

Local adaptation to habitat-specific herbivory and light levels in *Cardamine cordifolia*

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Abstract

In this study I examined the effects of phenotypic plasticity and local adaptation to light environment in the crucifer *Cardamine cordifolia*, at the Rocky Mountain Biological Laboratory, in Gothic, Colorado. Previous work by Louda and colleagues showed that plants in sun environments were subject to heavy herbivore attack, whereas plants in shaded environments essentially escaped herbivory. Louda et al.'s reciprocal transplant experiments in adjacent sun and willow-shade habitats showed that sun and shade plants exhibited phenotypic plasticity in response to low and high sun environments and that they were not locally adapted to these habitats. However, plant genotypes in deep shade habitats (under spruce trees) that have potentially been subjected to many generations of low light and low herbivory rates are likely to be locally adapted to this environment, as opposed to plants in high light, high herbivore environments. This dichotomy has not been studied previously and was the aim of my summer research project. I conducted a common garden, reciprocal transplant experiment using plants from sun and deep shade environments and added an herbivory component as well. I planted a

total of 520 individual rhizomes from nine sun source sites and nine deep shade source sites in 3 shade and 3 sun gardens. Plants in the shade source habitats had significantly higher rates of herbivory, high canopy coverage, and received significantly lower photosynthetically available radiation than plants from sun sources. After planting in common gardens, I found that plants from sun and shade environments exhibited phenotypic plasticity when placed in the opposite habitat. I also found, however, that plants exhibit evidence of local adaptation. In general I found that plants in shade gardens tended to have bigger leaves, longer petioles, fewer leaves and lower chlorophyll concentration than plants in sun gardens. I also found that plants from sun habitats tended to grow more quickly than shade plants regardless of which garden they were planted, suggesting local adaptation. One interpretation of this pattern is that because plants living in sun habitats exhibit 10-40x higher rates of herbivory than plants in shade habitats, this could select for a more rapid growth rate in plants in the sun than in the shade because more rapid growth rate could increase plant fitness in the face of high herbivore pressure. We are currently finishing experiments in which we added herbivores to each rhizome in order to determine how plants in sun and shade gardens from each source habitat (sun or shade) respond to herbivores. I predict that plants in shade gardens will be less well-defended than sun plants and that plants from shade sources will be less well defended than sun plants.

Introduction:

Most multicellular organisms are either plants or insects, and interactions between plants and their insect herbivores are thought to be a major driver of this diversity (Ehrlich and Raven 1964). In addition to the tremendous amount of variation in these interactions at the species level, there is habitat-specific variation in interactions between even a single host plant and its herbivore community. The system that I studied – *Cardamine cordifolia* and the

community of nearly 30 insects that feed upon it (Louda & Collinge 1992) – has been studied extensively at RMBL, and is a classic example of how the strength of interactions between plants and herbivores can vary with local environment (Collinge & Louda 1988). Collinge and Louda (1988) showed that there is a higher rate of herbivory on plants in the sun than plants in willow shade. In their studies, they also found that plants in the sun have lower reproductive output than plants in the shade (Louda & Rodman 1996). When plants in the sun and willow shade were treated with insecticide, both plants had similar reproductive and survival success. These results suggested that differential herbivory levels in the sun and shade account for lower reproductive success in sun plants. *C. cordifolia* is more abundant in shaded environments and it is likely that high activity of the herbivore community in the sun has driven the plant into the shade (Louda and Rodman 1996).

An interesting unanswered question in this system is whether consistent herbivory pressure that varies between sites based on light environment has led to differences in defense phenotypes between plants from sun-exposed and shaded habitats. Because there are tradeoffs between allocating resources to defense or to growth and reproduction (Louda & Rodman 1996), plants that are consistently exposed to different levels of herbivory might be expected to be genetically differentiated in defense traits due to differential natural selection in the two environments. Louda and her colleagues' plants were shaded by short stands of willows and were adjacent to sun plants, which may not provide a consistent differences (e.g., extreme low-or high-light environment). In my study, I went a step further and used plants from environments that represented the extremes in light habitats, where herbivory pressure is predicted to differentiate the most. Accordingly, I focused my attention on populations of *C. cordifolia* deep within old-growth spruce/fir stands and in large, open, sunlit meadows.

To investigate whether there are heritable differences in anti-herbivore defenses between populations of *C. cordifolia* in sun-exposed and deeply shaded habitats, I examined interactions between *C. cordifolia* and one of its most abundant herbivores, the drosophilid fly *Scaptomyza nigrita*, which is specialized on this plant species at RMBL (Andrew Gloss, unpublished data). The leaf mining larvae of this fly can consume about 75% of the leaf area in sunny conditions in a single year (Collinge 1987), which exceeds damage levels known to reduce plant fitness and reproductive output (Collinge and Rodman 1996). Similar to the herbivore community of *C. cordifolia* as a whole, *S. nigrita* is dramatically more abundant in the sun (Louda and Rodman 1989b). Specifically, I used *S. nigrita* larval performance as a bioassay to test for adaptation to differential herbivory in different habitats. In addition, I compared growth, leaf morphology, and chemical defenses to determine how sun- and shade-collected plants performed under different light conditions. All of this work was conducted in the context of six common gardens I created in sun and shade habitats, in which I grew plants collected from nine sun and nine shade habitats in the East River Valley.

Hypothesis 1: I expect the sun plants to exhibit stronger defenses because they have experienced constant, high rates of herbivory over many generations.

Hypothesis 2: I expect that the plants will have different phenotypic characteristics depending on light conditions in each experimental garden, differences because of garden to source interaction, and source habitat related differences in phenotype.

Source Populations

In order to test my hypotheses I selected nine sun and nine shade sites along the 401 trail and Copper Creek drainages. I therefore used rhizomes from 18 different source populations along the East River Valley. From each source site, I sampled at least 30 rhizomes that were to be used later on in a common garden experiment. I attempted to use the same ‘clone’ at each site in order to minimize within-size variation. The 30 rhizomes were planted into six common gardens (described below)—I planted 5 rhizomes from each source site into each garden. I separated individual ramets by cutting away above-ground biomass (AGB) and cutting underground rhizomes to approximately the same amount of biomass. Prior to trimming away AGB from each rhizome, I measured herbivory levels in 10 plants at each source site and recorded the number of *S. nigrita* adult female feeding punctures in the first two (most basal) leaves of each flowering stalk, the number of mines in each of these leaves, and leaf length and width in these two leaves (Figure 1). Since the common gardens were designed to test for both phenotypic plasticity and local adaptation to differences in light/herbivore conditions, that meant that I needed nine of the sources to be from deep shade under spruce shade with a history of at least 50 years of shade and the other nine sources to be from extreme conditions of sunlight for the most part of the day. In order to quantify photosynthetic activity I measured PAR (photosynthetic active radiation) using a light meter (Spectrum Technologies, Inc.). This allowed me to show on a micrometer scale how much light energy plants were getting between sun and shade locations (Figure 2). I also measured canopy density above each source site (how much vegetation was directly over the site, Figure 3) and diameter at breast height of the four largest trees within four meters of the source site (DBH, Figure 4). Quantifying canopy density using a densitometer allowed me to show how much sunlight a site actually received and DBH

showed me that there was a historically natural sun or shade environment at the source I was sampling. I also measured elevation at each site (figure 5). When values for each of these variables was averaged across the nine sun vs. nine shade sites, the sun source vs. shade source sites were highly significantly different from one another (using t-tests) with the exception of elevation.

Common Gardens

From each of the 18 source sites I collected 30 rhizomes. To avoid pseudoreplication, I randomly placed five rhizomes from each sample source into every garden. Therefore, I planted five rhizomes from each source (x 18 sources) into each of six gardens, which totaled 540 rhizomes planted. I created three sun gardens with conditions comparable to where I sampled from sun locations, and the other three gardens located in deep spruce shade on the west side of the East River across from the Gothic Town Site. In each garden, I planted 4 rows of up to 23 rhizomes. They were placed about 16 cm apart and were completely randomized. There were 90 rhizomes planted per garden. The gardens were watered enough as to keep the ground moist and plants were caged using fine mesh cloth to prevent herbivory. The cages allow me to conduct an herbivory assay on plants that had not been induced by other herbivores. After 2.5 weeks and at the end of the experiment, I measured petiole length, number of leaves >10 millimeters in length, leaf width, chlorophyll concentration on each plant that emerged from the rhizome, and I have performed one *S. nigrita* larval transfer (see Whiteman et al. 2011) in each plant >10 mm. This will allow for quantification of differences in plant phenotype due to light environment, garden conditions, and source by garden differences. Because leaves are growing from rhizomes (meristematic tissue) in these gardens, differences in

phenotype among plants from different collection localities reared in the same garden will likely reflect heritable differences.

Herbivory Assay

This bioassay will serve as a test for differences in anti-herbivore defenses in plants collected from different collection localities grown in common sun and shade habitats. I first collected larvae from leaves of *C. cordifolia* from one particular site at Copper Lake in Colorado to use for larval transfers into the gardens. I brought these leaves to the common gardens and extracted a single larva and placed it into the largest leaf of each available rhizome in each garden. I implanted a total of ~280 larvae into the gardens. After 72 hours of mining, I will remove the larvae, measure leaf area mined and weigh each larva. I expect (1) that larvae in shade gardens will eat more than larvae in sun gardens (regardless of source habitat) given that plants in sun gardens are likely to have more robust defences (given the literature) and (2) that larvae reared in shade source plants will feed more than larvae reared on sun source plants. I expect this in part because plants from the shade are likely to produce a constitutive shade avoidance syndrome phenotype, which is associated with a decreased in sensitivity to jasmonate-dependent defenses.

Results:

Source habitat differences: Using t-tests comparing values across the nine sun vs. nine source sites, I found that, on average, plants from the shade sources were in significantly shadier (DBH and PAR) environments and suffered significantly less herbivory than plants from sun habitats (Table 1), and there was no difference in elevation, on average, for each source habitat (sun vs. shade).

Common gardens: Preliminary data was taken from the gardens 2.5 weeks after planting, where I measured a number of important variables. I measured petiole length, number of leaves >10 mm, and leaf width. To compare values across garden types and source types, I used a nested ANOVA approach with source type (sun or shade) nested within garden type (sun or shade) (Table 2). Comparing all plants in sun vs. shade gardens, I found that plants in shade gardens had significantly longer petioles than plants in sun gardens, but that plants in sun gardens had wider leaves and more chlorophyll and leaves than plants in sun gardens. Within a garden type, however, I found that sun source plants had higher values for all these traits than shade source plants *within* a garden type. Results are expected soon for the herbivore assay but they are not yet available.

Discussion

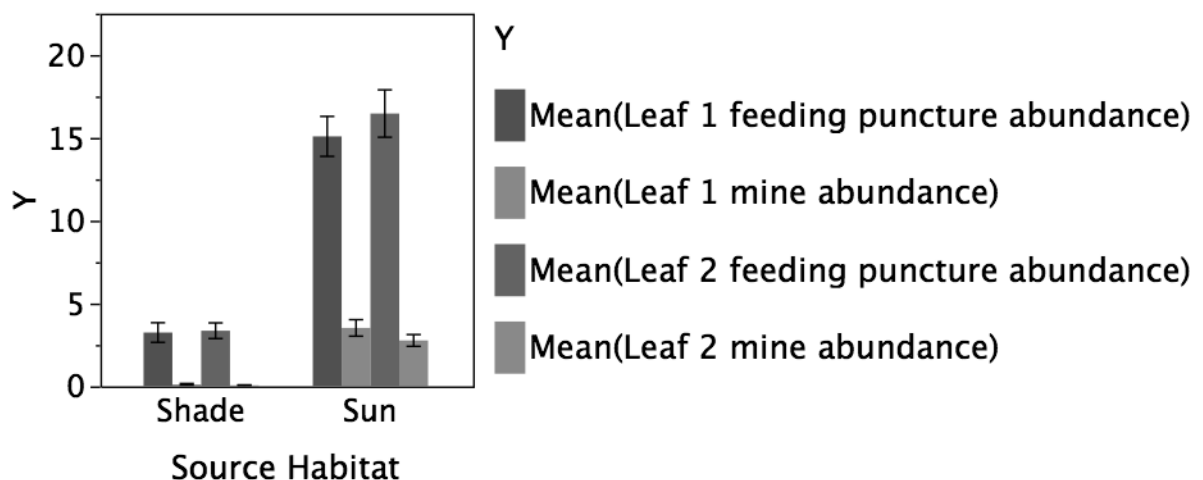
The results that I have obtained so far have shown that phenotypic responses of plants reared in sun vs. shade gardens accounted for most of the differences in the phenotypic traits that I measured. Plants responded to the light environment as I predicted—plants reared in sun gardens had shorter petioles than plants in shade gardens and plants in sun gardens had higher chlorophyll content. Leaf width was also greater in sun vs. shade gardens, which surprised me, but this was likely due to the fact that the shade plants probably grow more slowly, but will ultimately have larger leaves than sun garden plants. The second major finding is that *within* a garden type, I found evidence for source habitat effects. Although these were minor compared to the phenotypic plasticity, they were statistically significant. I found that plants from sun habitats typically had higher values for all traits measured compared to plants from shade habitats, regardless of garden type. This suggests that plants from the shade simply grow more slowly than plants from the sun. This may be due to differential natural selection in sun vs.

shade habitats, particularly with respect to herbivory. I would like to speculate that when I harvest data from the herbivore bioassay that there will be significantly higher leaf area mined in shade habitats and in shade plants than in sun habitats and in sun source plants. This, I think, will be due to the fact that jasmonic acid signaling and downstream glucosinolate production will be lower in plants from the shade than the sun.

Literature Cited

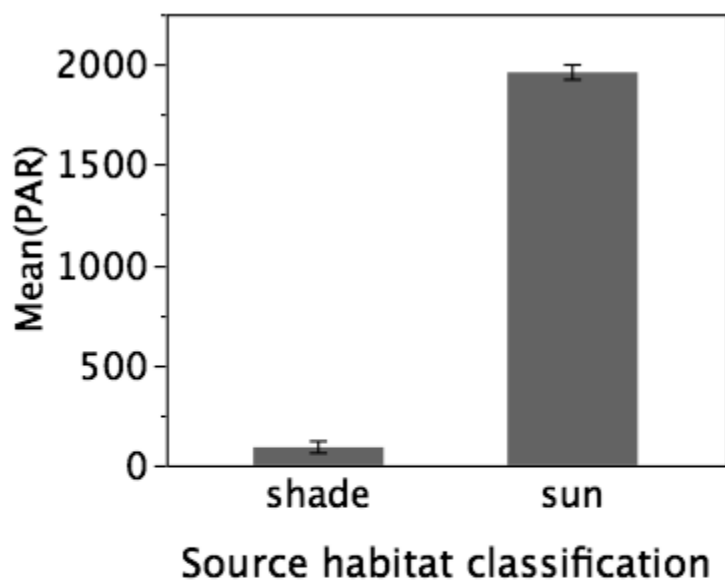
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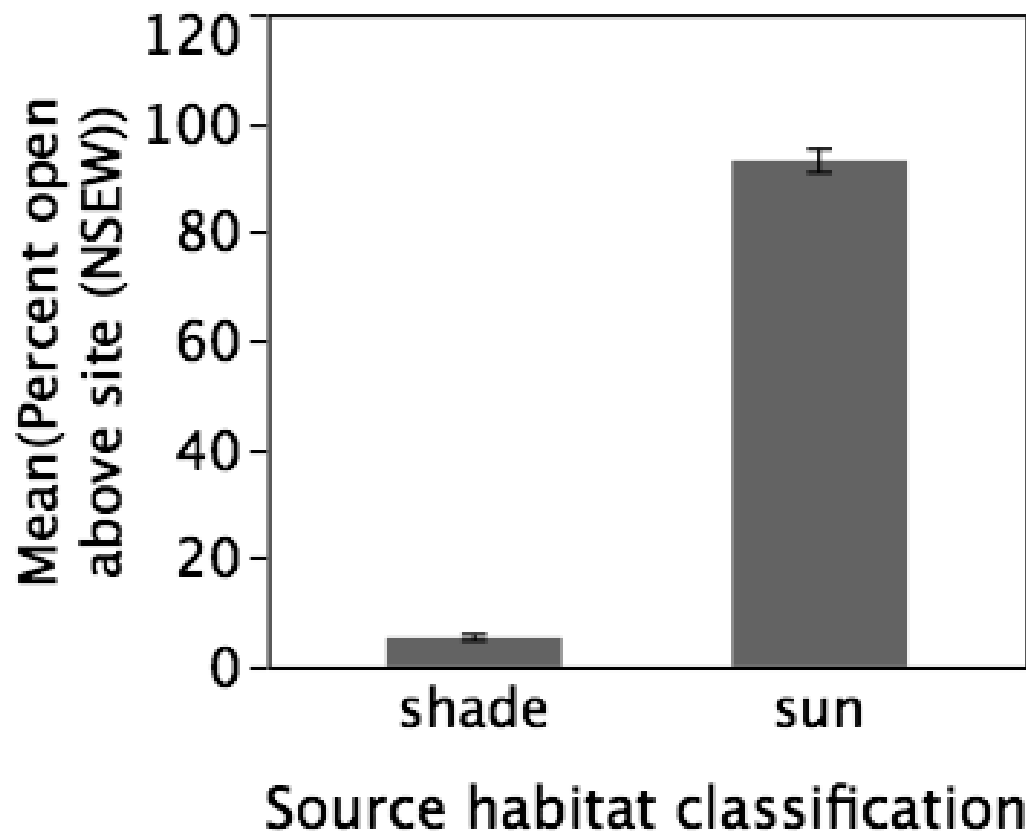
Each error bar is constructed using 1 standard error from the mean.

Figure 1



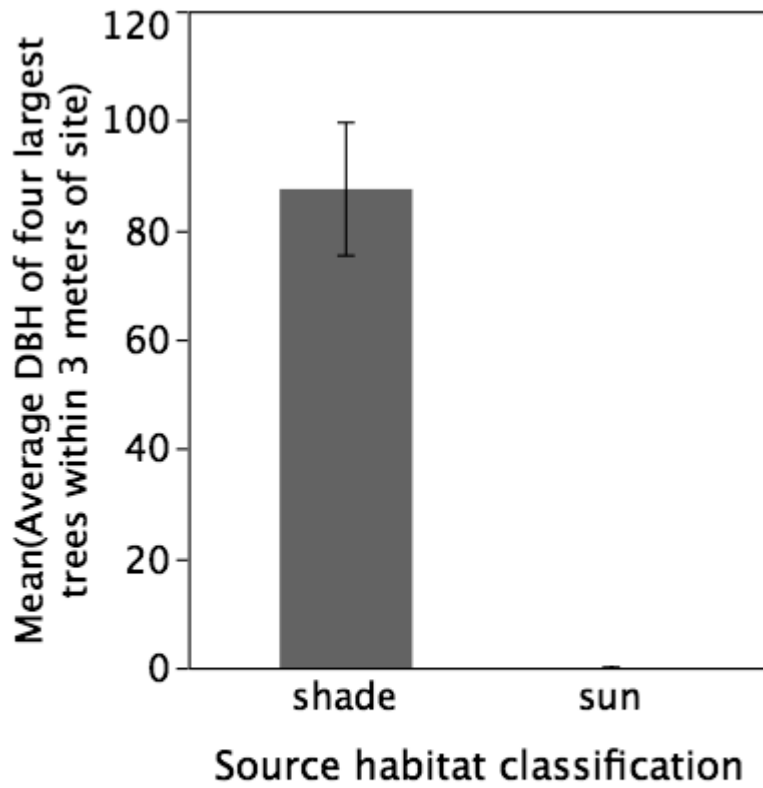
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Figure 2



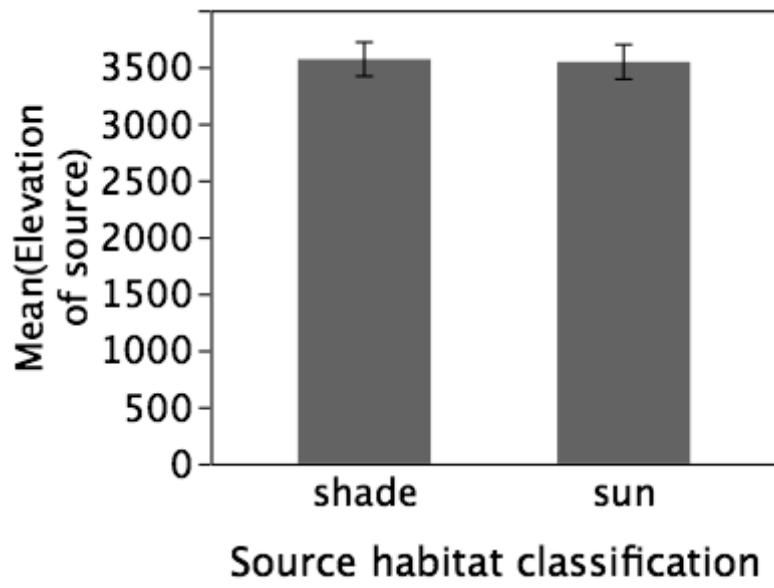
Each error bar is constructed using 1 standard error from the mean.

Figure 3



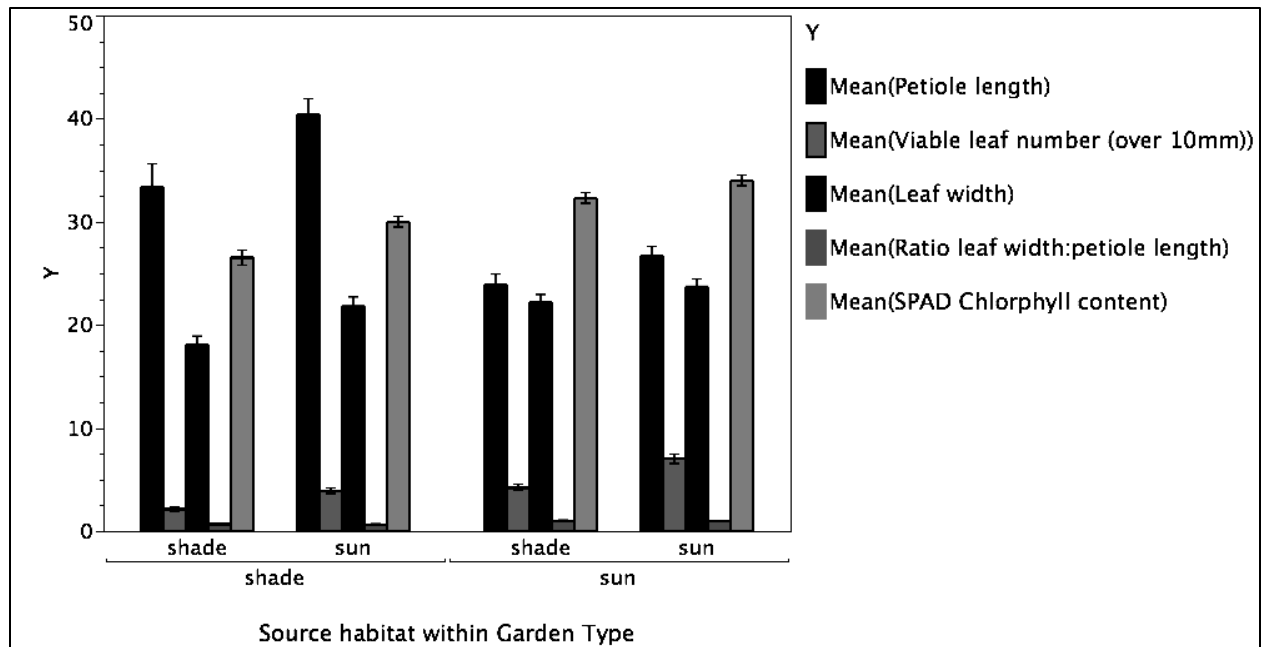
Each error bar is constructed using 1 standard error from the mean.

Figure 4



Each error bar is constructed using 1 standard error from the mean.

Figure 5



Caption for this figure: Average differences in phenotype traits among gardens, with source habitat nested within gardens. Overall ANOVA results are given in Table 2. Results for each variable are in Table 3.

**Table 1. Source
site comparative
data**

	Sun (N=9)		Shade (N=9)		<i>P</i> value (t-test) Sun vs. shade
	<i>Mean</i>	<i>St. Dev.</i>	<i>Mean</i>	<i>St. Dev.</i>	
<i>PAR</i>	1959.67	111.09	89.89	88.20	0.000000
<i>% canopy open</i>	93.05	6.52	5.34	1.68	0.000000
<i>Max dbh</i>	0.00	0.00	87.47	36.47	0.000002
<i>Elevation</i>	3543.96	431.67	3567.54	450.18	0.913975
L1 Mines	3.56	4.43	0.11	0.47	0.000000
L2 Mines	2.73	3.11	0.06	0.23	0.000000

For L1 and L2
mines:
*N=79 plants for
herbivory from
sun*
*N=68 plants for
herbivory from
shade*

Table 2. Nested ANOVA results comparing source habitat effects nested within common garden effects.

Overall Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	12447.525	6223.76	40.4364
Error	321	49406.712	153.91	
Total	323	61854.237		P < 0.0001

Table 2. Nested ANOVA results comparing source habitat effects nested within common garden effects for each variable (abbreviated results given here).

Variable	DF	F ratio	P value
Petiole length	2, 321	40.44	P<0.0001
Leaf number	1, 319	36.51	P<0.0001
Leaf width	2, 321	4.833	P<0.01
SPAD	2, 281	26.98	P<0.0001