

Origins of polyploidy and their impact on adaptation in *Andropogon gerardi*

Alyssa Phillips

3rd year PhD student

Department of Evolution and Ecology, University of California, Davis

PI: Jeffrey Ross-Ibarra

BSA sectional affiliations: Genetics, Ecology

Abstract

Polyploidy is prevalent across the green tree of life and is thought to play a key role in speciation and rapid adaptation. Many studies have shown polyploidy can affect environmental response through alterations of plant biochemistry, physiology, and morphology. Changes in environmental response can ultimately lead to niche differentiation between polyploids and their progenitors. Although progress has been made in understanding the functional impacts of polyploidy, it's unclear how this translates to niche differentiation at the population-level. I will use population genetics, a common garden, and ecophysiology to study the effects of polyploidy on local adaptation in *Andropogon gerardi* Vitman, commonly known as big bluestem. This study will be the first to connect the demographic history of polyploid origins to functional trait differences between cytotypes. More broadly, it will contribute to the understanding of how whole genome duplications contribute to adaptation.

Keywords: polyploidy, *Andropogon*, population genetics, local adaptation, ecophysiology

Background

Whole genome duplication (WGD) events have helped diversify the green tree of life (Landis et al., 2018; Stull et al., 2021). It's estimated that at least 35% of plant species are polyploids suggesting an important role in speciation (Wood et al., 2009). WGD have also been implicated in diversifying plant specialised metabolism, altering cell physiology, and increasing overall resilience to extreme environments (Edger et al., 2015; Van de Peer et al., 2017; Roddy et al., 2020).

Although polyploidy is prevalent and may be advantageous, there are many short-term challenges new polyploids must overcome in order to establish. Polyploids should theoretically be rapidly eliminated due to minority cytotype exclusion but it is estimated that 10% of monocots are mixed-ploidy species, meaning they contain multiple cytotypes (Wood et al., 2009). Many studies have reported niche differentiation between cytotypes but did not examine their functional trait differences so it is unclear how the cytotypes differed in their environmental response (Lumaret et al., 1987; Duchoslav et al., 2010; Ramsey, 2011). Other studies have tested functional trait differences between polyploids but did not test whether these traits are under selection (Pavliková et al., 2017; but see Thébault et al., 2011). As a result of the disconnect between cytotype functional trait differences and their selection pressures, we know little about how the functional impacts of polyploidy alter population-level processes and adaptation.

I will use population genetics and ecophysiology to study the effects of polyploidy on local adaptation in *Andropogon gerardi*, commonly known as big bluestem. *A. gerardi* is the dominant grass species in endangered North American tallgrass prairies, an important type of grassland (Weaver and Fitzpatrick, 1932). It is also found abundantly in shortgrass prairies, mixed grass prairies, and wooded grasslands (Figure 1). The species is composed of two cytotypes hexaploid ($6x=2n=30$) and enneaploid ($9x=2n=90$) that are both common but are not equally distributed across populations (McAllister et al., 2015) (Figure 1). Western populations are dominated by enneaploid individuals while northeastern populations are predominantly hexaploid (McAllister et al., 2015). The distribution of cytotypes may be explained by unequal distribution of WGD events or by niche differentiation between cytotypes.

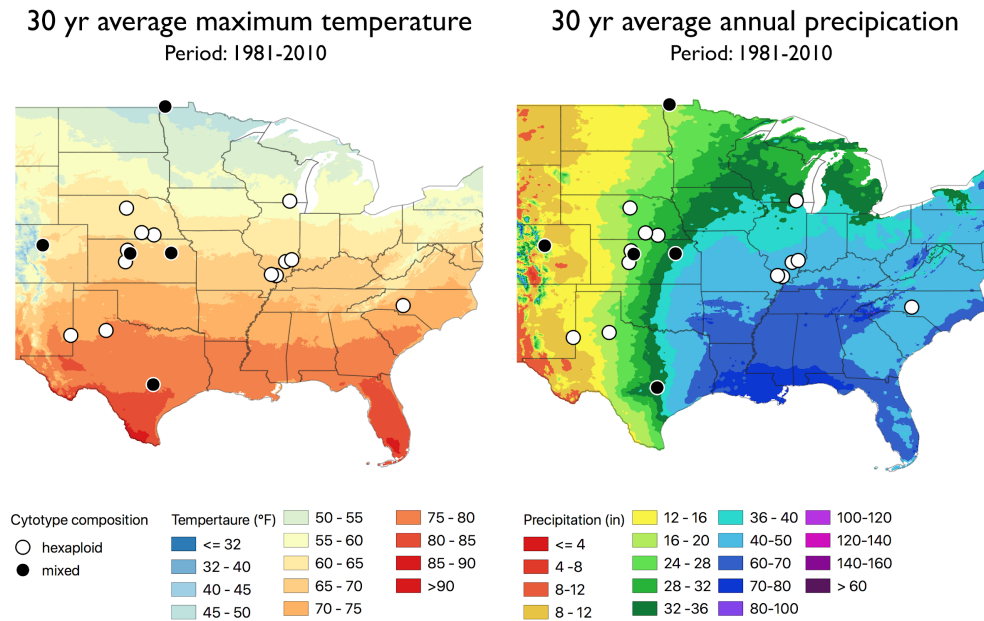


Figure 1. Locations of sampled *A. gerardi* populations and their respective cytotypic composition. Climate rasters are from PRISM Climate Group at Oregon State University.

Approach

Objective 1: Characterize the origins of intraspecific polyploidy.

The evolutionary history of the two cytotypes must be understood in order to contextualize their potential ecophysiological differences. For example, two cytotypes found in the same location may have similar ecophysiology. This pattern could result from the enneaploids having been produced from locally adapted hexaploids. Alternatively, the enneaploids may have been produced from foreign hexaploids and subsequently converged to a similar ecophysiology. I will use population genetics to evaluate whether enneaploids originated from a single WGD event or multiple WGD events (Figure 2).

Preliminary Research: *A. gerardi* was collected from 16 remnant prairies spanning the full species range (Figure 1). The ploidy of each sampled individual was determined using flow cytometry and whole genome re-sequencing was completed for all individuals. Additionally, our collaborators at Iowa State University and UC Riverside have recently assembled the first *A. gerardi* genome for use in my research.

First, I will cluster genotypes into genetic groups using principal component analysis and NGSadmix (Skotte et al., 2013). Genetic groups composed of a single cytotypic would indicate individuals are more closely related to members of the same ploidy and suggest origin from a single WGD event (Figure 2A). Alternatively, groups composed of multiple cytotypes would suggest multiple independent WGD events (Figure 2B). Additionally, I will quantify genetic differentiation between populations using the statistics F_{ST} , Rho (Ronfort et al., 1998), and analysis of molecular variance.

I will more explicitly test the number of origins by evaluating population tree topology using Treemix (Pickrell and Pritchard, 2012) and F_4 statistics. Treemix focuses on the relationship between populations rather than individuals. F_4 statistics test the topology of all possible mixed-ploidy quartets and can be used to clarify relationships not resolved by Treemix.

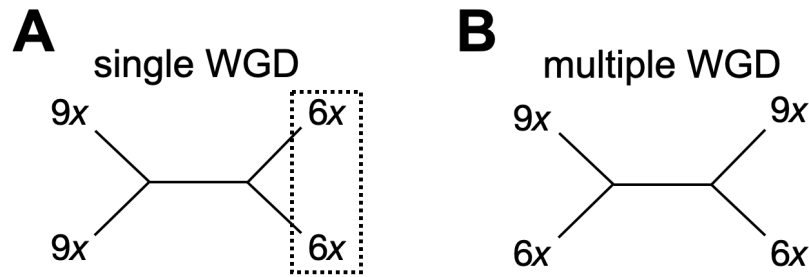


Figure 2. Two hypotheses for the origin of *A. gerardi* enneaploids (9x) displayed as unrooted trees. **(A)** Genetic groups are composed of a single cytotype 9x suggesting a single WGD. **(B)** Genetic groups are composed of multiple cytotypes suggesting multiple independent WGD events. An example of a genetic group is surrounded by the dashed box.

I will infer the minimum number of enneaploid origins from the number of independent enneaploid genetic clusters. Overlaying genetic group identity on geographic sampling location will allow me to infer how many origins occurred in each geographic region.

Objective 2: Identify ecophysiological differences between cytotypes and populations.

I will use a common garden experiment to evaluate ecophysiological differences between the two cytotypes and populations.

Preliminary Research: ‘Ecophysiology’ is a broad term that covers morphological, physiological, reproductive, growth, and phenological traits. I conducted a phenotyping trial in Fall of 2020 with a subset of individuals to identify ecophysiological traits of interest and narrow the scope of this study. Traits were retained if they had a significant population effect ($p < 0.05$) and were heritable ($H^2 > 0.25$). For example, I found guard cell length to have high heritability ($H^2 = 0.37$) and vary significantly across populations ($p = 0.002$) (Figure 3). My results suggest populations in hotter climates with less precipitation have larger stomata.

The common garden will be planted in May of 2021 in Columbia, MO using *A. gerardi* individuals from 14 populations. Each individual will have 3 biological replicates that have been produced from clonal propagation. In September of 2021 and 2022, each individual will be phenotyped for 13 traits evaluated in the phenotyping trial previously described.

I will test whether ecophysiological traits vary between cytotypes and populations using a linear mixed model. Ecophysiological trait differences between populations could result from genetic drift, local adaptation, or whole genome duplication. A significant cytotype effect would suggest whole genome duplication contributes to trait variation. Extensive research has shown correlation between genome size and stoma size (Hodgson et al., 2010), therefore variation related to ploidy in some ecophysiological traits would not be unexpected.

Objective 3: Evaluate whether trait differentiation is driven by selection or drift.

Finally, I will test whether population trait differences are due to habitat-specific selection (i.e. local adaptation) or neutral processes using a Q_{ST} - F_{ST} framework implemented in Driftsel (Ovaskainen et al., 2011). Q_{ST} is a statistic that measures the degree of genetic differentiation in a quantitative trait among populations. It is compared to F_{ST} , a metric of neutral genetic

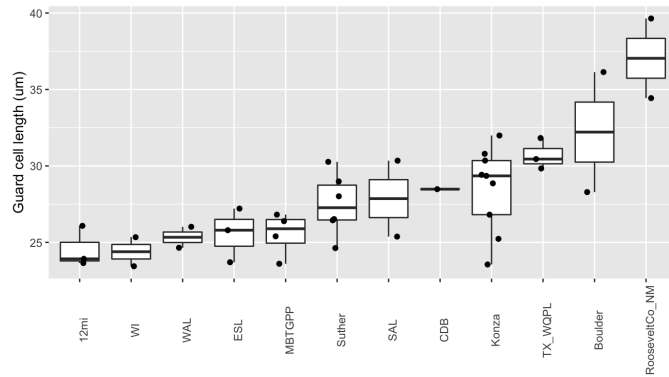


Figure 3. Mean guard cell length for each individual grouped by population. Populations are ordered by population mean guard cell length.

differentiation. If Q_{ST} is greater than F_{ST} , the degree of trait variation amongst populations is greater than expected by neutral processes therefore the trait is under selection.

Using this approach, I will test for selection on single and bivariate trait spaces. Many traits, like stomata size and density, are likely to be correlated due to biophysical constraints and pleiotropy therefore it's important to consider bivariate trait spaces. Ploidy will be considered in this analysis depending on the results from Objective 1 and 2. For example, if enneaploids have a single origin and there is a significant cytotype effect, I will test for selection independently in the two groups as they have separate evolutionary trajectories.

Significance

Current understanding of polyploid formation and selection in mixed-ploidy species has been limited by genomic technologies and lack of population genetics tools for polyploids until now. I will utilize whole genome sequence data and a new polyploid genome to conduct the first study, to the best of our knowledge, that tests for selection on ecophysiological traits in a mixed-ploidy species. This study will better our understanding of how niche differentiation occurs after polyploid formation. Additionally, it will provide data on genetic and ecological diversity that can be applied to conservation of the threatened remnant *A. gerardi* populations.

Proposed Budget

Additional funding for this project has been awarded by the Davis Botanical Society (\$2000) for the cost of the common garden field space. I'm requesting funding to partially cover costs for whole genome sequencing of 18 *A. gerardi* genotypes. This will allow an increased population sample size from 12 to 14 populations and increase my average within-population sample size.

Item	Description	Expense
Library preparation	\$20 per sample	\$360
Sequencing	NovaSeq lane	\$5400
	Total Sequencing Expenses	\$5760

Bibliography

- Duchoslav, M., L. Safárová, and F. Krahulec. 2010. Complex distribution patterns, ecology and coexistence of ploidy levels of *Allium oleraceum* (Alliaceae) in the Czech Republic. *Annals of botany* 105: 719–735.
- Edger, P. P., H. M. Heidele-Fischer, M. Bekaert, J. Rota, G. Glöckner, A. E. Platts, D. G. Heckel, et al. 2015. The butterfly plant arms-race escalated by gene and genome duplications. *Proceedings of the National Academy of Sciences of the United States of America* 112: 8362–8366.
- Hodgson, J. G., M. Sharafi, A. Jalili, S. Díaz, G. Montserrat-Martí, C. Palmer, B. Cerabolini, et al. 2010. Stomatal vs. genome size in angiosperms: the somatic tail wagging the genomic dog? *Annals of botany* 105: 573–584.
- Landis, J. B., D. E. Soltis, Z. Li, H. E. Marx, M. S. Barker, D. C. Tank, and P. S. Soltis. 2018. Impact of whole-genome duplication events on diversification rates in angiosperms. *American journal of botany* 105: 348–363.
- Lumaret, R., J.-L. Guillermin, J. Delay, A. Ait Lhaj Loutfi, J. Izco, and M. Jay. 1987. Polyploidy and habitat differentiation in *Dactylis glomerata* L. from Galicia (Spain). *Oecologia* 73: 436–446.
- McAllister, C., R. Blaine, P. Kron, B. Bennett, H. Garrett, J. Kidson, B. Matzenbacher, et al. 2015. Environmental correlates of cytotype distribution in *Andropogon gerardii* (Poaceae). *American journal of botany* 102: 92–102.
- Ovaskainen, O., M. Karhunen, C. Zheng, J. M. C. Arias, and J. Merilä. 2011. A new method to uncover signatures of divergent and stabilizing selection in quantitative traits. *Genetics* 189: 621–632.
- Pavlíková, Z., D. Holá, B. Vlasáková, T. Procházka, and Z. Münzbergová. 2017. Physiological and fitness differences between cytotypes vary with stress in a grassland perennial herb. *PloS one* 12: e0188795.
- Pickrell, J. K., and J. K. Pritchard. 2012. Inference of population splits and mixtures from genome-wide allele frequency data. *PLoS genetics* 8: e1002967.
- Ramsey, J. 2011. Polyploidy and ecological adaptation in wild yarrow. *Proceedings of the National Academy of Sciences of the United States of America* 108: 7096–7101.
- Roddy, A. B., G. Thérout-Rancourt, T. Abbo, J. W. Benedetti, C. R. Brodersen, M. Castro, S. Castro, et al. 2020. The Scaling of Genome Size and Cell Size Limits Maximum Rates of Photosynthesis with Implications for Ecological Strategies. *International journal of plant sciences* 181: 75–87.
- Ronfort, J., E. Jenczewski, T. Bataillon, and F. Rousset. 1998. Analysis of population structure in autotetraploid species. *Genetics* 150: 921–930.
- Skotte, L., T. S. Korneliussen, and A. Albrechtsen. 2013. Estimating individual admixture proportions from next generation sequencing data. *Genetics* 195: 693–702.
- Stull, G. W., X.-J. Qu, C. Parins-Fukuchi, Y.-Y. Yang, J.-B. Yang, Z.-Y. Yang, Y. Hu, et al. 2021. Gene duplications and genomic conflict underlie major pulses of phenotypic evolution in gymnosperms. *bioRxiv*: 2021.03.13.435279.
- Thébault, A., F. Gillet, H. Müller-Schärer, and A. Buttler. 2011. Polyploidy and invasion success: trait trade-offs in native and introduced cytotypes of two Asteraceae species. *Plant Ecology* 212: 315–325.
- Van de Peer, Y., E. Mizrahi, and K. Marchal. 2017. The evolutionary significance of polyploidy. *Nature reviews. Genetics* 18: 411–424.
- Weaver, J. E., and T. J. Fitzpatrick. 1932. Ecology and Relative Importance of the Dominants of Tall-Grass Prairie. *Botanical gazette* 93: 113–150.
- Wood, T. E., N. Takebayashi, M. S. Barker, I. Mayrose, P. B. Greenspoon, and L. H. Rieseberg. 2009. The frequency of polyploid speciation in vascular plants. *Proceedings of the National Academy of Sciences of the United States of America* 106: 13875–13879.

Biographical Sketch

Alyssa Phillips

Department of Evolution and Ecology, University of California, Davis
arphillips@ucdavis.edu • (952) 451-0856

A. Education

University of California, Davis	PhD in Plant Biology	2018-Present
Appalachian State University	BS in Biology	2014-2018

B. Selected Awards and Fellowships

Davis Botanical Society Student Research Grant (\$2000)	2020
Dean's Distinguished Graduate Fellowship, UC Davis	2018
Outstanding Senior for Biology, Appalachian State University	2018
Undergraduate Research Assistantship, Appalachian State University	2017
Appalachian State Honors College Research Grant (\$500)	2017

C. Publications

Phillips AR*, Seetharam A*, AuchBuchon-Elder T, Gillespie LJ, Soreng RJ, Kellogg EA, Romay MC, Buckler ED, Ross-Ibarra J, Hufford MB. Genome assembly and annotation of the economically important turfgrass *Poa pratensis*. Manuscript in preparation.

* authors contributed equally to this work

D. Selected Service and Outreach

Girls Outdoor Adventure in Leadership and Science (GOALS)	2020-Present
– GOALS is a free summer science program for high school girls and gender-expansive youth to learn science hands-on while backpacking through Sequoia National Park.	
Holistic Review Admissions Committee	2020-Present
Plant Biology Graduate Group, UC Davis	
– Currently developing a Holistic Review admissions system for the Plant Biology Group.	
Plant Biology Graduate Group First-Year Mentor, UC Davis	2019-Present
– Provide guidance and advice to a first-year graduate student on how to make the most of their lab rotations and general graduate school strategies.	
Co-chair of Advocacy Committee	2019-2020
Plant Biology Graduate Group, UC Davis	
– One of two elected in the Plant Biology Graduate Group to serve as representatives in the Graduate Student Association.	
Cell Lab Volunteer, Science Museum of Minnesota	2016
– Lead children through hands-on science experiments such as identifying unknown bacteria and examining their cheek cells.	

Diversity and Inclusivity in Botany

Although I was drawn to mathematics and the sciences in high school, it wasn't until I had my first science class taught by a woman teacher in my junior year that I considered pursuing a STEM career. During my undergraduate, I learned this was a common experience for women and historically underrepresented groups. As a result, I became involved with multiple initiatives focused on increasing diversity and inclusion in STEM.

One organization that I currently volunteer with is GOALS¹ (Girls Outdoor Adventure in Leadership and Science), a free summer science program for high school girls and gender-expansive youth to learn science hands-on while backpacking. GOALS works to empower our diverse scholars as leaders, scientists, and outdoors people. I have helped develop curriculum for this year's virtual program, update program admissions process, and create an informational video for program advertisement.

Additionally, I am working with fellow graduate students in the UC Davis Plant Biology Graduate Group to restructure our admissions process to be a holistic review that emphasizes leadership skills, rewards service, and acknowledges adversity applicants may have needed to overcome.

As shown through the examples above, I am committed to increasing diversity and improving inclusivity in Botany and STEM.

¹ <https://girlsoutdoorscience.com/>