

GEORGIA INSTITUTE OF TECHNOLOGY

Density-dependent Stiffness in Petiole Biomechanics

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Research Option in the School of Biology

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Abstract

Morphological features of plants vary with increasing size. This relationship between different physical characteristics and size is referred to as allometry. Recently allometric research focused on plants and in particular plant leaves due to their importance in nutrient flow and gas exchange. Allometric plant research aims to enhance our understanding of the ontogeny of plants and offers a tool for ecological modeling. Previous allometric models have glanced over the influence of biomechanics on leaf form and function. This research will test if density-dependent stiffness of petioles is variable or if it scales with leaf mass. In order to test the variance of stiffness in leaf petioles, Young's modulus (E) was measured by modeling the petioles as simply loaded beams. Young's modulus was shown to vary among different species, even those of the same genus. Density-dependent stiffness varied with leaf size for some herbaceous species but not for other woody tree species. Future research should investigate the biomechanical role of a possible redistribution of structural tissue and how this rearrangement would affect petiole stiffness and overall petiole function.

Introduction

Allometry is the study of how a part scales in relationship to the whole or another part and often deals with the study of the consequences of size on organic form and function. Allometric relationships are often the result of selection for the optimal form of an organism. The term allometry (Greek *allos*, "other," and *metron*, "measure") was first proposed by Julian Huxley in 1932, although the idea of allometry dates as far back as Galileo (Niklas, 1994). After studying the physical proportions of horse bones, Galileo stated

Nature cannot produce a horse as large as twenty ordinary horses or a giant ten times taller than an ordinary man unless by miracle or by greatly altering the proportion of his limbs and especially of his bones, which would have to be considerably enlarged over the ordinary.

Galileo's work is one of the first examples in the history of allometry.

All living things share the challenges of distribution and transportation of nutrients while being structurally limited by physical and environmental constraints. Previous studies have shown that relationships exist between an organism's vascular network structure and the allometry of different morphological characteristics. Much of this research has focused on plants because they are relatively easy to study in comparison with live animals.

One of the most influential models in plant allometry is the West, Brown and Enquist (WBE) fractal model that suggests allometric relationships are linked mechanistically to leaf vein network structure and resource exchange surfaces. This model characterizes the network geometry of the vascular system by using three parameters: branch lengths, branch radii, and tube radii. The model makes predictions of quarter-power scaling of allometric relationships in organisms containing a fractal, space-filling, vascular network (West et al., 1999).

The WBE model has sparked debate among biologists. Some people doubt the mathematical foundation and biological relevance of the model (Kozłowski, 2004). One of the largest criticisms originates from the fact that the WBE model often cannot account for any discrepancies between observations and predictions. Some criticisms are based upon a misinterpretation of the model (Brown et al., 2005). However, it has been shown that relaxing some of the biologically related assumptions of the model allows for more accurate predictions of allometric relationships between different physical properties of some plant species (Price and Enquist, 2006).

The WBE model makes four assumptions: 1) the network is volume filling, 2) the minimum work to move fluid through the network corresponds to the minimal

hydrodynamic resistance in a tube, 3) the properties of any individual leaf are independent of whole plant size, and 4) biomechanical constraints are uniform. Limited empirical data can be found in the literature pertaining to the fourth assumption, although Karl Niklas, a well-respected expert in plant allometry, has stated that density-dependent stiffness is probably variable. However, it has been seen that density-dependent stiffness in some green wood tree species is indeed uniform (McMahon, 1973). In this study I will evaluate the fourth assumption, which states that biomechanical constraints are invariant, by determining if density-dependent stiffness of petioles is invariant as assumed by the WBE model or if it scales to overall leaf mass. In addition, data will be collected to compare stiffness between different species and determine if any phylogenetic patterns can be observed.

Young's modulus (E), or elastic modulus, is a measure of the stiffness of a material and can be quantified as the slope of a stress-to-strain graph, where stress is the load applied to the material, and strain is the deflection caused by the applied load. A stress-to-strain graph has several important regions and can be used to determine different

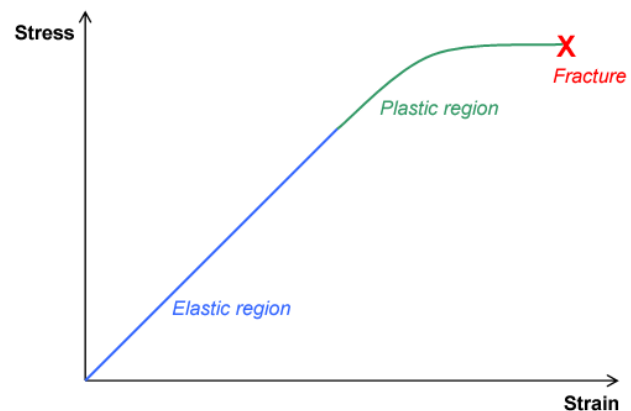


Figure 1. A basic stress-to-strain curve. The area under the blue line represents the elastic region, and the area under the green line represents the plastic region. The yield point is where the blue line changes to green, and the failure point or fracture is shown in red.

properties of a material. The most important region for this study is the elastic region, which is defined as the area under the curve where the slope is constant. The elastic region can also be described as the region in which the material returns to its original

position when the stress or load is removed, hence the term elastic. Young's modulus is the slope of the line in the elastic region. A steeper slope in the elastic region translates to a stiffer material. At a certain point, the addition of more stress prevents the material from returning to its original point. This point is referred to as the yield point. The region under the curve after the yield point is called the plastic region. Eventually, the material will break or fracture. This point is referred to as the ultimate failure or point of fracture.

This study focuses on the elastic region because this area of a stress-to-strain graph is the most biologically relevant. In nature, leaves must deal with different dynamic forces, such as rain and wind, and static forces such as self-loading. Therefore, leaf petioles must be stiff enough to support the whole leaf weight and allow for maximal light exposure but still be flexible enough to deal with perturbing forces caused by the environment (Niklas, 1999). The elastic region and Young's modulus are important because they reflect the influence of perturbing forces that do not cause permanent damage to the leaf and thus may be under optimizing selection.

Methods

Plant species were selected based on availability and reasonable petiole length (> 20mm). Twenty species were selected to investigate between species variation in petiole stiffness. Six species were selected for further investigation of the allometric relationship between leaf size and density-dependent stiffness. For the interspecific investigation, five mature leaves were selected from each species, and 20 leaves were collected for the intraspecific investigation. All leaves were collected from Georgia

Institute of Technology's campus and the Atlanta Botanical Gardens and brought into the lab within 20 minutes of being removed from the plant.

Petioles were removed from the leaf and any bulging ends were removed to make the petiole diameter as uniform as possible. Petiole length and mass were measured. The ends of the petiole were coated with petroleum jelly to prevent desiccation. Two orthogonal measurements of petiole diameter were made in three places along the length of the petiole in order to estimate petiole volume. Petiole density was determined based on the mass and estimated volume. The petioles were modeled as mid-point loaded, simply supported beams. This type of beam was selected because more accurate measurements can be obtained from simply loaded beams as opposed to cantilevered beams due to possible variations in the placement of the load or stress. The petiole was suspended at both ends, the vertical position of the petiole was noted, and weight was slowly added. The weight required to cause a notable deflection was recorded. Care was taken not to overstress the petiole by only causing deflections of 5% or less of the total petiole length. This was done three times for each petiole to allow time for the petiole to return to its original position between weights.

A single *Ginkgo biloba* petiole was used to create a stress-to-strain graph. This was done in order to evaluate if the slope of the stress-to-strain graph was actually constant in the elastic region (see fig. 1). Twelve increasing loads were added to the petiole, and the strain or deflection was noted for each.

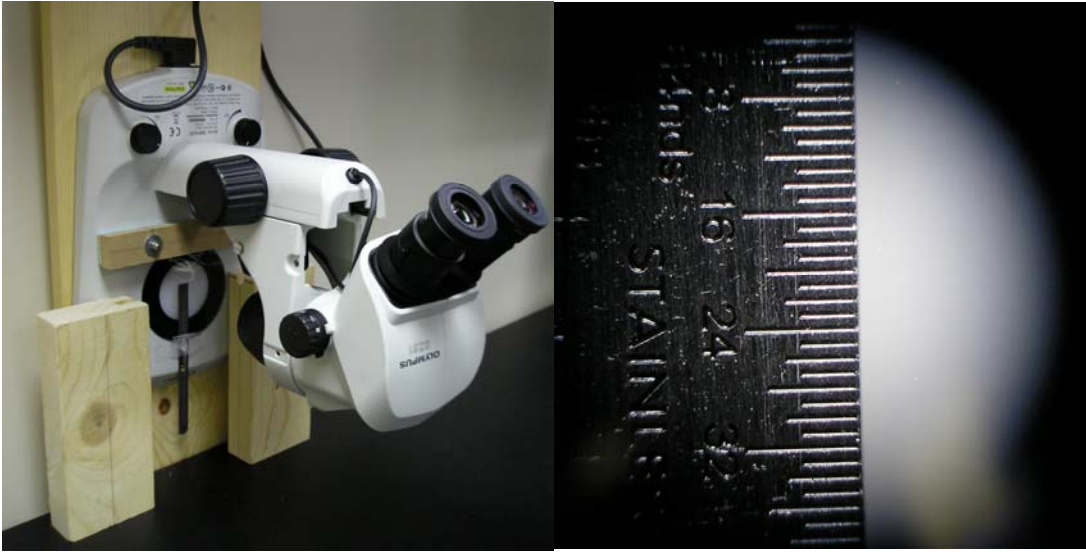
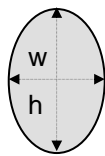


Figure 2. The apparatus used to measure the deflection of the petiole. The picture on the left shows the view through the microscope (scale $1/64^{\text{th}}$ of an inch). The right picture shows the microscope mounted horizontally in order to view the petiole supported by the two wooden blocks shown.

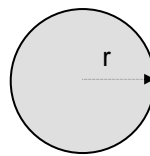
Young's modulus was calculated using the following formula for a simple mid-point loaded beam:

$$E = \frac{FL^3}{48I\delta}$$

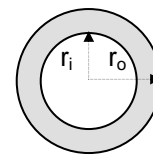
where I is the second moment of area, F is the force, δ is the deflection and L is the length. I is dependent on the shape of the cross section of the beam, as illustrated below in Figure 3. The following formulas may be used to calculate I :



$$I = \frac{\pi wh^3}{4}$$



$$I = \frac{\pi r^4}{4}$$



$$I = \frac{\pi}{4}(r_o^4 - r_i^4)$$

Figure 3. The cross section of different petiole shapes for an elliptical beam, cylindrical beam and a hollow cylindrical beam, and the corresponding formulas for I (second moment of area).

Results

Species	Average E (MPA)	SD
<i>Acer negundo</i>	47.90	± 14.41
<i>Acer rubrum</i>	66.33	± 18.65
<i>Acer saccharum</i>	185.13	± 52.73
<i>Carya illinoensis</i>	45.21	± 4.82
<i>Catalpa speciosa</i>	29.31	± 5.20
<i>Cercis canadensis</i>	28.40	± 3.80
<i>Ficus spp.</i>	9.71	± 2.41
<i>Fraxinus pennsylvanica</i>	54.73	± 6.49
<i>Ginko biloba</i>	32.04	± 8.27
<i>Hydrocotyle bonariensis</i>	5.28	± 0.69
<i>Liquidambar styraciflua</i>	45.64	± 8.26
<i>Liriodendron tulipifera</i>	92.39	± 11.16
<i>Oxalis stricta</i>	8.45	± 2.82
<i>Platanus occidentalis</i>	35.54	± 5.01
<i>Prunus spp.</i>	3.22	± 0.61
<i>Quercus acutissima</i>	27.00	± 4.64
<i>Quercus falcata</i>	11.74	± 2.73
<i>Quercus shumardii</i>	48.77	± 14.29
<i>Tilia americana</i>	17.57	± 4.40
<i>Trifolium repens</i>	12.00	± 1.67

Table 1. Average E for 20 different species. Species of the same genus (*Acer* and *Quercus*) show very little correlation.

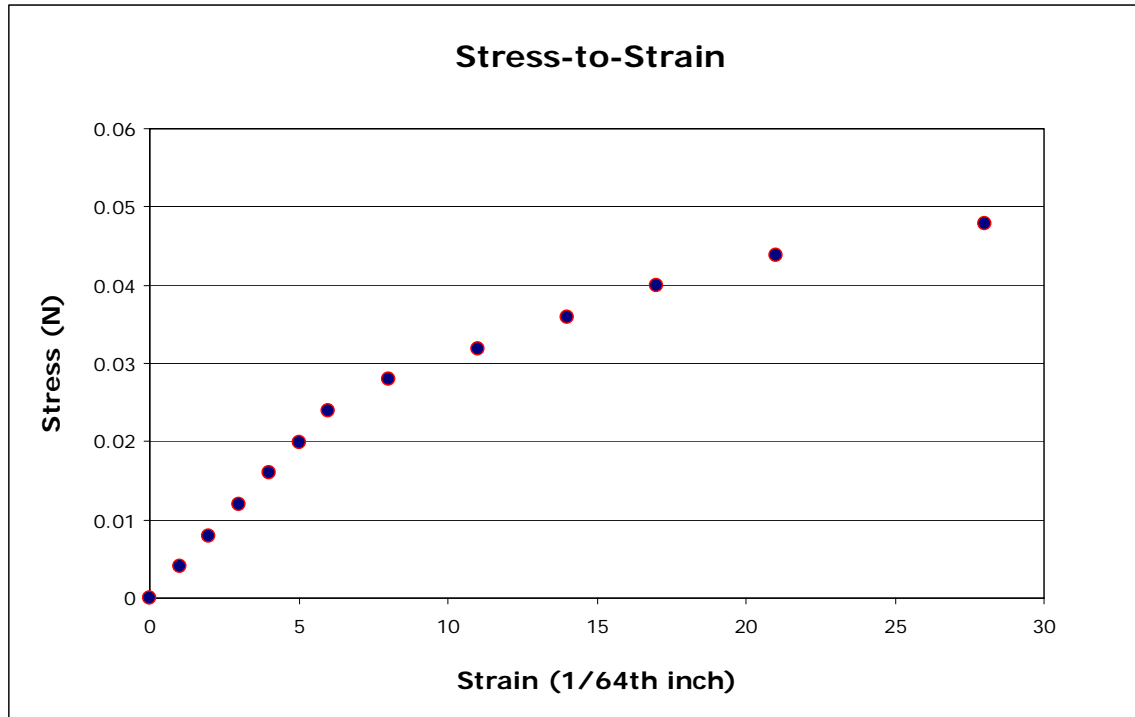


Figure 4. Stress-to-strain graph of a single *Ginkgo biloba* petiole using 12 increasing loads or stresses. Strain was measured in 64ths of an inch deflections for each of the 12 loads. A clear transition from the elastic region to the plastic region (yield point) can be seen after 0.02 N.

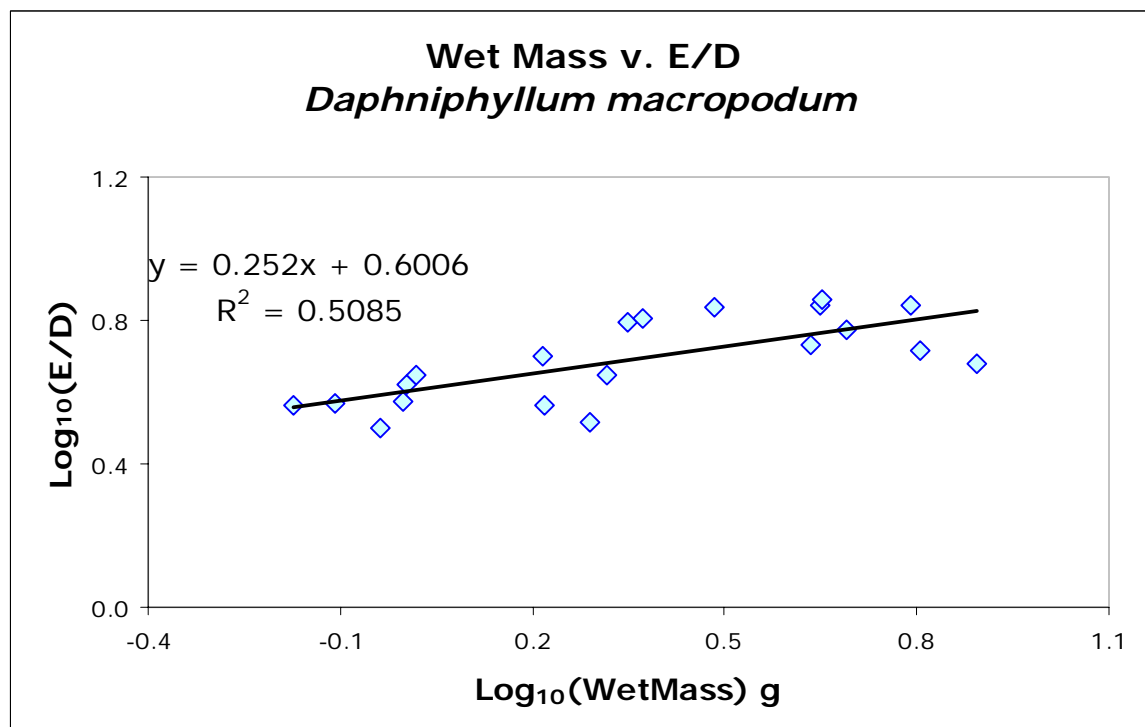


Figure 5. *E/D* scaled to mass. The blue dots represent each of the twenty leaves measured. The text in the top left corner shows the equation of the best fit line, where the slope represents the allometric scaling exponent. The R^2 value of 0.51 indicates that this equation explains about 50 percent of the variance in the data.

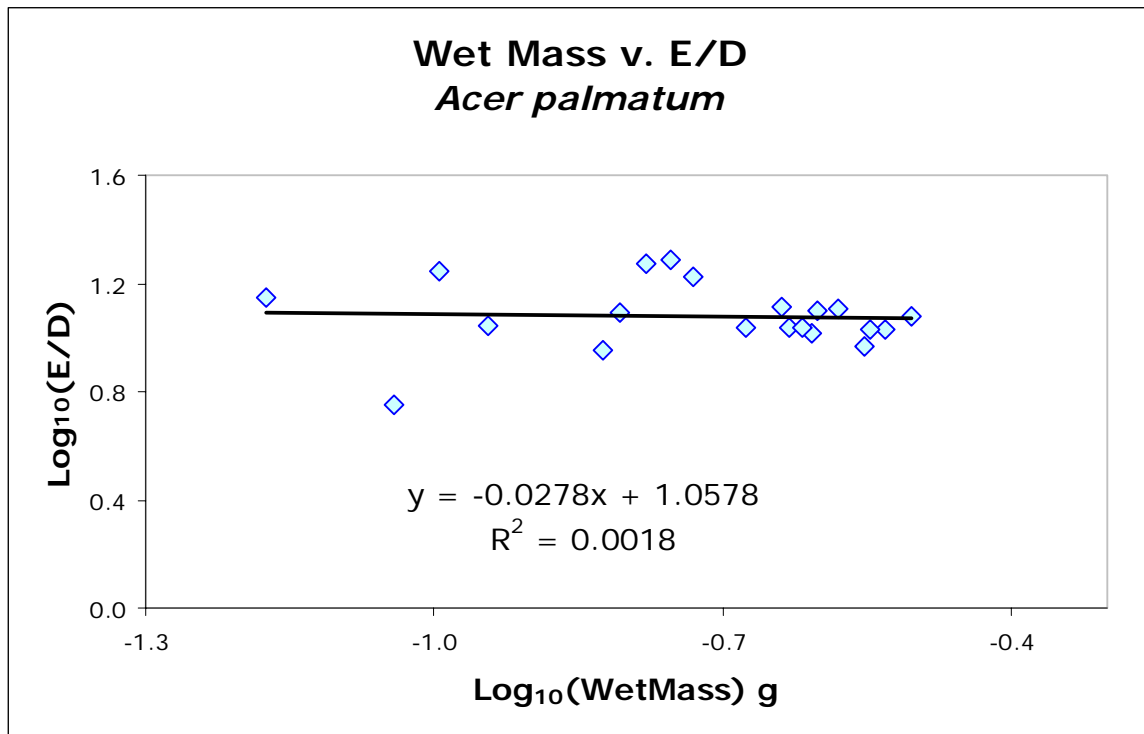


Figure 6. E/D scaled to mass. $R^2=0.00$

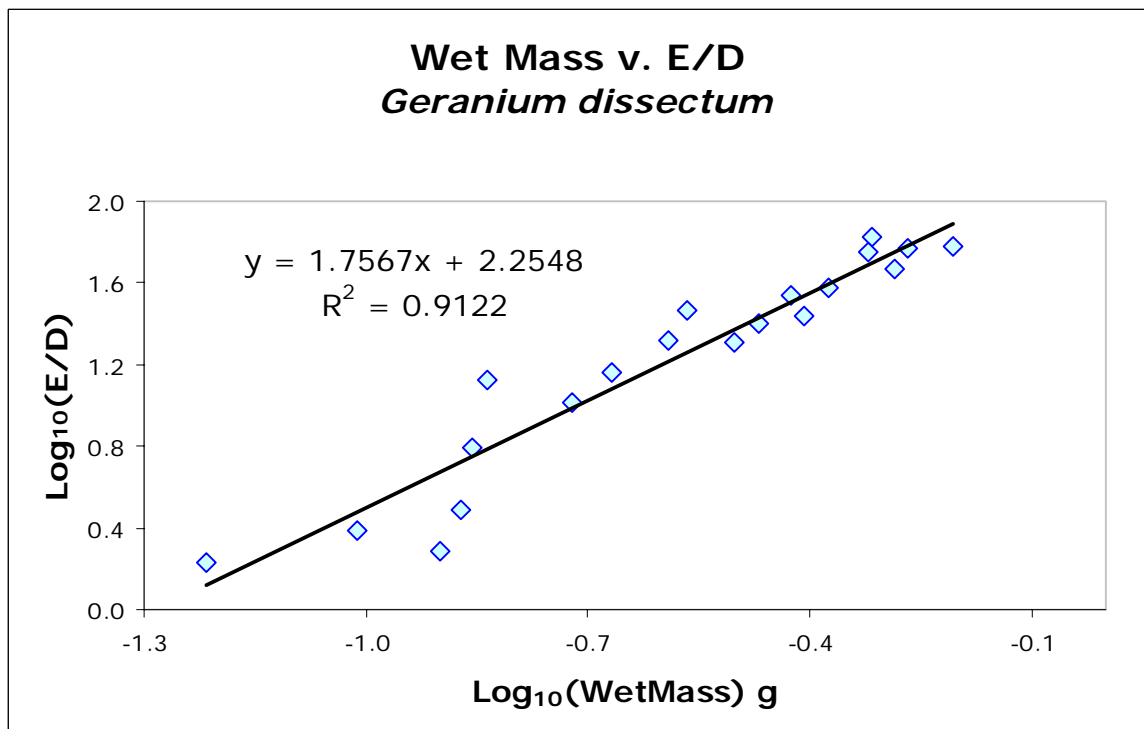


Figure 7. E/D scaled to mass. $R^2=0.91$

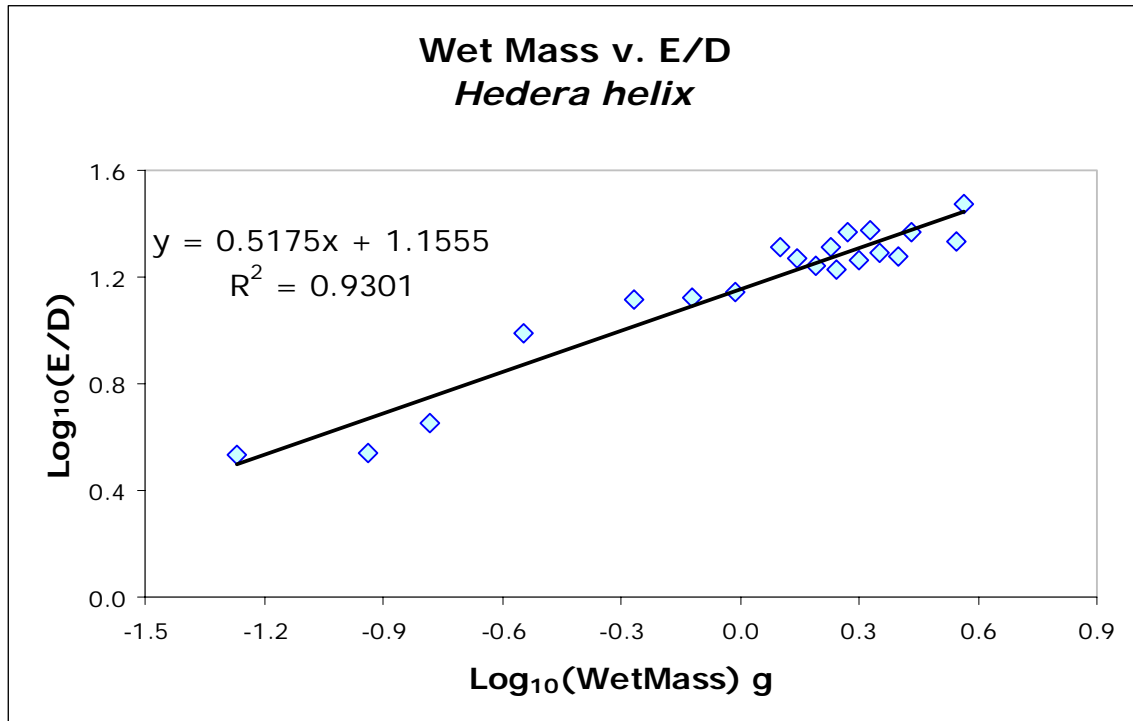


Figure 8. *E/D scaled to mass. $R^2=0.93$*

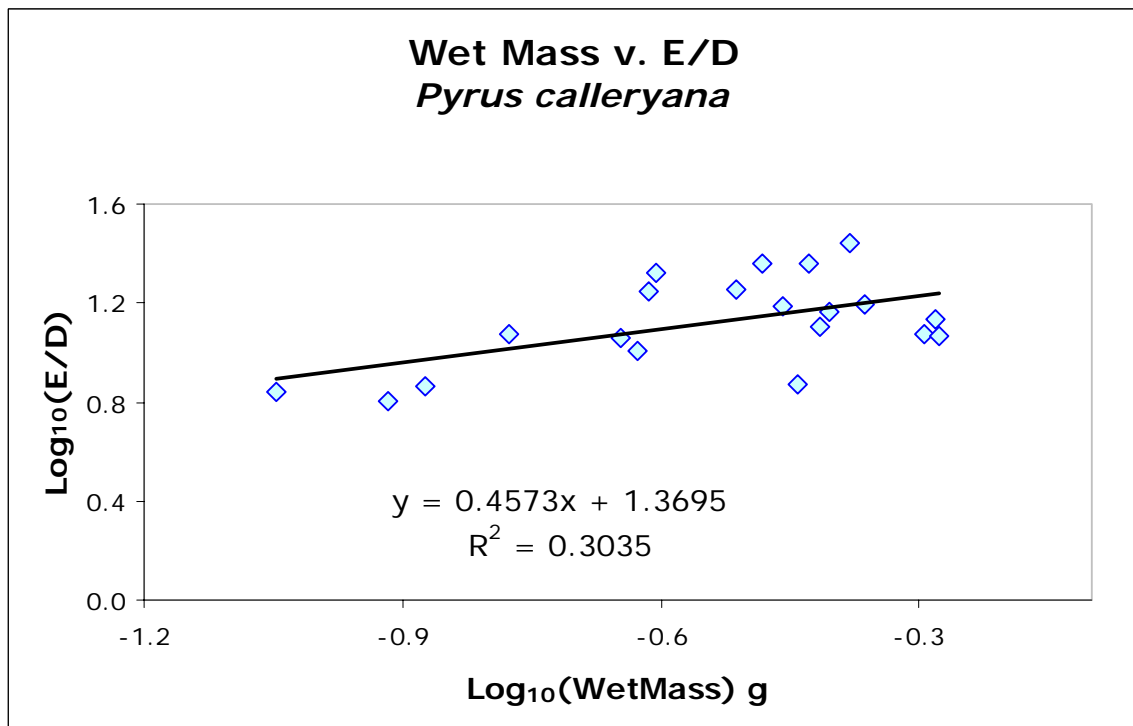


Figure 9. *E/D scaled to mass. $R^2=0.30$*

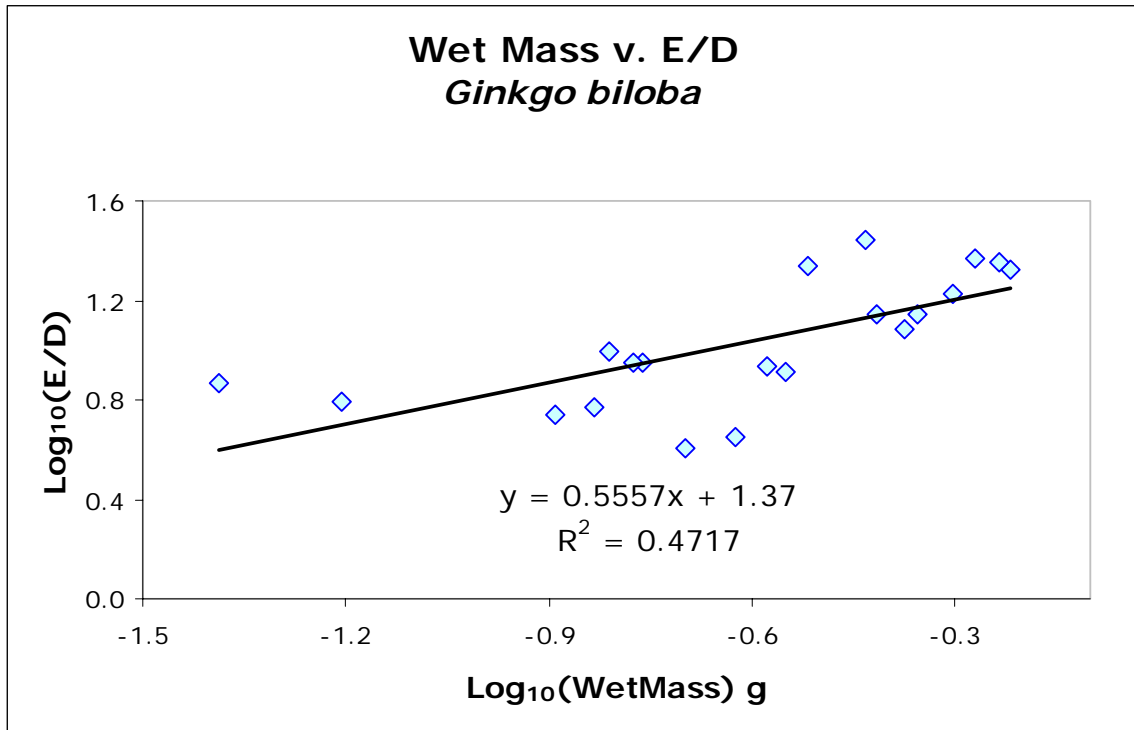


Figure 10. *E/D scaled to mass. $R^2=0.47$*

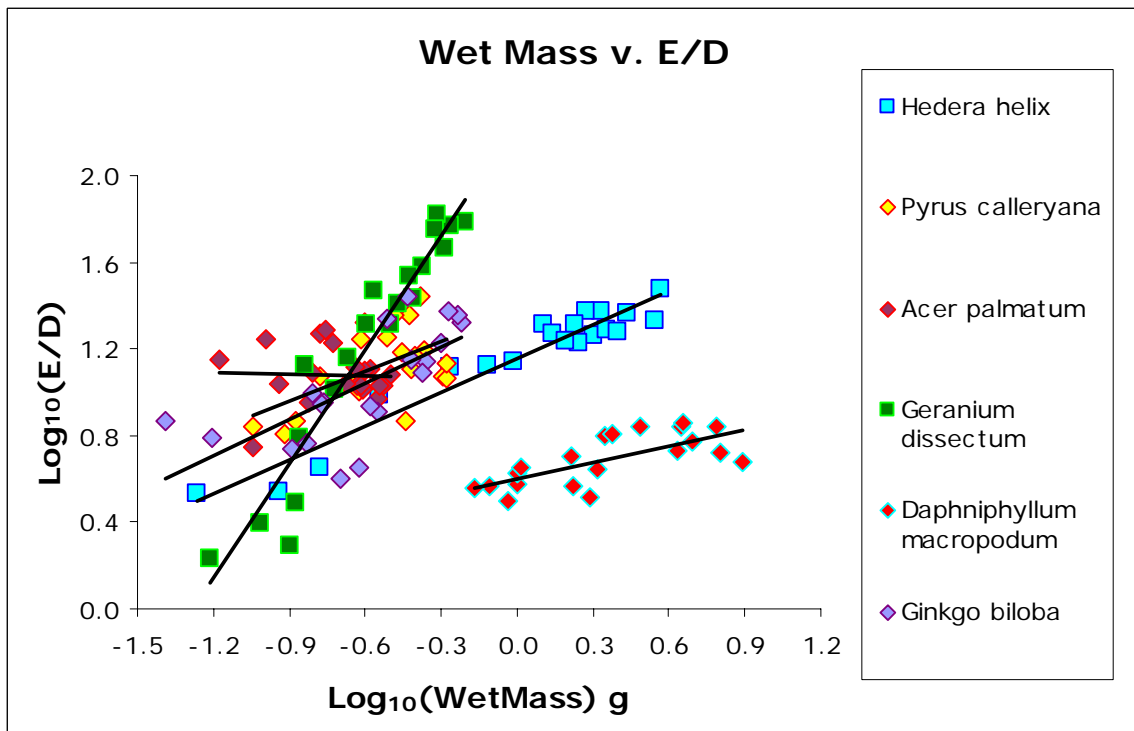


Figure 11. *E/D scaled to mass for all 6 species. Squares represent herbaceous species and diamonds represent woody species.*

The interspecific results of this study show that E varies across species, even those closely related (*Acer* and *Quercus*) (table 1). Density-dependent stiffness scaled well with overall leaf mass for *Hedera helix* ($E \propto M^{0.52}$, $R^2 = 0.93$) (fig. 7) and *Geranium dissectum* ($E \propto M^{2.20}$, $R^2 = 0.94$) (fig. 6). The relationship between mass and density-dependent stiffness was not as strong for the other species. *Acer palmatum* ($E \propto M^{0.07}$, $R^2 = 0.01$) (fig. 5) showed the most invariance with leaf mass. The stress-to-strain graph created using a *Ginkgo biloba* petiole displays a clear transition between the elastic region and the plastic region at approximately 0.02 N (fig. 4).

Discussion

Petioles serve two main functions: 1) to transport nutrients to and from the leaf lamina and 2) to serve a biomechanical support function. Petioles must be structurally sound to support a leaf's weight and prevent buckling from self-loading and dynamic weather forces. Previous allometric models have focused on the first function of the leaf petiole and often have neglected the second. This study attempts to shed light upon the importance of the biomechanical function of leaf petioles and how allometric models must account for the selection for optimal mechanical stability. The results show that density-dependent stiffness is not invariant as previously assumed and that Young's modulus and density-dependent stiffness scale with overall leaf mass for some herbaceous species.

Karl Niklas and other botanists have doubted the invariance of density-dependent stiffness in plants (1999), while others argue that density-dependent stiffness is indeed invariant (McMahon, 1973). This study supports the idea that the last assumption of the

WBE model, stating that biomechanical constraints are invariant, may not be true in all plants. The results show that for herbaceous plant species density-dependent stiffness appears to scale very well with leaf mass. However, for woody species, density-dependent stiffness does not scale as well as for herbaceous species. Some of the species show a weak variance with leaf mass, while others are completely invariant. It appears that the consistency of biomechanical properties with leaf mass depends on the type of plant. The WBE model's assumptions appear to hold true for woody tree species but not for smaller herbaceous plants.

More species should be investigated to better understand the role of phylogeny in determining density-dependent stiffness. Furthermore, this study only states that density-dependent stiffness scales to mass for some plants and not in others. The question of why density-dependent stiffness is not invariant needs to be addressed. Future studies should determine if the noted size dependency is caused simply by the changing shape of the petiole as it grows or if the tissue composition itself is changing. It is possible that petioles rearrange their structural tissue as they grow. As a leaf increases in size, the composition of structural tissue changes from being homogeneously distributed throughout the cross section of the petiole to being more prevalent on the outside edge. This reorganization would increase the stiffness of the petiole without substituting lignified tissue for hydraulic conductive tissue. Lignified staining procedures for tissue composition of the petioles were attempted but were inconclusive. A better staining method is needed to quantify the composition of structural versus vascular tissue in the petioles. Future studies should be conducted to more rigorously evaluate the validity of the assumption of invariance in the biomechanical properties of leaf petioles and

investigate why and how petioles increase their density-dependent stiffness as leaves increase in size.

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References

- Brown, James H., Geoffrey B. West, and Brian J. Enquist. "Yes, West, Brown and Enquist's Model of Allometric Scaling is Both Mathematically Correct and Biologically Relevant." Functional Ecology 19 (2005): 735-738.
- Kozłowski, J. & Konarzewski, M. "Is West, Brown and Enquist's model of allometric scaling mathematically correct and biologically relevant?" Functional Ecology 18, (2004): 283–289.
- McMahon, Thomas. "Size and Shape in Biology." Science 179 (1973): 1201-1204.
- Niklas, Karl J. "Research Review: a Mechanical Perspective on Foliage Leaf Form and Function." New Phytologist 143 (1999): 19-31.
- Niklas, Karl J. Plant Allometry: the Scaling of Form and Process. Chicago: The University of Chicago P, 1994.
- Price, Charles A., and Brian J. Enquist. "Scaling of Mass and Morphology in Plants with Minimal Branching: an Extension of the WBE Model." Functional Ecology 20 (2006): 11-20.
- Price, Charles A., Brian J. Enquist, and Van M. Savage. "A General Model for Allometric Co-variation in Botanical Form and Function." PNAS 104 (2007): 13204-13209.
- Vogel, Steven. Comparative Biomechanics: Life's Physical World. New Jersey: Princeton UP, 2003.
- West, Geoffrey B., James H. Brown, and Brian J. Enquist. "A General Model for the Structure and Allometry of Plant Vascular Systems." Nature 400 (1999): 664-667.