	0.0009	0.78	0.80	0.79	0.177
Co	0.0050	0.29	0.25	0.34	1.115
Cr	0.0030	0.17	0.15	0.20	0.438
Cu	0.0009	0.91	0.93	0.89	0.605
Fe	0.0021	1	1.00	1.00	
K	0.0940	1	1.00	1.00	
Li	0.0021	0.29	0.30	0.29	0.029
Mg	0.0003	0.92	0.92	0.93	0.058
Mn	0.0003	0.99	0.98	1	1.327
Mo	0.0040	0.69	0.63	0.75	1.855
Na	0.0070	0.94	0.97	0.91	1.640
Ni	0.0030	0.78	0.75	0.82	0.880
P	0.1600	1	1.00	1.00	
Pb	0.0200	0.29	0.28	0.30	0.057
S	0.1100	1	1.00	1.00	
Se	0.0330	0.18	0.18	0.18	0.004
Si	0.0040	0.83	0.82	0.84	0.104
Sr	0.0003	1	1.00	1.00	
U	0.0850	0.22	0.18	0.27	1.192
V	0.0012	0.29	0.28	0.30	0.057
Zn	0.0006	1	1.00	1.00	

Table 4. Differences between log-transformed mean concentrations of elements in which ANOVA found $p < 0.05$ for species means; reported means are back-transformed. Species sharing the same letter in the Significance Group column have means which are not significantly different according to the Tukey-HSD test.

<i>Element</i>	<i>Species</i>	<i>Mean concentration (ug/g)</i>	<i>Standard Error (ug/g)</i>	<i>Significance Group</i>
Boron	<i>Thlaspi</i>	114.690	25.545	A
	<i>Draba</i>	53.517	13.979	A, B
	<i>Fragaria</i>	38.901	8.164	A, B
	<i>Potentilla</i>	35.981	4.675	B
	<i>Salix</i>	43.463	12.318	B
	<i>Lupinus</i>	21.919	2.981	B
Barium	<i>Thlaspi</i>	743.300	317.39	A
	<i>Salix</i>	216.454	72.92	A, B
	<i>Draba</i>	296.065	159.25	A, B
	<i>Potentilla</i>	387.288	147.24	A, B
	<i>Fragaria</i>	53.424	12.76	A, B
	<i>Lupinus</i>	163.035	133.70	B
Nickel	<i>Thlaspi</i>	66.407	34.094	A
	<i>Salix</i>	51.555	28.782	A, B
	<i>Potentilla</i>	31.755	14.002	A, B
	<i>Lupinus</i>	15.566	7.805	A, B
	<i>Draba</i>	15.411	11.327	A, B
	<i>Fragaria</i>	4.568	3.292	B
Phosphorus	<i>Thlaspi</i>	11598.730	2430.7	A
	<i>Draba</i>	5832.487	1368.7	A, B
	<i>Lupine</i>	5839.589	1541.2	A, B
	<i>Fragaria</i>	5056.134	1271.1	A, B
	<i>Salix</i>	4797.100	1262.6	A, B
	<i>Potentilla</i>	3737.530	481.9	B
Potassium	<i>Thlaspi</i>	56091.773	12155	A
	<i>Draba</i>	28849.418	6385	A, B
	<i>Fragaria</i>	22093.175	5293	A, B
	<i>Lupinus</i>	18161.836	5472	A, B
	<i>Potentilla</i>	17121.582	2232	B
	<i>Salix</i>	11827.650	2702	B
Sodium	<i>Thlaspi</i>	608.479	284.18	A
	<i>Salix</i>	407.648	237.73	A, B
	<i>Draba</i>	174.879	50.98	A, B
	<i>Potentilla</i>	202.892	70.11	A, B
	<i>Fragaria</i>	84.257	15.16	A, B
	<i>Lupinus</i>	107.396	50.61	B
Sulfur	<i>Thlaspi</i>	15645.125	3901.0	A
	<i>Draba</i>	5995.254	1021.2	A, B
	<i>Lupinus</i>	6159.268	1501.1	A, B
	<i>Fragaria</i>	6025.431	1561.2	A, B
	<i>Potentilla</i>	4702.927	716.6	B
	<i>Salix</i>	4110.664	935.6	B

Table 5. Site means and standard errors for plant height and floral display size of three species. N=10 for subgroups. Within a species and variable, the same letter indicates distributions that are not statistically different by the Tukey-HSD test.

Species	Site	Plant Height (cm)			Floral Display		
		<i>mean</i>	<i>S.E.</i>		<i>mean</i>	<i>S.E.</i>	
<i>Draba</i>	Mine A	26.700	2.775	A	40.520	8.541	
	Mine B	19.870	2.261	A	29.020	10.109	
	Control A	38.830	1.170	B	96.700	33.069	
	Control B	39.483	14.340	B	102.000	88.580	
<i>Lupine</i>	Mine A	33.570	2.065	A	16.935	8.844	A
	Mine B	27.546	3.101	A	5.833	2.632	A
	Control A	57.988	2.831	B	70.400	14.420	B
	Control B	58.150	2.668	B	78.475	18.582	B
<i>Thlaspi</i>	Mine A	10.780	0.999		12.633	2.780	A
	Control A	13.054	0.877		5.150	0.686	B

Table 6. Site means and standard errors for total fruit set and seed set per fruit for three species. N=10 for subgroups. Within a species and variable, the same letter indicates distributions that are not statistically different by the Tukey-HSD test.

Species	Site	total fruit set			seeds set per fruit		
		<i>mean</i>	<i>S.E.</i>		<i>mean</i>	<i>S.E.</i>	
<i>Draba</i>	Mine A	148.400	56.025	A	4.800	0.788	A
	Mine B	60.400	18.947	A B	5.060	1.025	A B
	Control A	172.100	26.751	A B	7.840	1.931	B
	Control B	287.400	81.320	B	8.640	0.811	B
<i>Lupine</i>	Mine A	48.500	22.468		1.509	0.117	A
	Mine B	16.100	8.522		1.390	0.288	A
	Control A	40.000	10.370		0.657	0.143	A
	Control B	32.000	6.799		1.674	0.162	B
<i>Thlaspi</i>	Mine A	22.100	6.366		1.968	0.208	
	Control A	13.600	2.522		2.011	0.145	

Table 7. Significance of factors in models explaining variation in total fruit set for *Draba aurea*, *Lupinus argenteus*, and *Thlaspi montanum*. Other potential explanatory factors had been excluded if their variance inflation factors (VIF's) were greater than 9.

	Source	DF	VIF	F Ratio	Prob > F
<i>Draba</i>	Site Type	1, 20	2.19	0.6709	0.4224
	plant height (cm)	1, 20	2.28	5.8316	0.0254
	floral display	1, 20	1.69	3.9818	0.0598
	log time per plant visit	1, 20	4.15	0.0171	0.8972
	log time per flower visit	1, 20	3.83	0.0212	0.8858
	log pollinator visitation rate	1, 20	2.02	0	0.9962
	plant height (cm)*Site Type	1, 20	1.60	1.0038	0.3284
<i>Lupinus</i>	Site Type	1, 34	4.339	15.9233	0.0003
	plant height (cm)	1, 34	5.679	2.0685	0.1595
	floral display	1, 34	4.589	17.8468	0.0002
	plant height (cm)*Site Type	1, 34	1.531	1.3956	0.2457
	floral display*Site Type	1, 34	2.73	5.1057	0.0304
<i>Thlaspi</i>	Site Type	1, 5	3.596	0.2725	0.624
	plant height (cm)	1, 5	3.372	1.4243	0.2862
	floral display	1, 5	2.808	0.0513	0.8297
	log time per plant visit	1, 5	7.829	0.3543	0.5776
	ln avg time per flower (s)	1, 5	6.51	0.5082	0.5078

Table 8. Significance of factors in models explaining variation in set set per fruit for *Draba aurea*, *Lupinus argenteus*, and *Thlaspi montanum*. Other potential explanatory factors had been excluded if their variance inflation factors (VIF's) were greater than 9.

	Source	DF	VIF	F Ratio	Prob > F
<i>Draba</i>	Site Type	1, 16	3.17	3.5527	0.0777
	plant height (cm)	1, 16	2.96	0.1075	0.7472
	floral display	1, 16	8.92	0.067	0.799
	log time per plant visit	1, 16	4.81	1.5025	0.238
	log time per flower visit	1, 16	4.44	0.2021	0.659
	log pollinator visitation rate	1, 16	2.63	0.1717	0.6841
	plant height (cm)*Site Type	1, 16	2.05	0.0591	0.8109
	floral display*Site Type	1, 16	7.40	0.3122	0.5841
	log time per plant visit*Site Type	1, 16	3.28	0.0079	0.9304
	log time per flower visit*Site Type	1, 16	3.42	0.252	0.6225
	log pollinator visitation rate*Site Type	1, 16	1.70	0.2042	0.6574
<i>Lupinus</i>	Site Type	1, 28	4.02	0.9154	0.3469
	plant height (cm)	1, 28	4.69	4.2561	0.0485
	floral display	1, 28	4.16	0.0307	0.8621
	plant height (cm)*Site Type	1, 28	1.56	0.4927	0.4885
	floral display*Site Type	1, 28	2.83	0.0098	0.9218
<i>Thlaspi</i>	Site Type	1, 5	5.11	0.2329	0.6498
	plant height (cm)	1, 5	3.15	4.3333	0.0919
	floral display	1, 5	2.89	1.8654	0.2302
	ln avg time per flower (s)	1, 5	1.12	0.6646	0.452
	plant height (cm)*Site Type	1, 5	1.63	0.0932	0.7725

Table 9. Means and standard errors for pollination rates by site for *Draba* and *Thlaspi*. N=10 for subgroups. Within a species and variable, the same letter indicates distributions that are not statistically different by the Tukey-HSD test.

Species	Site	time/plant visit			time/flower visit		visitation rate		
		<i>mean</i>	<i>S.E.</i>		<i>mean</i>	<i>S.E.</i>	<i>mean</i>	<i>S.E.</i>	
<i>Draba</i>	Mine A	4.060	2.099	A B	1.048	0.321	2.890	1.761	A
	Mine B	7.999	3.341	A	3.225	2.174	3.752	1.403	A
	Control A	20.096	10.151	A B	2.397	0.975	9.322	4.299	A B
	Control B	48.962	13.283	B	6.350	1.394	19.650	5.384	B
<i>Thlaspi</i>	Mine A	0.400	0.147		0.350	0.133	0.048	0.017	
	Control A	1.298	0.811		0.474	0.229	0.212	0.350	



Figure 1. Map of study area showing Standard Mine, Peanut Mine, Virginia Mine, and Paradise Basin mine sites and associated control sites. Image created using Google Earth.

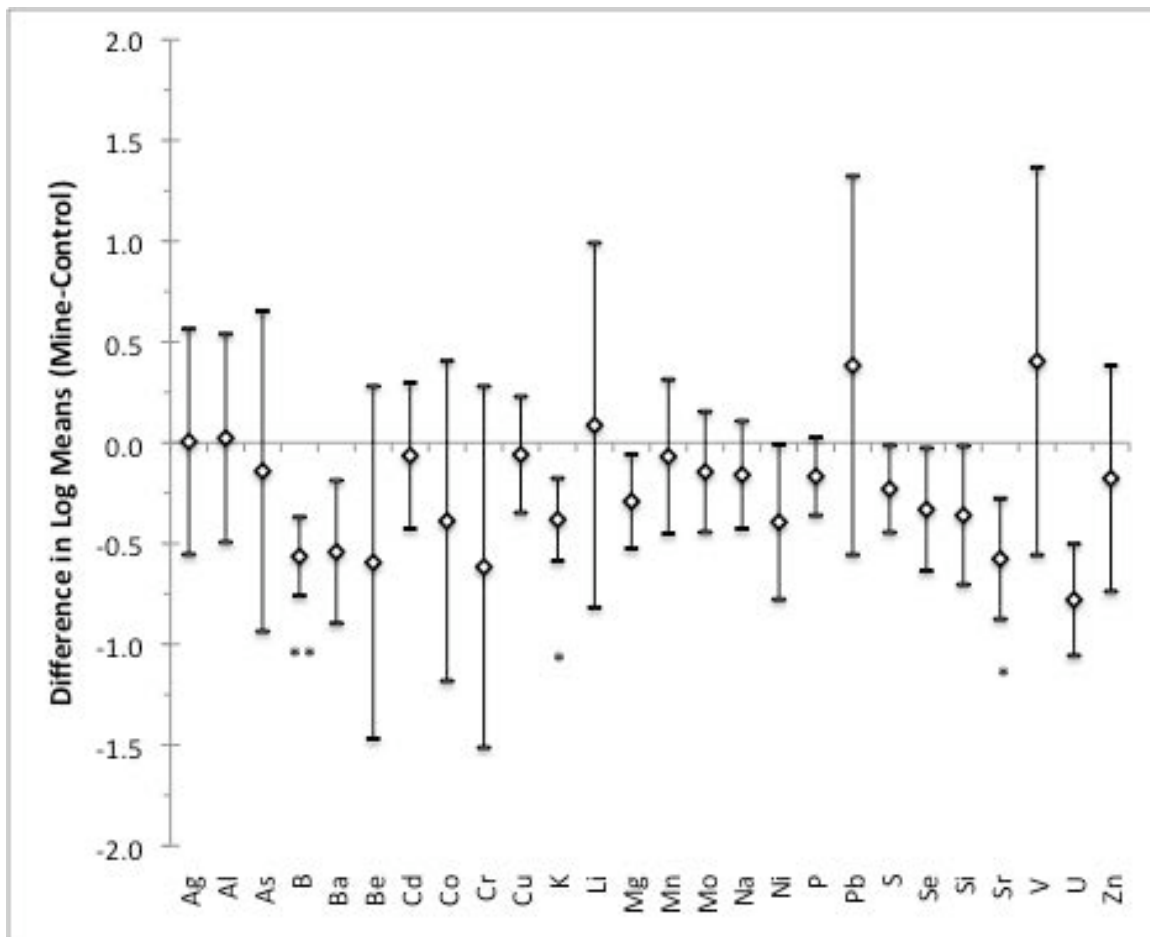


Figure 2. Differences between means of log-transformed element concentrations in plant tissue at mine sites and control sites, with bars representing ± 1 standard error. ** indicates significantly different means ($p < 0.05$) and * indicates marginally significantly different means ($p < 0.10$).

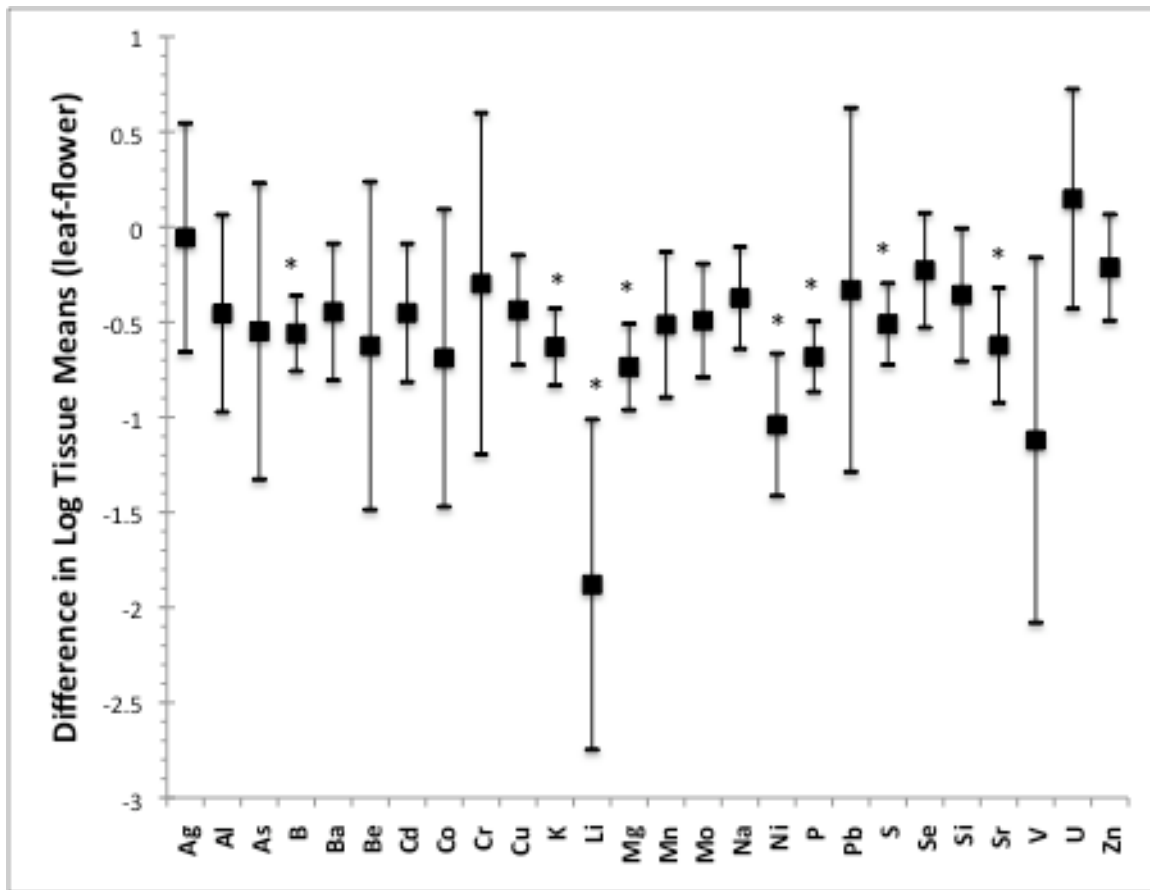


Figure 3. Mean differences in log-transformed concentrations of elements in leaf and floral tissue. Bars represent ± 1 SE. Significant differences (detected using one-way ANOVA, $\alpha = 0.05$) are indicated with an * above the error bar.

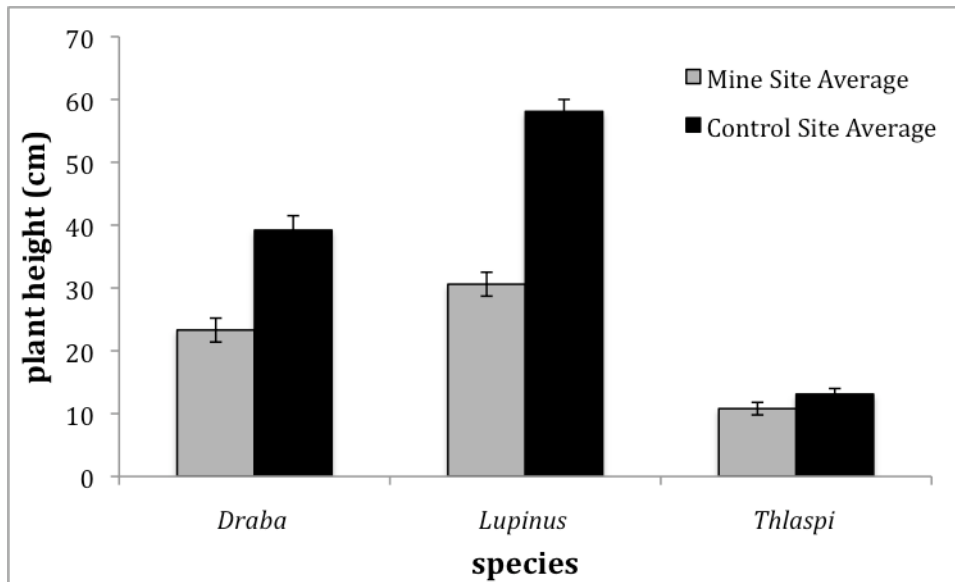


Figure 4. *Draba aurea* and *Lupinus argenteus* plants were significantly taller at mine sites than at control sites, while there was no significant difference in plant height for *Thlaspi montanum*. Bars represent $\pm$ one standard error.

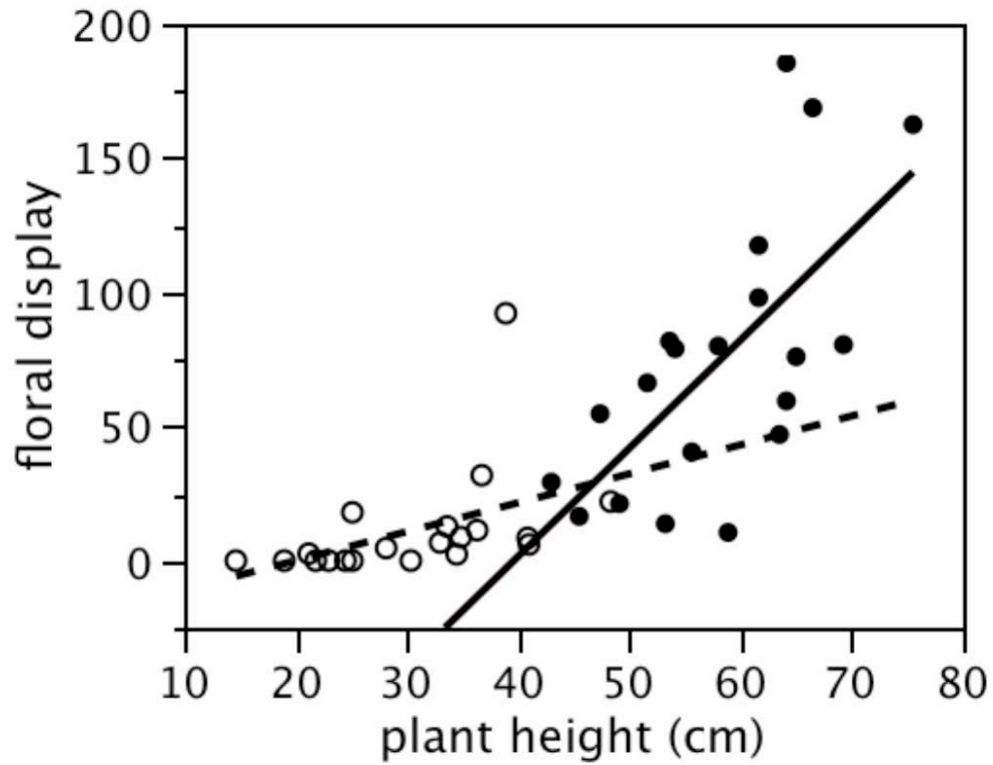


Figure 5. Variation in the floral display size (average number of open flowers during flowering season) of *Lupinus argenteus* was explained by an interaction between site type and plant height ($F_{1, 36} = 6.371$, $P < 0.025$). The solid line and filled circles represent mine site plants, while the dotted line and empty circles represent control site plants.

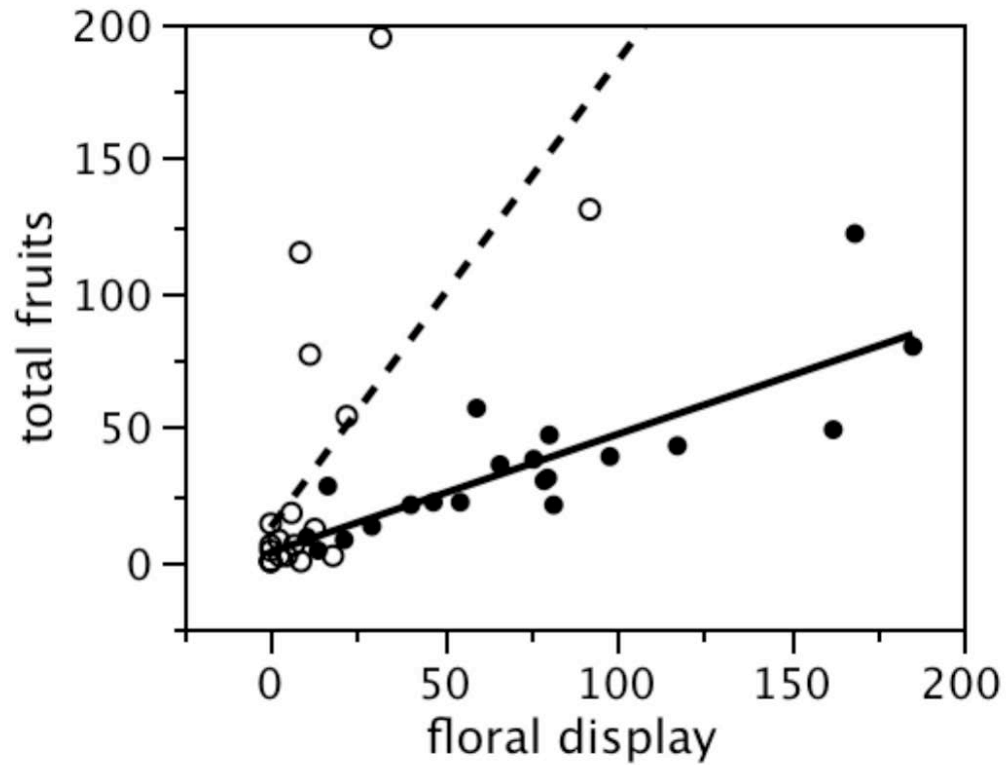


Figure 6. Variation in the total fruit set of *Lupinus argenteus* was explained by an interaction between site type and floral display size (average number of open flowers during flowering season) ($F_{1,34} = 5.1057$, $P = 0.0304$). The solid line and filled circles represent mine site plants, while the dotted line and empty circles represent control site plants.

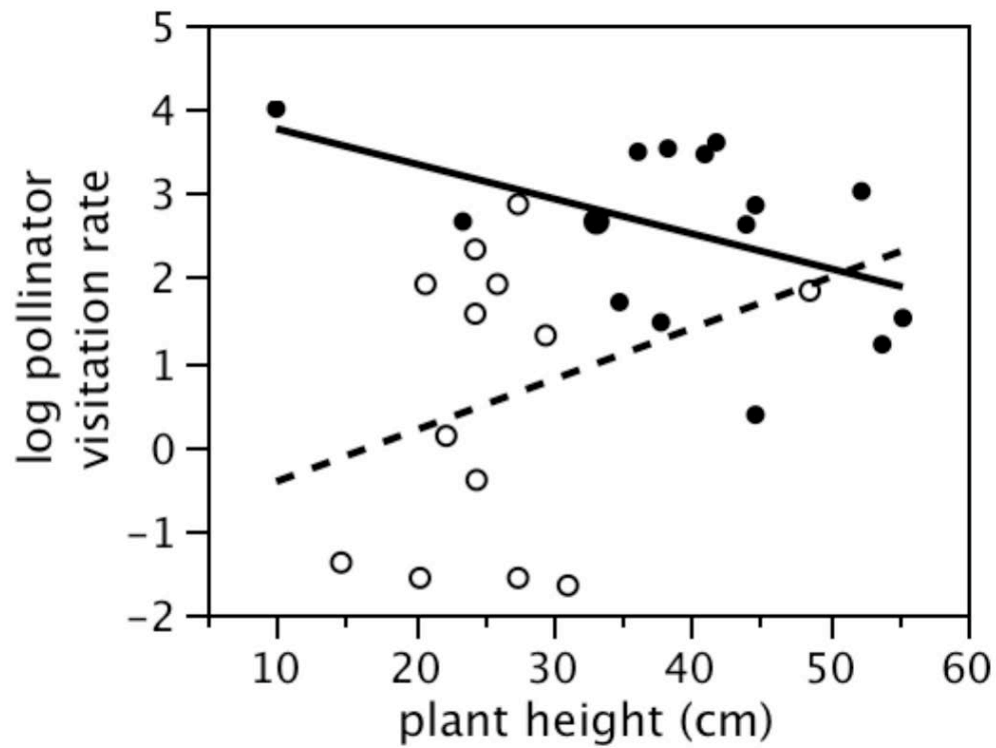


Figure 7. The variation in observed pollinator visitation rate was explained by site type ($F_{1, 24} = 7.8417$, $P = 0.0099$). There was a marginally not significant interaction between site type and plant height ($F_{1, 24} = 3.085$, $P = 0.0918$), which helped explain variation in visitation rate for *Draba*. The solid line and filled circles represent mine site plants, while the dotted line and empty circles represent control site plants.

Appendix I: Maps of Spatial Variation

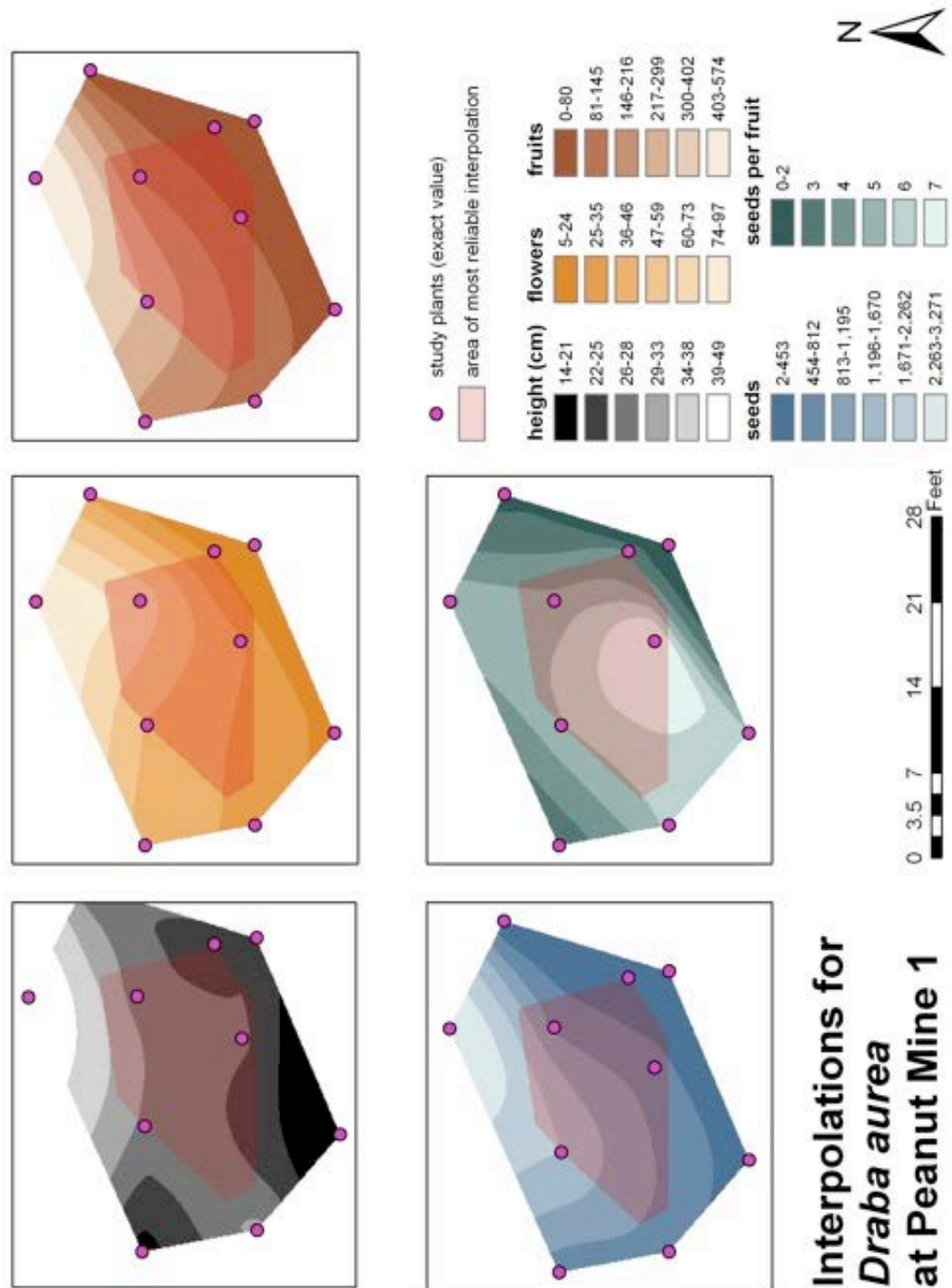


Figure 8. Interpolated spatial variation in five growth and reproductive measures for *Draba aurea* at Peanut Mine 1.

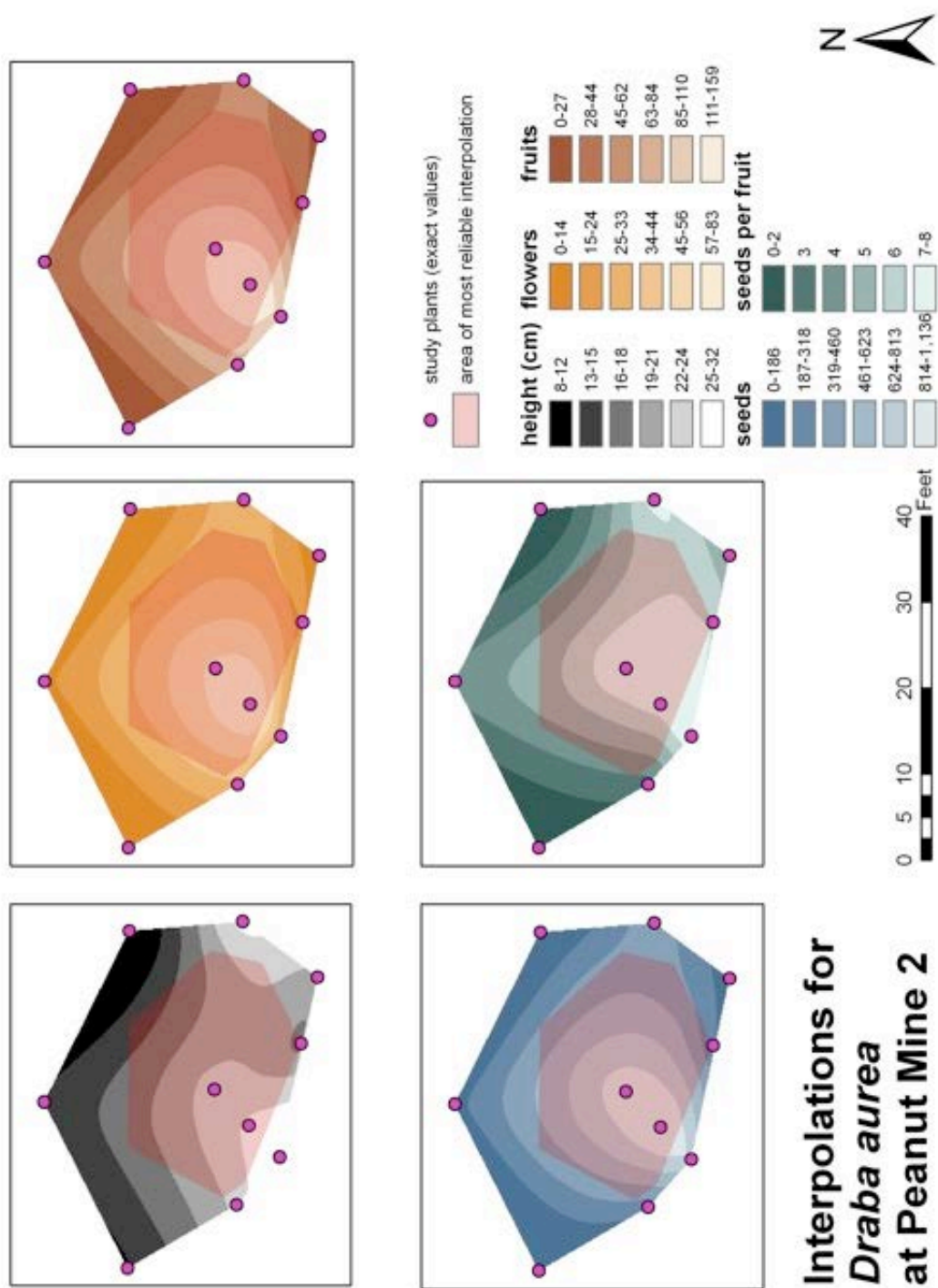


Figure 9. Interpolated spatial variation in five growth and reproductive measures for *Draba aurea* at Peanut Mine 2.

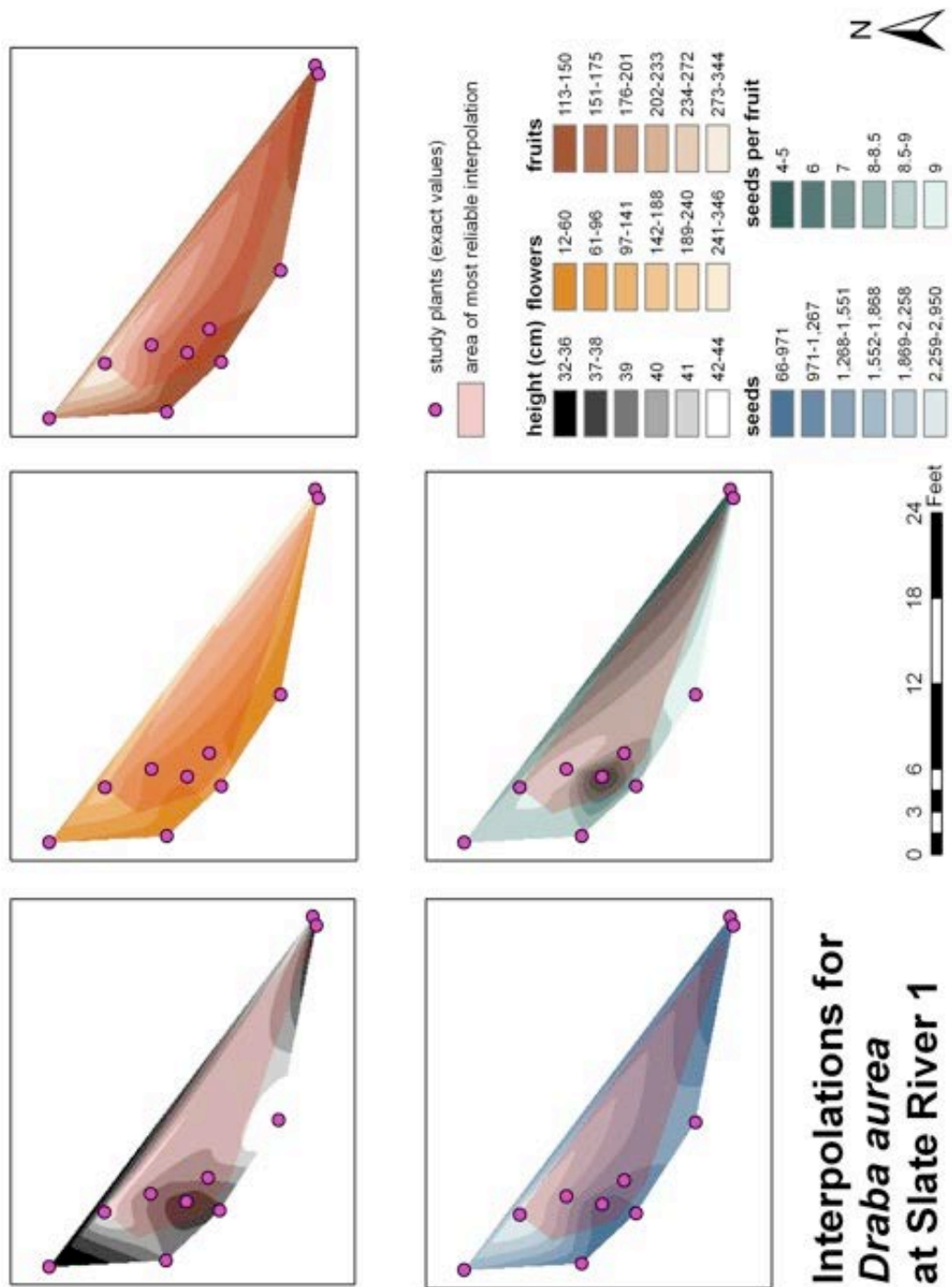


Figure 10. Interpolated spatial variation in five growth and reproductive measures for *Draba aurea* at Slate River 1.

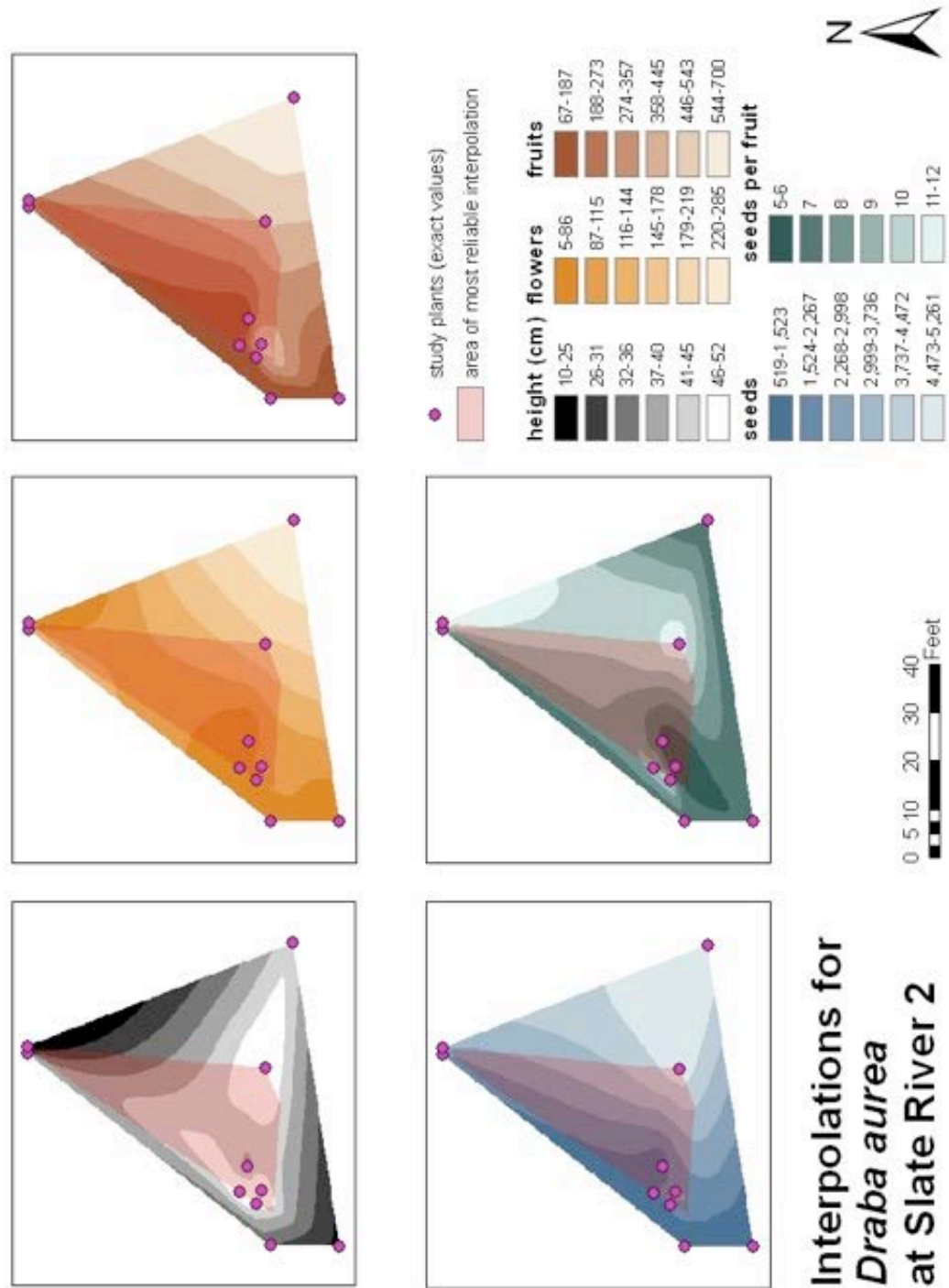


Figure 11. Interpolated spatial variation in five growth and reproductive measures for *Draba aurea* at Slate River 2.

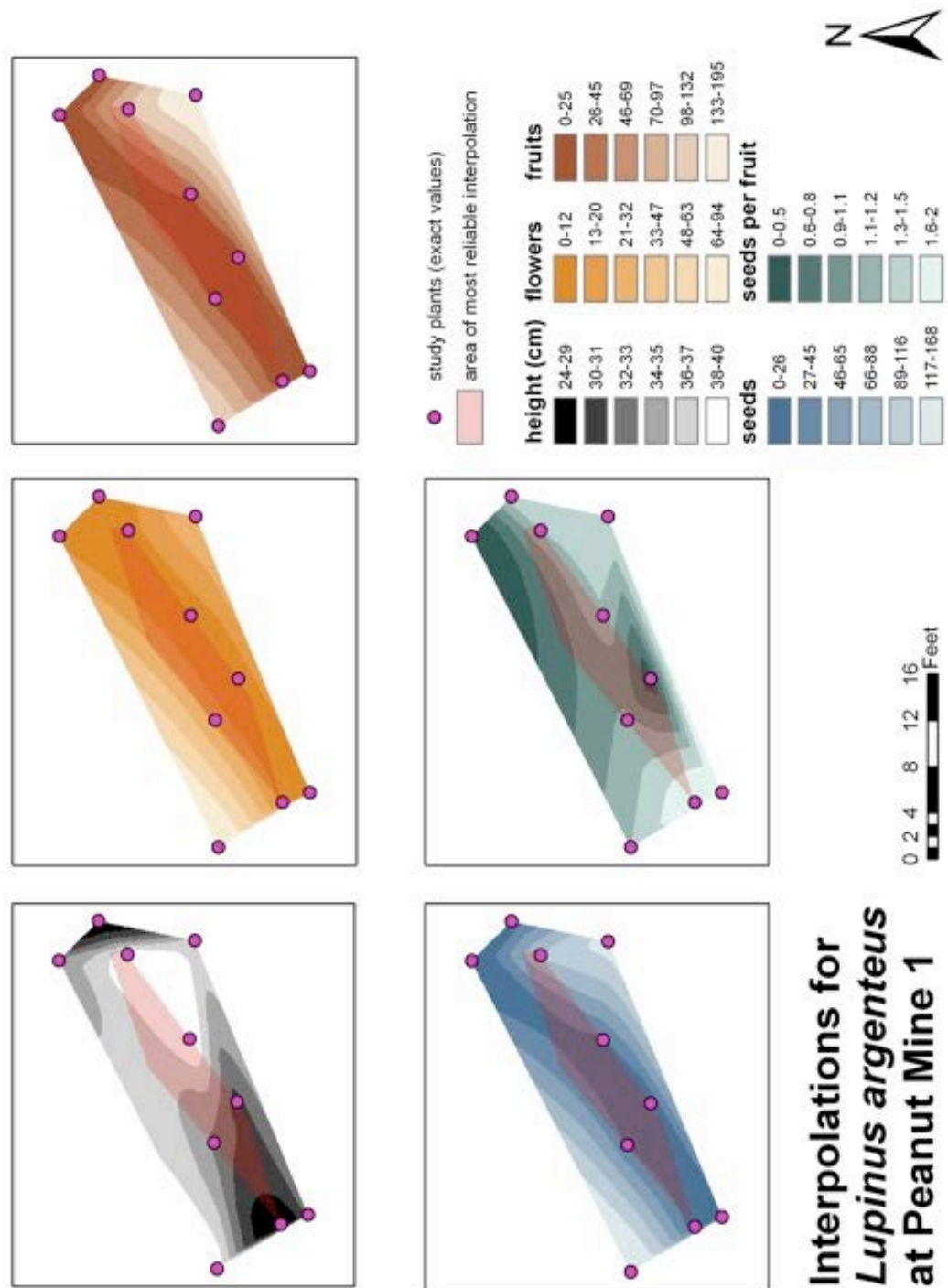


Figure 12. Interpolated spatial variation in five growth and reproductive measures for *Lupinus argenteus* at Peanut Mine 1.

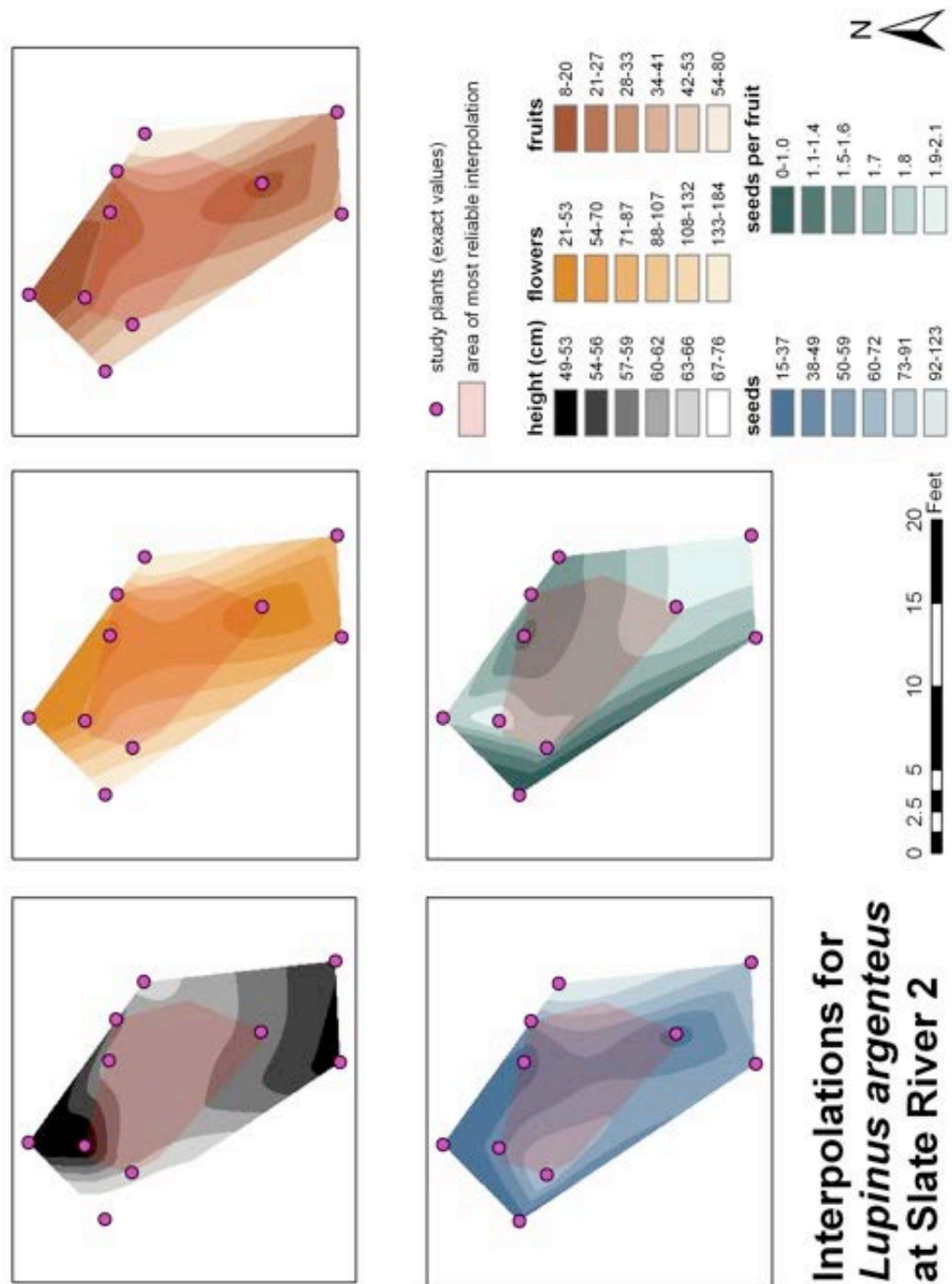


Figure 13. Interpolated spatial variation in five growth and reproductive measures for *Lupinus argenteus* at Slate River 2.

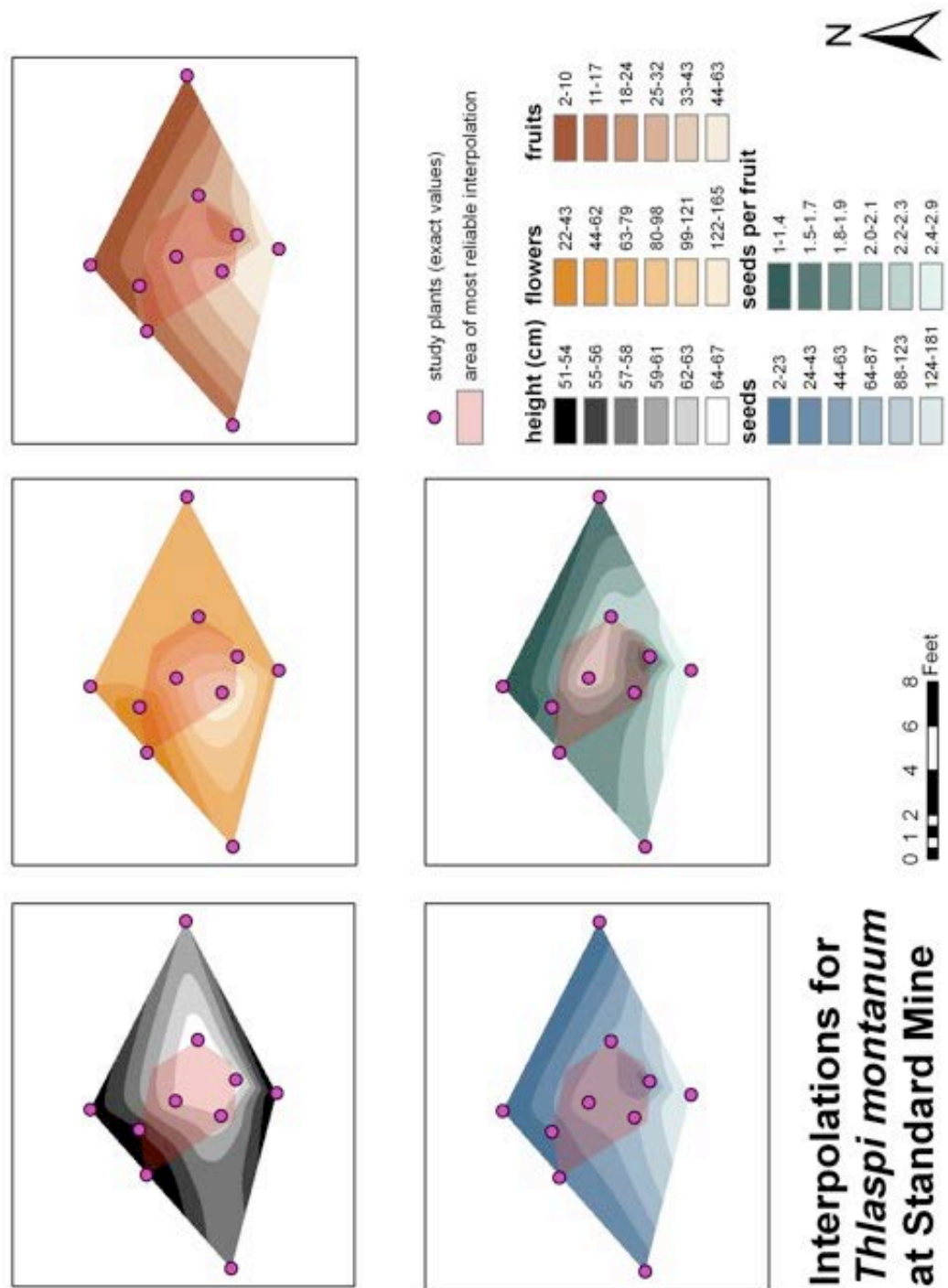


Figure 14. Interpolated spatial variation in five growth and reproductive measures for *Thlaspi montanum* at Standard Mine.

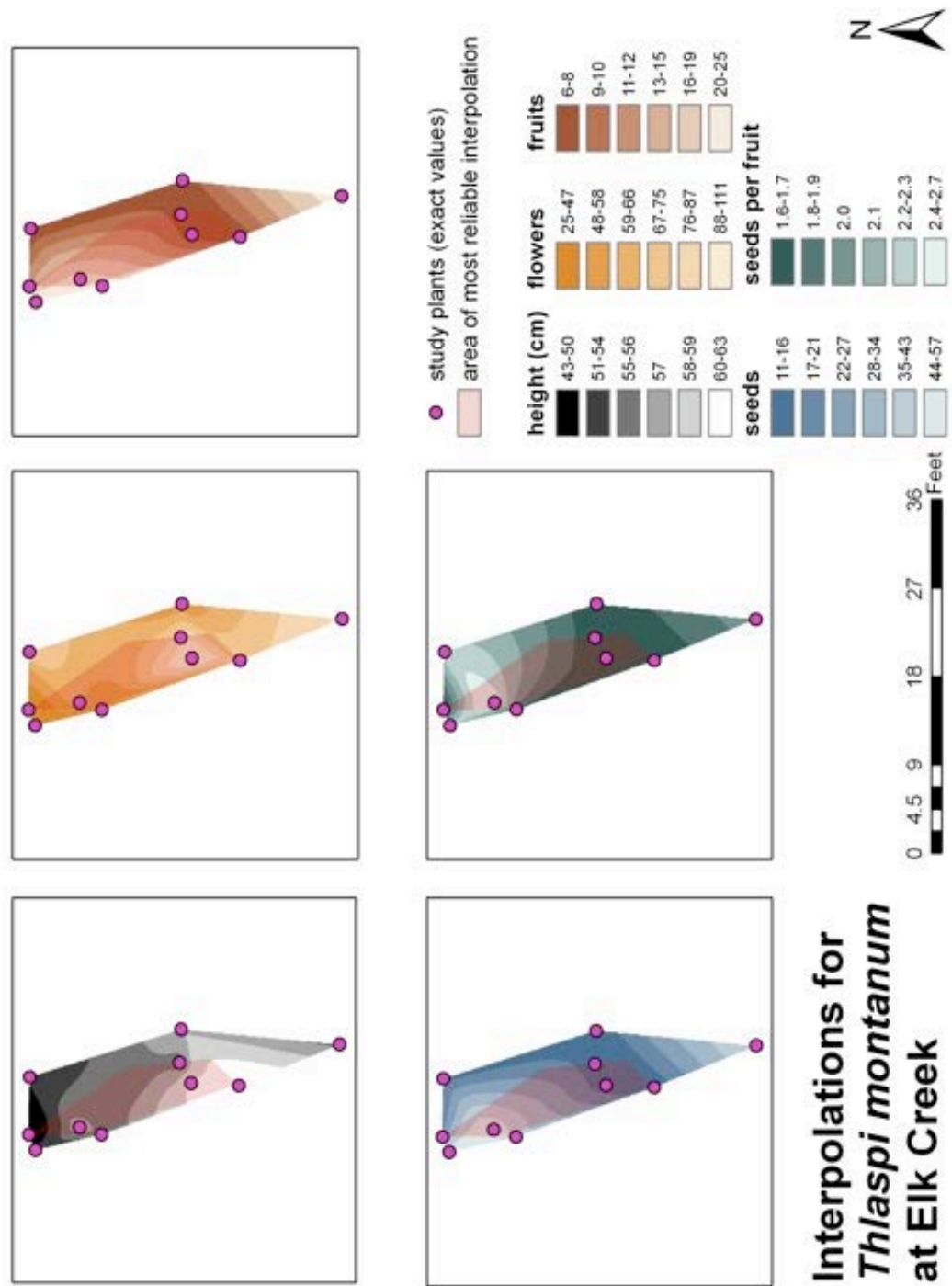


Figure 15. Interpolated spatial variation in five growth and reproductive measures for *Thlaspi montanum* at Elk Creek.