Leaf Herbivory and Symmetry

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Introduction

A variety of components influence the relationship between trees and insects; the morphology of the tree, chemical repellents and attractants. These repellents are produced by plants to help prevent insect and fungal activity. Variation in compensation under naturally stressful conditions has yet to be thoroughly investigated (Levine et al., 2004). Fending off herbivorous attacks requires deviating resources away from regular leaf processes. One such process is the growth of new leaves. Many leaves are asymmetric, which could be the result of diverting resources to prevent herbivorous activity.

The concept of symmetry is pervasive in nature. In many plant species, symmetry creates the opportunity for more efficient tissue usage in structural production, respiration, and the production of secondary chemicals tied to development (Møller, 1995). Symmetry aids in keeping leaves healthy because it creates even wind drag across the leaf and reduces wind induced damage (Møller 1995). This suggests symmetry is an indicator of tree health, but symmetry's relationship to herbivory is not well understood.

Populous tremuloides, the North American Aspen, reproduce both sexually and asexually via cloning. As a result, aspen often grow together in genetically identical clonal groups, and hence provide a practical model for this study. The clonal groups allow for the isolation of genetics as a variable.

Plants possess what is known as phenotypic plasticity; they are able to change their phenotypic expressions in response to environmental changes (Schlichting, C. D.; Pigliucci, M). Asymmetry in leaves is the result of trees diverting resources away from growth to focus on environmental factors like resistance to herbivory. Given that bilateral symmetry in the leaves is

controlled by a single set of genes susceptible to phenotypic plasticity, asymmetry may indicate a tree's susceptibility to herbivory (Møller, 1995). Hence, the purpose of our research is to determine whether or not there is a correlation between asymmetry and increased insect/microbial activity. If there is a correlation between asymmetry and increased herbivory, then we can conclude that asymmetry could be a phenotypic marker of a plant's susceptibility to herbivory.

Methodology

Leaves were collected by Silver Lake in Brighton of Big Cottonwood Canyon in September of 2014. The DNA from one leaf per tree was extracted, then analyzed using microsatellite genotyping. This data was compiled in a mass dataset for the entire grove.

For measuring asymmetry, the most healthy looking leaves from each tree were selected, and their image thresholded. The asymmetry of each leaf was determined using Image-J. First, the centroid of each leaf was found. Then, we measured radii at equal angles from the centroid at multiple angles. The ratio of these radii were averaged, and this value denotes the asymmetry of the leaves. In order to factor out the influence of leaves with huge amounts of leaf damage, we will only use the top 75% most symmetric leaves in our data set.

Leaf damage will be measured on a scale of 0 to 3 where 0 is no damage and 3 is extremely damaged. Each group measured and recorded these values independently, which does mean there is room for error as the values may not be consistent across groups.

In R, we will first examine the relationship between damage and clonality. If there is a correlation, we will then compare the level of asymmetry, the year the leaf samples were collected, the length/width ratio, and clonality with the level of leaf herbivory. By using many

covariants, we can systematically reduce the amount of confounding variables and gain a better understanding of the relationship, or the lack of relationship, between asymmetry and damage. Additionally, the intersecting nature of the many covariates can be examined. For example, the length/width ratio and asymmetry will both be employed together, as, when tested separately, they explain similar percentages of variation in leaf damage.

Anova Results

Input	**Total % Damage variation explained**	**p-value**
Damage ~ clone	11.4	.057
Damage ~ clone+asym75	22.15	0.0007145
Damage~ clone+asym75+ratio	26.26	0.0001771
Damage~ clone+year+ratio+asym75	26.81; Asym explains 7.75%	0.0002939
Damage~ clone+asym75+year+ratio	26.81; Asym explains 10.75%	0.0002939
Damage~ clone+year+asym75+ratio	26.81; Asym explains 8.83%	0.0002939

Conclusion

When both clonality and the year the leaves were collected, asymmetry and the length width ratio were accounted for, they explain 26.81% of variation in leaf damage. Our results are statistically significant because there is only a .02% chance of this relationship occurring

randomly. Furthermore, leaf asymmetry can account for up to 10.75% of variation in leaf damage. Interestingly, changing the order of the covariants in R changes the percent of variation in leaf damage that asymmetry accounts for. From these results, a clear correlation between damage and asymmetry forms.

Because asymmetry is related to leaf damage, we can conclude more symmetric leaves tend to have lower damage ratings than asymmetric leaves. However, we cannot currently determine whether leaf damage causes asymmetry due to the diversion of resources from leaf development to leaf protection or if more asymmetric leaves are more susceptible to damage.

There are multiple opportunities for future studies. The parameters for determining leaf damage could be refined to delineate between insect activity and microbial growth. Additionally, establishing a more uniform, quantitative procedure for assessing leaf damage would increase the veracity of the results. Understanding the mechanisms at work is a natural next step. Further studies on the chemical repellents that help regulate herbivory could give insight into one possible mechanism. In addition, auxiliary studies determining whether asymmetry causes damage or if damage causes asymmetry would greatly augment our understanding of the relationship between asymmetry and leaf damage.

Works Cited

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