Environmental impact on overall leaf morphology between *Acer rubrum* individuals

Jackie Berens, Sarah Gizzi, Rose Popma
University of Michigan Biological Station
EEB 392: Natural History and Evolution
Summer 2017
Professor: Dr. J. Jordan Price

ABSTRACT

Environmental variation can have a significant impact on phenotypic differences among individuals. The red maple is the most widely spread tree in eastern North America, and can survive in a large variety of microenvironments. Additionally, the leaves of the red maple are the most variable in shape of any maple. The objective of this study was to determine if the habitat variability of the red maple and the morphological variation in red maples leaves are linked. We collected soil, light, and size data from ten red maple trees, all located on UMBS property, and picked five leaves from each tree for morphometric analysis. Both our statistical and morphometric analyses were performed in R. The results found no statistically significant relationships between any of the environmental variables and leaf morphology, implying leaf shape in red maples is not determined by environmental factors. Replications of this study with larger sample sizes and more environmental factors should be performed, and tree genetics should be analyzed to determine the heritability of leaf morphology.

INTRODUCTION

Differences within a phenotypic trait are not determined by genotypic or environmental variation alone. Instead, they are caused by a combination of the two, though the exact ratio of genetic to environmental influence depends on the particular trait under investigation. The proportion of phenotypic variance due to differences in genotype is known as heritability and dictates the ability of phenotypic variations to be passed on to offspring. Some traits are almost completely non-heritable. For example, the color of a lion's mane is determined by nutrition and mean annual temperatures during development (20). This is not an indication that genetics have no influence on lions' manes, but rather that the observed phenotypic differences are not due to genetic variation.

The red maple (*Acer rubrum*) is one of the most abundant and widespread trees in eastern North America (8), and can persist in a wider range of microenvironments than any other forest species in the region (5). Its range extends along the eastern coast of the U.S. and southern Canada, and stretches as far west as the Great Plains (12) (Figure 1). The red maple's abundance can be attributed to its fast growth and high yielding seed crops (1), which allows it to become one of the dominant tree species in a forest ecosystem following a major disturbance(5).

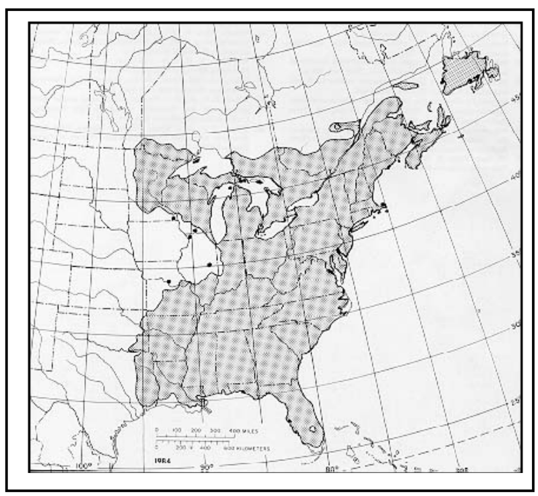


Figure 1: Range of the red maple tree (18).

In addition to its extensive range, red maples display an unusual amount of variation in leaf morphology. Within most maple species, there is little variance in leaf shape found within a single tree or between trees of the same species – most, if not all leaves have five lobes, with minimal to moderate serration. Red maple leaves, however, differ greatly within individual trees, possessing anywhere between three and five major lobes with varying levels of serration. Previous studies have shown that most of this variance within an individual tree is due to differences in light exposure between canopy layers (10). The effect of varying light levels on morphological differences of leaves between individual trees, however, is largely unknown.

Due to the diversity of red maple habitat and leaf morphology, we chose to study environmental influences on red maple leaf morphology. We studied ten red maples found in two different locales on University of Michigan Biological Station property. We believe that differences in light exposure will lead to differences in overall leaf morphology between *A. rubrum* individuals.

METHODS

Tree selection and leaf collection

Ten trees were selected, all located on UMBS property. Five trees were selected on Pine Point Trail east of camp, and five trees were selected on Grapevine Point Trail west of camp. A haphazard sampling method was used to select the five trees from each path.

Five leaves were collected from each tree. The leaves were randomly selected from the lowest branch of each tree, excluding damaged leaves. After leaf collection, each leaf was photographed against a white background with a scale reference, and the photographs were compiled into a .tps file using tpsUtil64,

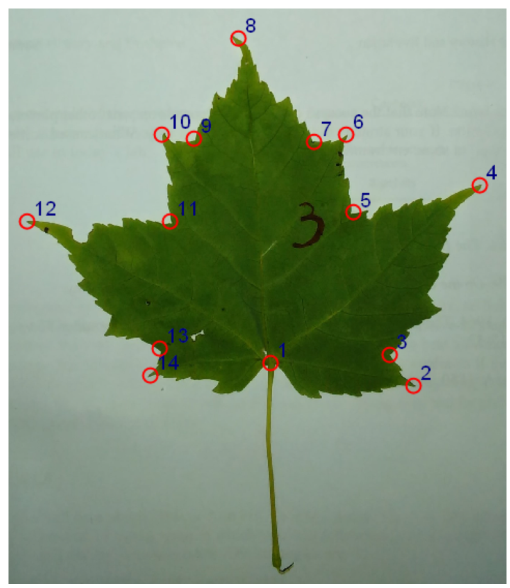


Figure 2: Landmarking scheme.

version 1.74 (15). Each photograph was landmarked in tpsDig232, version 2.30 (14), using a standard landmarking scheme (Figure 2) based on the scheme used by Chitmood and Otoni (6). The leaves were each photographed within an hour of being removed from the tree to avoid size change due to dehydration.

Field Measurements

Tree size and age influence the average size of the leaves of an individual tree (16). To account for this effect, we measured the diameter at breast height (DBH), a proxy for tree size, at 1.5 meters above ground for each tree. At the base of each tree, a soil corer was used to take a sample of the top six inches of soil to measure the nitrogen (4) and moisture level (11). After collection, we stored each soil core sample in a whirl pack and shook it to evenly distribute the soil. Each whirl pack was divided into two samples: one for carbon:nitrogen (C:N) analysis, and one for moisture analysis. We processed the soil samples analyzed for C:N through a PerkinElmer Series II CHNS/O analyzer. To analyze the other group of soil samples for moisture levels, the soils were weighted, baked in a FisherScientific Isotemp® 500 Series oven for 24 hours at 105˚C and weighed again. We used the weight difference to calculate the moisture content of each sample. All chemistry was done in the University of Michigan Lakeside Chemical Laboratory.

Light measurements were taken above the lowest branch of each tree, the source of the leaves, using a Digital Lux Tester YF-1065. The lowest branch was used because the upper layers of most adult trees in a forest experience direct sunlight, but the lower layers are subjected to environmental factors that influence the amount of light they receive, such as the canopy density and tree density of the surrounding area. Therefore, the lowest branches experience more variance in light exposure, allowing a clearer relationship between light exposure and leaf phenotype to be seen. Light measurements were taken at each tree over three days (August 4, 6, and 8, 2017). All three measurement days were sunny with partial cloud cover (19), and all data gathering occurred between 12:00 - 14:00. The consistent timing and the distributive days of data sampling were selected to reduce confounding variables and ensure that any variations found in light measurements were due to actual differences in light exposure, not differences in time of day or type of weather.

Analyses

All of our analyses were performed in R, version 3.4.0 (13). We calculated the mean light exposure of each tree from the three sets of light measurements. In addition to mean light exposure per tree, we calculated the overall mean light exposure and compared the individual light measurements to this value. The average difference from the overall mean for each tree was then found, providing a metric for light variation between trees (Equation 1). Soil moisture was calculated by taking the difference between the wet and dry masses and then dividing by the wet mass (Equation 2). Using our leaf landmark data, we calculated the surface area of each of the 50 collected leaves (17) and then found the mean surface area for each of the ten trees. Surface area was then plotted against DBH, average light exposure, average light variance, soil moisture, and C:N. We also ran a linear regression on each of these relationships.

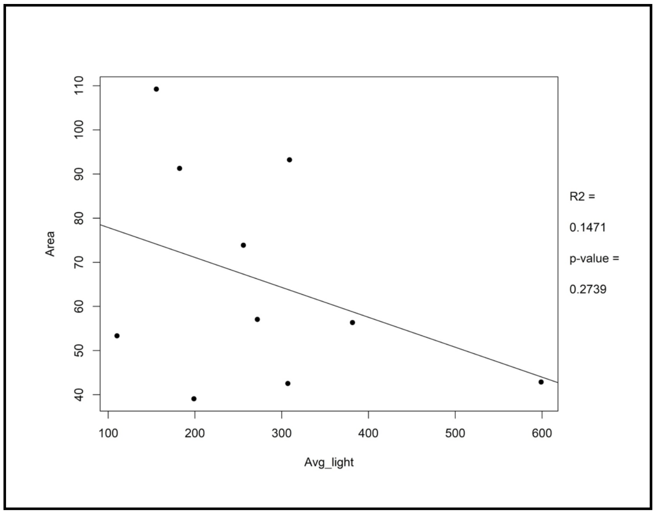
$$light\ variance = \frac{(light_{overall} - light_1) + (light_{overall} - light_2) + (light_{overall} - light_2)}{3} \quad \text{Eqn 1}$$

$$soil\ moisture = \frac{mass_{wet} - mass_{dry}}{mass_{wet}} \quad \text{Eqn 2}$$

In addition to surface area, we used our landmark data to perform a general Procrustes analysis on all 50 sets of landmarks (3). A general Procrustes analysis “translates all specimens to the origin, scales them to unit-centroid size, and optimally rotates them (using a least-squares criterion) until the coordinates of corresponding points align as closely as possible" (2). This process creates a new set of coordinates for each landmark according to its new location. We then ran a principal components analysis on the new sets of landmarks. A principal components analysis takes the input data, which are made up of possibly related variables, and creates a vector space of *n*-dimensions, where *n* is the number of variables in the input data. The values of the original variables are placed in a vector *x*, which is then subjected to a finite number of independent linear combinations. Each linear combination is used to derive a factor, known as a principal component (PC). The principal component that describes the linear combination with the most variation is denoted as the first principal component (PC1); the principal component that describes the linear combination with the second most variation is the second principal component (PC2); and so on (9). Applying this method to our data provided us with 24 principal component scores per leaf, with the first principal component accounting for 54.29% of the variation among leaf shapes. As such, we determined that the PC1 values were an adequate metric of leaf shape variability. We calculated the mean PC1 value for each tree and plotted these average values against DBH, average light exposure, average light variance, soil moisture, and C:N. A linear regression was run on each of these relationships.

RESULTS

Graphs created in R, version 3.4.0 (13), revealed no significant differences between average light, light variance, average leaf size, leaf variance, DBH, or C:N (Figures 3-12). The p-values calculated by the linear regressions represent the probability of rejecting the null hypothesis, which states that there is no statistically significant correlation between the two variables of each test. In order to confidently reject the null hypothesis, the p-value must be below 0.05. In our study, the p-values calculated ranged from 0.1945 (soil moisture vs. leaf average surface area) to 0.8214 (light variance vs. leaf variance). Therefore, none of the p-values were low enough to confidently reject our null hypothesis that changes in environment would affect the leaf variance between *A. rubrum* trees.



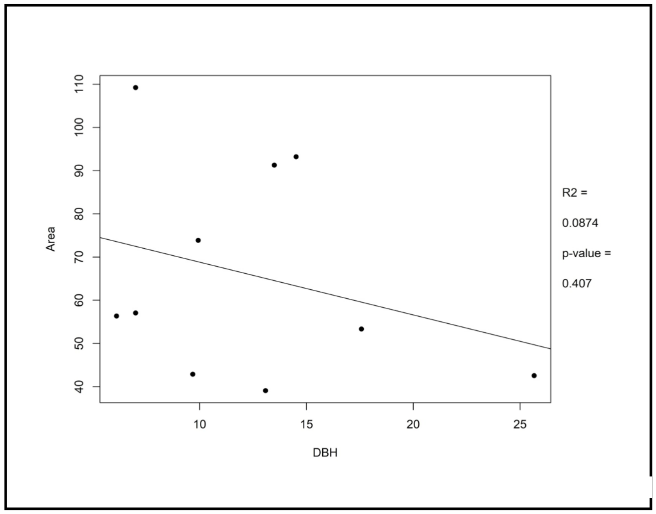


Figure 3: Average light vs. average leaf surface area per tree. p-value = 0.2739, r^2 = 0.1471

Figure 4: DBH vs. average leaf surface area. p-value = 0.407, r^2 = 0.0874

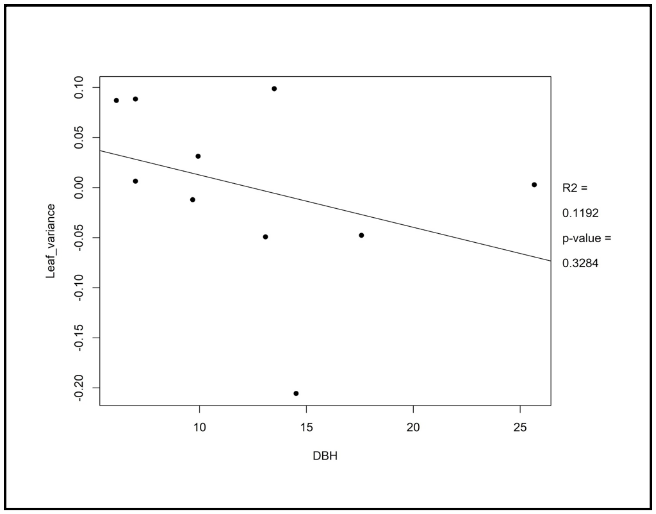


Figure 5: DBH vs. leaf variance. p-value = 0.3284, r^2 = 0.1192

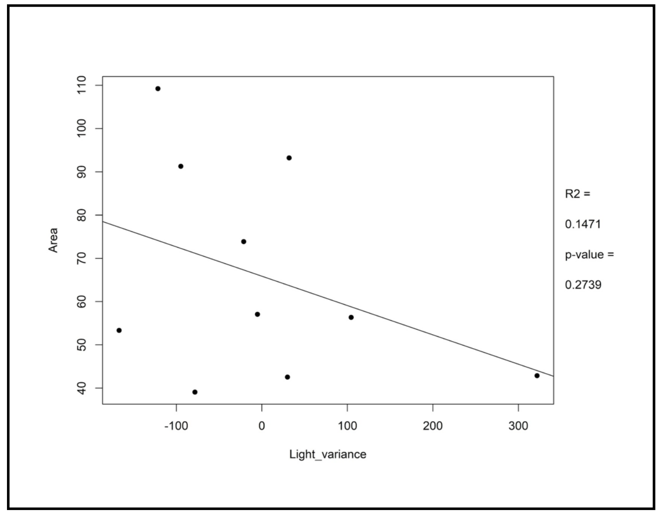


Figure 6: Light variance vs. average leaf surface area. p-value = 0.2739, r^2 = 0.1471

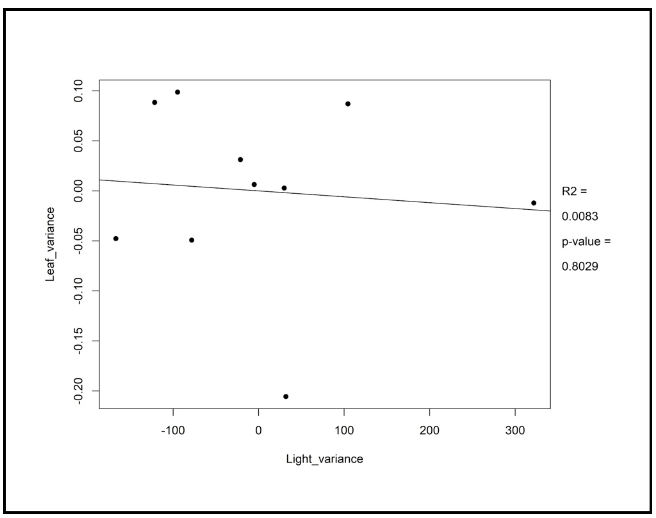


Figure 7: Light variance vs. leaf variance. p-value = 0.8029, r^2 = 0.0.0083

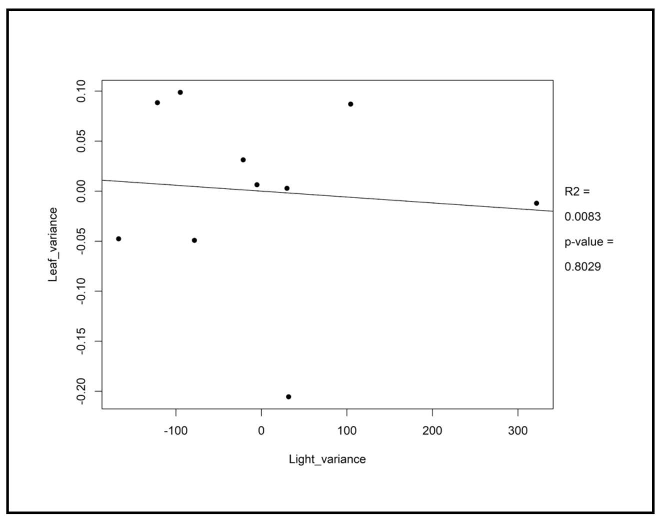


Figure 8: Moisture vs. average leaf surface area. p-value = 0.1945, r^2 = 0.2004

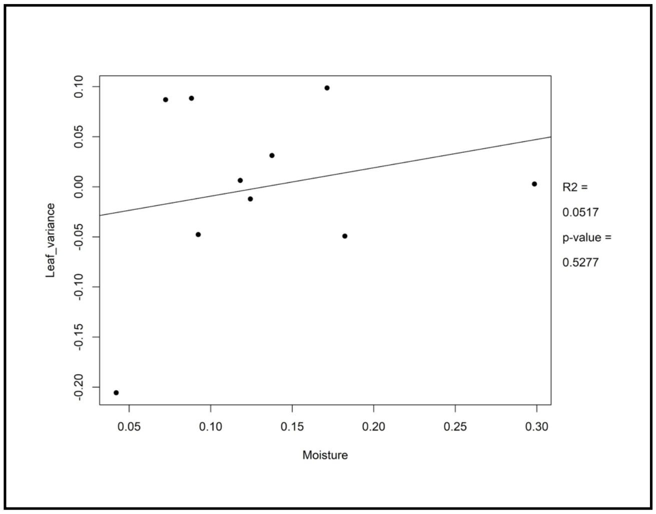


Figure 9: Moisture vs. leaf variance. p-value = 0.5277, r^2 = 0.0517

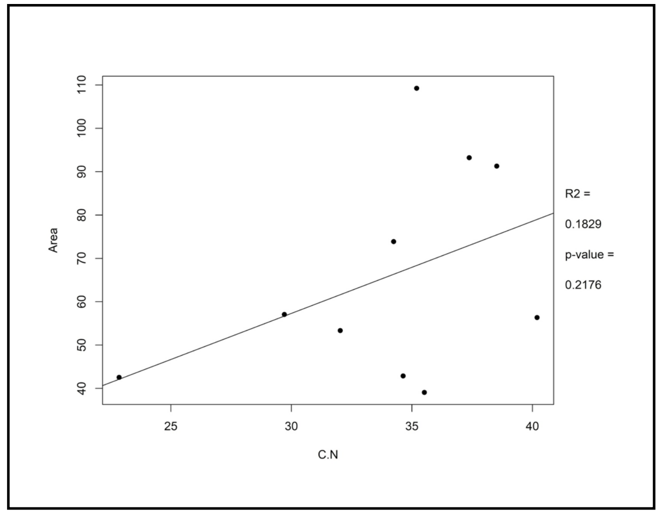


Figure 10: Carbon : nitrogen ratios vs. average leaf surface area. p-value = 0.2176, r^2 = 0.1829

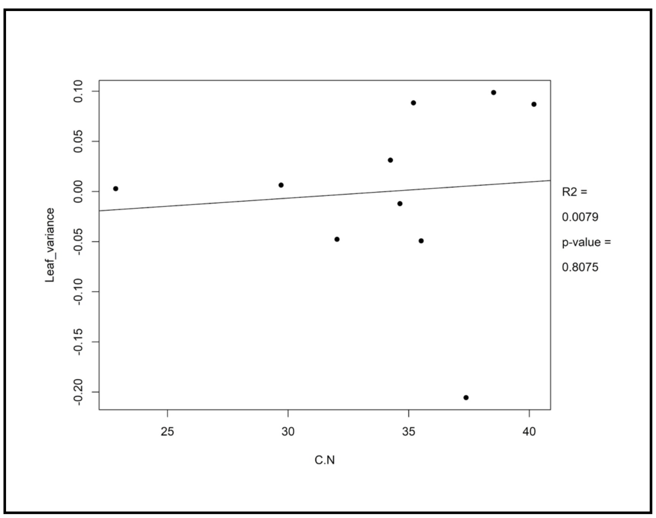


Figure 11: Carbon : nitrogen ratios vs. leaf variance. p-value = 0.8075, r^2 = 0.0079

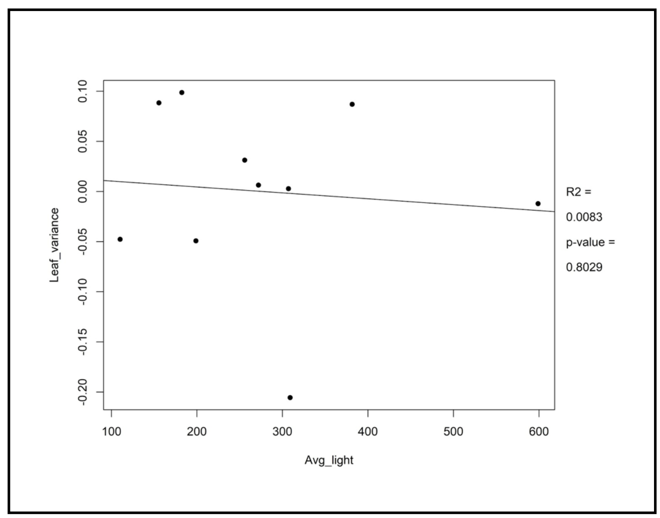


Figure 12: Average light vs. leaf variance. p-value = 0.8029, r^2 = 0.0083

DISCUSSION

Our results showed no significant influence of the environmental factors tested and variation in leaf morphology between red maples. However, this does not mean that leaf morphology between red maples is not influenced by environmental factors. There could be many environmental factors that influence variation in leaf morphology that we did not test. For example, surrounding plant and animal species, average rainfall, and CO_2 content in the atmosphere could all have an effect. Our light measurements may have also been inadequate because we only tested light levels above the leaves from 12:00-14:00 instead of testing light coming from all angles throughout the day as the sun changed position in the sky. In future studies, sensors could be arranged on each tree to continually record the amount of light each area of the tree is exposed to. In addition, the environmental conditions experienced by the parent tree (i.e. drought or malnutrition) could have an impact on leaf development in offspring. Since our study was limited to five environmental factors, there may be components not tested that correlate to variance in red maple tree leaf morphology.

However, if all the environmental factors were tested and no significant correlation was found between environment and leaf morphology, the variance could be due to genetics, indicating that leaf morphology is a highly heritable trait. Since heritability is a genetic component, genetic analysis of the leaves over multiple generations would be necessary.

CONCLUSION

The differences in organisms phenotypes are often a combination of environmental and genetic factors. In the case of the red maple, the variation in leaf morphology could be attributed to many environmental factors, from soil conditions to the degree of light exposure experienced by the parent tree. On the other hand, it could be a highly heritable trait influenced primarily by genetics. From the results of our study, we suggest further research using a larger population size, more environmental factors, and a longer time-period to determine if environmental factors had a significant relationship with red maple leaf morphology between trees. If environmental factors were found to be uninfluential, we suggest studies on the genetics behind red maple leaf morphology and its heritability.

ACKNOWLEDGEMENTS

We would like to thank Jordan Price and Donna Hollandsworth, our professor and teaching assistant, respectively, in natural history and evolution at the University of Michigan Biological Station for advice on the methods of our experiment and publication of our research. Thank you to Tim Veverica and Katlyn Hogan for their assistance in processing and analyzing our soil samples. We would also like to acknowledge Sherry Webster for assistance in gathering necessary equipment for our research.

LITERATURE CITED

1. Abbott, H. G. (1974). Some characteristics of fruitfulness and seed germination in red maple. *Tree Planters' Notes,25*(2), 25-27. Retrieved August 4, 2017, from
2. Adams, D., Collyer, M., Kaliontzopoulou, A., Sherratt, E. (2017). *Package 'geomorph'*. The Comprehensive R Archive Network. Retrieved from: https://cran.r-project.org/web/packages/geomorph/geomorph.pdf
3. Adams, D.C., and E. Otarola-Castillo. 2013. geomorph: an R package for the collection and analysis of geometric morphometric shape data. Methods in Ecology and Evolution. 4:393-399.
4. Aribal, L. G., Bonggay, J. G., & Fernando, E. S. (2017). Leaf size indices and structure of the peat swamp forest. Global Journal of Environmental Science and Management, 3(1), 63-74. doi:10.22034/gjesm.2017.03.01.007
5. Burns, R. M., & Honkala, B. H. (1990). *Silvics of forest trees of the United States*(Vol. 2). Retrieved August 5, 2017, from

https://play.google.com/books/reader?id=bMnRqCA3uzwC&printsec=frontcover&output=reader&hl=en&pg=GBS.PR1

6. Chitwood, D. H., & Otoni, W. C. (2017). Morphometric analysis of Passifloraleaves: the relationship between landmarks of the vasculature and elliptical Fourier descriptors of the blade. GigaScience, 6(1), 1–13. http://doi.org/10.1093/gigascience/giw008
7. Hadley Wickham (2007). Reshaping Data with the reshape Package. Journal of Statistical Software, 21(12), 1-20. URL http://www.jstatsoft.org/v21/i12/.
8. Hutnick, Russell J., and Harry W. Yawney. 1961. Silvical characteristics of red maple (Acer rubrum). USDA Forest Service, Station Paper 142. Northeastern Forest Experiment Station, Upper Darby, PA. 18 p.
9. Jolliffe, I. T. (2002). *Principal Component Analysis* (2 ed). New York.: Springer-Verlag.
10. Koike, T., Kitao, M., Maruyama, Y., Mori, S., & Lei, T. T. (2001). Leaf morphology and photosynthetic adjustments among deciduous broad-leaved trees within the vertical canopy profile. *Tree Physiology,21*(12-13), 951-958. Retrieved August 4, 2017, from https://academic-oup-com.proxy.lib.umich.edu/treephys/article/21/12-13/951/1626169/Leaf-morphology-and-photosynthetic-adjustments.
11. Kolodziejek, Jeremi & Michlewska, Sylwia (2015). Effect of soil moisture on morpho-anatomical leaf traits of Ranunculus Acris (Ranunculaceae). Polish Journal of Ecology, 63(3), 400-413. doi:10.3161/15052249PJE2015.63.3.010
12. Little, Elbert L., Jr. 1979. Checklist of United States trees (native and naturalized). U.S. Department of Agriculture, Agriculture Handbook 541. Washington, DC. 375 p.
13. R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

14. Rohlf, F. J. (n.d.). TpsDig232 (Version 2.30) [Program documentation]. Retrieved August 16, 2017, from life.bio.sunysb.edu/morph/sof-dataacq.html
15. Rohlf, F. J. (n.d.). TpsUtil64 (Version 1.74) [Program documentation]. Retrieved August 16, 2017, from life.bio.sunysb.edu/morph/soft-utility.html
16. Steppe, K., Niinemets, U., & Teskey, R. O. (2013). Chapter 9 Tree Size- and Age-Related Changes in Leaf Physiology and Their Influence on Carbon Gain. In F. C. Meinzer, B. Lachenbruch, & T. E. Dawson (Eds.), *Size- and age-related changes in tree structure and function*(pp. 235-253). Dordrecht: Springer.
17. Vincent Bonhomme, Sandrine Picq, Cedric Gaucherel, Julien Claude (2014). Momocs: Outline Analysis Using R. Journal of Statistical Software, 56(13), 1-24. URL http://www.jstatsoft.org/v56/i13/.
18. Walters, R. S., & Yawney, H. W. (n.d.). Acer rubrum L. Red Maple. Retrieved August 16, 2017, from https://www.na.fs.fed.us/spfo/pubs/silvics_manual/volume_2/acer/rubrum.htm
19. Weather History for KPLN - August, 2017. (n.d.). Retrieved August 09, 2017, from https://www.wunderground.com/history/airport/KPLN/2017/8/4/DailyHistory.html?req_city=&req_state=&req_statename=&reqdb.zip=&reqdb.magic=&reqdb.wmo=&MR=1
20. West, P., & Packer, C. (2002). Sexual Selection, Temperature, and the Lion's Mane. *Science, 297*(5585), new series, 1339-1343. Retrieved from http://www.jstor.org/stable/3832107