

Intraspecific Variation of Specific Leaf Area Along an Elevational Gradient

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ABSTRACT

We measured Specific leaf area for 23 plants across six elevational sites spanning a total of 610 meters. Within each site, we sampled twenty individuals from each species that was present to determine intraspecific variance in SLA. Nine species showed a significant positive relationship with elevation, while three species displayed a negative relationship. These results demonstrate that intraspecific changes in SLA for most species parallel shifts in mean community SLA values at different elevations. This finding indicates that intraspecific variation in SLA may be a large contributor to changes in community mean SLA values, in addition to species turnover between different elevations. Abundance measurements were taken with five 1.3 X 1.3 meter plots to estimate abundance values of species at all sites. There was no relationship between intraspecific variance and within or across community abundance measurements; however, greater intraspecific variance was positively correlated with species that occurred at greater elevational ranges. These findings indicate that species that exhibit a higher degree of plasticity are able to locally adapt to more abiotic environmental factors.

INTRODUCTION

Ecologists have a mandate to predict how species, ecological communities, and ecosystems will respond to climate change. As a result, there is an increasing need to understand what factors define the distribution of species and communities. Insights from functional ecology have shown that knowledge of environmental conditions that shape the assembly and dynamics of a community are critical in predicting a community's spatial distribution (Keddy 1992). The emerging paradigm views the abiotic environment as a series of filters that winnow out species that do not express the correct combination of traits to survive a set of environmental conditions (Keddy 1992, Grime 2006). Those species that tolerate the abiotic environment can then potentially colonize and survive in that location. Abiotic filtering selects for traits that are most adaptive to the specific environment, resulting in communities that display similar traits. Functional traits determine how demographic rates and growth vary in different environments and thus determine which species are filtered out of communities.

Studies have demonstrated the importance of functional traits at the community level (Kraft et al. 2008, Lavorel & Garnier 2002); however, few studies have addressed

how intraspecific trait variation may affect community structure and conspecific distribution and fitness (Akerly et al. 2002). Comparing functional traits across natural gradients is an effective method to understand what factors dictate how each community or species changes across a variety of conditions, and how they may respond to climate change (Cornwell & Ackerley 2009, McGill et al. 2006, Messier et al. 2010, Bryant et al. 2008). Functional ecologists have identified a series of traits that best predict variation in demographic rates, life history times, and growth (Westoby et al. 2002; Enquist et al. 2007). The traits of greatest importance are those that can be used to infer the range of conditions that a species can tolerate, while continuing to propagate and persist in a given environment. A general list of some of the most informative plant functional traits includes: the specific leaf area (SLA, calculated as leaf area divided by dry leaf mass; mm^2/mg), seed size, plant height, wood density, and N:P ratios in leaves (Westoby & Wright 2006, Weiher et al. 2009). Specific leaf area is representative of a plants investment in the leaf tissue. Higher SLA values are correlated with shorter leaf life span, and poor nutrient retention (Reich et al. 1997).

In this study, we quantified intraspecific variation of SLA for multiple species across an elevation gradient. We assessed how the degree of intraspecific variation reflects a species ability to span an elevational gradient as well as conspecific contributions to shifts in community trait values. Measures of trait variance can ultimately be attributed to genotypic differences, phenotypic plasticity, or stochastic differences between individuals (Bolnick et al. 2011). However, in this study, we were unable to differentiate between these separate phenomena. Nonetheless, our results enable us to assess if the magnitude of intraspecific variation due to these processes are important to community composition and species distributions. This project addresses two prominent hypotheses in functional trait ecology:

The first hypothesis is that intraspecific trait variation should match interspecific trait variation. Specifically, for a given environment, there is an optimal phenotype characterized by a trait value that maximizes organismal fitness or growth rate. By construction, environmental change results in a change of the optimal trait, which necessitates a shift in the traits within a given community. This assumption builds upon the central paradigm in ecology (Whittaker et al. 1973) and evolutionary biology (Levins

1968) where phenotypic and species compositional changes across gradients is thought to reflect which phenotypes maximize performance in differing environments. Thus, this hypothesis predicts that, along a given gradient, due to environmental filtering and biotic interactions, if one observes a shift in the mean community trait, then in order for a species to continue to remain in the community, there must be a corresponding shift in intraspecific traits.

The second hypothesis, originally articulated by Darwin (1859) in ‘The Origin of Species’ states that species that express a higher degree of functional trait variation compete more effectively within as well as across plant communities. As a result, species with a higher degree of trait variation should be more abundant, and will occur over a wider elevational range. Considering this prediction, our general hypothesis is, if species express greater variance in conspecific trait measurements due to multiple genotypes or phenotypic plasticity, then those species will compete more effectively at an intra- and inter-community level, and will also exhibit higher abundances within and across communities. Studies have also shown increasing and decreasing intraspecific trends with SLA and increasing elevation (Bowman et al. 1999, and Taguchi 2001). We hope to assess how species with opposite trends in SLA differ when compared across an elevation gradient.

METHODS

Study Sites: The study was conducted in Washington Gulch, which is a valley approximately six miles long, with a gradual elevation increase located just north of Crested Butte, Colorado. Samples were collected at six primary sites with roughly 100 meters of elevation difference between each site (Figure 1). An additional two higher elevation sites were selected to further assess intraspecific trends of early season plants (*Claytonia lanceolata*, *Erythronium grandiflorum*, and *Mertensia fusiformis*). All sites were open meadows on south facing slopes.

Functional Trait Measurements: In accordance with Holshof & Swenson (2010), we sampled twenty individuals of each species that was present at each site to evaluate a range of intraspecific trait values. To ensure that each plant was within the same stage of development, we only sampled leaves from individuals that were flowering. While sampling each species, we used a haphazard sampling technique to avoid sampling only

those individuals that were most visible. This sampling process involved randomly throwing a ruler and then sampling the nearest individual to where the ruler landed.

We removed one leaf (including petiole) per plant to calculate SLA in the lab. All leaves we collected were fully developed and exposed to the sun. To minimize wilting, we placed each leaf in a separate envelope that was stored in a cooler until we completed leaf processing in the lab. Each leaf was scanned to determine leaf area (mm^2) using ImageJ software (NIH Bethesda, Maryland). All leaves were placed in a drying oven set at 65 degrees Celsius for a minimum of 72 hours. After the leaves were dry, we measured dry weight and calculated SLA by dividing leaf area by dry mass (mm^2/mg).

Abundance Measurements: At each site, we selected five 1.3 X 1.3 meter plots to calculate abundance of each species at different elevations. Since each site was oriented along a South-facing slope, we placed the plots from the top to the bottom of the slope, as to include species that grow at different locations along the slope. All plants, excluding graminoids, within each plot were counted and identified to species level. For most species, we determined abundance by counting the number of stems. However, we calculated abundance for species that had clumped growth forms with multiple stems associated with one individual by counting the number of clumps that were separated by bare ground or other plants.

Statistical Analyses: All statistical analyses were computed in R (GNU General Public License). We used linear model analysis to compare elevation to intraspecific SLA measures to determine if there were more species with upward or downward trends with increasing elevation. Linear model analysis was also used to compare intraspecific variance in SLA measurements to within and across community abundances. To compare the intraspecific variance to the elevational range in which each species was present, we averaged intraspecific community variance values for each species. By averaging the variance values for each species across all sites in which the species was present, we avoided biasing the variance values due to sample size. These values were compared to the elevational range (i.e. the difference between the highest and the lowest site in which the species was present) with linear model analysis.

RESULTS

The six primary sites spanned a total of 610 meters with the two higher elevation

sites extending an additional 270 meters (Figure 1). Through the course of the study, we sampled 23 species and a total of 1,785 individuals across all sites. Twenty-two of those species were herbaceous plants and one was a woody shrub (Table 1). Eighteen of the species were sampled at three sites or more.

Functional Trait Measurements: Twelve of the 23 species demonstrated a significant trend between SLA measurements and elevation (Figure 2). Three species showed a significant decrease in SLA measurements with increasing elevation, and ten species increased significantly, which was consistent with patterns of community shifts in SLA (Figure 2 and Figure 3). The coefficient of determination ranged from 0.068 for *Potentilla gracillis* to 0.60 for *Hymenoxia hoopesii*. Two of the three species that expressed a decrease in SLA were early season perennials (*Claytonia lanceolata* and *Erythronium grandiflorum*).

Abundance Measurements: There were no significant trends in intraspecific variance of SLA to abundance within sites (Figure 4) ; however, site 5 (3155m) demonstrated a positive correlation between SLA variance and abundance that approached significance ($P = 0.077$, Figure 4). In fact, most sites showed the opposite trend to the initial hypothesis, whereby species with lower variance in SLA were more abundant (e.g. sites 1, 3, 4, and 6). Similarly, there was no significant trend between total intraspecific variance and total abundance across the six primary sites ($r^2 = 0.038$, $P = 0.37$ Figure 5). However, mean intraspecific community variance was positively correlated with the elevational range in which each species was present ($r^2 = 0.27$, $P = 0.011$, Figure 6).

DISCUSSION

The purpose of this study was to investigate the role of intraspecific variation in the distribution and composition of plant communities in Crested Butte, Colorado. Consistent with our first hypothesis, our results indicate that most species show intraspecific shifts in SLA that parallel changes that are seen in mean community level SLA. We reject the hypothesis that intraspecific variation is positively correlated with abundance. However, we did find a positive relationship between a species' average community variation and range in which the species was present.

Nine out of the twelve species with significant trends between elevation and SLA showed positive correlations (Figure 2). Similar trends in community level shifts in SLA have been shown along the same elevational gradient in Washington Gulch. Low elevation sites were characterized by low community mean SLA values and high elevation sites had high community mean SLA values (Figure 3 Henderson unpublished data). These findings support our first hypothesis, demonstrating that intraspecific shifts in SLA values at different elevations parallel community level shifts in SLA.

Observed differences between mean community trait values at different elevations are attributable to species turnover and/or intraspecific trait shifts in species that are present within multiple sites. Since conspecific changes in SLA values follow similar trends to community mean SLA values, our results indicate that intraspecific variation in SLA may be a large contributor to changes in community mean SLA values, in addition to species turnover between sites. Intraspecific variance can range drastically depending on the species and the site. For example, *Claytonia lanceolata* and *Erythronium grandiflorum* both had very high variance values at site 5 (3155m); however both displayed relatively low variance at site 4 (3008 m, Table 1). These results indicate that calculating intraspecific mean and variance values are important for each study community because there is such variability in trait values within a small spatial area.

The observed patterns in mean community and intraspecific SLA are consistent with the functional significance of this trait. Specific leaf area is a useful representation of a plant's investment in the leaf. Higher SLA denotes greater surface area per unit mass but is also correlated with a shorter leaf-life span (Reich et al. 1997). In this sense, the plant receives greater photosynthetic return for every unit mass that the plant invests in the leaf, but the leaf dies quickly. With this reasoning, it is advantageous to have higher SLA at higher elevations where the growing season is much shorter. Plants at lower sites invest more energy in growing thicker leaves that last longer because it is beneficial to have a longer-lived leaf through a longer growing season.

Three species demonstrated negative relations between elevation and SLA (Figure 2). *Claytonia lanceolata* and *Erythronium grandiflorum* are two of the first plants to emerge after the receding snowpack (Kimball et al. 1973). One potential reason that these species show the opposite trend of most species along the gradient is because they are

exposed to a different set of abiotic conditions through their growing season. Higher elevations have greater climatic variability than lower elevations, especially during the transition between winter and summer. For example, late season snowfalls are generally more severe at higher elevations. Low SLA signifies that the leaves are thicker, and therefore, have greater insulation (Ansaria & Loomis 1959). Since *Claytonia lanceolata* and *Erythronium grandiflorum* at high elevations are more likely to experience sub-freezing temperatures and potential snow during the early season, it is beneficial to have thicker, more insulated leaves. After early season plants have completed their growing cycle, the daily temperatures rise, and the probability of frost damage decreases for late season plants. At this point, it appears as if other factors such as growing season length dictate the most successful growth strategies.

Each elevational site is characterized by a unique set of abiotic conditions. Different abiotic conditions favor certain traits, which results in communities that display traits that are most adapted to that environment. For any set of environmental conditions, there is an optimal phenotype that maximizes organismal fitness and growth rate. Mean community trait values are representative of the optimal trait at a given location. Intraspecific changes in traits can be attributed to genotypic differences between separate populations or plastic responses to varying environmental conditions. Since SLA values for a species follow similar trends to community mean SLA values, our results indicate that an individual species demonstrates intraspecific responses in SLA across the elevational range in which it occurs that allows it to persist in all sets of abiotic conditions across its range. With this reasoning, a species that demonstrates a greater degree of intraspecific variance should also occur across a greater range of conditions. This prediction is supported by figure 5, which illustrates a positive relationship between average intraspecific variance for a species at each community and elevational range for that species. These two findings support the conclusion that species that exhibit a higher degree of plasticity or genotypic differences are able to locally adapt to more abiotic environmental factors.

Our data suggest that there is no trend between intraspecific variation in SLA and abundance within or across plant communities (Figure 3 and Figure 4). Since

intraspecific variance in SLA does not relate to abundance, other plant traits or biotic factors such as competition might be more influential to abundance.

This study demonstrates that intraspecific variation in SLA for most species parallels similar trends seen in community mean SLA across an elevational gradient. We found that species with greater variability in SLA also occur in communities across a greater elevational range. These findings indicate that plants with a higher degree of plasticity or genotypic variation are able to grow within a greater range of communities that are representative of different abiotic conditions. Intraspecific variation varies drastically between species and between sites, indicating that conspecific variation heavily contributes to community trait shifts across different environments.

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TABLES AND FIGURES

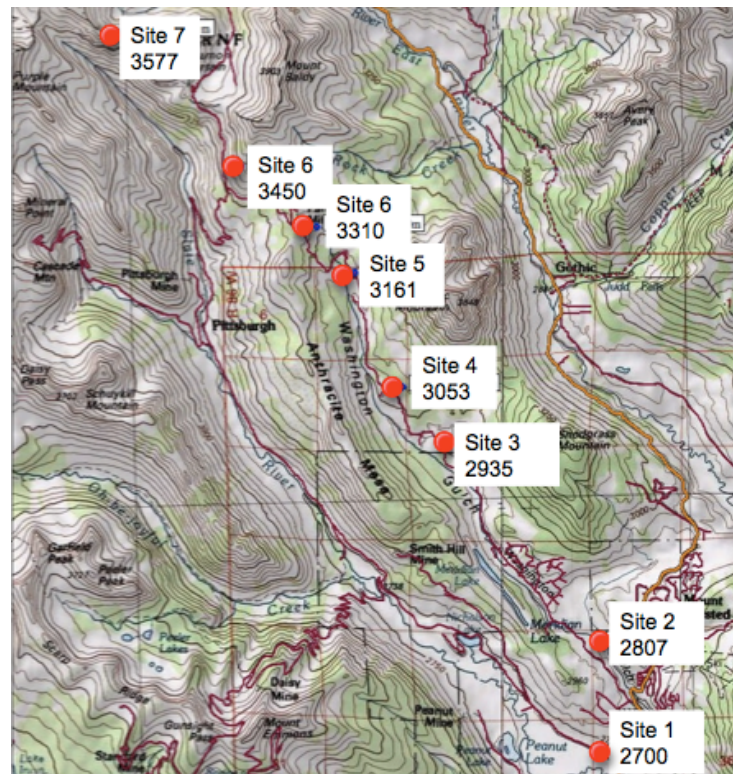


Figure 1: All sites are marked as red dots with elevation in meters.

	Sites						
Species	2710 m	2810 m	2906 m	3008 m	3155 m	3380 m	Total species variance
<i>Achillea millefolium</i>	7.575843311	11.73324815	2.55196283	3.563874446			8.373918244
<i>Claytonia lanceolata</i>			40.83004024	11.95470602	206.6987771	12.39369979	134.5231818
<i>Collomia linearis</i>	4.680067135	30.83947459	6.386364753	20.29395886			48.95847205
<i>Corydalis caseana</i>				36.25926396	26.37193004	20.58130622	29.08570756
<i>Delphinium barbeyi</i>				11.37396985	2.818396466		8.031068312
<i>Delphinium nuttallianum</i>	6.39966586	24.84996328	2.962764425	153.9197344	4.399370797		78.382244
<i>Eriogonum umbellatum</i>		1.699472443	2.256676114				1.998715011
<i>Erythronium grandiflorum</i>			22.81852116	6.672009096	358.0108903	7.610597895	132.8354702
<i>Gayophytum racemosum</i>		4.067247384	2.233333246				3.232132311
<i>Geranium viscosissimum</i>		0.944844384					0.944844384
<i>Geum triflora</i>	6.537320082	0.881737945	0.97760791				4.037573825
<i>Hymenoxia hoopseii</i>	3.885577674	2.173275754	2.468462342	4.192727173	10.32189192	13.37710249	17.316527
<i>Lathyrus lanszwertii</i>		4.756901062	16.42346711	12.5372941	3.941484409		13.05798727
<i>Lupinus polyphyllus?</i>	1.829719871	2.909150899	71.21810603	38.24631518	13.05523608		48.44003262
<i>Mertensia ciliata</i>				17.07669736	29.67397379	33.87778084	38.12454616
<i>Mertensia fusiformis</i>	5.842852144	39.51759984	4.76248	3.641786601	96.19127927	3.010185845	82.39542221
<i>Potentilla fruticosa</i> (shrub)	2.644844298	121.0710378	3.245559146	5.789274967			33.61481443
<i>Potentilla gracilis</i>	29.48071019	1.188470429	3.868697102	1.928317982	10.66536857	2.790780324	9.952667789
<i>Taraxacum officinale</i>	7.922953777	5.393324023	12.08949675				8.923425106
<i>Tragopogon dubius</i>	7.001427295		17.75413516				8.502482238
<i>Valeriana acutiloba</i>	4.719321237	30.47720341	8.058249682	15.00740791		42.39556686	93.0749025
<i>Veratrum californicum</i>		3.512082564	1.042872918		8.43517094		4.86845223
<i>Vicia americana</i>	11.02069914	5.593044529	29.02432124	13.19466859	19.13136279		38.14599181

Table 1: Total and site-level values of intraspecific variance in specific leaf area (SLA) for each species. Blank boxes indicate sites in which a species was not sampled or did not occur.

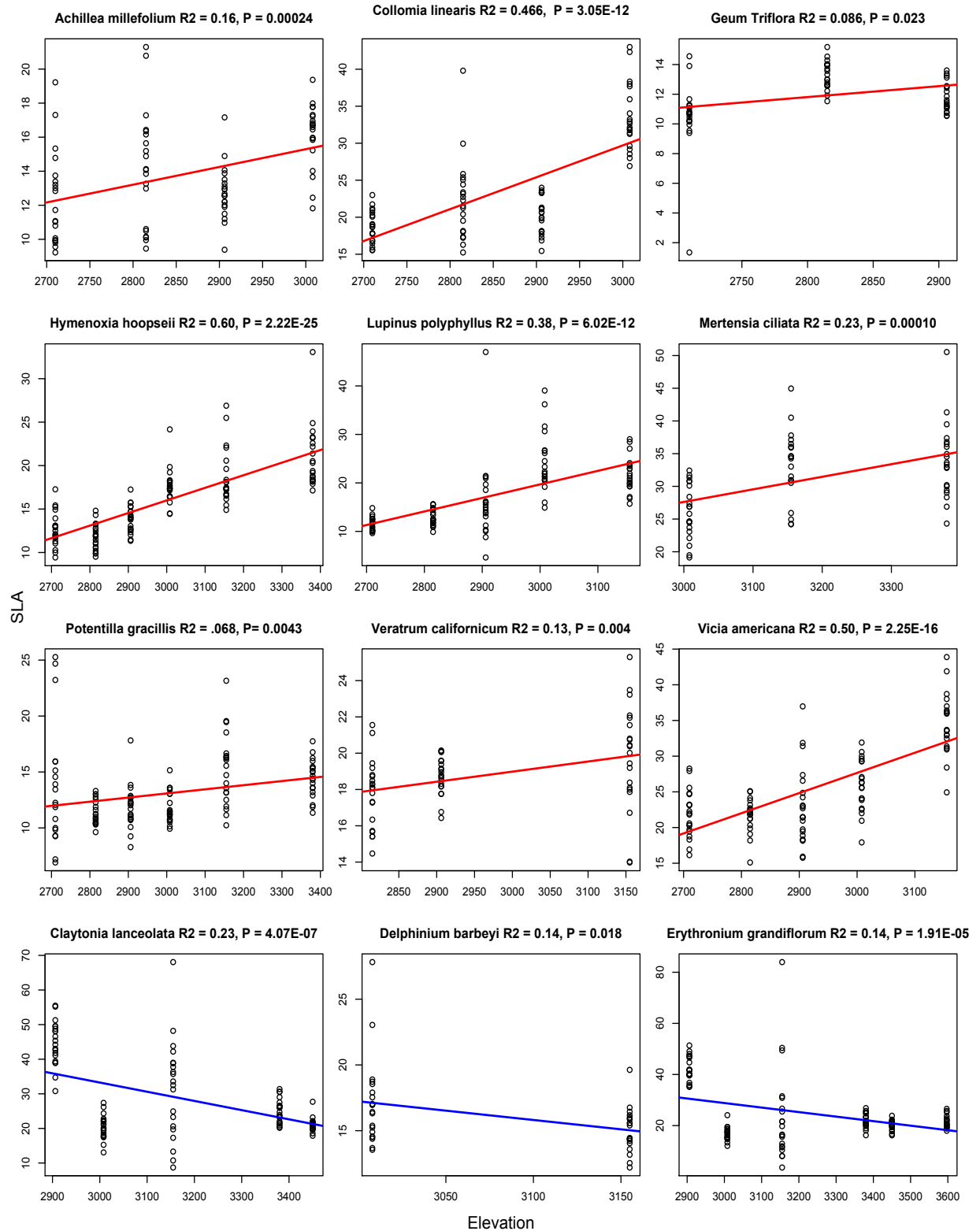


Figure 2: Linear regressions for species that show significant trends ($P > 0.05$) between Elevation and SLA. Red lines represent significant positive correlations and blue lines represent significant negative correlations.

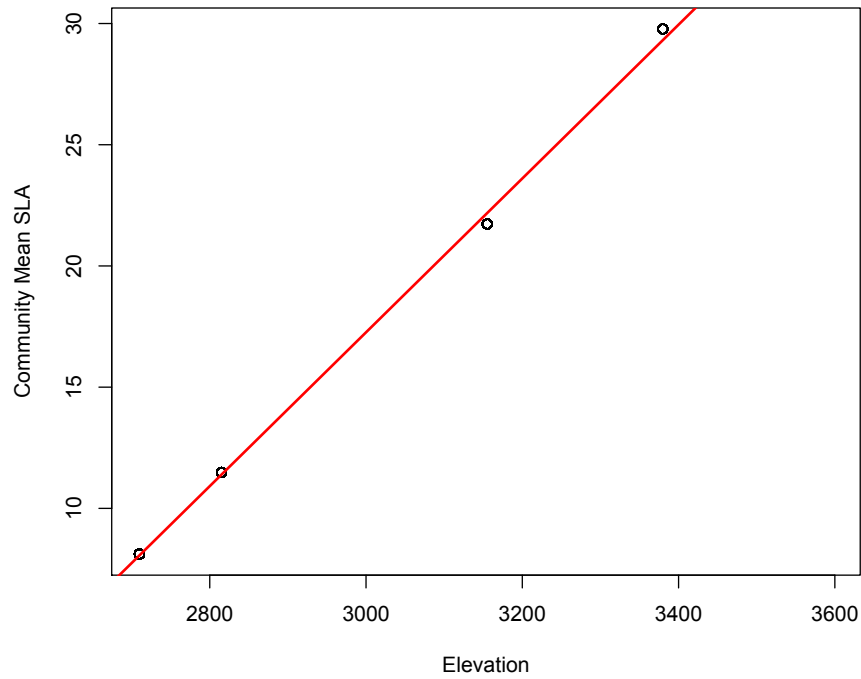


Figure 3: Mean community shifts in SLA across Washington Gulch in 2010 (Henderson unpublished data).

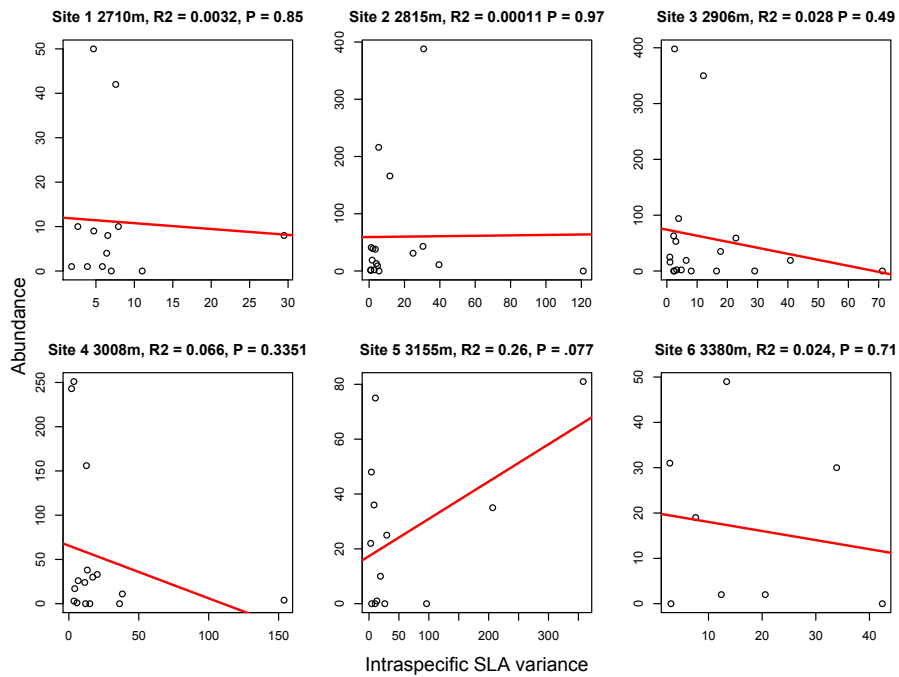


Figure 4: Intraspecific variance in SLA vs. community abundance. These data do not support any trend between intraspecific variance in SLA and community abundance.

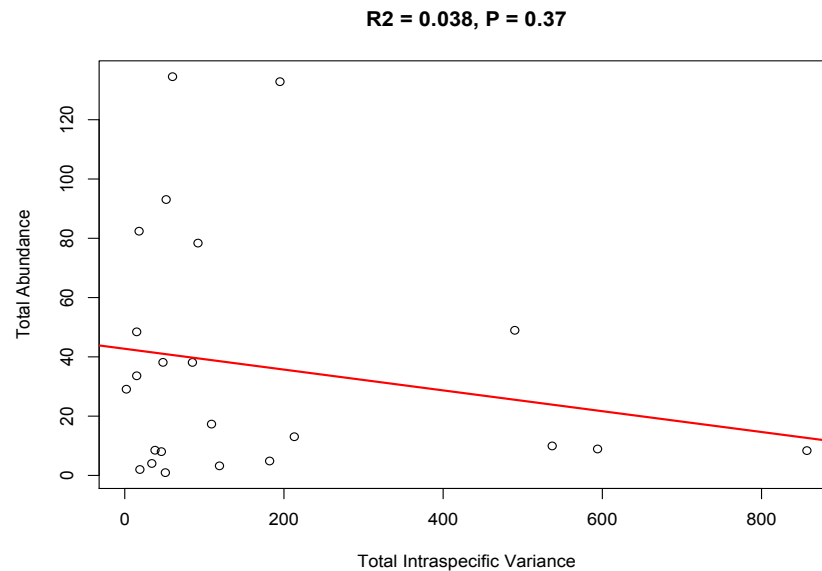


Figure 5: Regression analysis for total intraspecific variance in SLA and total abundance for a species across all sites. There is no significant trend for this analysis ($r^2 = 0.038$, $P = 0.37$).

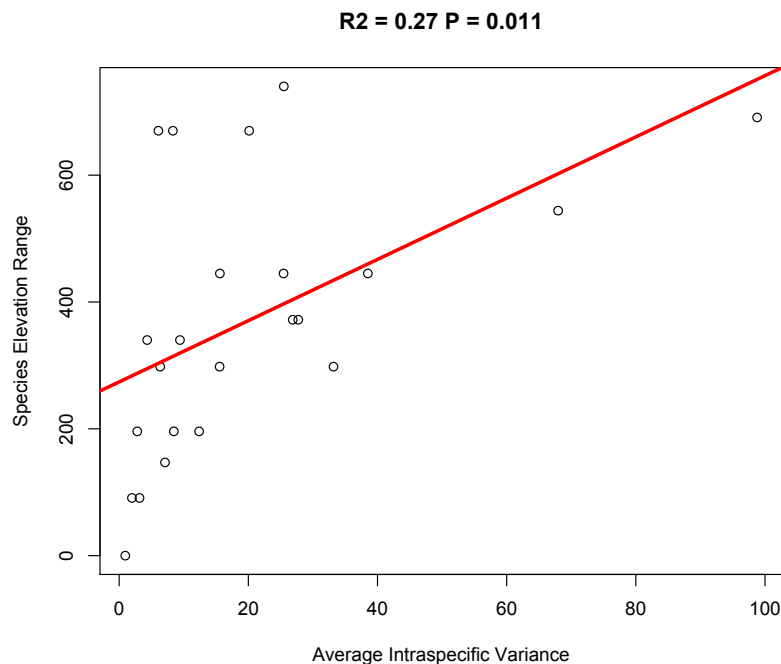


Figure 6: Regression analysis for average intraspecific community variance of SLA and the elevational range in which each species was encountered ($r^2 = 0.27$, $P = 0.011$). Average intraspecific variance was calculated by averaging the variance values for each species at every site in which the species occurred. Elevational range was calculated by subtracting the highest elevation site in which the species was found by the lowest elevation site that the species was found.