

COVER SHEET

TITLE: Investigation of the Physiological Changes Behind the Range Expansion of the Polyploid Hybrid, *Polystichum scopulinum*

AUTHOR'S NAME: Lucas Lin

MAJOR: Biology

DEPARTMENT: Biology

MENTOR: Christopher Krieg

DEPARTMENT: Botany

MENTOR(2):

DEPARTMENT(2):

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ABSTRACT

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In plants, polyploidy has been thought to play a key role in evolution as it can generate more genetic variation. However, we do not have a strong understanding of how polyploidy affects physiology, which limits our understanding of trait evolution and species ecology. To investigate this, we focused on an allopolyploid hybrid, *Polystichum scopulinum*, to understand how polyploidy may have enabled its range expansion in relation to its progenitors, *P. imbricans* and *P. lemmonii*. We show that the leaf thickness (LT) of the polyploid hybrid is similar to *P. imbricans*'s LT but significantly higher than *P. lemmonii*'s LT, and that the hybrid's leaf hydraulic conductance (K_{leaf}) is significantly lower than both progenitors. These results suggest that *P. scopulinum*'s physiological differences may be linked to differences in its ecology.

Lucas Lin/Biology

Author Name/Major

Lucas Lin

Author Signature

Christopher Krieg/Botany

Mentor Name/Department

Chris Krieg

Mentor Signature

05/02/2022

Date

Investigation of the Physiological Changes Behind the Range Expansion of the Polyploid Hybrid, *Polystichum scopulinum*

Lucas Lin¹, Christopher P. Krieg¹, Katherine A. McCulloh¹

1 - University of Wisconsin, Madison

Abstract

In plants, polyploidy has been thought to play a key role in evolution as it can generate more genetic variation. However, we do not have a strong understanding of how polyploidy affects physiology, which limits our understanding of trait evolution and species ecology. To investigate this, we focused on an allopolyploid hybrid, *Polystichum scopulinum*, to understand how polyploidy may have enabled its range expansion in relation to its progenitors, *P. imbricans* and *P. lemmonii*. We show that the leaf thickness (LT) of the polyploid hybrid is similar to *P. imbricans*'s LT but significantly higher than *P. lemmonii*'s LT, and that the hybrid's leaf hydraulic conductance (K_{leaf}) is significantly lower than both progenitors. These results suggest that *P. scopulinum*'s physiological differences may be linked to differences in its ecology.

Introduction

Of the many drivers of plant evolution, whole-genome duplication (WGD), also known as polyploidy, is thought to play an important role in the evolution of morphological and physiological traits that allow new species to distribute themselves across new ecological niches (Soltis *et al.*, 2003). Polyploidy occurs when an organism has more than two complete sets of chromosomes, which can come from within the species (autopolyploidy) or from different species (allopolyploidy). This extra genetic information comes with diverse effects, such as increased cell size, which can affect the growth rate and reproduction of the organism (Otto and Whitton, 2000). In addition to the increased cell size, if the organism is the hybrid of two species, there is a greater chance of new beneficial traits emerging (Otto

and Whitton, 2000). These new characteristics could allow polyploid progeny to withstand more stressful environments, such as drought-prone areas, as the polyploid may be able to express the gene copy and phenotype that has a more optimum response to the stressor (Hegarty and Hiscock, 2008). Using this ability, the progeny can potentially expand its ecological range into novel habitats compared to its progenitors. This range expansion of polyploid species has often been studied at a broad level with explanations of this expansion citing reproductive or genetic differences to the progenitors (Coughlan *et al.*, 2017; Hijmans *et al.*, 2007; Marchant *et al.*, 2016). While these studies have offered numerous explanations for the expansion for hybrid species' ecological ranges, few have attempted to examine these species' range expansion using physiological data.

Here we focus on the ecological range expansion of an allopolyploid fern species, *P. scopulinum*, which is a hybrid of *P. imbricans* and *P. lemmonii* (Soltis *et al.*, 1991). These two diploid parental species are found on the

western coast of the United States (Fig. 1). In this region, *P. imbricans* tends to more mesic habitats but can also be found on rocky slopes, and can be found in many locations scattered across the western coast (Wagner, 1979). *P. lemmonii* prefers rocky outcrops and is restricted to serpentine soils (Wagner, 1979).

The two parental species do not co-occur anywhere within their range, however, the hybrid species, *P. scopulinum*, can be found with either parent, including on serpentine soil (Soltis *et al.* 1991). In general, *P. scopulinum* has a greatly expanded range covering most of the parental species' range and extending into

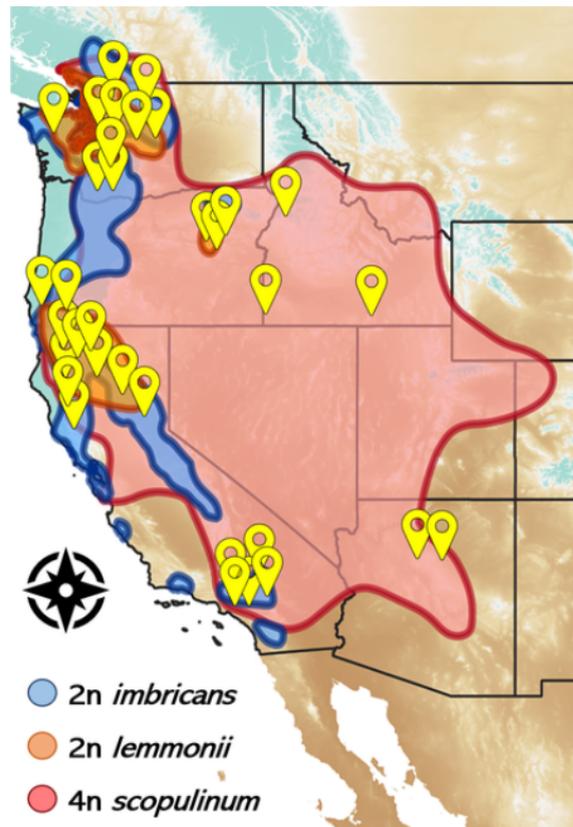


Figure 1: Distribution map of *P. imbricans* (blue), *P. scopulinum* (red), and *P. lemmonii* (orange) across the western United States.

the Rocky Mountain region (Wagner, 1979) (Fig. 1).

Although we can see that the polyploid hybrid, *P. scopulinum*, can survive in a greater variety of habitats, it is unknown whether this expansion is caused by the potential ability to adaptively display parental trait phenotypes, the expression of intermediate phenotypes, and/or the expression of phenotypes that exceed the parental phenotypes. Due to the apparent differences in distribution being most related to dry climates, we suspect that traits involving water usage will be key in understanding *P. scopulinum*'s greatly expanded range. In relatively dry environments, plants may need to increase hydraulic conductance in their leaf vasculature (K_{leaf}) to compensate for the increased transpiration while maintaining water use efficiency and photosynthetic capacity (Zwieniecki and Boyce, 2014). This increase can be achieved in multiple ways, including by decreasing the vein-to-vein distance (dx), decreasing the vein-to-epidermis distance (dy), or increasing leaf thickness (LT) (Fig. 2) (Brodribb, Feild, and Jordan, 2007).

In order to compare these physiological characteristics to the climate at each site, we used a drought index (Di) created by McCulloh *et al.* (in review), where higher values represent lower water availability and higher relative drought stress. In response to changes in Di at different sites, we expected that the allopolyploid hybrid, *P. scopulinum*, would express intermediate anatomical phenotypes between both progenitors, *P. imbricans* and *P. lemmonii* to change its K_{leaf} to an optimal value in order to maximize photosynthetic capacity while minimizing water loss due to transpiration.

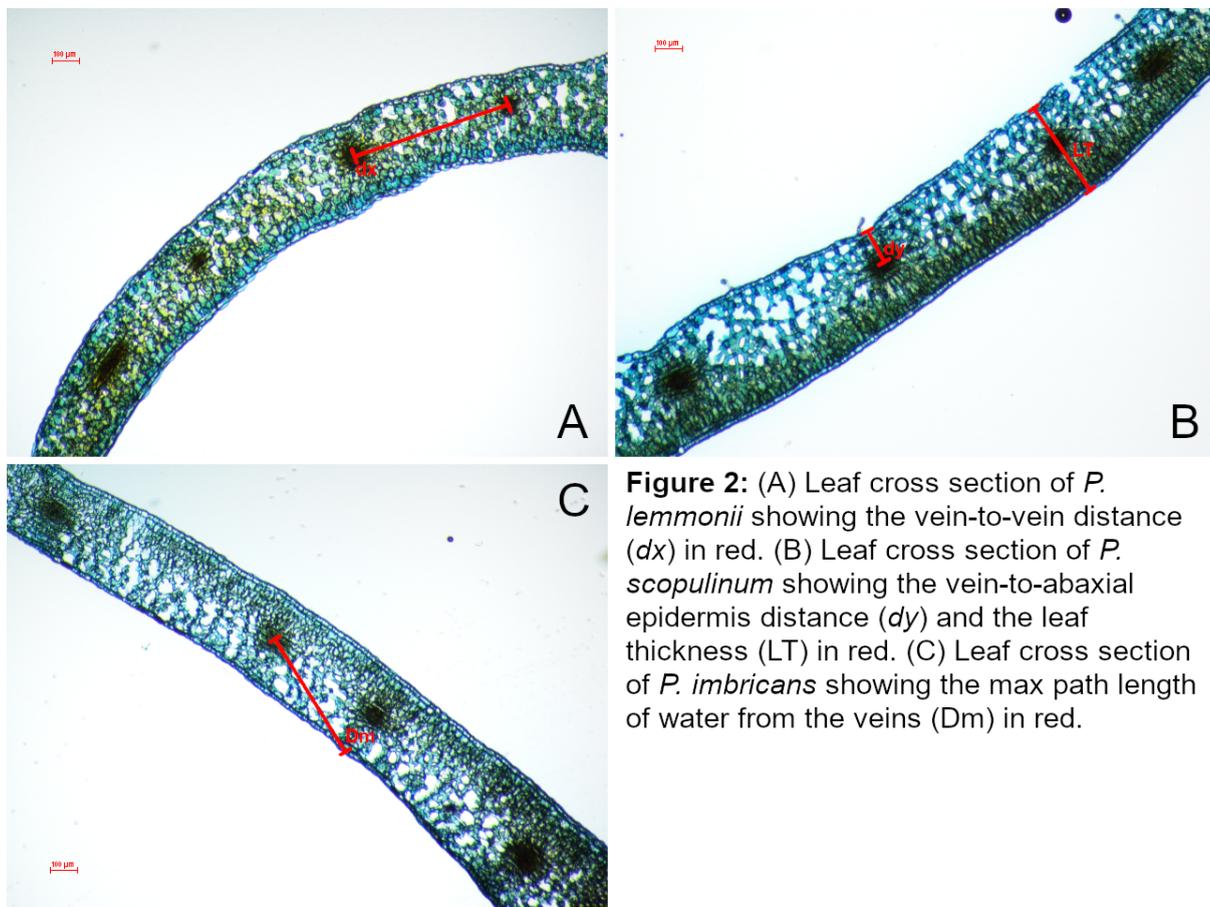
Methods

Sample collection: Fronds were collected from ferns located at 34 sites throughout their natural habitat along the western coast of the United States (Fig. 1). At each of the designated sites, several samples were taken from the upper third portion of a healthy, mature frond and preserved in 50% ethanol before analysis.

From 3-6 samples per species per site, we obtained the cross-sections of the pinna by freehand using razor blades to cut perpendicular to the pinna costa on the pinna near the

base of the frond. We then stained these cross-sections in toluidine blue and subsequently imaged them at 4x magnification on a Nikon Eclipse Ci microscope with a Nikon DS-Fi2 camera for analysis in ImageJ (Schneider *et al.*, 2012).

The dx distance was measured from the center of the xylem conduits in adjacent veins to obtain 3 measurements (Fig. 2A). Similarly, the dy distance was measured from the center of the xylem conduit to the abaxial epidermis using the shortest path to obtain 3 measurements (Fig. 2B). To measure the leaf thickness (LT), we took the distance between the adaxial and abaxial epidermises at 4 points, 2 across veins and 2 across areas without veins (Fig. 2B). The mean of each group of these anatomical measurements was used for the final measurements in each of the 3-6 site replicates. In all our measurements, we avoided the area near the pinna costa due to the significant changes in leaf thickness. From these measurements, we calculated K_{leaf} using the equation (1) described by Brodibb *et al.* (2007).



$$K_{leaf} = \left(\frac{LT^2}{4} + \left(0.078 \cdot \left(\frac{dx}{dy} \right)^2 \right) (LT)^2 \right)^{0.63} \quad (1)$$

The max path length of water from the veins (Dm) was also calculated from the dx and dy measurements (Fig. 2C). In addition to the anatomical measurements, we obtained environmental data in the form of the Di index at each sampling site using QGIS (QGIS.org, 2022).

Statistical Analysis: For each fern species, we used the linear model function in R to create a separate linear regression analysis of Di vs dx , dy , LT, and Dm (R Core Team, 2022). Using the full dataset, we used single-factor ANOVA tests followed by Tukey HSD tests in R to determine whether there were significant differences in these anatomical and environmental measurements between species (R Core Team, 2022).

Results

In our comparison among these three species, we found that there was a significant difference in the leaf anatomy of *P. scopulinum* affecting its K_{leaf} , which was found to be significantly different across all three species. For K_{leaf} , *P. scopulinum* was found to be the lowest compared to both *P. imbricans* ($p < 0.001$) and *P. lemmonii* ($p < 0.001$). *P. lemmonii* also had a larger K_{leaf} compared to *P. imbricans* ($p < 0.001$). One of the anatomical measurements involved in the calculation of K_{leaf} is dx , which we found was significantly lower in *P. lemmonii* compared to both *P. imbricans* ($p < 0.001$) and *P. scopulinum* ($p < 0.001$). However, there was no significant difference between the dx in *P. imbricans* and *P. scopulinum* ($p = 0.240$). This is unlike the dy measurement, where we found all three species were significantly different from one another. The dy in *P. lemmonii* was found to be the lowest compared to *P. imbricans* ($p < 0.001$) and *P. scopulinum* ($p < 0.001$) while the dy measurement in *P. imbricans* was larger than in *P. scopulinum* ($p = 0.011$). Analysis of Dm yielded similar results as all three species were significantly different from one another despite the similarity in dx

between *P. imbricans* and *P. scopulinum*. Of the three species, *P. lemmonii*'s Dm was the lowest compared to *P. imbricans* ($p < 0.001$) and *P. scopulinum* ($p < 0.001$) while *P. scopulinum* was the greatest compared to the next highest Dm in *P. imbricans* ($p = 0.035$). Despite the significantly different results across the three species for the *dy* and Dm measurements, the LT in *P. imbricans* and *P. scopulinum* were not significantly different from each other ($p = 0.908$) while *P. lemmonii* exhibited significantly lower LT than both *P. imbricans* ($p < 0.001$) and *P. lemmonii* ($p < 0.001$).

In addition to these comparisons of the anatomical measurements across the three species, we used regression analyses to compare these same measurements with Di. We found that Di was only significantly different between *P. imbricans* and *P. lemmonii* ($p = 0.044$), and in our regression between Di and *dy*, we also found a strong negative correlation in *P. scopulinum* ($r^2 = 0.525$; $p < 0.001$) that was not present in either of the progenitor species (Fig. 3A). There was a similar negative correlation between Di and LT ($r^2 = 0.502$; $p < 0.001$) in *P. scopulinum* (Fig. 3B). However, a regression of the relationship between Di and *dx* yielded only a weak negative correlation ($r^2 = 0.160$; $p < 0.01$) in *P. lemmonii* (Fig. 3C). Despite not having any significant correlation between Di and *dy* and only a weak correlation between Di and *dx* in *P. lemmonii*, we found that only *P. lemmonii* had a significant correlation ($r^2 = 0.167$; $p < 0.01$) between Di and Dm, albeit a weak negative correlation (Fig. 3D).

Discussion

Our physiological analysis of the allopolyploid hybrid, *P. scopulinum*, has revealed that it is exhibiting some traits within the phenotypic range of its progenitors, *P. imbricans* and *P. lemmonii*. In particular, the *dx* and LT in *P. scopulinum* were found to be significantly greater than that of *P. lemmonii* but not significantly different from that of *P. imbricans*. This suggests that the mechanism stipulated by Hegarty and Hiscock (2008) may be correct as *P. scopulinum* could be expressing the most optimum gene copy and phenotype for the given environmental conditions. This ability to choose and express the optimum phenotype is also

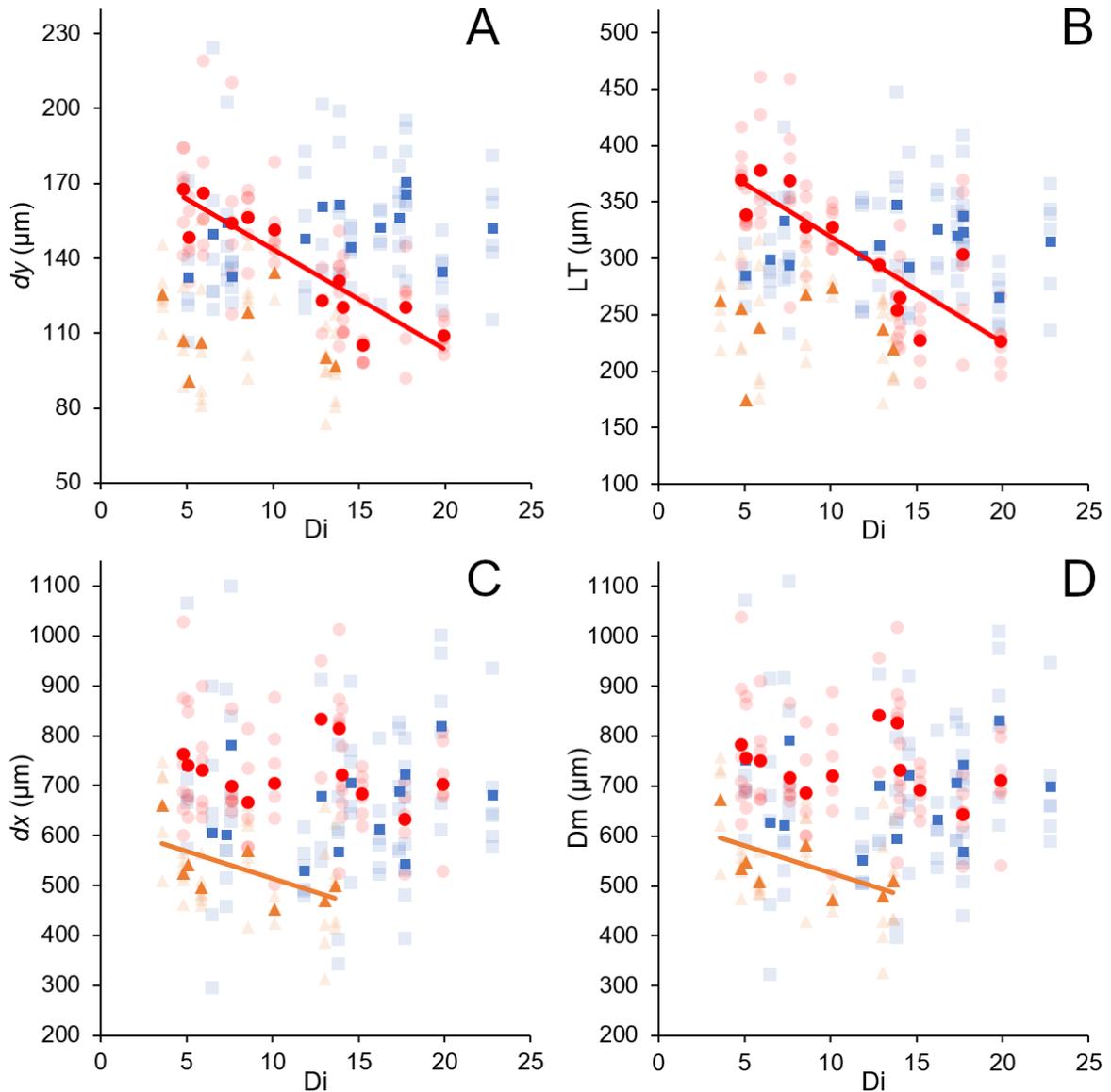


Figure 3: Scatterplots comparing Di with *dy*, LT, *dx*, and Dm in *P. imbricans* (blue squares), *P. scopulinum* (red circles), and *P. lemmonii* (orange triangles). Individual measurements are shown as semi-transparent points and site averages are shown in solid points. Regression lines are shown using individual measurements. (A) Di plotted against *dy*. A linear regression was fitted to *P. scopulinum* ($r^2= 0.525$, $p<0.001$). (B) Di plotted against LT. A linear regression was fitted to *P. scopulinum* ($r^2= 0.502$, $p<0.001$). (C) Di plotted against *dx*. A linear regression was fitted to *P. lemmonii* ($r^2= 0.160$, $p<0.01$). (D) Di plotted against Dm. A linear regression was fitted to *P. lemmonii* ($r^2= 0.167$, $p<0.01$).

supported by the results of the *dy* measurement as all three species were significantly different from another, and *P. scopulinum*'s value was the intermediate between both parental species. These results suggest that the polyploid nature of the hybrid species has allowed it to adapt to the aridity across its ecological range. This adaptation is best visible

when comparing Di with *P. scopulinum*'s dy (Fig. 3A) or LT (Fig. 3B) as both show a strong negative correlation with Di (dy : $r^2=0.525$; LT: $r^2=0.502$).

The negative correlation between Di and dy is also consistent with the correlation between Di and LT. LT consists of the dy distance in addition to the remaining vein-to-axial distance, so a change in dy has a direct effect on LT, which appears to be the case in *P. scopulinum*. Despite the decrease in dy and LT with increasing Di, K_{leaf} was significantly lower in *P. scopulinum* than both *P. imbricans* ($p<0.001$) and *P. lemmonii* ($p<0.001$) (Fig. 4). This differs from our expectation that *P. scopulinum* would increase K_{leaf} with higher Di and show K_{leaf} values higher than their parents. However, the lower K_{leaf} shown in the hybrid species is still transgressive (beyond that observed in the parental species) and could be a novel phenotype driven by polyploidization.

Still, the lack of correlation between Di and K_{leaf} shows that the three species may not be primarily adapting to the drought stress of each location. However, when comparing only the K_{leaf} between species, it is clear that all three species have significantly different K_{leaf} values (Fig. 4). In particular, the K_{leaf} in *P. scopulinum* is the lowest of the three species, meaning that it is taking on a conservative approach to water transport in its leaves, either by selection on adaptive phenotypes, and/or constraints imposed by genome duplication. This result was unexpected as *P. scopulinum* has a similar Di compared to both *P. imbricans* ($p=0.465$) and *P. lemmonii* ($p=0.344$) (Fig. 5). Given the similarity of *P. scopulinum*'s Di with *P. imbricans*' Di, which is the highest of the three species, it was expected that *P. scopulinum* would have a similar K_{leaf} to *P. imbricans*. This discrepancy could mean that there are additional factors that influence K_{leaf} beyond Di.

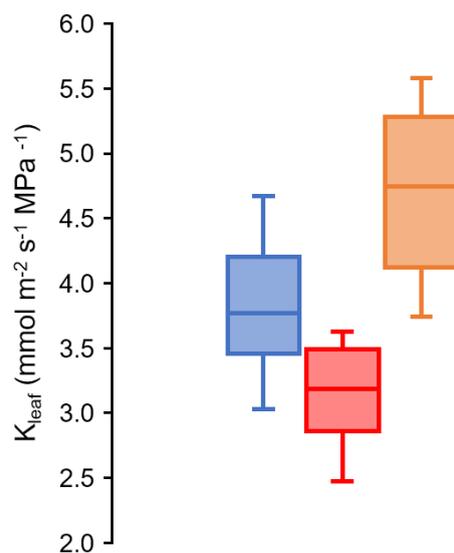


Figure 4: Box and whisker plot of the K_{leaf} of *P. imbricans* (blue), *P. scopulinum* (red), and *P. lemmonii* (orange) from left to right.

Although we can see that some of the anatomy in *P. scopulinum* is correlated to drought stress, namely d_y and LT, the overall result of these changes in anatomy, K_{leaf} , has no clear correlation with D_i . This means that drought stress is likely not the only environmental factor that influences the anatomy of these three species. One other potential environmental factor that may impact plant function is leaf area index (LAI), which is the total leaf area per unit ground area (Chen

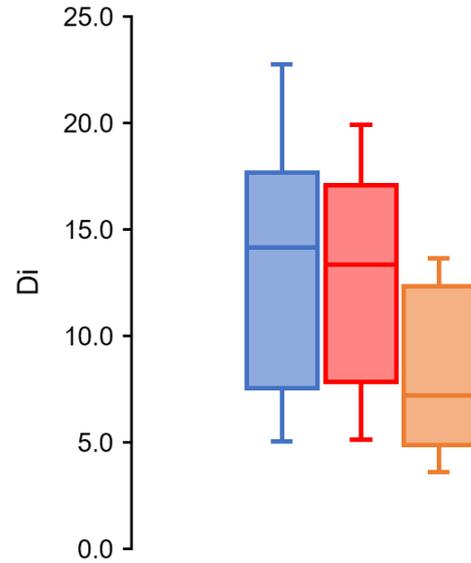


Figure 5: Box and whisker plot of the D_i of *P. imbricans* (blue), *P. scopulinum* (red), and *P. lemmonii* (orange) from left to right.

and Black, 1992). As the leaf area index increases, the additional shade could allow for plants with lower photosynthetic rates and hydraulic characteristics to thrive (Zwieniecki and Boyce, 2014). Preliminary results investigating the role of LAI have shown that there could be a moderate correlation between LAI and several of these anatomical measurements (unpublished data).

While other environmental factors like LAI may have some effect on the anatomy of the leaves in these species, another important source of phenotypic variation is the ploidy of the parental and hybrid species. We have seen that most of our anatomical measurements of the leaves in *P. scopulinum* have remained within the range of its parental species, *P. imbricans* and *P. lemmonii*, meaning that the hybrid could be expressing some genes from both parents to achieve the optimal phenotype. However we cannot definitively say whether this ability is caused by the hybridization or polyploidization, or both. This is because hybridization of the parental species results in partial genetic donation to a hybrid offspring but with the same or similar genome size, in contrast to polyploidization, which results in the donation of complete parental genomes and significantly increases the genome size of the polyploid offspring (Simonin and Roddy, 2018). To better understand the effects of this

increased genome size in polyploid hybrids and how it defines *P. scopulinum*'s phenotypes, further investigation of the role of polyploidy is required.

The results of this physiological analysis of these three fern species has shown that the allopolyploid hybrid, *P. scopulinum*, potentially has the ability to have both intermediate phenotypes (e.g. dx , dy , and LT) and phenotypes beyond that of its progenitors (e.g. Dm , K_{leaf}). *P. scopulinum* also shows that it is capable of changing some of its leaf anatomy, dy and LT, but not others, like K_{leaf} , to correspond with different amounts of drought stress. The drought stress of *P. scopulinum*'s habitat appears to have some effect on its physiological characteristics, but other environmental factors like LAI and genetic factors like the hybrid's tetraploid genome likely play important roles in the ecology of this polyploid complex.

Literature Cited

- Brodribb, T.J., Feild, T.S., and Jordan, G.J. (2007). Leaf maximum photosynthetic rate and venation are linked by hydraulics. *Plant Physiology* 144(4), 1890-1898.
<https://doi.org/10.1104/pp.107.101352>
- Chen, J.M. and Black, T.A. (1992). Defining leaf area index for non-flat leaves. *Plant, cell and environment* 15, 421-429. <https://doi.org/10.1111/j.1365-3040.1992.tb00992.x>
- Coughlan, J.M., Han, S., Stefanović, S., and Dickinson, T.A. (2017). Widespread generalist clones are associated with range and niche expansion in allopolyploids of Pacific Northwest Hawthorns (*Crataegus* L.). *Molecular Ecology* 26, 5484-5499.
<https://doi.org/10.1111/mec.14331>
- Hegarty, M.J. and Hiscock, S.J. (2008). Genomic clues to the evolutionary success of polyploid plants. *Current Biology* 18(10), R435-R444.
<https://doi.org/10.1016/j.cub.2008.03.043>
- Hijmans, R.J., Gavrilenko, T., Stephenson, S., Bamberg, J., Salas, A., and Spooner, D.M. (2007). Geographical and environmental range expansion through polyploidy in wild potatoes (*Solanum* section *Petota*). *Global Ecology and Biogeography* 16, 485-495.
<https://doi.org/10.1111/j.1466-8238.2007.00308.x>
- Marchant, D.B., Soltis, D.E., and Soltis, P.S. (2016). Patterns of abiotic niche shifts in allopolyploids relative to their progenitors. *New Phytologist* 212(3), 708-718.
<https://doi.org/10.1111/nph.14069>
- Otto, S.P. and Whitton, J. (2000). Polyploid incidence and evolution. *Annual Reviews* 34, 401-437. <https://doi.org/10.1146/annurev.genet.34.1.401>
- QGIS.org, 2022. QGIS Geographic Information System. QGIS Association.
<http://www.qgis.org>
- R Core Team, (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
- Schneider, C.A., Rasband, W.S., and Eliceiri, K.W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 9(7), 671–675. <https://doi.org/10.1038/nmeth.2089>

Simonin, K.A. and Roddy, A.B. (2018). Genome downsizing, physiological novelty, and the global dominance of flowering plants. *PLoS Biol.* 16(1), e2003706.

<https://doi.org/10.1371/journal.pbio.2003706>

Soltis, D.E., Soltis, P.S., and Tate, J.A. (2003). Advances in the study of polyploidy since *Plant speciation*. *New Phytologist* 161(1), 173-191.

<https://doi.org/10.1046/j.1469-8137.2003.00948.x>

Soltis, P.S., Soltis, D.E., and Wolf, P.G. (1991). Allozymic and chloroplast DNA analyses of polyploidy in *Polystichum* (Dryopteridaceae). I. The origins of *P. californicum* and *P. scopulinum*. *Systematic Botany* 16(2), 245-256. <https://doi.org/10.2307/2419277>

Wagner, D. 1979. *Systematics of Polystichum in Western North America North of Mexico*.

The American Fern Society.

Zwieniecki, M.A. and Boyce, C.K. (2014). Evolution of a unique anatomical precision in angiosperm leaf venation lifts constraints on vascular plant ecology. *Proc. R. Soc. B* 281:20132829. <https://doi.org/10.1098/rspb.2013.2829>