Group 7

Genotypic and Phenotypic variation of *Populus tremuloides* Wood Density

Water conductivity and tree strength, both influenced by the density of a tree's wood, play an integral role in a tree's development and survival. The *Populus tremuloides*, commonly known as the quaking aspen, exists abundantly in the Wasatch Mountains of northern Utah. Aspen trees tend to reproduce asexually, resulting in unique sets of clones. They also possess the ability to reproduce sexually, creating more variation within the genetic pool. While many aspen trees share the same characteristics, closer examination shows tremendous variation in phenotypic expression between diploid and triploid trees. The variation between these trees can be attributed to heritability, as well as environmental factors. Unfortunately, the exact role that each factor plays is still unknown. Leaf and trunk sizes highlight the general phenotypes studied. Individually, this research will examine the variation of density and the role it plays in the interaction of tree strength and water conductivity.

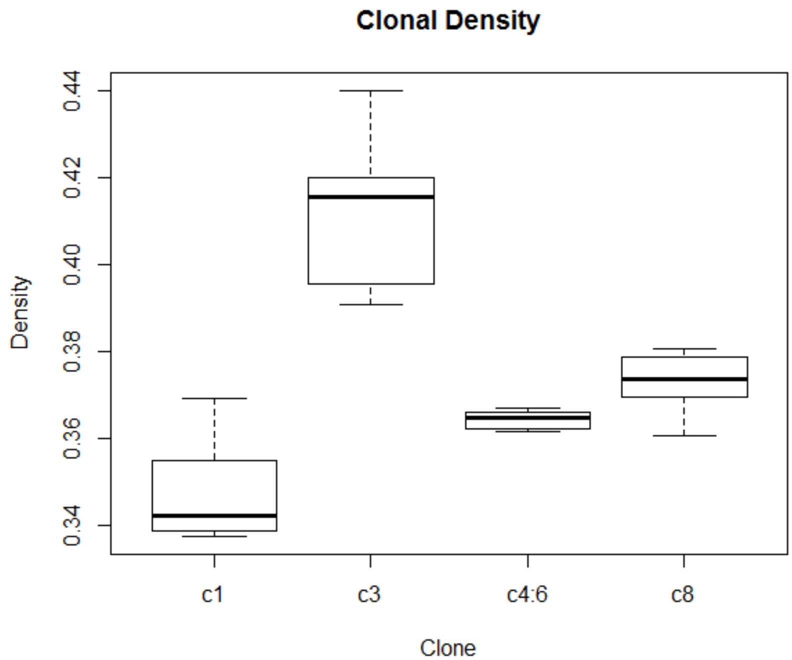
Our endeavor was to investigate the trends that occur between phenotypes. The possible relationship between two phenotypes provokes the question of whether density and tree size, and density and leaf size, trend similarly. Research done by Fabien Scholz and Sandra Bucci has suggested the relationship between density and leaf size in other systems (Scholz & Bucci, 2008). In an earlier publication, they identified an inverse relationship between these two attributes. Henry Horn's publication on tree elasticity, which is the flexibility of the branch of a tree, and its impact on size may also suggest a relationship between tree size and wood density (Horn, 2000). This is especially true given the implicit link between stiffness and wood density. Employing genotypic data collected from the Silver Lake ecosystem, statistical calculations would provide insight into the impact that heritability and environmental factors each contributes to density.

A large correlational study of leaf measurements was created from samplings of a variety of aspen trees within the Silver Lake ecosystem. Trees were selected based on proximity to each other, as well as physical attributes that may indicate genetic similarities or differences. Traits such as size, color, and trunk circumference were all taken into account. Each tree sampled was meticulously tagged, which made the identifying process much more efficient throughout the study. A total of ten trees were sampled, with approximately twenty leaves from each tree being randomly selected for size measurements. Leaf length, leaf width, tooth size, and damage quantification were the initial values taken for each leaf. For each sample, one leaf was selected and genotyped. PCR was first used to amplify the desired DNA segment. Gel electrophoresis was then employed to prepare the segment. Once the sample had been manipulated, Peak Scanner software was used to analyze the yielded results. The genetic makeup from a variety of leaves within the Silver Lake ecosystem was cross-examined, and aspen clones were identified.

The Silver Lake ecosystem is located within the Wasatch National Forest, which restricts the destruction of flora within the protected area. Thus, the same trees from which leaf measurements were gathered could not be used for wood samples. This required us to sample trees that had fallen from natural causes. Although this was an easy inconvenience around which to operate, it did limit our accessibility to higher amounts of density data. Ultimately, wood from five downed trees was extracted. Three circular cross sections were taken from four of these trees, with only two cross sections being available for the fifth tree. After being removed by chainsaws and other cutting materials, these samples were transported to the University of Utah. After being thoroughly dried in an oven, the samples needed to be further manipulated before having their densities measured. In order to insure accurate results, the bark was sliced off of each sample. Using handsaws, a belt sander, and a band saw, 2 smaller samples were taken from each cross section. For the tree that provided only two cross sections, 3 smaller samples were taken. In order to comply with the density measurements to be taken later, the samples were manipulated into a cube with edge length of approximately 0.5 inches.

Archimedes principle was used to acquire volume. Given that the density of an object is equal to its mass divided by its volume, the mass of the dried sample was first taken. The samples were then submerged in a large beaker of water. This was a pivotal step, as each sample needed to be wet when placed in the cup of water. A small cup of water was placed on a balance. These samples will be extracted by chain saws before being transported back to the University of Utah. After employing an oven to dry the wood, the pith and the bark will be removed from the cross section. These parts of the tree have differing densities, and could skew our data regarding wood density (Rosell, 2013), (Kampe, 2013). The remaining wood will be submerged in a water-filled container that has been placed on a balance. Using Archimedes principle, the mass of the water displaced will equal the density of the submerged wood. These findings will be statistically processed to see if density is influenced by genetics or environment. A computer-based software will be used to interpret our data. Statistical calculations will be made to determine the genotypic and environmental influence on density, as well as leaf size.

The results of our project were astounding. Using the program r we found statistically significant aspects of our project including connecting phenotypic variation to genes and making a compelling argument similar to those made in the Scholz and Bucci study and Horn study, that high tree density leads to high strength and low water conductivity. First density was compared to genotype using genotype as a factor. Graphing clone type against density we found a surprising trend within the data.



Looking at the graph above we see that clone type seems to play a part in the density of the tree. In addtion to the differences between each clone compared to each clone it is also interesting to look at them as groups. When genotyped it was discovered that clone c1, c8, and c4:6 were all triploids while clone c3 was a diploid. The graph shows clearly a statistical diffence between the densities of diploid tree and the denisites of triploid trees. Since clone type seems to play a role in density we used the analysis of variants (ANVOA) which compares the statistical probability of the relationship between the two factors occurring simply because of chance. In our ANOVAs we compared the role that genotype plays on density. Below is a table that ANOVA.

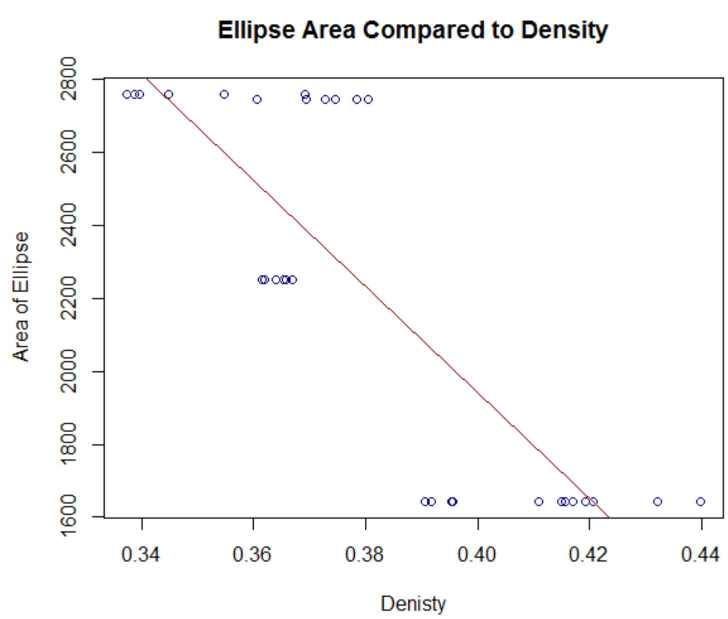
```
Call:
lm(formula = Density ~ clone, data = cube)

Residuals:
       Min         1Q     Median         3Q        Max 
-0.0213529 -0.0067037  0.0005487  0.0056242  0.0278555 

Coefficients:
            Estimate Std. Error t value Pr(>|t|)    
(Intercept) 0.347380   0.004945  70.247  < 2e-16 ***
clonec3     0.064627   0.006056  10.671 5.39e-11 ***
clonec4:6   0.016866   0.006993   2.412  0.02324 *  
clonec8     0.025297   0.006993   3.617  0.00126 ** 
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.01211 on 26 degrees of freedom
Multiple R-squared:  0.8425, Adjusted R-squared:  0.8243 
F-statistic: 46.36 on 3 and 26 DF,  p-value: 1.419e-10
```

The most important number on this table is the p-value in the lower right hand corner. That value of 1.419e-10 is the statistical probability that results obtained were purely based on chance. Cleary this is such small percentage that we can say a tree's clone type heavily impacts a trees density. This knowledge however has a high degree of fallibility because the program assumes that the clones are independent of each other which untrue in many respects. The connection is still very important because it plays a role in the next ANOVA that we did which was comparing the area of leaves to density. The following graph is a visual look at density compared to leaf area.



This graph shows an inverse relationship between density and area. In addition to the scatterplot a red line was superimposed on the data to demonstrate this inverse relationship. Clearly this graph indicates that density seems to play a big role in leaf area. However to see if the relationship was statistically significant we used the same ANOVA program used in the previous step.

```
Call:
lm(formula = ae ~ Density, data = cube)

Residuals:
    Min      1Q  Median      3Q     Max 
-433.43 -193.02  -66.09  214.08  518.28 

Coefficients:
            Estimate Std. Error t value Pr(>|t|)    
(Intercept)   7751.0      697.7  11.109 8.99e-12 ***
Density     -14523.0     1823.1  -7.966 1.12e-08 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 283.7 on 28 degrees of freedom
Multiple R-squared:  0.6939,	Adjusted R-squared:  0.6829 
F-statistic: 63.46 on 1 and 28 DF,  p-value: 1.124e-08

>
```

As in the first analysis the most important number is the p-value: 1.124e-8. This small number again indicates that the likelihood of the relationship between density and leaf area being totally due to chance. Cleary, the density of the tree has an impact on the leaf area of the tree. Both these analyses are useful at implicating important facts.

First, using the information presented by Scholz and Bucci, that “Consistent changes in stand level biophysical traits observed along the gradient of increasing tree density included a decrease in weighted-average wood density, and increases in leaf surface area per plant, leaf specific hydraulic conductivity, specific leaf area and stomata conductance.” Clearly our results trend in the same direction as their results. When the density of the wood goes up the leaf area goes down. What is fascinating about our research is in the Scholz/Bucci study they found that the species of wood trended in this manner when comparing leaf areas. However in our study we found that the density of the wood trends the same way but within a single species. This implicates that its possible tree species does not affect leaf area, but instead that tree density regardless of species impacts leaf area. The realization leads to our next important conclusion.

In Horns paper he references a study that found that the strength of the wood correlated to the density of the wood, in addition to the elasticity of the wood fiber. Disregarding elasticity, the paper shows that an increasing density trends to increasing tree strength, which would then lead to a greater resistance against the physical environment. These conclusions facilitate the conclusion that those tree with genotypes creating favorable tree densities balances lead to more adaptable and sustainable trees. This knowledge allows us to assume that the trees with higher density resisted the physical environment much more effectively than ones who don't.

Work Cited

Horn , H. (2000). *Twigs, trees, and the dynamics of carbon in the landscape*. Oxford University Press.

Rosell, J. A. (2013). Bark functional ecology: Evidence for tradeoffs, functional coordination, and the environment producing bark diversity. *New Phytologist*,

Wu, F. (2013). Genotypic parameters of wood density and fiber traits in triploid hybrid clones of populus tomentosa at five clonal trials. *Annals of Forest Science*, *70*(7), 751-759. doi: 10.1007/s13595-013-0307-7

Kampe, A. (2013). New insights into heartwood and heartwood formation. *Plant Cell Monographs*, *20*(2013), 71-95. doi: 10.1007/978-3-642-36491-4_3

Scholz, F. G., & Bucci, S. J. (2008). Plant- and stand-level variation in biophysical and physiological traits along tree density gradients in the cerrado. *Brazilian Journal of Plant Physiology*, *20*(3), 217-232. Retrieved from http://www.scielo.br/scielo.php?pid=1677-0420&script=sci_serial