

The Genetic and Environmental Effects on the Leaf Venation of *Populus tremuloides*

Group 3

Introduction

For the purposes of this class, we have been investigating how a specific variable within a population changes in relation to the genetic and environmental variables. To evaluate the effect of these two factors, we chose Quaking Aspen (*Populus tremuloides*) as our research subjects. Quaking Aspen made an ideal subject group because of their clonal nature and close proximity to the lab. By clonal nature, we mean that aspens often reproduce asexually, resulting in large forests of aspens that share a large amount of genetic material. Our particular investigation sought to explore the differences in leaf venation across clonal groups. Leaf venation is an important part of plant development, especially since they represent a way in which the organism responds to their environment (Dale, 1981). Veins are the transportation system of leaves. They import and export materials; the xylem imports water from the roots and the phloem exports photosynthetic products. The higher the vein density, the more efficient each individual leaf can be. Thus, venation patterns allow us to visualize the general organization and specialization of leaves.

For our particular investigation, we aimed to analyze the relationship between leaf venation and clonal groups. We wanted to see how genetics and environment interact with this variable. To quantify leaf venation, we counted the number of secondary veins in each of the leaves sampled. Since leaf size varies as well, we compared it to the length of the leaves. This comparison adjusted for any variation due to leaf size. In early leaf development, the leaf primordium undergoes a slow limited expansion phase and a dramatic expansion phase in which the cells both multiply and expand in size. Secondary veins typically grow during the “slow stage” of early leaf development (Dale, 1981). This allows us to conclude that primary and secondary veins are more related to the genetic component of growth, while tertiary veins (veins that branch off of the secondary veins) are more determined by environmental factors. We used this information in part with the genotypes of our sampled trees to determine if this ratio is determined by genetic variation, the environment, or a combination of the two.

Questions

There are several topics that were explored throughout this study. We quantified leaf venation and determined clonality by genotyping, meaning that we could then analyze other relationships amongst this Silver Lake aspen population. Our main investigation aimed to examine the relationship between the genetics and leaf venation. We looked at whether vein scaling differs between clones or if venation is the same within the same clone of aspens. Ultimately, this forced us to ask the question of whether these differences amongst clones are due to environmental factors or biological factors. Fortunately, the differences indicated a statistical relevance so that we were able to draw some conclusions about the population. In further research, we would like to explore the role of polyploidy in leaf venation patterns and whether these factors, namely leaf venation, change the health and success of a tree. This is the impact of this research, if more veins relate to overall success, encouraging these characteristics in domestic plants could make them more successful.

Materials and Methods

Populus tremuloides, the quaking aspen, was chosen for this study because it reproduces both sexually and asexually. In the aspen forest at Silver Lake, Utah, it's easy to see possible clone groups because of similarities in tree color, bark color, and other characteristics. About ten trees from each of the potential clone groups were tagged with aluminum and sampled for leaves using slingshots and extended clippers. The leaves were then scanned into a database, numbered, and measured. Next, the DNA was extracted from each tree, and multiple loci were amplified by PCR to determine both the tree's clonality and ploidy. After this, we were able to use the genetic data to divide the sampled trees into nine different clones.

Our group counted the number of secondary veins branching off the first order vein in each leaf. The secondary veins are defined for our purposes as large veins directly connected to the primary vein that branch out at least two-thirds of the way to the perimeter of the leaf. Furthermore, secondary veins lose their significance in the purposes of this experiment once they lose their white coloring towards the tip of each leaf. The ratio of veins to length will be compared to clonality through ANOVA in R, a computational statistics program. The outcome allows us to conclude the effect of genetic components, environmental components, as well as both genetic and environmental factors on leaf venation.

Results

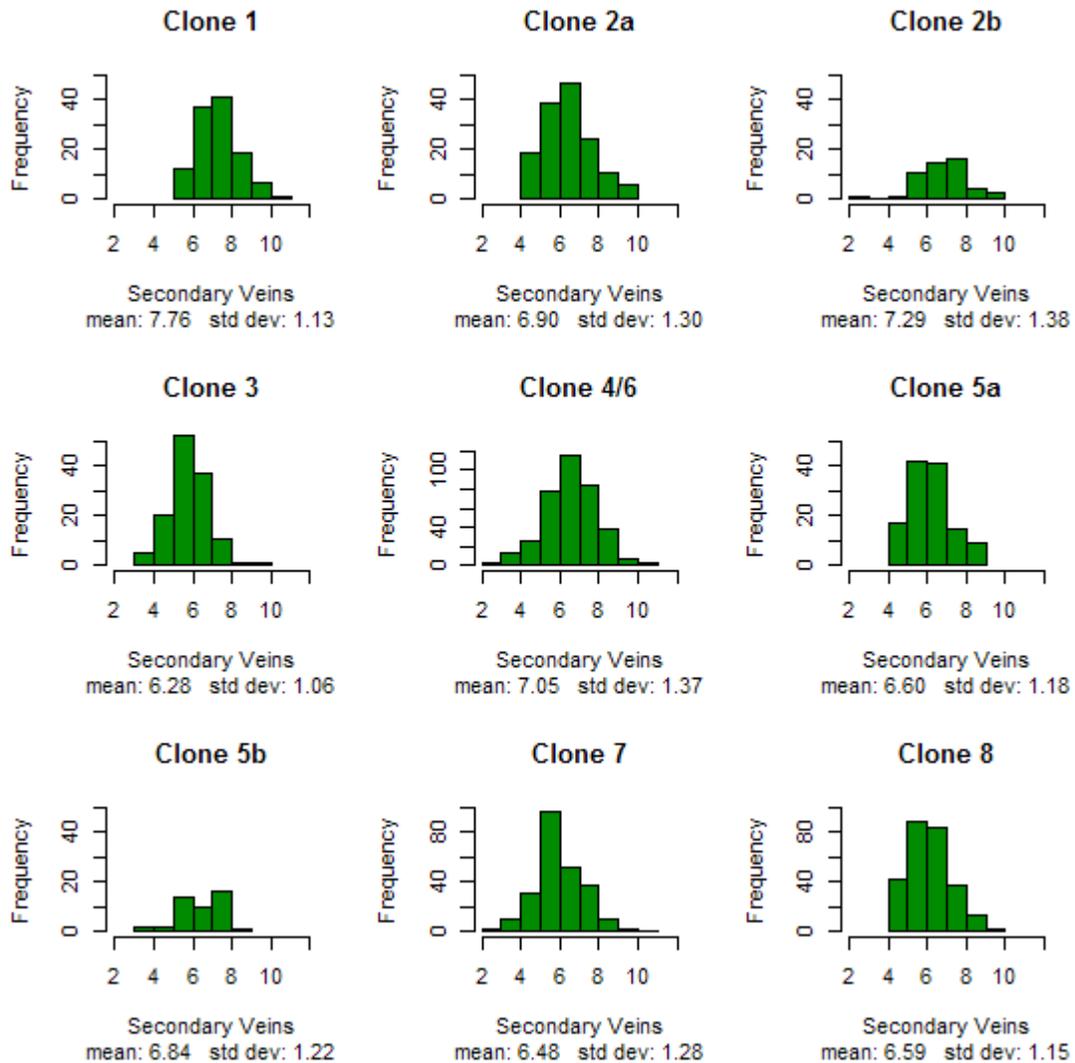
Our results indicate that there is a relationship between the three tested comparisons. The first, represented by the first row of the table, analyzes the connection between the number of veins on a leaf as compared to the length of the leaves. The second is the affiliation between clone patterns and vein count. The third and final is the correlation between vein counts and the ratio between leaf length and clone patterns. All of these comparisons were proven to be significant by ANOVA testing in the R program. These significance values may be seen below.

SIGNIFICANCE TEST	P-VALUE
Vein to Length	2.2×10^{-16}
Vein of Clone	2.2×10^{-16}
Vein:Length to Clone	2.2×10^{-16}

The table above shows that the relationship between the number of secondary veins to the length of the leaf, the relationship between the number of secondary veins to the specific clone of trees, and the relationship between the ratio of the number of veins to the length of the leaf to clones are all highly significant. We conclude this significance because all of the P-values listed above are less than our assumed alpha-level of 0.05.

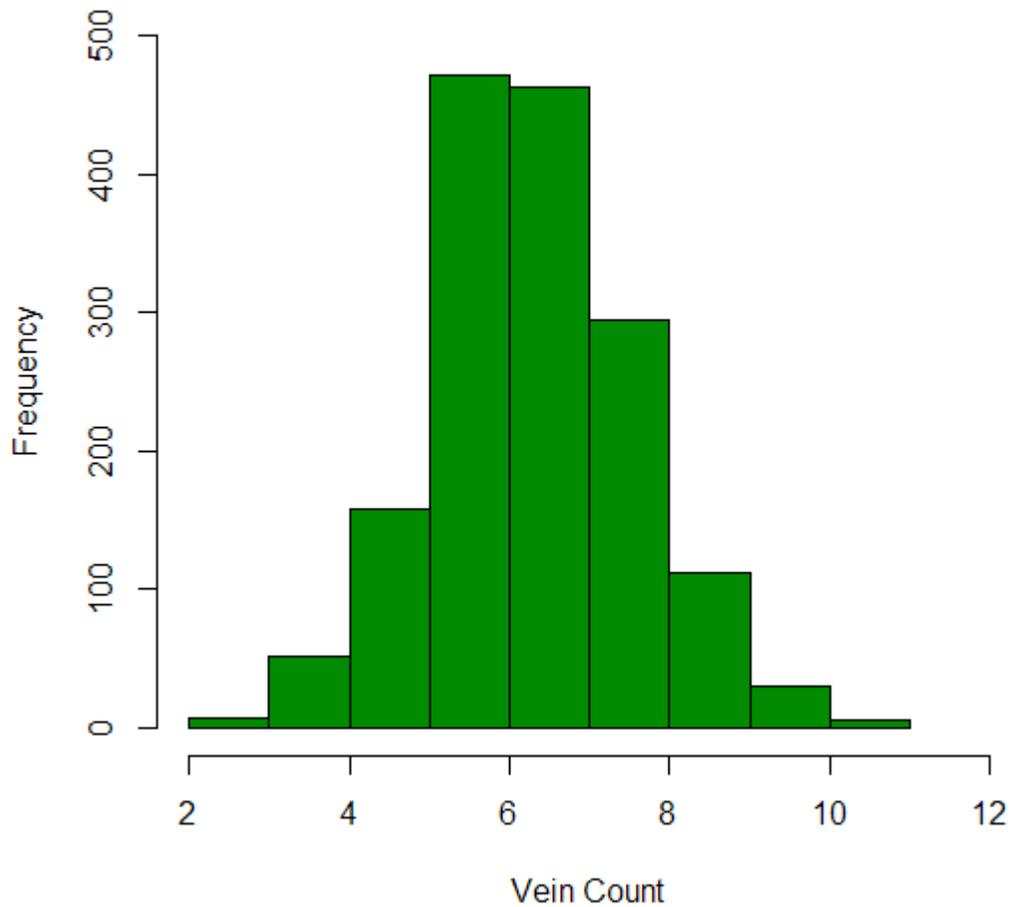
The following graphs provide some visuals of how our leaf vein count differs amongst clones and the population, as well as the relationship between leaf vein and leaf length.

HISTOGRAMS OF FREQUENCIES OF VEIN COUNTS



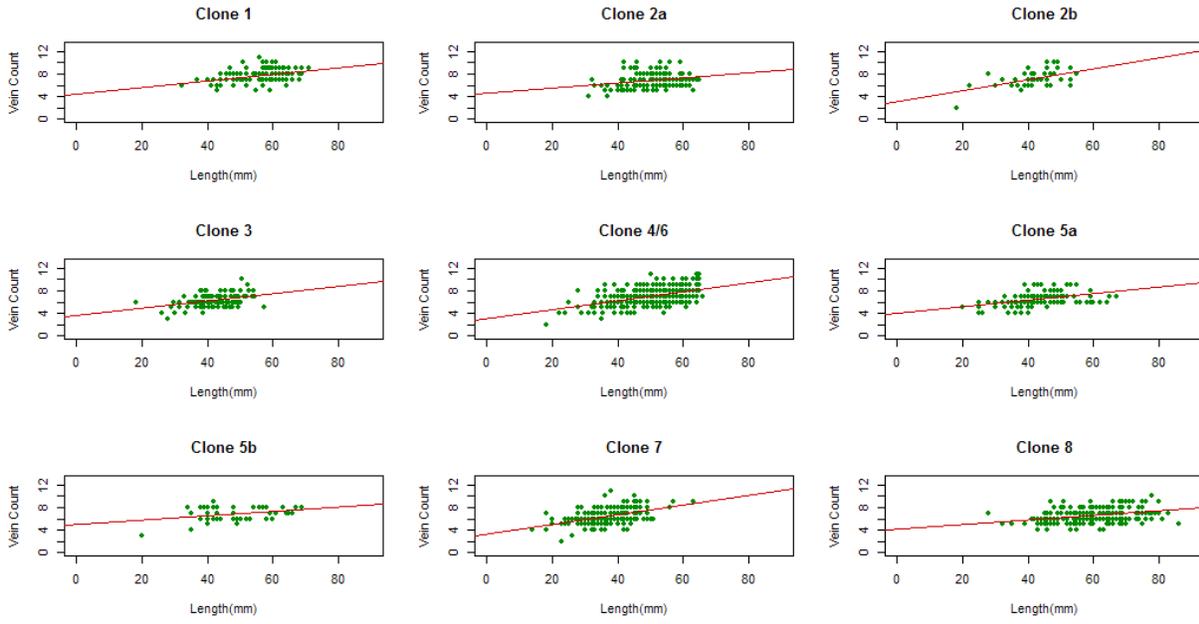
The above histograms show the frequency of the number of veins amongst the different clones. Clearly, most of the distributions appear approximately normal, indicating that there is a center for the number of secondary veins among the leaves of the clones. The centers for the graphs vary between 5 and 8. Below, there is a summary graph showing the frequency of vein count of all the clones together. Similarly, the distribution below appears approximately normal. It seems to be centered at 6 secondary veins per leaf.

Frequencies of Vein Count for All Clones



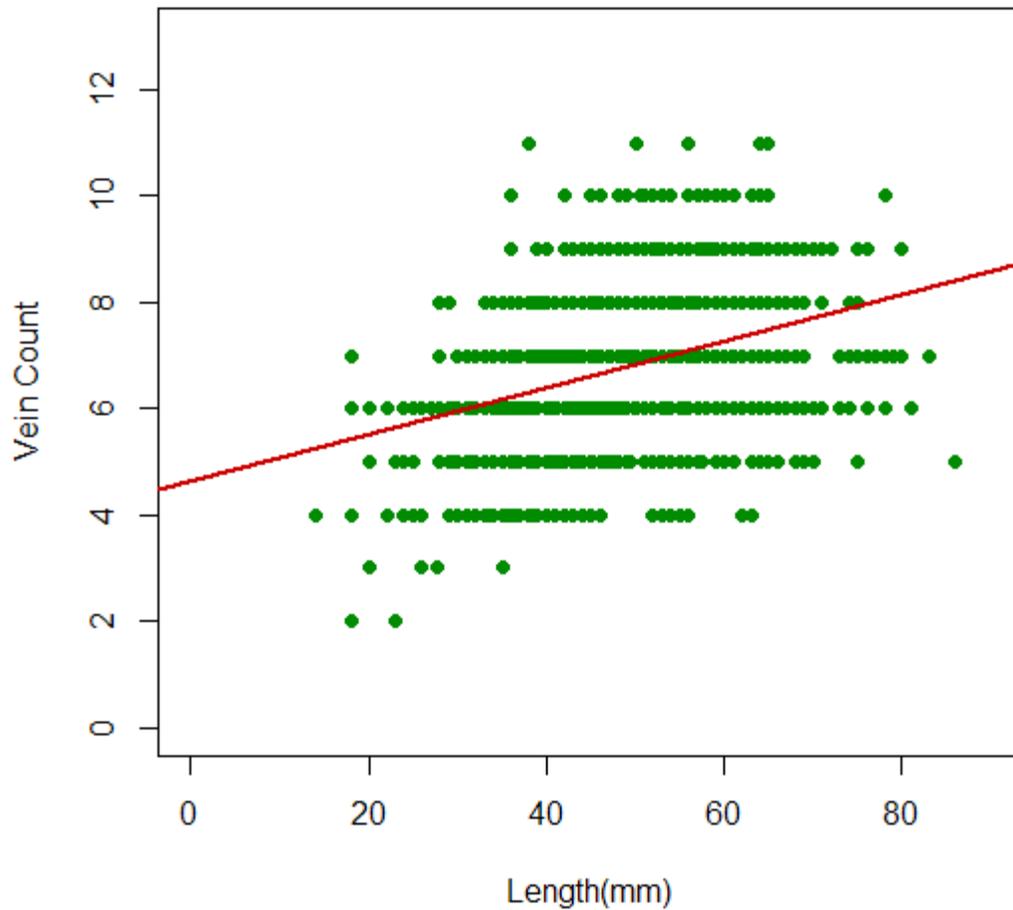
The graph above shows the vein count for all leaves collected by every group in the class. The total amount of leaves ended up numbering nearly 1600. The data creates a very nice bell curve, while skewing a little left, showing a tendency towards a lower vein numbers per leaf. Overall, we can state that this distribution is approximately normal.

VEIN COUNT TO LENGTH RATIO BY CLONE



The above graphs show the relationship between the number of secondary veins and the length of each individual aspen leaf, divided into the 9 clonal groups that were determined through gene extraction and PCR. A line of best fit was then calculated and graphed over the scatter plot. Each clonal group tends to have the individual leaves close to each other in clumps, and there is a positive correlation between vein count and leaf length in all nine clones. Each clone has a visibly unique length to vein count ratio, which reflects its heritability.

Relationship Between Leaf Length and Vein Count for All Trees



A positive correlation between leaf length and secondary vein count is clear in the graph above. Unlike the graph above it, it clumps all of the clones together so that we can view the entire population together. Here, the same trend is apparent. As leaf length increases, the number of secondary veins tends to increase as well. However, there is a wide range of vein counts for each length of leaf. For example, for leaves with a length of 40mm range from about 3 to 11 veins per leaf overall, but Clone 8's 40mm length leaves have a vein count from 4 to 7 while Clone 1's ranges from 5 to 9.

Conclusion

The purpose of this investigation was to evaluate the effect of genetic and environmental factors on our chosen variable: leaf venation. We expected that both components would contribute to variation in leaf venation equally. In the end, we found that our data disproved our hypothesis. The heritability of the leaf length to vein count ratio was found to be 98.67%. This suggests that the number of secondary veins is not a reflection of the environment in which they were developed. We performed several ANOVAs that related the number of veins to clones and the leaf length to clones, as well as relating the vein-length ratios to clones. Across all tests, we found that these relationships were significant. Thus, we can conclude that there is a large genetic component to the formation to leaf venation. There is, however, a small amount of variation within each clonal group. This indicates that genetics are not the only factors in determining leaf vein development. The rest of the variation must be due to various environmental factors. Overall, we conclude that both biological and environmental components have an effect on leaf venation. Further research should explore the influence of the environment on tertiary veins, and overall effect of specific environmental factors, such as climate and soil composition, on the leaf vein to length ratio.

Literature Cited

- Bonan, Gordon B.. "Chapter 9-Leaves and Plants." *Ecological climatology: concepts and applications*. 2nd ed. Cambridge: Cambridge University Press, 2008. 14-15. Print.
- Callahan, C M, Rowe, C A, Ryel, R J, et al. (2013). Continental-scale assessment of genetic diversity and population structure in quaking aspen (*populus tremuloides*). *Journal of biogeography* 40(9), 1780-1791
- Dale, J. E., and F. L. Milthorpe. *The Growth and Functioning of Leaves: Proceedings of a Symposium Held Prior to the Thirteenth International Botanical Congress at the University of Sydney, 18-20 August 1981*. Cambridge [Cambridgeshire: Cambridge UP, 1983. Print.
- Mock, K. E., C. A. Rowe, M. B. Hooten, J. Dewoody, and V. D. Hipkins. "Clonal Dynamics in Western North American Aspen (*Populous Tremuloides*)." *Molecular Ecology* 17.22 (2008): 4827-844. Print.
- Mock, Karen E., et al. "Widespread Triploidy In Western North American Aspen (*Populus Tremuloides*)." *Plos ONE* 7.10 (2012): 1-10. *Academic Search Premier*. Web. 13 Nov. 2013.
- Sack, Lawren, Christine Scoffoni, Athena McKown, Kristen Frole, Michael Rawls, J. Christopher Havran, Huy Tran, and Thusuong Tran. "Developmentally based scaling of leaf venation architecture explains global ecological patterns." *Nature Communications* 3 (2012): n. pag. *Nature*. Web. 23 Oct. 2013.