The Effect of Geography on Variations within Clones of *Populus Tremuloides*

by Kevin Yang, Kacey Davis, Taylor Briggs, and Naveen Rathi

Introduction

At large scales, variation is a direct effect of both environment and genetics (Brunet, *et. al*). The clonal nature of aspen trees, or *Populus tremuloides*, gives us a rare opportunity to examine the effects of the environment on phenotypic traits while controlling genetic variables. Environmental factors such as changes in altitude may influence traits expressed in various trees of the same clone, including leaf size (length*width) and leaf shape (length/width) (Polle, *et. al*). Using two years of data from three clones in Big Cottonwood Canyon, we can investigate how altitude affects variation among trees.

Previous studies suggest there is variation because of geography. One article indicates that as populations of aspens spread north-west across Minnesota to central Alberta, trees become 34% taller and have 84% more biomass than local sources (Schreiber, 2013). This suggests that on large scales, geography has a direct effect on aspen tree phenotypes. Another study suggests that factors such as the year the plant was grown have a much larger effect on amino acid composition than geography (Vilanova, M., *et al*.). Our study will account for year of data collection (2013 or 2014) as well as clone while determining the effect of altitude, which is directly proportional to longitude in our specific data collection site. The previous year's (2013) study of aspen trees at the University of Utah by Jon Seger's Biol 2005 class demonstrated that leaf shape varies with longitude, as shown in Figure 1 (Seger, *et. al*).

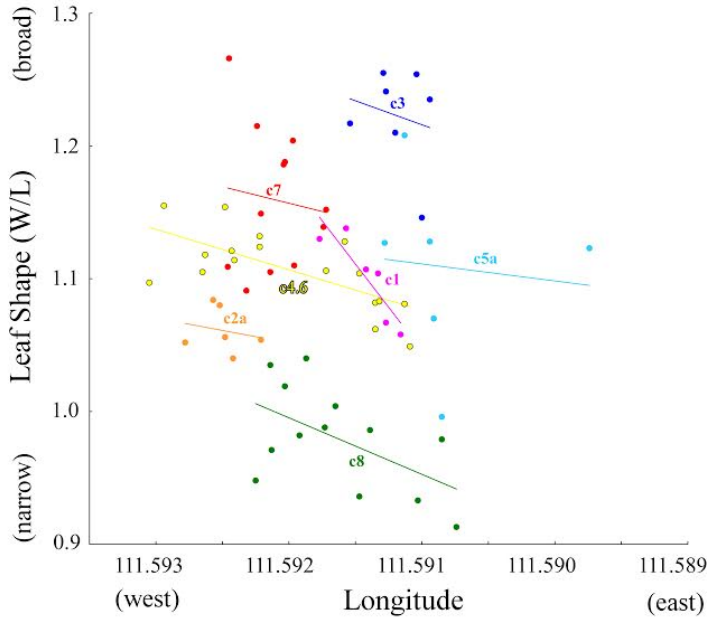

Figure 1

Our study will expand on these results by looking at leaf size and shape compared to altitude and year. Since geography is known to affect some qualities such as the height and biomass of trees, we have reason to believe that within clones, aspen trees will display environmental variation because of the difference in altitude. One study suggests that on plain ground, longitude is a better measure of location than altitude (Kuzemko). However, our site demonstrates a strong correlation between longitude and altitude and we hypothesize that altitude may be a better judge of location.

We hypothesize that there will be an inverse relationship between the length of the leaves and the altitude of the tree. Broader leaves may be less successful than longer leaves, so we predict that an increase in altitude will cause the trees to be less successful. Additionally, we believe that altitude will be a better judge of a tree's location and thus explain a larger percent of variance with respect to both leaf shape and size; although longitude and slope are highly

correlative, altitude would better assess areas of the site with slopes not explained through longitude.

Methods

The first part of the study involved going to Big Cottonwood Canyon to collect the leaves. The area for the study was chosen last year and groups split up to cover areas not visited by last year's groups. Leaves were collected from random trees and the geographic coordinates and circumference at chest height were recorded. The leaves were then arranged on paper and numbered to create leaf sheets. Scanning the leaf sheets into the computer allowed for digital data recording and manipulation. To determine its genetic information, PCR was performed on one leaf from each tree. The clones were determined by comparing eight loci patterns and those that were identical were declared to be of the same clone. From the scanned leaves, the length and width of the trees were determined using ImageJ, and the area was found by thresholding the leaves and using a python program written to find the area. The altitude was found by using Google Earth Pro. The coordinates of the individual trees are pinpointed on the area's map and the cursor was held over the spot to find the altitude associated with each tree.

By using R programming, the comparisons between leaf size and shape, altitude, and year were completed. Three clones were chosen, 4-6, 8, and 5a for their geographic variance and large clone size. To control for year variance, the clones were split between the years that their data was collected, 2013 and 2014, to compare the variance between the two years. The leaf size was taken by multiplying length by width and the shape was determined by the ratio of length over width. These of each clone per year were compared

to the tree's altitude to determine variance between trees of one clone of one year. This process was completed for each clone of each year and size and shape. A comparison between longitude and altitude was ran to determine which is a better indicator of variation.

Results

(see attached spreadsheet)

Discussion

The results show that both altitude and longitude are significant variables for leaf size and shape. The length of the leaves consistently decreased when altitude increased, following an inverse relationship. This could indicate that trees at higher elevations are faced with greater stress, affecting leaf length growth. These greater stresses may be caused by a temperature decrease at greater altitudes, or a lesser amount of water and nutrients received since they are transported downhill to the lower trees.

Longitude and altitude have the same same percent of variance explained, which demonstrates a strong correlation between altitude and longitude and indicates neither altitude nor longitude are a better measure of location in our specific site. Strangely, neither longitude nor altitude was a significant factor for shape; however, both were significant when considering size. Longitude has the same percent of variance explained (54.88% for shape and 39.03% for size) as altitude, leading to the conclusion that altitude and longitude are just proxies for an underlying factor such as soil quality, water content, pH, or toxic mineral concentration. One theory is that at higher elevations, the slope of the mountain is steeper, allowing more nutrients to wash down away from the trees up higher

and supply a greater amount of nutrients for trees at lower altitudes. But whatever the factor is, be it soil, nutrients, water, or slope, it does change with a change in altitude. This opens up more experiments to find the underlying cause of the correlation between shorter leaves at higher altitudes.

One problem we originally faced is that when testing asymmetry with longitude and altitude, no results were significant. This showed that leaf symmetry differs more by clone and not by location. Also, our sampling techniques gave no documentation of from where on the tree the leaves were taken, and the amount of sunlight that the leaves were exposed to could affect the results. Misrecording in ImageJ by measuring length then width would have majorly skewed the data as well.

Further research should be carried to explain the underlying factor behind the relationship between altitude and longitude. Two characteristics in particular that could explain this relationship is the concentration of metal ions or the slope of the hill as altitude increases. If one were to examine concentration of metal ions, sample the soil by each target tree and use a mass spectrometer to separate the metals in the soil based on the charge and mass ratio. If one were to examine the relationship of shape and/or size of the leaf with the slope of the ground at the tree, then measure angle of the ground at each target tree's trunk.

Bibliography

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Extended Results

Sample Code:

1. lvsA <- cbind(leaves, area = leaves$L*leaves$W)
2. c4_6 <- subset(lvsA, clone == "c4_6")
3. c4_6_2013 <- subset(leavesc4_6, year == "2013")
4. a <- lm(LW ~ alt_m + tree, data = c4_6_2013)
5. anova(a)

```
           Df  Sum Sq  Mean Sq F value   Pr(>F)
alt_m       1 0.04139 0.041392  7.3087 0.007197 **
tree       15 0.14598 0.009732  1.7184 0.045706 *
Residuals 350 1.98216 0.005663

alt_m       -0.0003303  0.0004461  -0.740   0.4596
```

see attached spreadsheet