

How do the Effects of herbivory and light gradients impact the overall plant fitness within Different Environments?

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Abstract:

The strength of natural selection on phenotypic traits as a result of herbivory can be measured within and among environments by quantifying spatial variation in fitness and herbivory levels in the different environments. High spatial variation among different light gradients can be expected to cause higher directional selection favoring habitat differentiation (local adaptation) among environments. In this study, I tested the hypothesis that light quality habitats influence fitness indirectly through variation in herbivory among habitats. I measured a suite of plant traits across a light quality gradient using the plant bittercress as a model. Plants were measured across 18 phenotypic traits. The correlation between fruit/seed number in response to leaves mined, leaf area mined, sum of leaf mined is expected to have negative correlation when herbivory is high. The results indicate that there is a negative correlation between herbivory between seeds per plant (reference figure 6). Secondly, that there is no significant difference between sun and shade as a factor of herbivory but willow shade ecotone had the least amount of herbivory.

Introduction:

Light quality gradients have been found to affect the fitness of plants directly and indirectly. Indirect mechanisms can include such factors as differential herbivory in sun and shade habitats due to intrinsic behavioral preferences of the herbivorous insects attacking these plants. Here, I tested the hypothesis that a light quality gradient was correlated with differential herbivory and that herbivory negatively influenced fitness. I tested this in the plant *Cardamine cordifolia* (bittercress), which is a model ecological plant. Previous research showed that herbivory is relatively low in bittercress plants living in shaded environments and high in bittercress plants living in sun habitats (Collinge and Louda 1989). Light gradients are defined as being different environments in which they can be classified as sun, shade, and willow shade environment. This developmentally could be a result of the sun plants developing earlier than those in the shade and willow shade (Collinge and Louda 1989). Sun plants are potentially more heavily damaged by herbivores because the female leaf miners could be actively looking for oviposition sites early in the season, which in turn, could allow hosts to be more available from sun environments, rendering them more susceptible to herbivory than shaded and willow shade plants.

Cardamine cordifolia A.Gray (bittercress) is a small (30-75cm tall), herbaceous, perennial mustard (Brassicaceae) plant, that occurs throughout the Rocky Mountains (Harper 1954). Bittercress often grows in wet, shaded areas along streams > 2,000 meters in elevation (Harrington 1954) and is found within different light quality habitats (Harper 1969, 1977; Louda & Rodman 1983a, b; Grime et al. 1988). Bittercress' rhizomes are in patches where the rosettes are created. Bittercress is also a monoecious plant that reproduces sexually. For over a decade, the system of bittercress leaf miner interactions was studied in context of what factors drive the local distribution of bittercress (Lande and Arnold 1983, Collinge and Louda 1988a, Collinge and Louda 1988 b, 1989, Louda and Rodman 1996). Bittercress is more abundant growing under the shade of willows rather than in the open sun (Collinge and Louda 1989). Differential, spatially-explicit herbivory between sun and shade habitats was found to be responsible for this skewed distribution. There was no evidence for ecotypic differentiation between the sun and shade derived plants (derived from rhizomes) in a reciprocal transplant experiment (Louda and Rodman 1996). However, in a common garden experiment using rhizomes derived from sun and deep, evergreen shade in 2011, Humphrey et al. (in review) found some evidence for potential ecotypic differentiation between habitats. It is possible that one mechanism explaining this is chronic herbivory in the sun and low herbivory in the shade, which could select for habitat specialization in this species. One

approach to relate differences between similar micro-habitats in bittercress to test for directional selection on plant phenotypic traits among different light quality micro-habitats.

Scaptomyza nigrita (a drosophilid leaf miner), is one of the major herbivores of bittercress, which it attacks by defoliating leaves, and drinking the nutrients exuding from the wounds in which it lays eggs. Eggs hatch and form leaf mines, consuming a large percentage of leaf area (Collinge and Louda 1988a, Collinge and Louda 1988 b, 1989). Bittercress is heavily attacked by leaf miners in sun-exposed areas, but less so in shaded areas. This study tested two hypothesis related to patterns of herbivory across light environments in *C. cordifolia*. I hypothesized that bittercress in shaded areas are likely to allocate resources into growth, development and production of fruits and seeds because herbivory is typically low. Contrastingly, in the sun, I hypothesized that bittercress is likely to allocate more of its resources in herbivore defense (either via resistance or tolerance mechanisms) due to the high abundance of leaf miners and other herbivores. Bittercress initiates flowering earlier in sun-exposed areas contrastingly then shaded areas because the shade has a reduced amount of resources compared to sun-exposed areas (Collinge and Louda 1989). Early availability of host plants in sun exposed areas (comparable to shade) maybe an influential factor of bittercress' susceptibility to herbivory and parasitism by leaf miner.

Lande and Arnold (1983), introduced the idea that measuring multiple phenotypic traits of an organism and relating these to plant fitness can give insight into which traits are most predictive of plant fitness. This is because a single isolated characteristic is not what defines an organism's direct and indirect effects from natural selection. Natural selection, a variable that is not easily accounted for, acts on multiple environmental factors which are represented in organismal phenotypes. The "directional aspect of selection" is measured by the effect of mean values of generational phenotypic characteristics (Lande and Arnold 1983). One such approach is based on a vector of "partial regression coefficients of individuals" to relate fitness with phenotypic characteristics (Lande and Arnold 1983). Characteristics of directional selection are an average gradient of the surface of individual relative fitness, weighted by the phenotypic distribution (Lande and Arnold 1983). Plant fitness can be quantified phenotypically by life span, fecundity, mating success, and parental effort, in a regression of total fitness. The "partial regression coefficients" of individual fitness on the organismal characters (traits) are defined as directional selection gradient (Lande and Arnold 1983).

Interactions between herbivory and light quality have been well studied in plant ecology, yet no definitive pattern has emerged relating the two factors (Collinge and Louda 1988a, Collinge and Louda 1988 b, 1989). Allocation theory predicts that plants must make trade-offs between growth and defensive characteristics in order to survive (Herms and Mattson 1992). So as result of allocation theory, one can postulate that plants in each microhabitat are locally adapted to their environment, whether evergreen shade, willow shade ecotone, or sun environment. This would explain differences in plant development and fitness in the sun, shade, willow ecotone environments where herbivore pressure varies between habitats. A classic phenotypic selection approach (Lande and Arnold 1983), was based on selection differentials and selection gradients of herbivores on plant within and among light quality habitats. Louda and her colleagues did not measure selection gradients or differential selection among the micro-habitats, which was noted by them that "impact of herbivores on plant fitness is not yet well described" (but see Louda and Rodman 1996). The goal of this proposal is to quantify the relationship between herbivory and fitness across a light quality gradient, using a phenotypic selection approach.

Methods

I measured a variety of plant traits to predict how differential selection pressures from herbivores may be a factor in sun, shade and ecotone environments plant fitness. The micro-habitats were studied between sun, shade and intermediate ecotonal areas (ie. willow shade ecotone). Plots were chosen in three microhabitats: open-sun exposed areas (5% shade based on densiometer), deep evergreen canopy areas (95% shade based on densiometer), and ecotonal areas (greater than 5% but less than 95% shade based on densiometer). The use of a densitometer was used to determine the percent of canopy cover per each patch. Patches were measured at least a distance of two meters apart, because clonal growth causes plants to be genetically identical within a patch. Photosynthetically active radiation (PAR) was measured with the use of a PAR meter (LightScout Quantum Light Sensor, Spectrum Technologies, Plainfield, IL) to quantify the amount of useable sunlight reaching plants in each patch at the same time each day. The overall spatial map of the experiment is given in Figure 1 below.

Selection of habitats, plots, patches (**All sites located along the lower 401**)

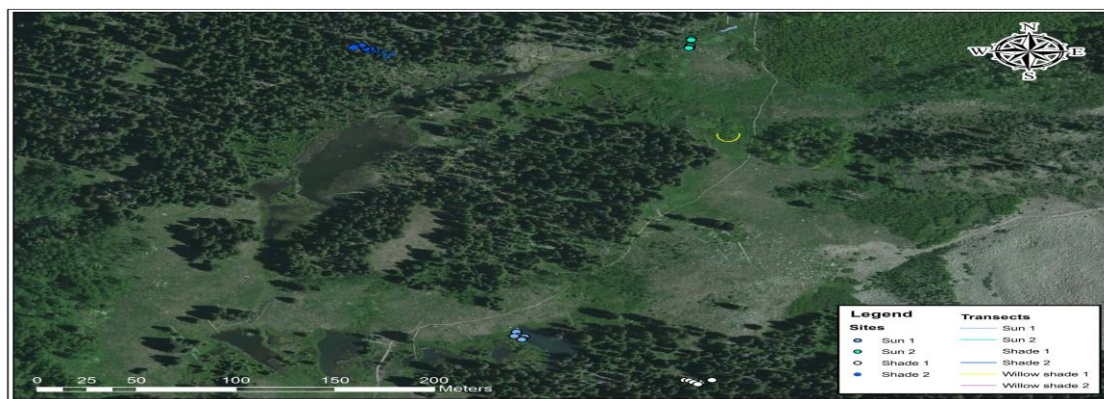


Figure 1 ArcGIS map of the study sites transects are denoted by lines, and satellite sites are denoted by circles.

Field sites and satellite sites were chosen near to the 401 forest service trail; all sites had a readily accessible source of water. Six sites were used, two of each light quality category (i.e. sun, shade, and willow shade ecotone) and also 28 satellite sites (used to supplement the fact if the 6 field sites had considerable damage from rain storms), please refer to figure 2. A satellite site is one that only contains five focal plants, located apart from the field sites. Within the six field sites, I selected five patches and five focal plants per patch. Focal plants were marked with flagging tape per patch/satellite site. Bittercress was opportunistically chosen within a given patch/satellite site on the basis of health and availability see figure 2.

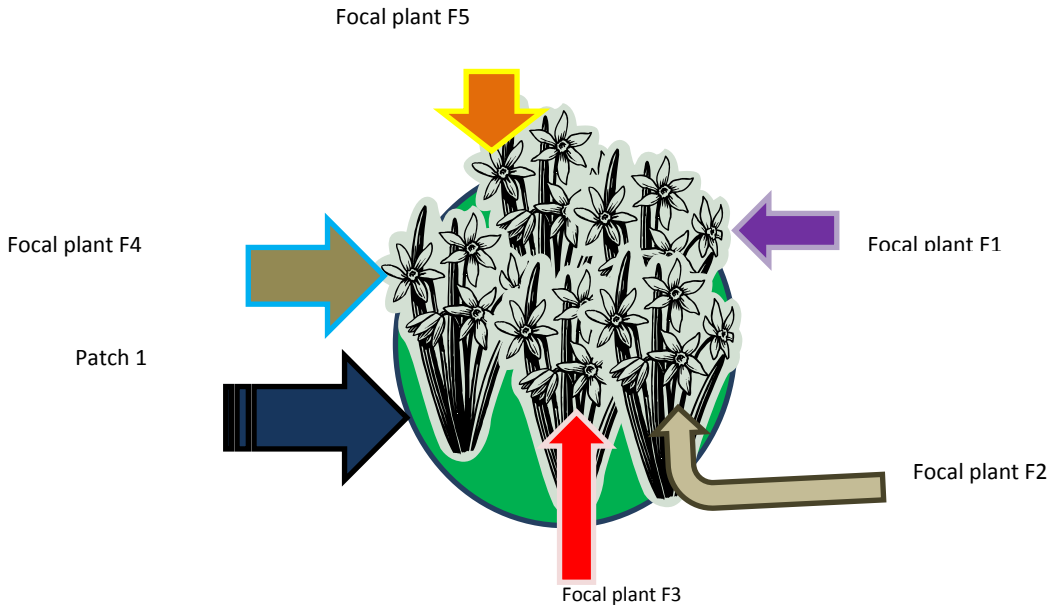


Figure 2 Diagram how focal plants were selected within a given patch in each micro-habitat or satellite site.

Trait measurements:

The phenotypic traits I measured were: plant height, petiole length, leaf number, number of mines, number of stipples, leaves mined per plant, number of leaves mined per plant, total number of mines per plant, leaf area removed by herbivores per two bottom basal leaf, number of leaves defoliated, leaf area mined by leaf miners, leaf area, number of flowers and number of fruits. These traits were measured at two different collection times during the study to notice any different levels of predation throughout the season. First, the plant height was measured with the use of a 3 meter measuring tape. Petiole length was measured from the basal leaf and internode length was measured from the distance of the basal leaf to the leaf directly above the basal leaf with the use of 30 cm ruler. The number of leaves was counted from the base of the plant (leaf 1) leaves to the most apical leaf of the plant. Number of mines and number stipples were counted from the two most basal leaves with the use of an LED light that projected light under the leaf to distinguish precisely if mines or stipples were present. Total number of mines per plant and number of leaves mined per plant was measured with a LED light to accurately assess the number of mines. Leaf area was determined during the final collection by photographing the two most basal leaves and was measured through the program Image J (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, <http://imagej.nih.gov/ij/>, 1997-2012). The leaf area mined per leaf was processed through the program Image J, as well as the leaf area of defoliation by other herbivores, using photographs of mined leaves all mined leaves were measured for leaf area mined. The number of fruits and seeds was collected by Kerri Miazgowiec in a separate study of plant fitness across microhabitats. The sample size was 290 focal plants per collection time in which I measured the phenotypic traits above and plant fitness throughout a season.

Statistical Analysis-

For this study, Photosynthetically Activating Radiation was taken to see if it predicted our classification of different habitats as sun, willow shade or evergreen shade. *Habitat as a correlation to Herbivory: a*

significant habitat and habitat x site interaction was measured through a Kruskal Wallis test, which does not assume a normal distribution and was appropriate for our over-dispersed dataset. Spatial (site) variation was compared with herbivory levels using a Kruskal Wallis test to see the differences among sites with the three levels of herbivory. A multiple regression analysis was done for each habitat as an independent variable and each of level of herbivory as a dependent variable.

Results:

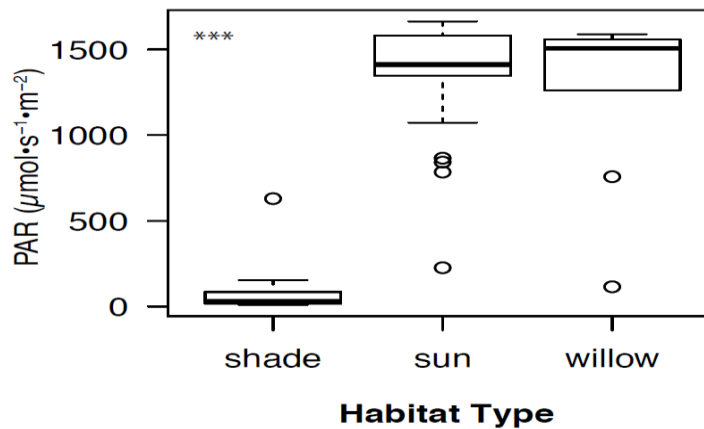


Figure 3 Spatial Variation by micro-habitat as a result of PAR (Photosynthetically Active Radiation). A Kruskal Wallis test was used to analyze the data. Light readings were recorded in $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$.

Spatial Variation by micro-habitat as a result of PAR (Photosynthetically Active Radiation):

Photosynthetically Active Radiation (PAR) was related to each habitat type to see if expected trends were present. The expected trends were that sun habitat would have the most PAR, followed by willow shade, then lastly shade. The three different environments were measured for PAR readings to distinguish that the sites were not overlapping in anyway and were definitive of what we expected. The expected outcome which was positively correlated with our prediction was that sun would have the most par then decrease to willow shade to and lastly to shade. According to studies by Louda, light gradients are expected to be a causal factor that leads into herbivory with the sun having the highest amount.

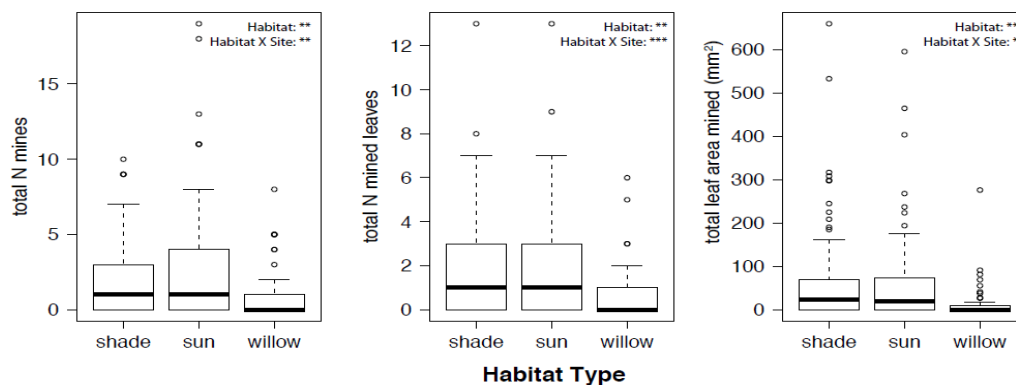


Figure 4 Habitat as a correlation to Herbivory: a significant habitat and habitat x site interaction. A Kruskal Wallis test was used for environments different in herbivory levels. Total number of mines

and number of mined leaves per plant were collected throughout the season but only the final data was used for analysis. Leaf area mined (mm^2) was measured at the end of the study so that removal of leaves did not lower the fitness of the plants.

The collection of the total number of mines and total number of mined leaves per plant were collected at different times then total leaf area mined. Three levels of herbivory were measured (from left to right total number of mines, total number of mined leaves, and total leaf area mined) in relation to the different environments. A Kruskal Wallis analysis was used for this analysis. Also habit is denoted differently according to each of the legend. In this graph, there is no significant difference between Sun and Shade environments but willow shade environments are represented to have the least amount of herbivory in each panel and herbivory was significantly lower in plants in willow shade relative to the other environments. Also the lowest sampled sites were the willow shade and that could be a confounding factor as to why the numbers are so low.

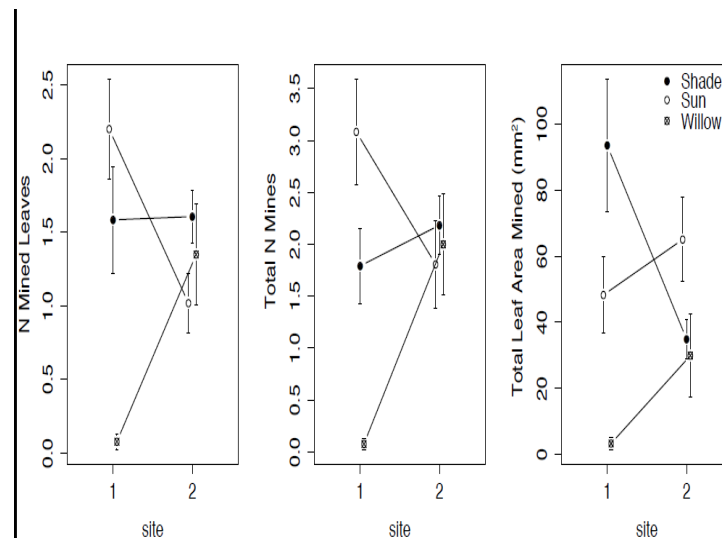


Figure 5A Kruskal Wallis test, was used to analyze spatial (site) variation as a result of herbivory. The three graphs represent if there any differences between site number as a defined by one factor of herbivory.

They are significantly higher herbivory levels for sun plants compared to plants in the two other habitat types within site 1, for the factors of number of mined leaves, and total number of mines. For the shade site 1, plants suffered significantly higher herbivory when the factor of total leaf area mined was tested. From site to site there is variation in the amount of herbivory, in only the first graph of number of mined leaves is consistent within the habitat of shade.

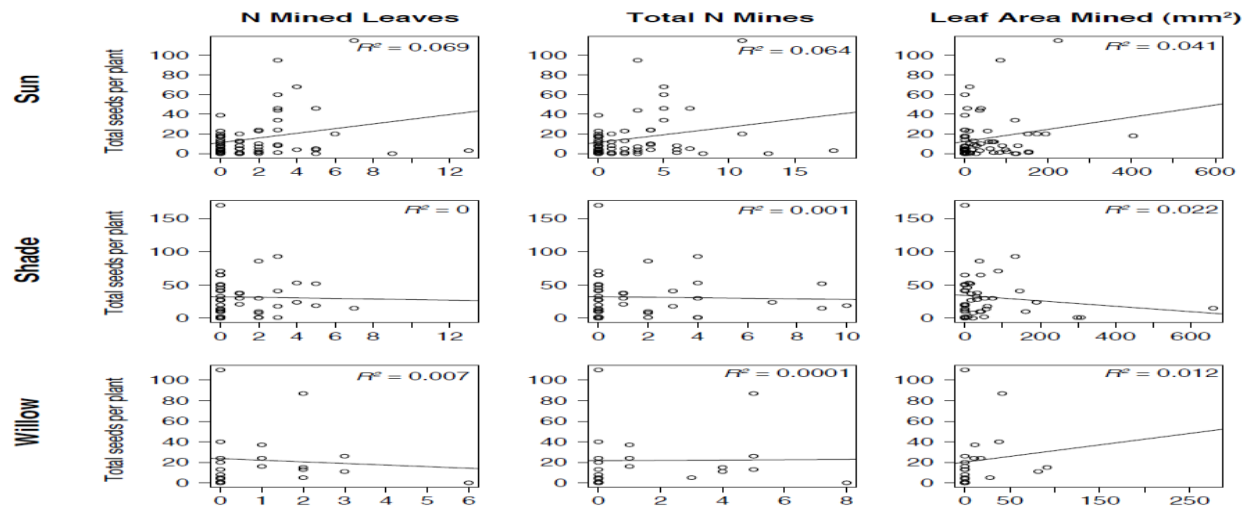


Figure 6 A multiple regression analysis assessed Total number of sets per plant in relation to the three characteristics of herbivory.

Total number of seeds per plant was used as a response variable to the different types of herbivory. The data points were widely dispersed which means that there is no significant correlation between the number of seeds and the amount of herbivory caused by leaf miners in any environment. A cause for there being no significant difference could be water stress at the beginning of the season or other environmental factors that were not accounted for.

The results indicate that there is not a significant difference between shade and sun, but willow shade had the lowest and was significantly different. Ecotypic differentiation is not present in these patterns between sun and shade light gradients, but there is some evidence of this for the willow shade sites. So in conclusion, based on the combination of the results, it can be said that for this data collection that herbivory is not a limiting factor in the seeds per plant.

Discussion:

There was no significant correlation between reproductive fitness of bittercress and leaf miner herbivory. There was a significant interaction between herbivory and light quality. Unexpectedly, the willow shade habitat suffered the lowest amount of herbivory. A significant correlation between the number of mines and leaves that were mined between environments showed a strong relationship. Leaf area mined however did not seem to have a strong correlation with leaves that were mined and number of mines within environments. The environment that had the most leaves mined per plant and number of mines per plant was the sun, followed by shade, then by willow shade habitat. The result of willow shade with low amounts of herbivory may be due to the fact that there were not that many willow shade habitat sites in comparison to the sun and shade habitats. Based on the observed patterns for this season, one hypothesis is that the snow melted faster this year, thus giving the leaf miners a choice between sun and shade habitat because the availability of sun plants are always emerging before shade plants. Future approaches that would be beneficial to the bittercress system are measuring the amount of water stress a plant has within a given season and relate to plant fitness. If this was an ongoing study a cyclic pattern could be studied between herbivory in different climatic to if there is any relationship between drought years and years of high snowmelt.

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