Proposal # 1

Color Me Impressed

Leaves are an integral part of trees and contribute significantly to a tree's health. Most importantly, they contain much of the trees active chlorophyll and as such are the structures in which most photosynthesis takes place. Although leaves have a pretty widespread structure, especially within tree species, they are sufficiently different in a variety of physical characteristics. Color is one of the most distinct factors about the appearance of a tree, yet it also serves as an indication of a plethora of factors about the tree. Research that has already been conducted leads to several assumptions: the darker the leaf, the more densely packed it is with chlorophyll because chlorophyll is a green pigment, darker colors absorb more heat so a smaller leaf size is more beneficial to prevent overheating, and higher elevation is indicative of less CO_2 in the atmosphere.

We are applying these factors to a group of aspen trees just northwest of Silver Lake in the Big Cottonwood Canyon to determine how leaf color vary among these factors: symmetry, diploidy/triploidy, latitude and longitude, and size of leaf. We hypothesize several ideas:

1. Darker leaves will likely be more symmetrical because they will be healthier on average and will therefore be under less stress (This assumes that symmetry is desired)
2. Darker leaves will also be smaller in terms of leaf height and leaf width than lighter leaves to lessen heat absorption.

3. Leaves at higher latitude will be darker to improve CO_2 conversion rates.

4. Triploid leaves are lighter because they have bigger cells therefore chlorophyll is less densely packed.

To compare tree color versus four different factors (symmetry, diploidy/triploidy, latitude versus longitude, and size of leaf), we need to conduct four different studies. To ensure that our data is as consistent as possible among the clones, we are going to utilize leaves collected from the same date rather than look at leaves collected throughout the course of the semester. The biggest challenge here is determining the scale for measuring or quantifying color. To do this, we can potentially formulate a gradient and compare the leaf color to that, or we can utilize Image J and determine the saturation of the leaf color and plot that against a variety of different variables. For symmetry, we are going to use image J to determine the area of half of the leaf and compare that to the other half. Then we can correlate that to the color quantities we obtain. Second, we can also plot the width versus height ratios, diploid and triploid clones, and latitude versus longitude data against the color data resulting in three distinct graphs. In total, we should have four graphs/ data tables, which we can then compare to ascertain how color is related to symmetry, diploidly/triploidy, latitude versus longitude, and size of leaf.

Proposal #2

Genetic variation in western aspen trees (Populus tremuloides) has been relatively unstudied. Through research of these aspen trees, we can come to a better understanding of genetic and environmental factors on the aspens' phenotypic traits. P. tremuloides is a clonal species, reproducing asexually for most of its existence, while only reproducing sexually by flowering after severe fires or other similar events. Aspen range from Alaska and Canada to New England and to the south in California and New Mexico (Mock).

In this particular study, we will examine a site in northern Utah in Big Cottonwood canyon near the Brighton Ski Resort. This location contains a large number of accessible aspen as well as a number of different clones. The fact that these trees are clonal makes them identical to each other in their genetic code so varying phenotypic traits can usually be attributed to environmental effects. An effect of certain environmental factors, such as soil-creep, snow, catastrophic events, and phototropism, is the aspen trunks growing curved (Harker).

We want to determine if curvature may affect tree fitness. To test this, we are going to specifically look at the symmetry of the leaves, where a ratio of length/width equal to one would show symmetry. We hypothesize that curvature is bad for a tree, thus the ratio of length/width would not be equal to one for curved trees, showing asymmetry. To look at fitness, we will also examine variation in length and width measurements, which we have data from a previous group in our study to suggest that there is more variation in the length of leaves than in the width.

We have focused our efforts on two compiled and simplified questions: (1) Does trunk curvature have an effect on leaf width/length ratio? In other words, do curvy trunks have more asymmetrical leaves? (2) Do trees with curvy trunks have leaves that follow previously noted patterns of more variation being in the length of leaves than the width for each tree?

Through analyzing data we will collect, we hope to determine if there is reason to believe that trunk curvature causes stress to the tree enough to affect its fitness.

To collect data, we will sample approximately 20-40 leaves from each selected tree. In addition to this, we will label the trees, measure the circumference of the trees at breast height, and take pictures of the trunks with meter stick as a scale. The leaves collected will then be brought back to the lab and placed and labeled on leaf sheets. Most leaves will be placed on leaf sheets, but those that aren’t will be genetically tested to discover which clone they belong to. By separating the DNA from the leaf material and running a gel electrophoresis, we are able to determine which of the known clones the tree belongs in or determine if the tree represents an unknown clone. The genetic testing also allows us to find out if the is a diploid or a triploid.

Data for each tree is categorized by its clonal type and its ploidy. Leaf sheets will be scanned into a computer database and uploaded into a cloud drive. From here will open the images using a measurement program called Image J. Every leaf will be given both a length and width measurement and a label number for each measure. Using the statistical analysis program R, we will compare the leaf measurement ratio of a curved tree to a non-curved tree of the same clone. Through the analysis of six pairs of trees with contrasting curvature, we hope to determine if the curviness of the trunk has an impact on the ratio of the leaves, showing symmetry or asymmetry, and also if it shows the same pattern of more variation between length measurements, thus seeing if the curvature has an impact on the overall health of the tree.

Works Cited

Curved Tree Trunks: Indicators of Soil Creep and Other Phenomena

R. Ian Harker

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Mock, Karen, C. Rowe, M. Hooten, J. Dewoody, and V. Hipkins. "Blackwell Publishing Ltd Clonal Dynamics in Western North American Aspen."*Molecular Ecology* 17.22 (2008): 4827-844. Print.

Proposal #3

The effect of geography on variations within clones of Populus tremuloides Introduction Aspen trees, or Populus tremuloides, are extremely useful in examining effects of environment because we are able to control for genetic variation. Specifically looking in the Big Cottonwood Canyon area, we can investigate environmental effects such as geography (longitude and latitude), altitude, and concentration of metal ions. The clonal nature of aspen trees gives us a rare opportunity to examine the true effects of environment.

Background & Significance:

The observance of traits such as bent trunks and variance in height are reasons to believe there is variation based on geography. For example, previous studies suggest that as populations spread north-west across Minnesota to central Alberta, trees are measured to be 34% taller and have 84% more biomass than local sources. Since variation in geography has been known to affect the height and biomass of trees, we have reason to believe that clones of aspen trees would display variation due to environment. Identifying a trend within clones based on environmental factors would help monitor the health and success of specific clones. Also, pinpointing distinct factors such as elevation or excessive levels of metal ions while controlling for genetic variation could help forest workers determine what types of environments contribute to the success of aspen trees.

Specific Aims

First, we believe that there will be an inverse relationship between the elevation on which the tree is located and the leaf size as determined by the length/width ratio. As the elevation increases, the length to width ratio decreases, because length measurements are closer in magnitude to width measurements. Second, we believe that there will be an inverse relationship between the L/W ratio and the distance from the New York Mine. Third, we believe that there will be an inverse relationship between the concentration of toxic elements, specifically metals such as Cadmium, and the distance from the New York mine. Finally, we predict that the the distance from the mine will have a greater effect on the length to width ratio of the leaves than the minor change in elevation and consequently slope, and a greater effect than whether the trees are diploid or triploid.

Within these micro elevations and changes in the characteristics of the geography of our samples, we will be monitoring the length to width ratio of the leaves, we will observe the effect of three different characteristics: proximity to the New York Mine, elevation, and the circumference of the trees considered.

We are questioning if the trees that are closer to the New York mine have a higher concentration of Cadmium or other toxic metals that could produce to be dangerous to the trees. In turn, the concentration of the toxic substance could affect the length to width ratio. Thus, this adds another element to the geography (coordinates, elevation) that we will be testing.

Another one of the characteristics we are trying assign to the geography is the length to width ratio of the leaves. We selected the length to width ratio of leaves because this number can be affected by the amount of leaves on a tree. Leave that are longer are more “privileged,” which means that the growth of the leaf has not been stunted. A smaller value in the ratio of length to width indicates that the leaf has been affected by the environment if longer leaves are more desirable.

Tree circumference is the other characteristic we are trying to assign to the geography. It is expected that the circumference is larger at lower altitudes because at higher elevations the slope of the mountain was higher. Since the slope of the mountain is steeper, we predict that more nutrients will be washed away from the trees and their surroundings, leading to a lower circumference of trees. On the contrary, the trees at lower altitudes are on a flatter slope so they have less washing away of nutrients.

Finally, we will use genetics for two purposes. The first one is to discover which trees are a part of which clone from the new samples that we collected this year. The second purpose is to finally analyze what effect the tree being diploidy or triploidy has on the length to width ratio of the leaves of the trees. This will be simply to ensure that the genetics do not have a larger effect than factors such as proximity to the New York mine.

**NOTE: We will select a clone(s) that is spread over a large geographic distance to ensure geographic variability within the sample.

Research Design & Methods

Our research would begin by selecting widespread clones. We would then cross-examine location of trees (using altitude, longitude and latitude) within the clone with respect to the size of the leaf and circumference of the tree. We will also send samples of the leaves to a professor in the geophysics department at the University of Utah to run a small-amount trace detection process to see what the concentration of toxic metals is inside the leaves from the clones we test. Thus by knowing the geographic location of the trees that we analyzed, we will be able to assess the effect that proximity to the New York mine has on the tree leaves. The database being used is all of the data collected from the groups 1 through 14. We will use R to see if there is a significant relationship between location of the tree within the clone with respect to the size of the leaf and circumference of the tree.

http://onlinelibrary.wiley.com/doi/10.1111/1365-2664.12102/pdf

Proposal #4

Abstract

It is both well-established and logical conclusion that the size of teeth and average serration of leaf edges is dependent on the size of the leaves themselves. Looking at a population of aspen trees in Big Cottonwood Canyon, Utah, the dependency of leave size to serration is to be analyzed. If found to have a linear correlation, determine a genetic difference between clones to describe the average rugosity of the leave edges as a function of clonal differences.

Introduction

Quaking aspens (*Populus tremuloides*) are a unique species of tree in that they have the capability to reproduce sexually and asexually. Much of the biomass of the aspens are in the leaves, and thus much of the phenotypic variation between the trees can be found in the qualities of the leaves, for example variation in leaf area, leaf color, venation, petiole length, and serration of edges. In this paper, we will be focusing specifically on diversity of leaf margin serration.

It is important to consider that there is variation between the leaves on a single tree, trees within the same genet, and trees from separate genets. Spatial distribution of ramets may also affect variation in serration of the leaf edges. As a result, analysis will be done to either eliminate or describe the relationship between location and serration.

Variation in serration leaves affects the ecology of the tree in several ways. Spatial patterning of serrations was shown to depend on the formation of auxin maxima, which are regions that have cells with higher concentrations of auxin (Kawamura). This shows that auxin may have a role in regulating the serration of the leaf. Auxin, however, also affects other processes in the leaf, including venation patterns (). Venation patterns affect the distribution of water through the leaf, which in turn affects the amount of water that can be transpired from the leaf (). Transpiration affects the thermodynamics of the leaves, showing how they expel excess heat absorbed throughout the day, and also how much water will be lost (). Therefore, serration of the leaf may be a phenotypic indicator of important ecological processes in the leaf.

Challenge

The study will be focused on the extent to which the leaf margin serration in *Populus tremuloides* can be attributed to genetic variation. To do so, a program will be ran that determines the average rugosity of leaf edges, and it will be applied to different clones. We predict that variation is most likely to be more significant between different clones and less significant within a single ramet or genet.

Methodology

In order to begin a study analyzing said *Populus tremuloides'* phenotypic diversity, the genotypic trends and environmental influences need to separated and analyzed. In order to separate the different factors of leaf shape, a statistical analysis of the rugosity (or average roughness) of the leaf edges will be conducted. An analysis program aimed to tabulate the rugosity will be written and coded and least-square regression lines will be fit to a plot built on the data outputs. These data will be described conclusions will be drawn.

Currently the exact program is in development. Using a database of leaves from various genets of *Populus tremuloides* a program will be written The main features of the program are intended to include an output providing rugosity, location (latitude, longitude), altitude, and surface area of the leaf (noting tree number and clone name). This will provide enough data accurately apply statistical tests to determine the source, be it genetic or environmental, of the phenotypic variation.

Proposal #5

The concept of symmetry is pervasive in nature. A multitude of studies propagate the idea that symmetry and balance often coincide with a higher degree of fitness, appeal, etc. It is known that a variety of components influence the relationship between trees and insects, such as the morphology of the tree, chemical repellents and attractants, among other factors. Aspens provide a unique opportunity to study the genetic influence on asymmetry, as they allow for the isolation of environment as a variable.

Plants, unlike animals, possess what is known as phenotypic plasticity. In essence, plants are able to quickly change their phenotypic expressions in response to environmental changes. Asymmetry in the leaves may be the result of the tree diverting resources away from growth to focus on things like herbivory resistance. Given that bilateral symmetry in the leaves is controlled by one set of genes susceptible to phenotypic plasticity, asymmetry may indicate a tree's susceptibility to herbivory.

Asymmetry could be an indicator of environmental stress, genetic stress, or structural instability, and hence a correlation between asymmetry and increased insect/microbial activity would suggest that asymmetric trees, on a clonal level are, for whatever reason, less able to deter predation.

Hence, the purpose of our study is to examine the possible correlation between aspen leaf asymmetry and clonality and the prospective relationship between leaf asymmetry and herbivory. To do this, we will be compiling the genotypic data of all the sampled trees, and comparing that to the determined asymmetry of the leaves. The asymmetry will be determined, most likely, using R to measure the area of each of two bilateral lobes of the leaves. Asymmetry will be scaled to the clonal level. Afterwards, should there be a correlation between clonality and asymmetry, we will then compare the relative level of asymmetry in each clone to the levels of microbial and insect herbivory, which is explained more in the methods section.

Proposal #6

Introduction

The petiole of a leaf, an integral part of any tree, bridges the gap between the leaf and the rest of the tree as well as facilitates gas diffusion through the pendulum effect, allowing them to swing freely in the wind, and elongate the leaf to enhance photosynthesis. Thus, leaf morphology, including the petiole, is substantial in the functionality of the leaf and its fitness because an elongation of the petiole allows the leaves to expose themselves to light more significantly (Tsukaya, Kozuka, & Kim, 2002). *Populus tremuloides* serves as an appropriate experimental unit since they exhibit varying characteristics that are qualitatively and quantitatively observable. They are ideal for genotyping because of their growth patterns in large clusters of genetically identical clones. They reproduce asexually, meaning there are a lot of trees in the same clone within proximity to each other that are easy to sample data within and among the clones to determine variation. In addition, there are a large amount of *P. tremuloides* relatively close to the University of Utah campus, facilitating data collection.

This investigation analyzes the leaf to petiole ratios within and among *P. tremuloides*, or Quaking Aspen trees, and variation with regards to ploidy among clones. We will take the leaf petiole length to area of the leaf ratio in order to find this variation. There has been research that has determined leaf petiole length is significantly correlated to specific genes (Tsukaya, 2005). Because of this research, our hypothesis is that there will not be any variation within clones regarding petiole length, however there will be a large variation among clones, especially when considering ploidy. If there is significant variation within clones, we can say that leaf petiole length is likely attributed to the environment, contradicting our hypothesis. If there is little variation within clones, but significant variation among clones, we can say this is attributed mostly to genetics. The factor of ploidy is interesting and valuable to further understand how triploid differs physiologically compared to diploid.

Method

Leaf samples will be taken from eight *P. tremuloides* that are suspected to be in the same clone. A genotype will prove whether or not the trees are in the same clone. This will be completed for each group with eight or more trees with between twenty-five to thirty-five leaves being collected for each tree. After determining the trees' genotypes and respective clones, an open-source image processing software, called ImageJ, will be utilized to analyze the differences between them. Measurements such as leaf petiole length and area will be taken (in millimeters) and a ratio will be created with the two measurements. This will be completed for fifteen to twenty-five leaves to find this ratio and then averaged among the clones in the order of tree number, clone, and then ploidy.

After the image processing procedure, next comes the statistical analysis portion of the investigation. An Analysis of Variance (ANOVA) will be performed in the open-source statistics language program R in order to find the variation within clones and among clones taking into consideration ploidy (separation of triploid versus diploid). If there is significant variations between clones, but retains similarities within each clone, it is likely that petiole size is attributed mostly to genetics. The values given by R can result in general implications to determine the degree by which genetics influence petiole length. If the variation occurs within the clones instead, the variation can then be possibly attributed to environmental factors.

Guidelines for reviews

Please address each of the OCAR elements:

Opening and Funnel
How well does it work, and why?
What could be improved, and how?

Challenge
Is it compelling? Why or why not?
How could it be improved?
Are the specific questions clearly articulated?
Can you suggest improvements or expansions?

Action
Does the proposal explain what will be done in enough detail that you can imagine doing it yourself?
Are the possible outcomes (findings) described?
Any suggestions for improvement?

Resolution
Are interpretations of the expected or possible findings described?
Is it clear how they relate to the opening and the challenge?
Any suggestions for improvement?

At the end, please mention any specific grammatical or sentence-structure issues that you think the authors should address.

Then please send your group's reviews to Andre:

a.kurlovs@gmail.com