**Preliminary Major Goals and Research Questions – GRFP Proposal Draft**

**Project Overview:**  
As part of the Ten Thousand Oaks Project, this research will investigate how **somatic mutations**, **standing genomic variation**, and **phenotypic plasticity** contribute to **drought adaptation** in *Quercus garryana* (Oregon white oak). Understanding how genetic diversity within and among individuals shapes adaptive potential is essential to predicting population responses to climate change and guiding future restoration and remediation strategies.

**Major Goals:**

1. **Detect and characterize somatic genetic variation** within individual oak trees and evaluate its potential contribution to local adaptation.
2. **Quantify genomic offsets** to assess how current genetic variation aligns with future climate conditions, particularly increasing drought stress.
3. **Disentangle the roles of genetic variation versus phenotypic plasticity** in shaping drought responses at early life stages.
4. **Inform admixture-based remediation strategies** by identifying genotypes and mechanisms that enhance drought resilience.

**Guiding Research Questions:**

* Do individual trees accumulate distinct somatic mutations across different branches, and could these mutations generate novel adaptive variation?
* How does genomic variation among seedlings influence their morphological and physiological responses to drought conditions?
* What proportion of drought adaptation is driven by fixed genetic differences compared to phenotypic plasticity?
* Can genomic offset analyses predict which populations or genotypes are most vulnerable under future climate scenarios?
* How might identified drought-resilient genotypes inform admixture-based conservation strategies for *Q. garryana*?

**Experimental Approach (brief):**  
Seedlings from natural populations will be grown under contrasting water regimes (drought vs. well-watered) to measure variation in morphological and physiological traits. Whole-genome and GBS sequencing (~96 individuals, ~4,000 loci) will link genetic variation, including somatic mutations, to observed drought responses. These results will help clarify the genetic basis of drought adaptation and support broader Ten Thousand Oaks Project goals related to restoration and long-term population persistence.

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Somatic mutations, traditionally regarded as errors of cellular replication, are increasingly recognized as potential biological indicators of adaptive capacity in long-lived organisms. In perennial trees such as the Oregon white oak (Quercus garryana), somatic mutations can accumulate across different branches over decades, producing intra-individual genetic variation that may contribute to resilience under environmental stress. By assessing somatic mutations as biological indicators, we can gain insight into microevolutionary processes that operate within individual trees, complementing population-level studies of genetic variation. This perspective is particularly relevant in the context of drought, a growing threat to forest ecosystems in the Pacific Northwest, where prolonged periods of water scarcity challenge the survival of keystone species like Q. garryana. Understanding the role of somatic variation in drought adaptation not only advances basic ecological and evolutionary knowledge but also has direct applications for conservation and management strategies aimed at enhancing climate resilience.

The Oregon white oak is a foundation species whose presence structures communities and supports biodiversity across its range in the Pacific Northwest. Despite its ecological importance, populations are increasingly threatened by drought, habitat fragmentation, and shifting climate patterns. Adaptation in long-lived trees is a slow process, often constrained by the timescales required for generational turnover, making it crucial to identify sources of genetic and phenotypic variation that allow trees to cope with rapid environmental change. While previous research has characterized population-level genomic diversity in oaks, the potential adaptive contributions of somatic mutations remain largely unexplored. At the same time, phenotypic plasticity—the ability of individual trees to adjust growth and physiology in response to environmental conditions—likely plays a complementary role in drought resilience. The Ten Thousand Oaks Project aims to integrate these perspectives by evaluating how standing genomic variation, somatic mutations, and phenotypic plasticity collectively shape the capacity of Q. garryana to withstand drought stress across its range.

This project is guided by four central questions. First, do individual trees accumulate distinct somatic mutations across branches, and can these mutations generate novel adaptations for drought resilience? Second, what is the relative contribution of standing genomic variation compared to phenotypic plasticity in observed drought tolerance? Third, can genomic offset analyses predict which population structures are most vulnerable under future climate scenarios? Finally, how can genomic offset analyses inform conservation strategies to enhance the climate resilience of Oregon white oak populations? Addressing these questions requires a multi-scale approach that combines fieldwork, controlled experiments, high-throughput genomic sequencing, and computational modeling.

To evaluate somatic variation, I will collect branch samples from individual trees across the species’ range, representing a diversity of climatic and geographic conditions. Partial-genome sequencing using Illumina technology will allow identification of somatic mutations, which will be mapped to functional genomic regions associated with drought tolerance. By comparing mutation patterns among branches within single trees, I can quantify intra-individual genetic heterogeneity and assess whether somatic mutations provide adaptive potential that could buffer against environmental stress. These data will provide a novel perspective on the evolutionary significance of somatic mutations in long-lived plants, positioning them as measurable indicators of an individual tree’s capacity to respond to drought.

Phenotypic plasticity will be assessed through greenhouse experiments in which seedlings from multiple populations are grown under controlled drought and well-watered conditions. Traits related to growth, water-use efficiency, and stomatal regulation will be measured to quantify plastic responses. This approach will enable the disentanglement of genetic versus environmental contributions to drought tolerance and help determine whether phenotypic plasticity can compensate for the limits of standing genetic variation. Integrating these experimental results with somatic mutation data will provide a comprehensive understanding of the mechanisms by which individual trees and populations respond to drought stress.

Genomic offset analyses will be applied to evaluate population vulnerability under projected climate change scenarios. By linking existing population genomic data with spatially explicit environmental variables derived from Geographic Information Systems (GIS), genomic offsets quantify the mismatch between current genetic variation and future climatic conditions. This approach identifies populations at greatest risk from climatic shifts and enables predictions of which genetic lineages may harbor the adaptive potential to persist. Importantly, incorporating information on somatic mutations into genomic offset models may reveal previously unrecognized sources of resilience within individual trees that could inform conservation priorities and management interventions.

The integration of high-throughput genomics, experimental phenotyping, and spatial modeling will be supported by high-performance computing resources, allowing large datasets to be processed and analyzed efficiently. This multi-disciplinary approach will produce a comprehensive view of adaptive capacity in Q. garryana, linking fine-scale somatic variation with population-level genetic structure and environmental vulnerability. By combining empirical and predictive approaches, this project will generate actionable insights for conservation management, such as identifying populations that could serve as genetic reservoirs for restoration efforts or guiding assisted migration strategies under climate change.

Expected outcomes of this research include a quantitative assessment of somatic mutation accumulation in long-lived trees, a detailed understanding of the relative contributions of standing genetic variation and phenotypic plasticity to drought resilience, and identification of populations most vulnerable to climate stress. Furthermore, this work will advance the broader application of genomic offset analyses in conservation biology by integrating intra-individual variation, which has often been overlooked in predictive models. Beyond scientific contributions, the project has broader societal implications, providing tools for forest managers, conservation practitioners, and policymakers seeking to maintain resilient ecosystems in the face of climate change.

In summary, the Ten Thousand Oaks Project seeks to illuminate the complex interplay of genetic and phenotypic mechanisms underlying drought adaptation in Oregon white oak. By treating somatic mutations as a biological indicator of adaptive potential, this research bridges molecular genetics, ecology, and conservation science. The project’s integration of field-based sampling, greenhouse experiments, high-throughput sequencing, GIS, and computational modeling provides a comprehensive framework to assess resilience across multiple scales. Ultimately, this work will enhance our understanding of how long-lived trees respond to environmental stress and inform strategies to preserve and restore keystone forest species under changing climatic conditions. By combining innovative genomics with applied conservation objectives, the Ten Thousand Oaks Project positions Q. garryana as both a model system for studying adaptation in long-lived plants and a focal point for climate-resilient forest management.

This project investigates how standing genomic variation, somatic mutations, and phenotypic plasticity interact to shape drought adaptation in *Quercus garryana* (Oregon white oak) across its range in the Pacific Northwest. The Oregon white oak is a keystone species that underpins the structure and function of oak savannas and woodlands throughout the region, yet it faces accelerating decline due to intensifying drought and habitat loss. Understanding the mechanisms that allow this long-lived species to persist under climatic stress is critical for both evolutionary biology and applied conservation. This study will focus on three primary questions: whether individual trees accumulate distinct somatic mutations across branches that may contribute to drought resilience, how much of the observed drought tolerance is attributable to standing genomic variation compared to phenotypic plasticity, and whether genomic offset analyses can identify populations most vulnerable under future climate scenarios.

The research will integrate genomic, environmental, and physiological data to reveal multi-level processes underlying adaptation. Field sampling will occur across the species’ full latitudinal and climatic gradient from southern British Columbia to northern California. Within each site, leaf and cambium tissues will be collected from individual trees, including multiple branches per tree to test for intra-organismal genetic differentiation. Environmental data such as precipitation, temperature, and soil moisture will be recorded for each location using GIS-based climate layers and on-site measurements. DNA will be extracted and sequenced using high-throughput Illumina platforms, targeting partial genomes to capture genome-wide patterns of polymorphism while maintaining efficiency across hundreds of samples. Read alignment, variant calling, and quality control will follow standard pipelines implemented in BWA and GATK. Within-tree variation will be analyzed to identify somatic mutations, and population-level variation will be examined using landscape genomic analyses to link allele frequencies with climatic variables associated with drought. Genomic offset models will be applied to evaluate the extent to which current population structures are mismatched with future projected conditions under climate change scenarios.

Complementary greenhouse experiments will assess the role of phenotypic plasticity in drought tolerance. Seedlings derived from genetically distinct populations will be grown under controlled drought regimes to measure variation in water-use efficiency, growth rates, leaf morphology, and photosynthetic performance. Combining these experimental data with genomic information will allow partitioning of drought resilience into genetic and plastic components, clarifying the degree to which adaptation in this species relies on evolutionary change versus physiological flexibility. The integration of high-throughput sequencing, environmental modeling, and experimental physiology will generate a comprehensive view of adaptive potential in a dominant tree species of the Pacific Northwest.

**Intellectual Merit**

This research will advance understanding of how multiple evolutionary mechanisms interact to influence adaptation in long-lived plants. While standing genomic variation and phenotypic plasticity are established contributors to adaptive capacity, the role of somatic mutation in generating functionally relevant variation within individual trees remains poorly understood. By explicitly quantifying somatic variation and its potential connection to drought resilience, this project extends evolutionary theory beyond population-level processes to include intra-organismal sources of novelty. Integrating genomic offset analysis provides a predictive framework for assessing vulnerability under climate change and contributes to methodological innovation in conservation genomics. The combination of bioinformatics, landscape ecology, and experimental physiology represents an interdisciplinary approach that strengthens the mechanistic basis of adaptation research. Findings will contribute to both basic evolutionary biology and applied conservation strategies, while all data and analytical workflows will be openly shared through public repositories to enable reproducibility and future collaboration.

**Broader Impacts**

The outcomes of this research have direct implications for forest conservation and climate adaptation across the Pacific Northwest. By identifying populations of *Q. garryana* with the greatest adaptive potential and predicting those most vulnerable to climatic shifts, this work will inform ongoing restoration and genetic remediation efforts coordinated through the Ten Thousand Oaks Project. Results will guide seed sourcing, admixture design, and assisted regeneration strategies aimed at sustaining oak ecosystems and the biodiversity they support. The project will also contribute to education and public engagement by involving undergraduate students in field collection, data analysis, and greenhouse experimentation, providing early-career training in genetics, ecology, and computational biology. Outreach activities in partnership with local conservation organizations will increase community understanding of how genomics informs ecosystem management. In addition to enhancing the scientific foundation for regional conservation, this research will build transferable models for integrating genomics into climate resilience planning across forest ecosystems. Through open data sharing, interdisciplinary training, and direct conservation application, the project will strengthen both the intellectual and societal foundations of adaptation genomics in a changing climate.

In plants, adaptive responses to environmental stress are shaped by three primary mechanisms: standing genomic variation, somatic mutation, and phenotypic plasticity. Standing variation provides the raw material for natural selection by maintaining allelic diversity across populations that experience heterogeneous environments. In tree species with long generation times and extensive gene flow, such variation can sustain adaptive potential even under rapid environmental change. However, selection alone may not act quickly enough to track the pace of climate change, prompting increasing interest in other sources of adaptive flexibility. One such source is phenotypic plasticity—the capacity of a single genotype to produce different phenotypes under variable environmental conditions. Plasticity can buffer populations against short-term stress, allowing persistence until genetic adaptation catches up. Yet the relative contribution of plasticity versus genetic differentiation to drought tolerance in Q. garryana has not been quantified.

A third, often overlooked mechanism is somatic mutation. In long-lived trees, somatic cells undergo thousands of mitotic divisions over centuries of growth, during which replication errors and environmental stress can introduce new mutations. These mutations may accumulate in different parts of the same individual, leading to genetic mosaicism within a single tree. If such mutations occur in tissues contributing to reproductive structures, they can enter the germline and contribute to heritable variation. While typically considered rare or neutral, recent evidence suggests that somatic mutations can produce functional changes that affect stress tolerance and fitness. This potential source of adaptive variation is especially relevant for species like Q. garryana, where individual trees can live for hundreds of years and experience multiple environmental regimes during their lifespan. Testing whether somatic mutations play a measurable role in drought adaptation could redefine how we understand the evolutionary dynamics of long-lived plant species.

***Intellectual merit***

The Ten Thousand Oaks Project—a large-scale collaborative effort led by Portland State University—provides the framework for addressing these questions. By integrating population genomics, environmental modeling, and experimental physiology, this research will examine how different adaptive mechanisms interact to shape drought resilience across Q. garryana populations. Field sampling will capture genetic and environmental variation across the species’ entire range, from the humid coastal regions of northern California to the dry interior valleys of southern British Columbia. Genomic data derived from high-throughput Illumina sequencing will enable detection of both population-level polymorphisms and within-tree somatic variants. Using environmental association analyses and genomic offset models, we will assess how existing genomic structures align with present and projected climate conditions, identifying populations most vulnerable to future drought stress. Complementary greenhouse experiments will expose seedlings from diverse genotypes to controlled drought regimes to measure physiological traits associated with water use efficiency and stress tolerance.

***Broader impacts***

Together, these approaches will provide an integrated view of how Q. garryana responds to drought at genetic, physiological, and ecological scales. The results will advance understanding of tree adaptation by disentangling the relative contributions of inherited genetic variation, newly generated somatic diversity, and plastic responses to environmental stress. Beyond contributing to evolutionary theory, this work will generate actionable data for conservation practitioners seeking to maintain or restore climate resilience in oak-dominated ecosystems. By identifying genotypes and populations most capable of persisting under future drought conditions, the research will directly inform management decisions aimed at safeguarding one of the Pacific Northwest’s most ecologically significant tree species.

***Literature cited***

**Workflow overview:**

* Field sampling of ~20 trees per population across climatic gradients.
* Multi-tissue sampling per tree for somatic mutation detection.
* DNA extraction and Illumina WGS or exome sequencing.
* SNP calling, somatic mutation identification, and quality control.
* Genotype–environment association and genomic offset analyses.
* Greenhouse drought experiments to assess phenotypic plasticity.
* Integration of genomic, environmental, and physiological data in statistical models.
* Functional annotation of adaptive loci and application to conservation planning.