DOES DROUGHT RESPONSE CHANGE WITH PARENTAL ANCESTRY ACROSS A

WHITE PINE HYBRID ZONE?

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ABSTRACT

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Hybrid populations of two white pine species, *Pinus strobiformis* and *Pinus flexilis*, are found throughout the southwestern United States. Understanding characteristics of hybrid populations will support efforts to conserve natural genetic diversity as trees adapt to climate change. Using the concept of a "dendrophenotype," or observed variation in tree-ring size that corresponds to a particular stress event in a tree's life, we calculated four indices of drought response using tree-ring data from nine hybrid geographic sites across Colorado, New Mexico, Arizona, and Texas. We used these indices in combination with hybrid index values (percentage of *Pinus strobiformis* ancestry inherited by a single hybrid) for sampled individuals to (1) examine relationships between climate, tree-ring sensitivity, and hybrid index, (2) test for relationships between tree-ring-based drought indices (resistance, recovery, resilience, and a Wet:Dry ratio) and hybrid index, and (3) develop multivariate models that predict drought response based on hybrid index and other variables. We found (1) significant correlation between monsoon index and hybrid index, monsoon index and tree-ring sensitivity, and hybrid index and tree-ring sensitivity in sampled populations, (2) little direct connection between hybrid index and four tree-ring-based drought indices, and (3) no predictive relationship between hybrid index and the drought indices, but stronger relationships may be found using different variables. Validation of a relationship between hybrid index and the annual monsoon cycle points to a future genotype-phenotype analysis that could use existing, fine-scale genetic data to further explore the effects of water availability on the inheritance of adaptive traits in hybrid populations.

Available literature indicates that the intersection of dendrochronology and genomics will likely be a useful tool for understanding inheritance in hybrid PIST / PIFL populations, especially for the purpose of mapping the genetic architecture of adaptive traits, creating landscape-level biosurveillance efforts, and developing strategic breeding programs.

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ACKNOWLEDGEMENTS

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CHAPTER ONE

INTRODUCTION

In the American Southwest, climate-disturbance relations influence the age structures and composition of woodland and conifer forests, as evidenced by tree-ring based reconstructions of drought, disturbance history, and tree demography (Swetnam and Betancourt 1998). For water-limited regions such as the Southwest, drought impacts in particular are severe for forest ecosystems (Allen and Breshears 1998). For southwestern white pine (*Pinus strobiformis),* predictions of warmer, drier climate with increasing drought frequency (Seager and Vecchi 2010) are concerning, given that competitors such as Douglas-fir (*Pseudotsuga menziesii)* and Ponderosa pine (*Pinus ponderosa)* are less sensitive to drought (Barton and Teeri 1993), and that southwestern white pine's disjunct, high-elevation distribution prevents that species from dispersing (Shirk et. al. 2018). Competitive exclusion of southwestern white pine from the Southwest would decrease biodiversity in mixed-conifer ecosystems of the Sky Islands, remove a critical food source for local wildlife (large, nutritious seeds), and increase watershed vulnerability (Geils, Hummer, and Hunt 2010). Therefore, it is crucial to develop methods for conserving adaptive traits in southwestern white pine so that ecosystem benefits conferred by that species are not lost in a drying future.

Southwestern white pine vulnerability may be reduced by its hybridization with the limber pine (PIST; *Pinus flexilis E. James),* a species that shares a portion of its range in the western United States (Tomback et. al. 2011; Steele 1990). The two species are primarily differentiated by mean average temperature, which is cooler in PIST's range (Menon et. al. 2021), but it is known that the hybrid zone is shifting northwards into PIFL's core range (Menon et. al. 2020). Hybrids are characterized as having generally PIST-dominant genomic

backgrounds that are bolstered by freeze and water-availability related SNPs of PIFL ancestry (Menon et. al. 2021). While PIST is known to use water efficiently under moisture stress (Goodrich et. al. 2015) and PIFL is a generalist ecosystem pioneer that survives on harsh, xeric sites (Schoettle & Rochelle 2000; Windmuller-Campione & Long 2016), it is unknown how varying ancestry throughout the hybrid zone influences hybrid performance in drought conditions.

To understand how hybrids will fare under a northward range shift, it is important to elucidate the mechanisms of drought tolerance in hybrids while considering how hybrid ancestry influences such mechanisms across the hybrid zone. One useful means of evaluating drought performance in trees is dendrochronology. Studying growth over time via tree rings allows researchers to understand how genetic composition, climate patterns, and local environment influence an individual tree over the course of its lifetime. For a stress event such as drought, having a lifelong time chronology of growth data is necessary given that there are often lagging consequences of drought that cannot be understood from drought-year growth alone. Pairing signatures of drought performance before, after, and during drought events with genotypic information relevant to water conservation or biological stress response can reveal patterns of inheritance of drought-adaptive traits in hybrid populations. These patterns can be used to understand seedling transfer guidelines, breed drought-resistant seedlings, and best plan for the management of PIST, PIFL, and hybrid offspring as the hybrid zone moves northward.

The purpose of this thesis is to analyze and compare hybrid drought response to five severe drought events from the past 75 years (1950 – 2015) among nine distinct populations within the hybrid zone. We used both relative and absolute measurements of hybrid performance during, before, and after drought, along with hybrid indices and environmental covariates, to

develop four models of hybrid drought response. The second chapter of this thesis is a literature review of information relevant to the development of Chapter Three, a manuscript intended for peer-reviewed publication, in which our study design and results are discussed. This thesis concludes with a fourth chapter that addresses management implications of our results.

CHAPTER TWO

ADAPTATION BY INTROGRESSION IN A CHANGING WHITE PINE HYBRID ZONE:

A REVIEW

INTRODUCTION

Given future challenges presented by increased drought severity, the threat of non-native pathogens, and changes in annual precipitation, the intersection of dendrochronology and genetics will provide a useful avenue for understanding the genetic basis of drought response in *Pinus strobiformis / Pinus flexilis* hybrids. Despite living in a future that brings unexpected climate changes and unprecedented dryness, certain species of trees will grow more successfully in altered conditions than others due to heightened adaptive capacity (Aubin et. al. 2016). Two *Pinus* species, southwestern white pine (*P. strobiformis;* PIST*)* and limber pine (*P. flexilis;* PIFL*)* produce hybrid offspring that exhibit characteristics intermediate of both parents (Menon et. al. 2018). Known hybrids span the Davis Mountains of western Texas to the San Juan Mountains of southern Colorado (Menon et. al. 2018). However, adaptive introgression, which drives the formation of new gene combinations in hybrid populations, facilitates a northward shift in the hybrid zone (Menon et. al. 2020). Although previous work has characterized PIFL and PIST as isohydric (Schoettle & Rochelle 2000; Bucholz et. al. 2020), it is unknown how degree of hybrid ancestry affects the drought response of mature hybrids living in natural conditions.

The subduction of the Farallon plate beneath the Pacific plate occurred roughly thirty million years ago (Baldridge 2004). Over time, the motion of these tectonic plates slowly formed what is thought of as the modern southwestern United States: the Colorado Plateau, the Rocky Mountain range, and "sky islands," or relatively small mountainous oases, punctuating vast desert. Climate-disturbance relations across what will be referred to as the Southwest are complex and have altered forest age structure and composition through time (Swetnam and Betancourt 1998). Precipitation varies seasonally across the Southwest, with variable precipitation in the late winter and early spring followed by a dry, late spring and early summer, monsoonal rains in July and August, and a dry autumn (Swetnam and Betancourt 1998). Plant communities in the Southwest experience patterns of periodic recruitment and mortality, which are disturbed by increasing drought length (Betancourt et. al. 1993).
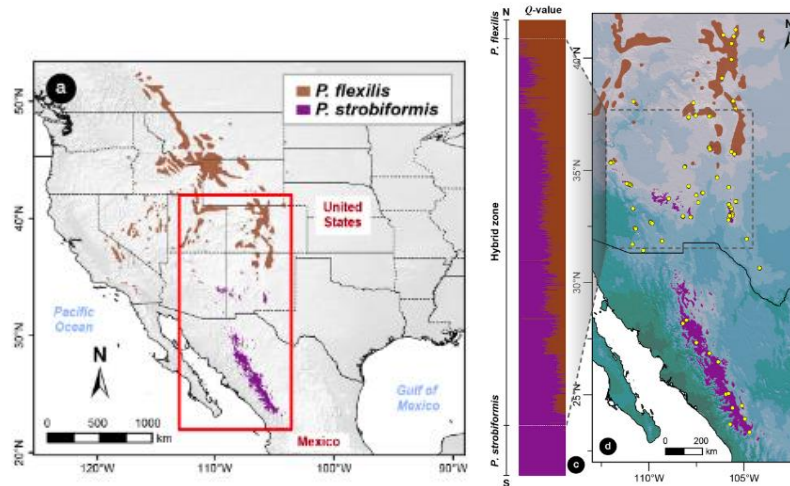
FIGURES 2.1a (L) / 2.1b (R). To the left, the contemporary ranges of *P. flexilis* and *P. strobiformis* are colored in brown and purple, respectively. The PIST / PIFL hybrid zone is boxed in red. To the right, the vertical bar shows the latitudinal distribution of hybrid ancestry, where brown indicates proportion of PIFL ancestry and purple indicates proportion of PIST ancestry.

Increased drought severity is a likely consequence of climate change in the Southwest, and contributes to climate vulnerability of southwestern forests (Thorne et. al. 2018). One useful tool for evaluating a forest's susceptibility to climate-driven change is forest genomics, through which genome- or exome- wide scans are implemented for the purpose of understanding variations in population-level genetic adaptation to climate and other biotic/abiotic stressors (Isabel, Holliday, & Aitken 2020). Understanding patterns of inheritance in forest trees has allowed researchers to perform biosurveillance and remote sensing operations in stands vulnerable to pest invasion, evaluate the effects of hybridization on a species' potential to experience a range shift, and unravel the details of conifer evolution (De La Torre et. al. 2019; Hamelin & Roe 2019; Menon et. al. 2019; Haagsma et. al. 2020). Overall, landscape genomics

will likely play a large role in forest conservation moving forward as it provides a means of rapidly evaluating the adaptive capacity of entire forests along with influential variables.

CHARACTERIZING PINUS STROBIFORMIS AND PINUS FLEXILIS

PINUS STROBIFORMIS

One species that may persist in a future that is climatically altered is southwestern white pine (*Pinus strobiformis;* PIST). PIST is commonly found in the Sierra Madre Occidental mountain range of western Mexico and the Rocky Mountains of Arizona and New Mexico (Shirk et. al. 2018). A member of the "high five," or a group of six five-needle white pine species native to western North America, PIST belongs to the genus *Pinus,* subgenus *strobis* (soft, pinyon and white pines)*,* which is characterized by female cone structure, seed morphology, and mode of seed distribution (Figure 1.2a**;** Geils, Hummer, & Hunt 2010; Vasilyeva & Goroshkevich 2018). The white pines of western North America, such as PIST, fill important ecological roles by distributing large, nutritious seeds to birds and mammals, increasing biodiversity, and protecting local watersheds (Geils, Hummer, & Hunt 2010). Unfortunately, all white pines of North America are also threatened by white pine blister rust (WPBR), a forest disease caused by the non-native fungal pathogen *Cronartium ribicola* (Conklin et. al. 2009). However, a major gene for resistance (Cr2) has been identified in PIST, which stops the progression of WPBR in the needles, preventing stem infection (Kinloch et. al. 2003; Kinloch et. al. 2004; Sniezko, Kegley, & Danchok 2008). In open, dry, mixed-conifer environments that are suitable for Douglas-fir (*Pseudotsuga menziesii)* or ponderosa pine *(Pinus ponderosa)* regeneration, PIST also regenerates well, especially in places where that species can already be found in the overstory

(Goodrich & Waring 2017; Goodrich et. al. 2018). Management of PIST has a negative impact on abundance of WPBR, likely due the capacity for silvicultural treatments to influence microclimate, which is important to the reproductive cycles of *Cronartium ribicola* (Geils, Hummer, & Hunt 2010; Goodrich et. al. 2018). Although the species is not heavily researched due to its low timber value and general rareness in mixed-conifer forests of the Southwest, it is known that PIST exhibits a variety of characteristics that make it well adapted for a warmer, drier future (Conklin et. al. 2009; Looney & Waring 2013). For one, mature PIST is as resistant to surface fire as *Pinus ponderosa* and may even survive incidences of fire from a young age (Sakulich & Taylor 2007). PIST also has been shown to use water more efficiently with increasing moisture stress, making it a compatible species for a drier future (Goodrich et. al. 2015).

PINUS FLEXILIS

Like PIST, PIFL is a five-needle white pine that exists across a broad latitudinal gradient from northern New Mexico to Alberta, Canada (Sindewald et. al. 2020). In the northern Rocky Mountains and further west of that range, PIFL is commonly found at low elevations with whitebark pine (Burns & Honkala 1990). In contrast, PIFL grows at higher elevations in the southern Rocky Mountains and hybridizes with PIST in the southern portion of that species' range (Menon et. al. 2019). Due to a wide range of physiological tolerance, PIFL often grows successfully regardless of surrounding environment and can survive on harsh, xeric sites (Schoettle & Rochelle 2000). PIFL trees have light grey bark that become scaly and ridged over time, as well as conic crowns that round with age (Little 1980; Kral 1993). The term "flexilis" comes from the flexibility of PIFL branches, and also reflects the species' ability to contort into krummholz form at timberline. PIFL also shares a commonality with PIST: susceptibility to

white pine blister rust (Sniezko et. al. 2016). In fact, PIFL was designated as endangered in 2014 in Canada by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC). Despite a future of health challenges provoked by WPBR, PIFL exhibits a range of advantageous qualities and may actually thrive in a changing climate. First, major gene resistance for WPBR has been identified in populations of PIFL in Canada, and the species is considered a generalist, meaning that it can tolerate both arid conditions and poorly developed soils (Windmuller-Campione & Long 2016; Moreno-Letelier, Ortíz-Medrano, & Pinero 2013). Furthermore, PIFL shares a mutualistic relationship with the Clark's nutcracker, which can cache PIFL seeds more than 20km from a source tree (Figure 2.2b; Tomback and Linhart 1990; Tomback et. al. 2005). Seed caching by the nutcracker fosters PIFL regeneration, which in turn creates high elevation tree islands that serve as nurse structures for other plants, influencing stand structure and composition (Myers and Harms 2011). PIFL is an excellent ecosystem pioneer, as its shade intolerance causes it to be easily replaced by other neighboring species, excluding quaking aspen *(Populus tremuloides)* and Rocky Mountain juniper (*Juniperus scopulorum)* (Layser & Schubert 1979). Relatively little research exists on PIFL because white pines have low timber value and therefore their growth and ecology has not been emphasized in past studies (Windmuller-Campione & Long 2016).

FIGURES 2.2a (L) and 2.2b (R): Immature cones of PIST (L; 2.2a) and PIFL (R; 2.2b).

COMPARING DROUGHT RESPONSES IN PIST AND PIFL

Although some effects of drought on hybrid growth can be inferred by understanding response to drought in parent species, more research is needed to clarify the influence of hybridization on drought response across the hybrid zone. Like many pine species, PIST is isohydric, or drought-avoidant, and exhibits greater drought avoidance with increased elevation. This is evidenced by decreasing water potential with decreasing elevation and low PISTΔ^{13}C in warm, dry conditions at low elevation (Bucholz et. al. 2020). Δ^{13}C, or carbon isotope discrimination, is the process of determining how much of the "heavy" isotope ^{13}C is present in matter. Generally, an inverse relationship is observed between water use efficiency and Δ^{13}C, indicating that greater water use efficiency leads to discrimination against ^{13}C (Farquhar et. al. 1982). In PIFL, stomatal density and per-leaf stomata count decrease with elevation (Schoettle & Rochelle 2000). Stomatal density is correlated with conductance of water vapor (Nobel 1983). Given a negative relationship between stomata count and elevation, PIST may face greater

challenges to moisture balance at low elevation. Furthermore, recent work using a genotype-environment association approach revealed signatures of adaptive evolution across gradients of water availability across the hybrid zone (Menon et. al. 2021), suggesting that one function of hybridization is bolstered drought performance.

IMPACTS OF BLISTER RUST AND TREATMENT AVENUES

As climate change threatens to alter the distributions of PIST, PIFL, and their hybrid offspring, it is important to understand existing pressures on PIST and PIFL for developing proper management in a compromised environment (Shirk et. al. 2018; Menon et. al. 2019). One such pressure, as mentioned previously, is the spread of the fungal pathogen *Cronartium ribicola* J.C. Fischer from Asia into North America (Geils, Hummer, & Hunt 2010). *C. ribicola,* which causes WPBR in susceptible white pine populations, requires two hosts to complete its life cycle: white pines, and plants of the genus *Ribes, Pedicularis,* and *Castilleja* (Spaulding 1911). After the initial stage of spermagonium formation occurs on an infected white pine, aeciospores are produced, which disperse from the pine to infect surrounding alternate hosts (Klebahn 1889). Infected alternate hosts may then release uredinospores, which infect other local alternate hosts. After several generations of uredinospore dispersal, hair-like telia form on the leaves of alternate hosts and release basidiospores, which re-infect white pines and initiate the infection cycle once again.

While management has greatly helped to control the spread of WPBR in eastern white pine populations, management for WPBR has not been as successful in white pine populations in the west (Kinloch 2003; Geils 2010). New-Deal-era *Ribes* eradication efforts of the early

twentieth century, although moderately successful, did not impactfully limit the spread of WPBR into additional populations of western white pine (*P. monticola)* or into populations of other white pine species in the west such as PIST, PIFL, or sugar pine (*P. lambertiana)* (Schwandt et. al. 2010; Zeglen et. al. 2010). Genetic research and silviculture are utilized today to successfully bolster some white pine populations such as *Pinus monticola* or *Pinus lambertiana* against the threat of *C. ribicola* (Schoettle & Sniezko 2007; Weiss et. al. 2020; Bronson et. al. 2018).

One positive achievement that has been made towards protecting white pines from unsustainable WPBR infection is the discovery of complete resistance. The Cr3 gene in southwestern white pine (Kinloch & Dupper 2002) and the Cr4 gene in limber pine (Schoettle et. al. 2014), as these major genes for resistance have been termed, confer a hypersensitive reaction to both species which confines WPBR to the needles, impeding stem infection (Sniezko et. al. 2014). Quantitative disease resistance to WPBR, which allows for fewer / latent stem infections and higher survival of cankered trees, has also been observed in seedlings of both PIST and PIFL, although the underlying genetic mechanisms of this type of resistance are less understood (Sniezko et. al. 2010; Stephan 1986; Sniezko & Kegley 2001). This type of resistance may be more durable in the long-term, as the pathogen *C. ribicola* cannot easily develop virulence to a polygenic form of resistance.

CONIFER EVOLUTION

Conifers are known for their adaptability in both temperate and boreal forest systems, as well as their provision of ecosystem services (Prunier, MacKay, and Verta 2016). The lifespan of a conifer may last thousands of years, a testament to the conifer's ability to exhibit trait plasticity

and resist biodegradation (Loehle 1987; Prunier, MacKay, and Verta 2016). Such longevity is also evident in the conifer's inability to eliminate gene copies or gene-like sequences that are useless (Nystedt et. al. 2013; Neale et. al. 2014; Morgante & DePaoli 2016). Therefore, it is not surprising that conifers evolve slowly due to such accumulation of genetic material, or that many contemporary conifer genomes contain much ancient genetic material (Pary et. al. 2012). The slow evolution and mutation rate of conifers is supported by a high degree of hybridization (particularly in North American pine populations), which shows that some modern pine species still lack reproductive isolation mechanisms that more quickly evolving tree species may possess (Leitch & Leitch 2012; Buschiazzo et. al. 2012).

CHARACTERIZING HYBRID OFFSPRING

The PIST / PIFL hybrid zone, an example of limited reproductive isolation, was first described in 1971 by Engelmann, and has since been corroborated by both morphological and genetic data (Figure 2.3; Bisbee 2014; Menon et. al. 2019; Tomback et. al. 2011). Although both species grow naturally at mid-to-high elevations, they occupy distinct geographic spaces, with PIST ranging from southern Colorado to southern Mexico and PIFL ranging from the southwestern U.S. to Alberta, Canada) (Windmuller-Campione & Long 2016; Tomback et. al. 2011). A study investigating the hybrid index of PIST / PIFL hybrids, defined from this point as the percentage of ancestry conferred to an individual hybrid by PIST, revealed that the average PIST / PIFL hybrid exhibits a majority of genetic ancestry derived from PIST (Menon et. al. 2019). This is unusual, considering that even hybrids that are geographically nearer to PIFL consistently receive mid-to-high hybrid index scores, as opposed to seeing a uniform gradient of hybrid index moving from PIST → PIFL (Menon et. al. 2019). Furthermore, the index of PIST /

PIFL hybrids is strongly influenced by latitude and climate, suggesting that there should be a

discernable relationship between level of hybridization (hybrid index), location of origin, and

climate variables such as precipitation (Aguirre-Gutierrez et. al. 2015; Menon et. al. 2020).
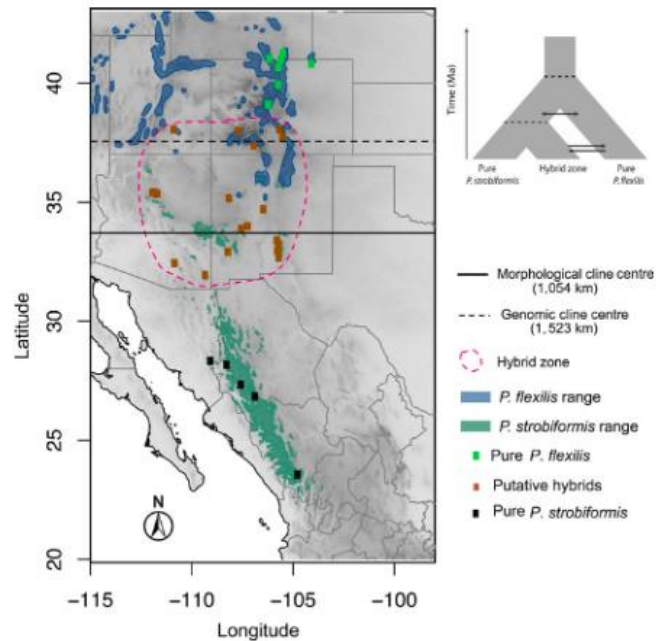
FIGURE 2.3: the overlapping ranges of PIST and PIFL in the Southwest, with the hybrid zone

represented by brown squares circled by a pink dashed line. The figure included to the upper

righthand corner represents the locations of the cline centres, estimated from morphological

(bold line) and genomic (dashed line) data (Figure from Menon et. al. 2020).

One complication that arises when disentangling the relationship between hybrid index,

provenance, and climate is evidence that the PIST / PIFL hybrid zone is not stagnant. In fact, the

hybrid zone is likely expanding northward due to adaptive introgression, leading to a prominence

of hybrids with PIST – dominant genomic backgrounds bolstered by ancestral PIST single-

nucleotide polymorphisms (SNPs)f related to gradients of water availability (Menon et. al. 2018; Menon et. al. 2020). This idea becomes more compelling given the rapidly changing climate of the Southwest, as well as the fact that PIST / PIFL hybrids are known to diverge along a drought gradient within the hybrid zone (Menon et. al. 2019; Allen & Breshears 1998; Gitlin et. al. 2006). Given this evidence, hybrids may exercise a drought advantage if the northward expansion of the hybrid zone will lead to a) wetter, more comfortable growing conditions for hybrids living in the southern portion of the hybrid zone, and b) genetic makeup that confers a combination of adaptive traits from both parent species.

DROUGHT, DENDROECOLOGY, AND GENETICS

Dendroecology, the application of tree-ring analysis for understanding the ecological forces that influence tree growth, links patterns of climate with variations in wood anatomy (Housset et. al. 2018; Cook & Kairiukstis 1990; Girardin et. al. 2016; Hartmann & Trumbore 2016). A tree core, which can be obtained non-destructively, provides a lifelong record of a tree's growth. By quantifying yearly variations in tree-ring width and relating those variations to existing records of climate (precipitation; temperature; topographic wetness; drought indices), dendroecologists may understand how a tree's growth varies with time-sensitive stress incidents. Ultimately, dendroecology identifies growth-limiting ecological factors and the exact times at which they strongly (or weakly) affect tree growth (Fritts 1976; Girardin et. al. 2012). Recently, researchers have started to consider variations in tree-ring growth in relation to the timing of climatic constraints as a new class of phenotypes. These phenotypes, termed "dendrophenotypes," can be used to associate variation in growth with heritable traits via quantitative genetics (Figure 2.4; Housset et. al. 2018; Heer et. al. 2018). Dendrophenotypes are

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useful for characterizing long-term growth traits in trees because no punctual measurements are required for dendrochronological analysis to ensue other than collection of cores, and because the occurrence of dendrophenotypes may be tested against the presence of single-nucleotide polymorphisms linked to stress response or climate (Housset et. al. 2018).
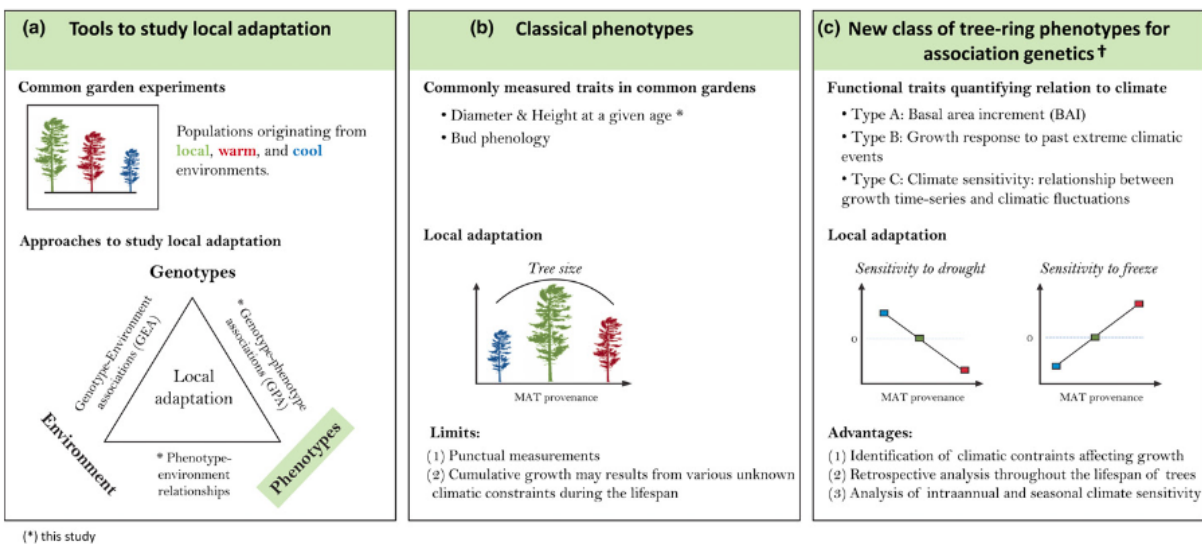
FIGURE 2.4: (a) tools for linking local adaptation, genetics, and environments (triangle adapted from Sork et. al. 2013); (b) dendrometric traits classically studied in dendroecology / forest genetics; (c) a new class of tree-ring phenotypes, to be used for association genetics & validation of candidate genes. (from Housset et. al. 2018). The novelty of this "new class of tree-ring phenotypes" is the application of such phenotypes to genetic sequences identified in the same populations from which tree rings measurements were derived.

Ultimately, a study that evaluates the effects of growth during a stress event such as drought would aim for a combination of methods that are ideal to use for growth analyses, take

into consideration growth rates prior to and after a stress event, and consider stress response from both relative and absolute perspectives. Drought has been studied frequently in dendroecology (e.g. Gonzalez-Casares et. al. 2016; Fuchs et. al. 2019; Bickford et. al. 2011), often studied alongside elevation (Bickford et. al. 2011; Adams & Kolb 2005; McDowell et. al. 2010). There are a variety of ways to characterize drought in dendrochronological studies (Eilmann et. al. 2006; Gonzalez – Casares et. al. 2016; Bickford et. al. 2011; Fuchs et. al. 2019; Hess & Fule 2020; Erickson & Waring 2014). Some studies use a drought index such as monthly SPEI (Standardized Precipitation – Evapotranspiration Index), which considers the effects of temperature on evapotranspiration rate, or PDSI (Palmer Drought Severity Index), a regional drought index based on precipitation and potential evapotranspiration (Palmer 1965; Vincente-Serrano et. al. 2010; Erickson & Waring 2014; Gonzalez-Casares et. al. 2016). Drought indices like PDSI are more useful than evaluations of drought based on precipitation alone because they incorporate the effects of time on the lagging consequences of a dry period on water storage. Others opt to use measurements of precipitation to define drought that are directly related to tree-ring growth based on the fact that ring growth is strongly influenced by the previous winter / current growing season's precipitation (Fuchs et. al. 2019; Hess & Fule 2020). Therefore, precipitation can by sorted by "water year" (spanning from October 1st – September 30th of the current growing year), and particularly dry or wet years are identified. There are also several ways to assess the effects of drought on ring growth in dendrochronology, one of which is a "wet:dry analysis" (Bickford et. al. 2011; Hess & Fule 2020). Through this method, the average RWI (ring width index) for a selected period of wet years is divided by the average RWI for a selected period of dry years for each tree included in a study (Fuchs et. al. 2019). Thus, large wet:dry values indicate a notable difference in the growth that occurs during wet years vs. during

dry years (Bickford et. al. 2011). Other studies opt to use an entire multi-decadal range of drought data to examine growth rather than selecting specific drought years (Gonzalez-Casares et. al. 2016).

THE INTERSECTION OF GENOMICS AND DENDROECOLOGY

Before the term "dendrophenotype" was introduced in 2018, researchers looked for ways to associate heterozygosity, or individual genetic diversity, with growth over time (Heer et. al. 2018; Babushkina et. al. 2016; Pluess & Weber 2012). The idea of individual homeostasis in a heterogenous environment suggests that individuals with higher heterozygosity (genetic diversity) will exhibit more stable growth patterns and experience less impact from environmental factors (Cannon 1929; Fridman 2015; Peirson 2015). Earlier attempts to relate genome-wide genetic markers with tree-ring growth drew weak positive relationships between average ring width / variance and heterozygosity of those markers, but failed to observe strong enough relationships between those two variables to draw strong conclusions (Babushkina et. al. 2016). However, these novel attempts gave way to an important collision of two disciplines: dendrochronology and population genomics. In 2018, two studies provided a solid foundation for the advancement of paired dendrochronology and genomics (Heer et. al. 2018; Housset et. al. 2018). Both studies address the linkage of dendrometric traits (termed "dendrophenotypes" or "a new class of phenotypes") and association genetics for understanding how genetic regions and climatic fluctuations influence growth during episodic stress scenarios. These studies are foundational because they (a) provide a framework for combining dendrochronology & genetics in both natural and common garden settings, (b) demonstrate how candidate genes related to stress responses can be validated via dendrochronological methods, and (c) provide well-

reasoned explanations for why and how dendrometric traits can be used as phenotypes in association genetics (Housset et. al. 2018; Heer et. al. 2018; Roschanski et. al. 2016; Lloret, Keeling, & Sala 2011). Although one study uses BAI (basal area increment) and another uses TRI (tree-ring index), to quantify changes in tree growth, both studies provide means for calculating dendrophenotypes that are replicable (Housset et. al. 2018; Heer et. al. 2018). Basal area increment, the area of wood added to the cross-section of a tree at breast height as a ring each year, is an absolute measurement. Tree-ring index, on the other hand, is a relative measurement that assigns a value to each ring in a tree core based on the average ring size across the entire core.

The advent of BAI-based Rs/Rc/Rt calculations and the identification of single nucleotide polymorphisms related to climate within the *Pinus strobus* genome have facilitated the integration of such variables for the purpose of understanding how individual growth response is related to the underlying genetic makeup of an individual tree (Nadeau et. al. 2016; Rajora et. al. 2016). Although higher frequency and intensity of stressful episodes threatens to push biological systems to their limits, the cumulative effects of stress episodes can be unravelled by studying "resilience" (Scheffer et. al. 2001; Folke et. al. 2004). Resilience, an ecosystem's capacity to recover after disturbance and regain its original structure and function, is broken down into four quantifiable parts for the purpose of studying long-term stress response in tree rings (Lloret, Keeling, & Sala 2011). These four terms and their associated calculations, which take into account BAI measurements from during, before, and after a stress event, are summarized in (Figure 2.5). Recently, groups have applied the idea of Rs/Rc/Rt in GPA (genotype-phenotype association) studies for the purpose of testing relationships between genotypes growing in a common environment and the resulting phenotypes of interest (e.g. Eckert et. al. 2015; Heer et.

al. 2018; Alexandre et. al. 2020; Depardieu et. al. 2020). Prior to 2017, such phenotypes derived

from dendrochronological analysis had not been used for quantitative genetics studies (Housset

et. al. 2017).
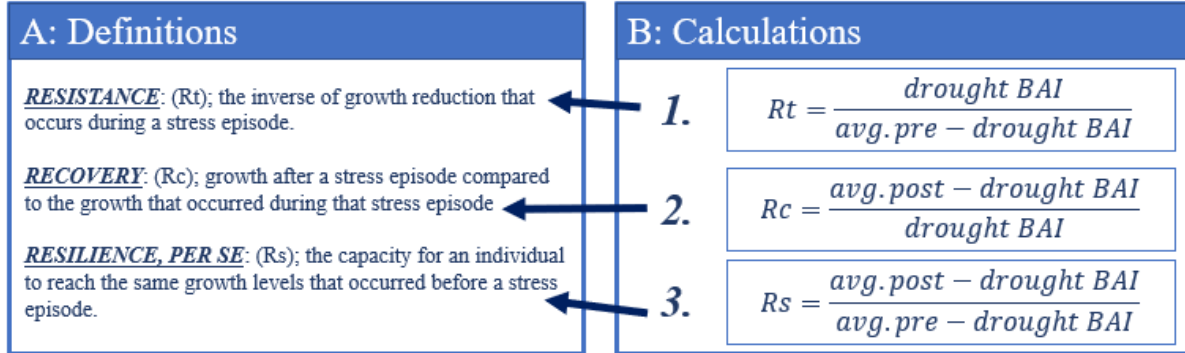
FIGURE 2.5: definitions and calculations under the umbrella of "resilience," defined by Lloret,

Keeling, & Sala 2011.

THEMES, FUTURE DIRECTIONS, AND CONCLUSIONS

Landscape-level genomics studies will likely be important in the future, especially for the

purpose of large-scale biosurveillance operations, evaluating adaptive traits that could benefit

species survival in a changing environment, and predicting stand response to drought events. An

application of a genome-wide study that may gain traction in the future is the combination of

dendrochronology and genetics. Dendrophenotypes, or time-sensitive responses to stress that can

be associated with the inheritance of certain genotypes, are an example of such an application

(Heer et. al. 2018). Finally, a third theme that appeared frequently in this review is the potential

for models that predict species resilience under increased drought stress and assisted breeding

programs for conserving species that may no longer survive in their changing native environments.

Based on these emergent themes, there is much potential for future research. For one, transplanting hybrid seedlings from various provenances north of the hybrid zone may be useful for the purpose of identifying physiological stress responses in hybrids. This may also reveal how hybrid seedlings with react under a northward migration scenario. Finally, a suspected northward migration of the PIST / PIFL hybrid zone means that hybrids will be exposed to cooler winter temperatures. However, it is not known how hybrid cold hardiness differs from that characteristic in either parent species, making this topic worth investigating in the near future.

Overall, there is much to be learned about patterns of inheritance in hybrid PIST / PIFL populations, specifically how hybrid index affects response of hybrids to stress events. One necessary research avenue seems is the combination of SNP data and dendrochronologically determined growth traits for the purpose of understanding how hybrids can inherit adaptive characteristics from their contributing parent species. Furthermore, it will be important to elucidate the relationship between hybrid provenance, hybrid index, and success in increasingly northern latitudes / higher elevations. It may be useful to create models that can predict hybrid seedling transplant success rate based on genetic makeup and provenance for the purpose of using strategic management to promote the growth of hybrid individuals.

PIST / PIFL hybrids are threatened by increased drought severity, a non-native fungal pathogen, and unknown capacity for adaptation to cooler environments. However, multiple research avenues lend predictive power to the question of how hybrids will fare in the future. For one, the threat of white pine blister rust encroachment in the hybrid zone may be monitored more easily moving forward thanks to the use of hyperspectral imagery and biosurveillance. Ongoing

work seeks to unravel quantitative gene in both PIST and PIFL and determine whether there are truly two distinct major genes for resistance given contemporary gene flow between the species. The consequences of warmer, frequent drought in the hybrid zone can be inferred by models that predict range shifts and contractions, and also by time series of hybrid growth around and during major drought events. Common garden and genetic studies elucidate the genetic architecture of drought response in hybrids by testing growth performance of seedlings removed from their provenances and identifying SNPs related to drought and freeze tolerance within hybrid populations. Finally, the availability of dendrochronological data within hybrid populations points to the possibility of comparing drought growth phenotypes to SNPs via a genotype-phenotype analysis.

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CHAPTER THREE:

DOES DROUGHT RESPONSE CHANGE WITH PARENTAL ANCESTRY ACROSS A

WHITE PINE HYBRID ZONE?

ABSTRACT

Hybrid populations of two white pine species, *Pinus strobiformis* and *Pinus flexilis*, are found throughout the southwestern United States. Understanding characteristics of hybrid populations will support efforts to conserve natural genetic diversity as trees adapt to climate change. Using the concept of a "dendrophenotype," or observed variation in tree-ring size that corresponds to a particular stress event in a tree's life, we calculated four indices of drought response using tree-ring data from nine hybrid geographic sites across Colorado, New Mexico, Arizona, and Texas. We used these indices in combination with hybrid index values (percentage of *Pinus strobiformis* ancestry inherited by a single hybrid) for sampled individuals to (1) examine relationships between climate, tree-ring sensitivity, and hybrid index, (2) test for relationships between tree-ring-based drought indices (resistance, recovery, resilience, and a Wet:Dry ratio) and hybrid index, and (3) develop multivariate models that predict drought response based on hybrid index and other variables. We found (1) significant correlation between monsoon index and hybrid index, monsoon index and tree-ring sensitivity, and hybrid index and tree-ring sensitivity in sampled populations, (2) little direct connection between hybrid index and four tree-ring-based drought indices, and (3) no predictive relationship between hybrid index and the drought indices, but stronger relationships may be found using different variables. Validation of a relationship between hybrid index and the annual monsoon cycle points to a future genotype-phenotype analysis that could use existing, fine-scale genetic data to further explore the effects of water availability on the inheritance of adaptive traits in hybrid populations.

KEYWORDS

dendroecology; resilience; forestry; genomics; dendrophenotype; *strobiformis; flexilis*

INTRODUCTION

In long-lived forest species like conifers, interspecific hybridization serves an adaptive purpose, preventing long generation length and slow mutation rates from inhibiting the accumulation of new genetic information (Aitken & Bemmels 2015; Hamilton, De La Torre, & Aitken 2015). In hybrid zones, recombinant genotypes provide a source of functional novelty (Rieseberg & Wendel 1993; Arnold 1997). When hybrid populations are subject to isolated, diverse ecological conditions such as the Sky Islands of the southwestern United States (Gottfried et. al. 2005), understanding characteristics of hybrids across hybrid zones can help to understand more about adaptive traits (Abbott, Barton, & Good 2016).

Southwestern white pine (PIST; *Pinus strobiformis* Engelm.) and limber pine (PIFL; *Pinus flexilis* James), two closely related species of high-elevation, five-needled white pine, share partially overlapping ranges in the western United States (Figure 3.1; Tomback et. al. 2011; Steele 1990). PIFL, named for the flexibility of its branches, exhibits wide physiological tolerance and will survive on harsh or xeric sites, where it may take on a krummholtz form (Schoettle & Rochelle 2000; Windmuller-Campione & Long 2016). PIST's native range includes Mexico and the southwestern United States, where the species is found within dry and mesic mixed-conifer ecosystems (Looney & Waring 2013). Across western North America, white pines fill important ecological roles by distributing large, nutritious seeds to birds and mammals, increasing biodiversity, and protecting local watersheds (Geils, Hummer, & Hunt 2010; Tomback & Achuff 2010).
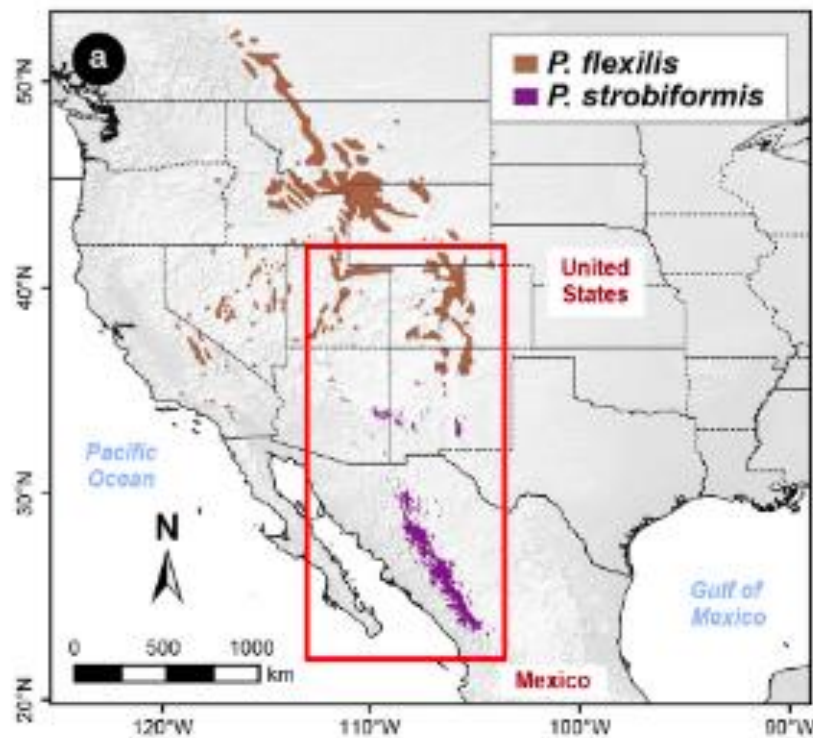
Figure 3.1: In brown, the core range of limber pine, PIFL overlaps partially with the core range of southwestern white pine, PIST in purple. The red box represents the extent of the hybrid zone where PIST and PIFL produce hybrid offspring.

Despite ecological divergence and speciation of PIST and PIFL, gene flow between PIST, PIFL, and their hybrid populations formed a hybrid zone. Hybrids were first recognized by intermediate morphological characteristics (Steinhoff & Andresen 1971) and recently confirmed by molecular genetic analysis (Moreno-Letelier & Piñero 2009; Menon et. al. 2018). Development and maintenance of species boundaries around the hybrid zone was likely facilitated by adaptive responses to drought stress because of (a) correlation between hybrid index (defined here as proportion of PIST ancestry), and variables linked to drought, and (b)

identification of candidate genes for drought stress related to divergent selection that drives

speciation among PIST, PIFL, and hybrids (Menon et. al. 2018). A broad latitudinal gradient of

ancestry exists within the hybrid zone (Figure 3.2; Menon et. al. 2018). To the north, hybrids are

more genetically similar to PIFL where the hybrid zone overlaps with PIFL's core range, while

the opposite is true in the central and southern portions of the hybrid zone. The degree of

hybridization is potentially related to a drought gradient within the hybrid zone based on

previous evidence that latitude and source population climate have significant effects on hybrid

index (Aguirre-Gutierrez et. al. 2014; Menon et. al. 2020).

Hybrid zones provide potential sources of genetic rescue for species at risk, as

demonstrated in selected breeding experiments on *Populus x canadensis* (Rajora & Rahman

2003). Due to the likelihood of increased drought severity throughout the hybrid zone in the near

future (Thorne et. al. 2018) and the potential for hybrid outplantings to react in unexpected ways

to new climates, it is worth investigating the adaptive capacity of hybrids across diverse climates,

particularly under drought conditions (Menon et. al. 2020). It is important to understand if

drought response is related to the degree of hybridization, especially if assisted gene flow is

required to conserve potentially adaptive traits such as drought tolerance (Aitken & Bemmels

2015).

Although some effects of drought on hybrid growth can be inferred by understanding

response to drought in parent species, more research is needed to clarify the influence of

hybridization on drought response across the hybrid zone. Like many pine species, PIST is

isohydric, or drought-avoidant, and exhibits greater drought avoidance with increased elevation.

This is evidenced by decreasing water potential with decreasing elevation and low PISTΔ^{13}C in

warm, dry conditions at low elevation in common garden studies, indicating efficient water use.

(Bucholz et. al. 2020). Throughout 65 sampled populations in the southwestern US and Sierra Madre Occidental of Mexico, PIST cone morphology and seed weight increased with decreasing latitude, where PIST grows in more humid and temperate climates moving south in its range (Leal-Saenz et. al. 2020). Cone morphology and seed weight were also well predicted by availability of precipitation during the growing season, as well as degree of hybridization with PIFL, although the combined effects of those variables on morphology were not disentangled by the aforementioned study. There is not sufficient evidence to call PIFL isohydric, but previous research on mature, natural populations of PIFL has shown that stomatal density and per-leaf stomata count decrease with increasing elevation, or are negatively correlated with elevation (Schoettle & Rochelle 2000). As density and count of stomata are indicators of greater control over water and carbon dioxide uptake, this suggests that PIFL may be more susceptible to challenges in maintaining appropriate moisture balance at higher elevations.

Studying the long-term growth response of hybrids during drought can be time-consuming given that multi-decadal time series are typically needed. Dendrochronology, the study of crossdated tree rings, is suitable for connecting a time-series of growth traits to genetic factors that are under selection (Heer et. al. 2018; Housset et. al. 2018). Using tree-ring measurements around and during drought events, "dendrophenotypes," or distinct individual growth signatures, can be related to the occurrence of genetic markers such as SNPs (single – nucleotide polymorphisms) within candidate genes that influence the effects of drought stress on growth. Prior work used a literature search method to identify candidate genes related to pollution-induced stress response before searching for SNPs from those genes present in an existing data set (e.g. Heer et. al. 2018). Other studies commenced with dataset-wide identification of any biallelic SNPs before examining correlation between water-availability

gradients across the PIST / PIFL hybrid zone and occurrence of those identified SNPs (Menon et. al. 2021). Several research groups have applied individual tree growth response to the onset of and years following major drought periods to changes in basal area increment (BAI), the wood that a tree adds to its cross-sectional area each year (Kohler et. al. 2010; Lloret et. al. 2011; D'Amato et. al. 2013). Application of this growth quantification to genotype-phenotype analysis studies created a means of linking genetic markers with dendrophenotypes (e.g. Eckert et. al. 2015; Heer et. al. 2018; Depardieu et. al. 2020). Recent work shows that hybrid populations benefit from background variants (SNPs from PIST) along gradients of water availability and recently introgressed variants (SNPs from PIFL) along freeze-related environmental gradients (Menon et. al. 2021).

In this study, we investigated the relationship between hybrid index and drought response in hybrid PIST / PIFL populations using four tree-ring-based indices of growth from nine geographic sites distributed across the hybrid zone from Texas to Colorado. We asked the following questions. Q1: At the scale of the hybrid zone across the southwestern US, is there a relationship between climate patterns (monsoon index), tree-ring sensitivity, and hybrid index? Q2: Do tree-ring based drought responses vary with hybrid index? And Q3: can we develop multivariate models predicting tree-ring-based drought responses based on hybrid index as well as tree, site, and geographical variables?

MATERIALS AND METHODS

FIELD SAMPLING

Menon et. al. (2021) sampled 22 populations of pure PIST, 12 populations of pure PIFL and 98 populations from the PIST / PIFL hybrid zone (Figure 3.2). Foliage was sampled at each population for destructive genetic analysis, which provided hybrid index data for sampled trees (Menon et. al. 2021). Collection trees were separated by at least 30 – 60m and 6-10 trees were sampled within each population. A gridded design of latitude and longitude with paired high-low elevation populations dictated core and foliage collections within the hybrid zone (Menon et. al. 2021).

STUDY AREA

In the present study, we selected a subsample of 30 hybrid populations from the original 98 that (a) represented a range of climatic conditions across the hybrid zone and (b) had enough cores for a reliable sample of each selected population (Figure 3.2). We then grouped populations together into nine geographic sites for analyses. These nine sites represent a diverse assortment of locations across the hybrid zone in terms of annual precipitation, temperature, and average hybrid index (Table 3.1).
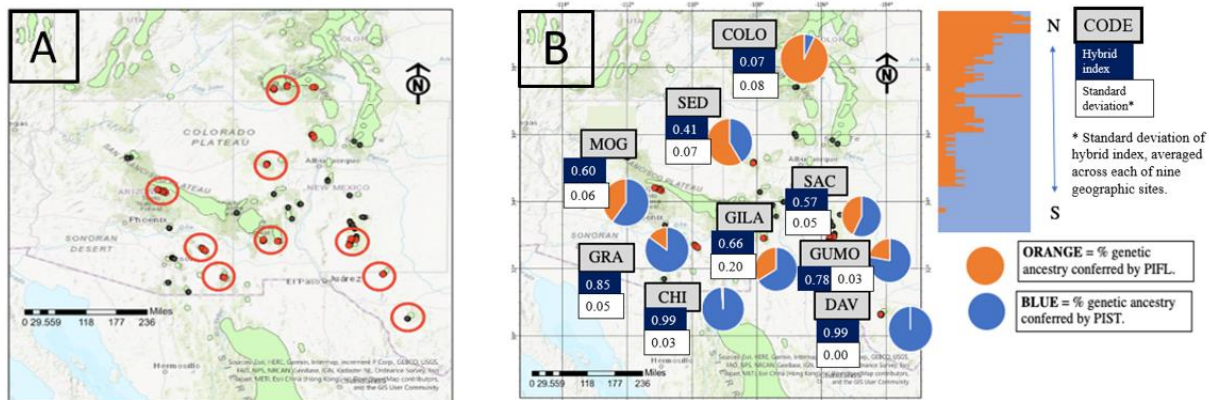
Figure 3.2a and 3.2b: In 3.2a, nine geographic sites within the hybrid zone, subsampled from an original 98 populations by (Menon et. al. 2021), are circled in red. In 3.2b, the same nine subsampled sites are labeled in grey and accompanied by corresponding ancestry pie charts (where blue represents hybrid index, or percent PIST ancestry, and orange represents percent PIFL ancestry).

For each tree sampled, we first collected an increment core on the uphill slope and a then collected a second core at a 90-degree angle from the first core to account for potential reaction wood. We froze each core for storage. Individual tree characteristics and environmental conditions at each population were recorded during the time of core collection. These characteristics included presence of white pine blister rust on individual sampled trees, diameter at breast height (DBH; cm), geographical coordinates, elevation (m), aspect, presence or absence of plants of the genus *Ribes* (which act as hosts for white pine blister rust), and white pine regeneration within each population.

LABORATORY METHODS

Climate Data

      Climate variables marked as potentially relevant to biological growth response in the literature and previous research were downloaded from ClimateWNA (see Supplemental Table 1) (Hamaan et. al. 2013; Goodrich et. al. 2016; Goodrich et. al. 2017; Bucholz et. al. 2020; Menon et. al. 2021). By dividing summer precipitation (PPT_sm) by mean annual precipitation (MAP), we calculated monsoon indices to understand the relationship between hybrid index and regional changes in water availability across the portion of the hybrid zone sampled for this study. Arizona and New Mexico receive up to half of annual rainfall from the summer monsoon season, which is driven by unbalanced heating of the southwestern US and Pacific Ocean (Guido 2008). Warm air in the southwestern US rises, causing moist, cool air to fill the low-pressure space that forms and deliver summer monsoon rain. Available SoilGrids250m data were also extracted at the site-level within each of the nine sites at depths of 0-5cm and 100-200m. (Hengl et. al. 2017). In SoilGrids, prediction models are fitted using more than 230,000 soil profile observations from the World Soil Information Service (WoSIS) and a series of environmental covariates. Soil property maps are generated at 250m spatial resolution at six sampling depths. Five soil properties were taken into consideration across the nine populations: bulk density of fine earth (kg / m^3), cation exchange capacity (cmolc / kg), soil organic carbon content (permille), pH measured in water solution, and pH measured in KCl solution.

Dendrochronology

      We thawed cores in 2020, dried them for 24 hours after removal from the freezer, extracted cores from storage straws, and glued them to wooden mounts. We surfaced cores with

sandpaper of increasingly fine grit until wood cells were clearly visible under magnification. We visually crossdated cores by comparing particularly narrow rings, or marker years, with existing chronologies from the International Tree Ring Database (Stokes and Smiley 1968). Then we scanned the samples at 1200 dpi resolution and used the program Coo Recorder v. 9.3 (Cybis Elektronik & Data A.B. 2019) to digitally measure the width of each tree ring. We checked the accuracy of crossdating using the program COFECHA (Holmes 1983). We dated a total of n=104 trees. We read raw ring widths into R using the dplR library (Bunn et. al. 2021) raw measurements were converted to BAI using the bai.out() function. Prior to calculation of RWI from raw ring data, each chronology must be detrended to remove age-related growth trends and climate-influenced signals. We used the detrend() function, also from the dplR library, to apply a cubic smoothing spline, which removes the age-related growth trend and other unwanted signals from tree-ring data, and converted raw measurements to RWI (Cook & Kairiukstis 1990).

Analysis

We implemented two methods for quantifying each individual tree's response to drought stress: a) a calculation of resistance (Rt), recovery (Rc), and resilience (Rs) and b) a Wet:Dry ratio. Resistance is defined as the inverse of the total growth reduction that occurs during a drought period, recovery is defined as the growth increase that occurs after a drought period in relation to growth rate during the drought event, and resilience is defined as a tree's capacity to reach pre-drought growth levels after that drought has subsided (Lloret, Keeling, & Sala 2011). The benefit of using such indices to understand growth response to drought conditions is that patterns of tree growth can account for past and present influences on an individual tree's ability to minimize change during a disturbance, then recover to a pre-disturbance state. A limitation of

the Rt/Rc/Rs indices is the temporal proximity of some drought years (for example, 2000 and 2002). We used such indices to capture lagged recovery that may occur due to drought legacy effects, which can lead to imprecise estimation of resilience (Ovenden et. al. 2020). The calculations of Rt/Rc/Rs (Equations 1-3) are ratios based on an absolute measurement (basal area increment; BAI) to identify three aspects of tree response to a stress event (in this case, a drought period).

$$\text{Equation 1: } resistance\ (Rt) = \frac{during\ drought\ BAI}{BAI\ averaged\ over\ 5\ years\ pre-drought}$$

$$\text{Equation 2: } recovery\ (Rc) = \frac{BAI\ averaged\ over\ 5\ years\ post-drought}{during\ drought\ BAI}$$

$$\text{Equation 3: } resilience\ (Rs) = \frac{BAI\ averaged\ over\ 5\ years\ post-drought}{BAI\ averaged\ over\ 5\ years\ pre-drought}$$

The Wet:Dry ratio (W:D) compares tree growth between major wet years and major dry years using RWI, and is useful because it provides an assessment of growth change in relation to limiting conditions, such as drought (Fekedulegn et. al. 2003; Bickford et. al. 2011; Hess & Fule 2020). Because W:D compares individual years rather than periods, it should be less susceptible to the legacy effects of temporally close droughts such as 2000 and 2002. RWI is a dimensionless unit that compares one year of individual radial growth to average annual radial growth across a tree's lifetime. We calculated W:D by dividing average RWI for the five wettest water years by average RWI for five driest water years at each site (Equation 4).

$$\text{Equation 4: } wet\text{:}dry\ (W\text{:}D) = \frac{Average\ RWI\ from\ 5\ wettest\ water\ years}{Average\ RWI\ from\ 5\ driest\ water\ years}$$

We calculated average sensitivity (average annual percentage change in ring width) of each site to address Q1. We also calculated correlations between all combinations of monsoon index, hybrid index, and sensitivity to address Q1 (Figure 3.4), as well as correlation coefficients

and coefficients of determination for hybrid index and (a) monsoon index, (b) relative spring humidity, (c) maximum temperature in autumn, and (d) annual precipitation as snow to identify relationships between hybrid index and regional water availability (Figure 3.5). To calculate drought indices for addressing Q2 and Q3, we selected drought years around which to calculate Rt, Rc, and Rs. We averaged longitude and latitude for each site and downloaded monthly precipitation and other climate data for those averaged coordinates for the period 1950 – 2015 (ClimateNA; Wang et. al. 2016). Time scale included the current "climate-change-type" drought from the mid-1990s to present (Allen & Breshears 1998) as well as the previous largest recorded southwestern drought, approximately 1951-56 (Swetnam & Betancourt 1998). All the tree-ring chronologies had adequate sample depth (minimum 8 trees) by 1950. Annual precipitation for the period 1950 – 2015 was calculated for each group by water year rather than by calendar year due to the close relationship between tree-ring growth and previous winter / current growing year precipitation (Yang et. al. 2014). A water year is the 12-month period extending from October 1st of year (X) to September 30th of year (X +1). For each group, we sorted water years and identified the five driest years within the period 1950 – 2015 (Table 3.2). We calculated indices of resistance, recovery, and resilience using BAI around each population's five driest water years.

For each individual tree, a total of five Rt measurements, five Rc measurements, five Rs measurements, and a single W:D was calculated. Finally, we created single values of each index per tree by averaging Rt, Rc, and Rs across all five driest water years for each individual tree. To test for a relationship between hybrid index and growth response to drought conditions and address Q2, we graphed each of our four drought indices versus hybrid index and used a Pearson's correlation matrix to identify multicollinearity and significant correlation between

hybrid index and covariates from sampled sites (where significant is defined as $p < 0.05$) (Figure 3.3).

Variable Selection and Model Development

We developed four linear a priori models using the lm() package in R for each of the four drought indices / dependent variables, Rt, Rc, Rs, and W:D for the purpose of addressing Q3. We included hybrid index values for each individual tree (Menon et. al. 2021), along with aspect, mean annual temperature and presence / absence of white pine blister rust as independent covariates in each a priori model. For our four final models, we initiated the building process with 26 environmental, 5 soils, and 15 tree-level independent variables (Supplemental Table 1). We chose to convert our categorical aspect values to numerical values, where N = 360° and NE = 45°. Prior to final model development, we used the Pearson's correlation matrix to assess correlation between independent covariates of interest (Figure 3.3). To develop final models, we used LASSO training in R's glmnet() package (Friedman et. al. 2010), which combines backwards and forwards model selection by shrinking the coefficients of significant covariates and setting non-significant covariate coefficients to zero while minimizing the residual sum of squares. We loaded our four model datasets into R before "training" LASSO with lasso.model() from the glmnet() package (Friedman et. al. 2010). We added a single random effect to the model to account for differences generated by individual hybrid sites before implementing the training procedure. Next, we carried out model selection in LASSO for each of the four response variables, or drought indices, using bootstrapped data and 1000 replicates, which allowed us to obtain coefficients for each independent variable included in the training procedure. We saved these coefficients from the training procedure and converted them to percentage of time included

in model selection for the total 1000 replicates (Supplemental Figure 2). We identified covariates that appeared in the four training models more than 50% of the time, and created final linear mixed-effects models using the lmer() package in R (Table 3.5). To see the relative fit of each of our four final models for Rt, Rc, Rs, and W:D, we used the BIC() function in R to obtain values from the Bayesian Information Criterion, by which low values indicate a better model fit (Table 3.6).

RESULTS

CHARACTERIZING HYBRID POPULATIONS

The total number of trees dated from each site ranged from 8 – 15 with an average site size of n=10 individuals (Table 3.1). The geographic sites at the highest and lowest average elevations (SAC and DAV, respectively) varied by 690m. Across the hybrid zone, we observed a 460mm range in precipitation. The wettest population, MOG, located at the Mogollon Rim of central Arizona, averaged approximately two times the mean annual precipitation exhibited at SED, the driest population, located in the Zuni Mountains of western New Mexico. Mean monsoon index was highest at DAV, which also provided the samples with the highest mean hybrid index, and lowest at COLO, which provided the samples with the lowest mean hybrid index. and lowest at COLO. COLO, the site at the highest latitude, also had the lowest mean annual temperature (6.66°C), while DAV, the site at the lowest latitude, had the highest mean annual temperature (13.32°C). The mean annual temperature at DAV was exactly two times larger than the mean annual temperature at COLO. On average, trees from GILA tended to have larger DBH (an average of 37.2cm), while trees with the smallest average DBH came from SED

(24.1cm), meaning that our site with the largest average DBH contained trees that were approximately 1.54 times larger than trees at our site with the smallest average DBH.

Table 3.1: Geographic sites (organized under "Site Code") are listed by percentage of southwestern white pine ancestry from least to greatest, with common names for each sampling location listed to the right for each code. Mean latitude, longitude, elevation, MAP (mean annual precipitation), MAT (mean annual temperature), and number of trees per site are summarized.

Site Code	State	Site Common Name	Mean Latitude	Mean Longitude	Mean Elevation (m)	MAP (mm)	MAT (°C)	n (# trees)	Mean Hybrid Index	Mean DBH (cm)	Mean Age	Mean Monsoon Index
DAV	TX	Davis Mountains	30.639461	-104.165240	2220	623	13.3	10	0.99	29.5	76	0.51
CHI	AZ	Chiricahua Mountains	31.850988	-109.302930	2550	832	9.5	11	0.99	31.7	75	0.45
GRA	AZ	Pinaleño Mountains	32.692026	-109.903090	2830	911	7.7	15	0.85	36.4	78	0.43
GUMO	NM	Guadalupe Mountains	31.945747	-104.861780	2410	690	10.9	10	0.78	30.9	95	0.49
GILA	NM	Gila National Forest	32.923673	-107.953820	2310	677	10.6	10	0.66	24.1	65	0.45
MOG	AZ	Mogollon Rim	34.332922	-110.948656	2280	916	9.9	8	0.60	29.9	61	0.25
SAC	NM	Sacramento Mountains	32.857712	-105.781180	2890	787	7.7	11	0.57	34.1	65	0.45
SED	NM	Zuni Mountains	35.162562	-108.104600	2710	456	7.8	13	0.41	37.2	73	0.31
COLO	CO	San Juan Mountains	37.356050	-107.805690	2430	614	6.7	10	0.07	28.2	58	0.25

Genetically, hybrid sites tended to resemble the more proximal parent species. For example, at COLO, where sampled hybrids were closer geographically to the core PIFL range, 93% of the average hybrid's genome is genetically similar to that of pure PIFL, while only 7% of genetic material resembles that of PIST. Conversely, at DAV, most trees sampled were characterized as pure PIST, with only a minute percentage of genetic ancestry conferred by PIFL (Figure 3.2). We observed a genetic gradient across the hybrid zone, with a gradual shift from almost pure PIFL ancestry to the north and pure PIST ancestry to the south.

Site conditions varied across the hybrid zone. While WPBR is known to occur in many locations within the hybrid zone, our sample only included a single site with infected trees, SAC (Sacramento Mountains). Plants from the genus *Ribes*, which serve as hosts for fungal pathogen *Cronartium ribicola*, were also observed at SAC. Although infection was only observed for one site of hybrids, *Ribes* plants were also observed at SED, GRA, and CHI, but it is worth noting that *Ribes* are abundant in the southwestern U.S. and simply may not have been close enough to sample trees to be included in the data collected in 2016.

Based on soils property predictions from SoilGrids, GUMO showed the most and GRA showed the least dense soil conditions. SAC was the site with the highest cation exchange capacity (28 cmolc / kg), 1.3 times the cation exchange capacity modeled at GRA, the site that exhibited the lowest modelled cation exchange capacity. Although SAC had high cation exchange capacity, it also exhibited the lowest modelled soil organic carbon content (18 per mille), while GILA, which had the highest soil organic carbon content, more than double that value (58 per mille). The soil organic carbon content at GILA was 3.2 times larger than the soil organic carbon content at SAC. The soil pH tests yielded slightly different numbers (for example, the H_2O measurement produced modeled values 1- or 2- units lower than the KCl

measurement), but sites were organized in the same ascending order by pH in both cases, with the highest soil pH modelled at COLO and the lowest soil pH modelled at GRA.

DENDROCHRONOLOGICAL DROUGHT RESPONSE

A total of seven common drought years appeared across sites (Table 3.2). The two most commonly-appearing drought years, 1956 and 2000, are located towards the beginning and end of the study period (1950 – 2015). Those years also appeared as common marker years across sites during the tree-ring dating process. We mapped each site alongside their respective average sensitivities and average tree rings. Our data revealed positive correlations between dendrochronological sensitivity, annual monsoon indices, and location within the hybrid zone in response to Q1 (Figure 3.4). We also observed statistically significant relationships between hybrid index and (a) monsoon index, (b) relative spring humidity, (c) maximum temperature in autumn, and (d) annual precipitation received as snow (Figure 3.5). Annual monsoon index and hybrid index exhibited a correlation of 0.15, while tree-ring sensitivity and monsoon index exhibited a correlation of 0.46. Hybrid index and tree-ring sensitivity exhibited the highest correlation of 0.78.

Table 3.2: A ranking of driest water years by site, in descending order by hybrid index.

Site Code	Driest Water Year	Precip (mm)	% of time appeared across all driest years?	2nd Driest Water Year	Precip (mm)	% of time appeared across all driest years?	3rd Driest Water Year	Precip (mm)	% of time appeared across all driest years?	4th Driest Water Year	Precip (mm)	% of time appeared across all driest years?	5th Driest Water Year	Precip (mm)	% of time appeared across all driest years?
DAV	2011	13	9	1956	28	13	1953	28	4	1971	28	4	1998	30	2
CHI	1994	38	2	2003	41	7	2000	42	11	1956	43	13	1970	47	2
GRA	2000	39	11	2003	41	7	2002	43	9	2004	46	2	1955	51	4
GUMO	2011	11	9	1953	21	4	1951	22	11	2002	22	9	2003	23	7
GILA	1951	22	11	1956	24	13	1955	28	4	2011	29	9	1971	31	4
MOG	2002	30	9	2000	39	11	2006	41	2	1959	48	2	1974	49	4
SAC	2011	31	9	1956	38	13	1951	39	11	1964	40	2	2000	41	11
SED	1951	19	11	1956	19	13	1950	21	2	2000	22	11	1989	23	2
COLO	2002	25	9	1977	27	2	1951	29	11	1974	30	4	1956	31	13

Variable Pair	Correlation Coefficient
Hybrid index and monsoon index	0.15
Hybrid index and tree-ring sensitivity	0.78
Monsoon index and tree-ring sensitivity	0.46

COLO
0.32
0.25

SED
0.38
0.31

MOG
0.31
0.25

GILA
0.36
0.45

GRA
0.25
0.43

GUMO
0.45
0.49

SAC
0.30
0.45

CHI
0.25
0.45

DAV
0.47
0.51

monsoon index

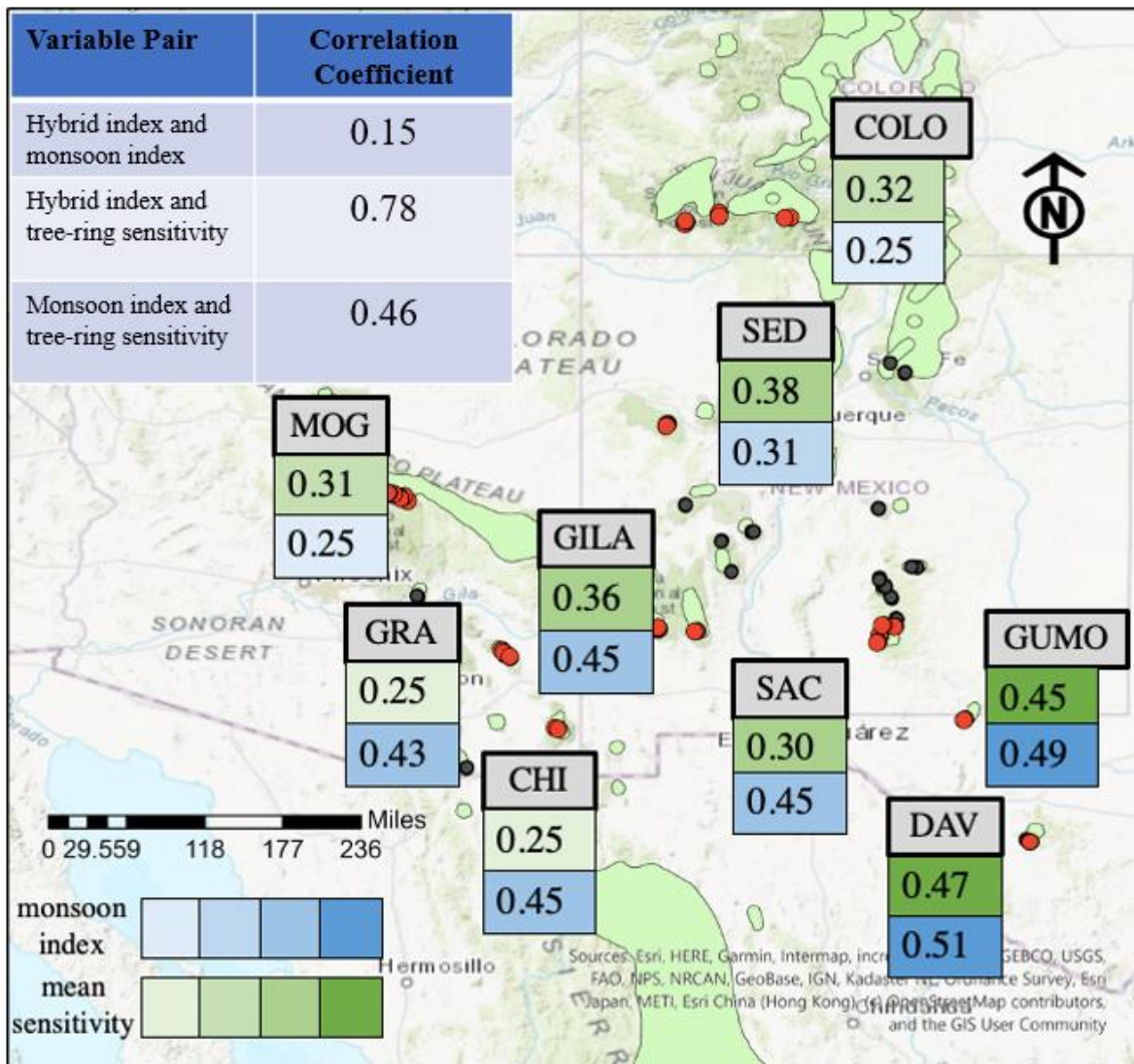mean sensitivity

Miles
0 29.559 118 177 236

Figure 3.4: Nine sampled sites (labeled with their codes in grey) within the hybrid zone, alongside their average monsoon indices (labeled in blue) and tree-ring sensitivity (labeled in green). Monsoon index and mean sensitivity gradients are indicated by increasingly dark shades of blue and green, respectively.

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The site with the least sensitive cores was MOG, which was consistent given that the highest mean annual precipitation was observed at that site (Tables 3.1 and 3.3). Across the nine populations, smallest BAI during the driest water year was observed at GILA. GILA had the second smallest chronology depth of the nine sites studied (89 years). Average sensitivity was highest at DAV, the site with the highest mean annual temperature of the nine sites studied. Series intercorrelation, the mathematical correlation between standardized tree rings within a chronology, was highest at SED.

Table 3.3: Chronology statistics for the nine geographic sites in this study.

Population Code	Chronology Length (Years)	Series Intercorrelation	Average Sensitivity	Number of Trees	Number of Cores
DAV	104	0.601	0.468	10	14
CHI	111	0.588	0.271	11	14
GRA	109	0.525	0.250	15	19
GUMO	130	0.661	0.452	10	13
GILA	89	0.613	0.364	10	15
MOG	90	0.657	0.248	8	8
SAC	116	0.670	0.298	11	18
SED	135	0.704	0.378	13	25
COLO	80	0.526	0.318	10	11

Resistance (Rt) was highest in DAV, the southernmost site with the highest mean annual temperature (Figure 3.6). Average Rt was lowest at GUMO, which scored 0.61, indicating that growth decreased by approximately 39% during each drought period for trees from that population. Recovery (Rc), on the other hand, was the lowest at COLO (1.42) and the highest at SED (2.13). Even COLO, which had the lowest average Rc of all populations, scored 1.42, indicating that trees from COLO exhibit an average 42% increase in growth rate following drought. In addition, this means that the average tree from SED was able to more than double its

growth rate following a drought event. In addition, SED, which produced the most easily

recovered trees, also produced the least resilient populations. In fact, the average tree from SED

scored an Rt value of 1, indicating that growth was generally consistent from pre- to post-

drought throughout that population. No site scored an average value of less than one for

resilience (Rs). Although most sites were unable to resist drought, or score 1 or higher for Rt,

every site was able to recover growth rate after the drought ended and maintain or increase pre-

drought growth conditions. Among the nine sites studied, COLO scored the lowest W:D ratio of

0.82, indicating that trees from this site grew faster during dry years than during wet years.

Conversely, DAV, the most sensitive population, scored the highest W:D ratio of 1.94, indicating

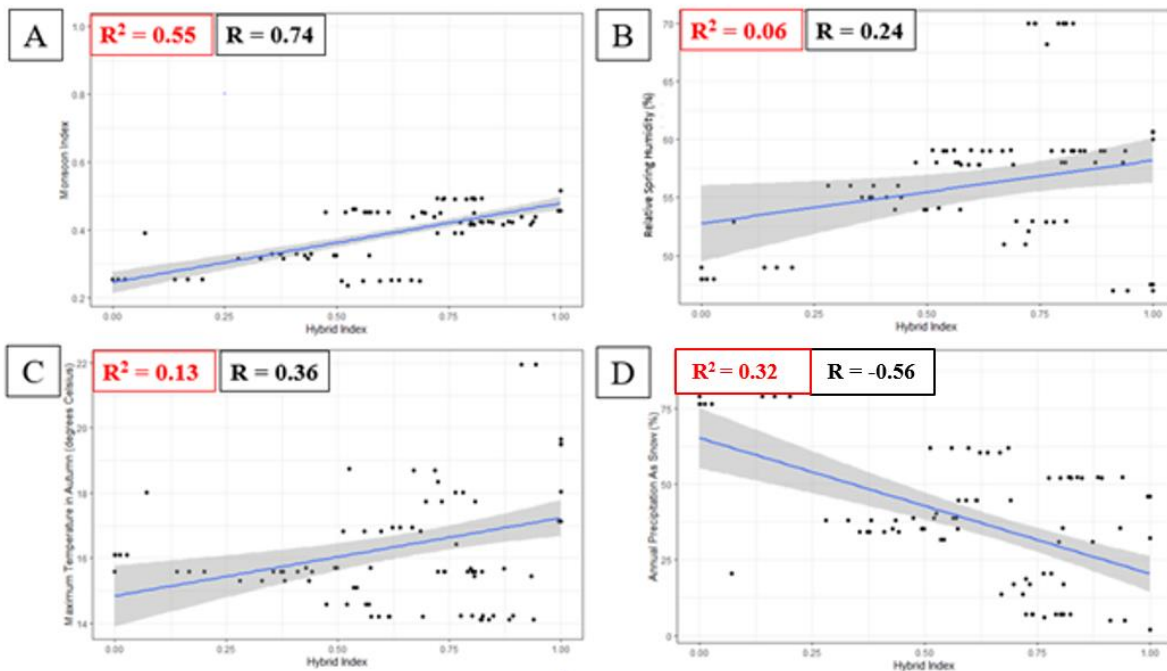that trees from this site grew almost two times faster in wet conditions than in dry conditions.

Figure 3.5: Four scatterplots showing correlation coefficients (R) and coefficients of

determination (R^2) for hybrid index and (A) monsoon index, (B) relative spring humidity, (C)

maximum temperature in autumn, and (D) annual precipitation as snow.
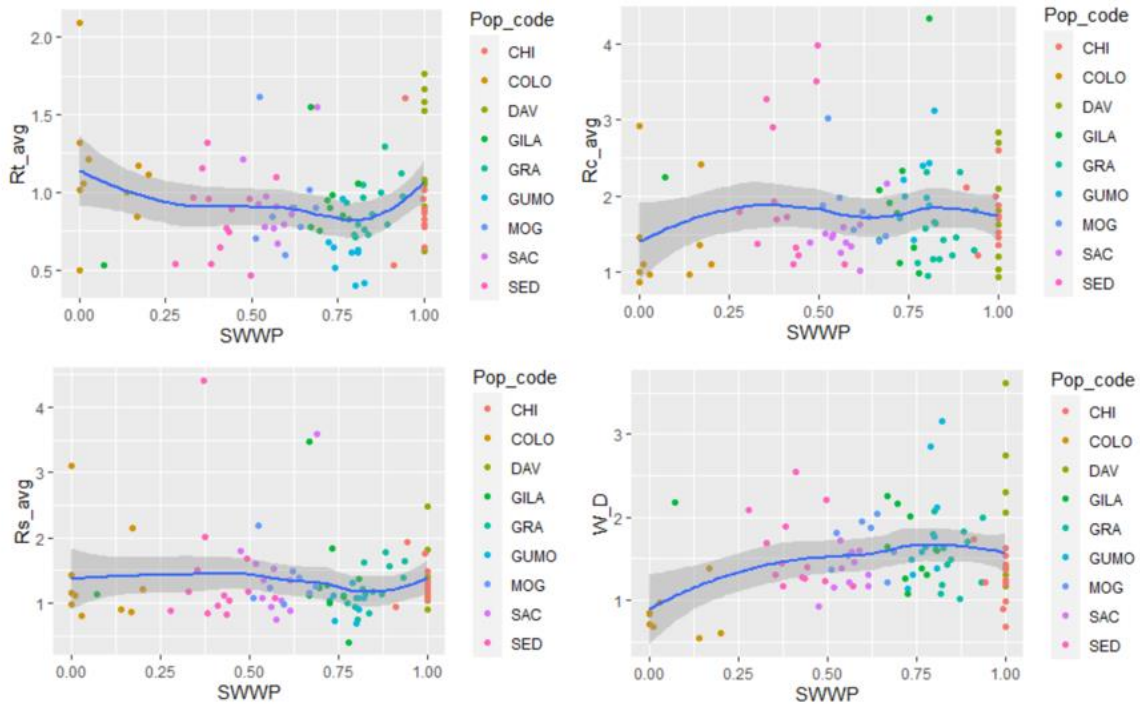
Figure 3.6: From left to right, top to bottom, hybrid index (SWWP) across all nine geographic sites is compared to four drought indices: (a) Rt, (b) Rc, (c) Rs, and (d) W:D. Blue ribbons show trends across each set of data. Data points are colored by geographic site.

MODEL RESULTS

The covariates that we selected for LASSO training of the four final multivariate models exhibited high multicollinearity (Figure 3.3). This required us to remove highly correlated covariates after a first rendition of LASSO training and re-train the four models of drought response to produce final versions of each model (Supplemental Figure 2). The removal of these highly correlated covariates resulted in four final multivariate models that each included less than half of the original covariates tested in LASSO trainings. Only a singular covariate (in turquoise; Supplemental Figure 2) was selected more than 50% of the time for inclusion in the model of Rs, making it the least extensive multivariate model of our final four.

Figure 3.3: Pearson's correlation matrix for all covariates tested in LASSO training, where red indicates a strong negative correlation and blue indicates a strong positive correlation.

Overall geographic site was selected by LASSO more than 50% of the time, so that covariate was included in all four of our final multivariate models. Aspect, which previous work has identified as a covariate of significance in hybrid regeneration efforts (Goodrich et. al. 2017), was selected more than 50% of the time by LASSO for Rt, Rc, and W:D, and was included in those final multivariate models (Supplemental Figure 2). DBH was also included in those three final multivariate models. Hybrid index, which we hypothesized would have a significant effect

on our four drought indices, was only selected more than 50% of the time by LASSO for Rc, so that covariate was only included in one of four final multivariate models. There were several covariates selected by LASSO for inclusion in final multivariate models that we did not expect to appear. For one, the presence of white pine blister rust, although only identified for 8 sampled individuals, was selected by LASSO for inclusion in the final multivariate models for Rt and Rc. In addition, bulk density of fine earth at sampling depths of 0-10cm was included as a significant covariate in the final models for Rc and W:D, and that same covariate but for sampling depths of 100-200cm was included in the final models for Rt, Rc, and W:D. Finally, relative spring humidity was included almost 90% of the time by LASSO in training models for Rt, so we included that covariate in the final multivariate model for Rt.

In response to Q3, of the four covariates that we included in the a priori models of Rc or Rs, none were significant predictors of their response variables (Table 3.4). For the W:D a priori model, however, mean average temperature (MAT) was identified as a significant predictor. MAT was also identified as a significant covariate for the model of Rt. The a priori model for Rt also included southern aspect as a significant covariate. Our main covariate of interest, hybrid index, did not have a significant effect on any of the four response variables, Rt, Rc, Rs, or W:D (Figure 3.6).

No strong relationships appeared between hybrid index and drought response alone (Figure 3.6). However, several weak, not statistically significant, relationships were apparent, notably a positive relationship between the W:D and hybrid index (Figure 3.6). There does not appear to be any relationship between Rs and hybrid index. As for the relationship between Rt and percentage of PIST ancestry, a weak negative relationship was observed.

Table 3.4: coefficients, t-values, and p-values for a priori models for the four drought indices used in this study, resistance (RT), recovery (RC), resilience (RS), and a wet:dry ratio (W:D). The covariates chosen for each a priori model include hybrid index (SWWP), mean average temperature (MAT), presence or absence of white pine blister rust (WPBR), and seven aspect categories. An asterisk indicates a significant p – value.

A Priori Covariates: RT	Coefficient	t-value	p-value
Intercept	0.47245	2.342	0.0216*
SWWP	-0.14603	-1.055	0.2946
MAT	0.04369	2.092	0.0394*
WPBR	0.02197	0.183	0.8551
Aspect N	0.21489	1.400	0.1652
Aspect NE	-0.03058	-0.205	0.8380
Aspect NW	0.10363	0.810	0.4201
Aspect S	0.27616	1.985	0.0504
Aspect SE	0.28302	1.747	0.0843
Aspect SW	0.20333	1.469	0.1455
Aspect W	0.18826	1.258	0.2117

A Priori Covariates: RC	Coefficient	t-value	p-value
Intercept	2.000414	4.798	6.88e-06*
SWWP	-0.141747	-0.495	0.6216
MAT	0.009037	0.209	0.8346
WPBR	-0.461443	-1.861	0.0662
Aspect N	-0.520465	-1.641	0.1046
Aspect NE	0.222149	0.721	0.4729
Aspect NW	0.040167	0.152	0.8796
Aspect S	-0.501216	-1.743	0.0849
Aspect SE	-0.194332	-0.581	0.5631
Aspect SW	-0.511970	-1.790	0.0770
Aspect W	-0.050189	-0.162	0.8714

Table 3.4, continued.

A Priori Covariates: RS	Coefficient	t-value	p-value
Intercept	1.50868	3.636	0.000477*
SWWP	-0.16094	-0.565	0.573453
MAT	-0.01167	-0.272	0.786477
WPBR	-0.12377	-0.502	0.617258
Aspect N	0.18762	0.594	0.553900
Aspect NE	-0.15172	-0.495	0.622048
Aspect NW	0.08629	0.328	0.743702
Aspect S	0.06055	0.212	0.832935
Aspect SE	-0.02610	-0.078	0.937750
Aspect SW	0.03036	0.107	0.915313
Aspect W	0.15701	0.510	0.611193

A Priori Covariates: W:D	Coefficient	t-value	p-value
Intercept	0.85681	2.496	0.0146*
SWWP	0.17744	0.791	0.4311
MAT	0.08411	2.394	0.0190*
WPBR	-0.10336	-0.533	0.5955
Aspect N	-0.38316	-1.555	0.1240
Aspect NE	0.05237	0.214	0.8309
Aspect NW	-0.16522	-0.797	0.4279
Aspect S	-0.31144	-1.384	0.1702
Aspect SE	-0.05949	-0.228	0.8199
Aspect SW	-0.28766	-1.294	0.1994
Aspect W	-0.44063	-1.837	0.0699

Our Bayesian Information Criterion values indicate that resistance was best modelled by the covariates selected using LASSO of the four final models of drought response that were produced (Table 3.6).

Table 3.5: Final models developed for our four response variables, resistance (RT), recovery (RC), resilience (RS), and the wet:dry ratio (W:D). Covariates are defined as follows: site code = overall site of nine total, SWWP = percentage of PIST ancestry, DBH = diameter at breast height, WPBR = presence of white pine blister rust, aspect = direction that growing environment faces, RH_sp = relative spring humidity, BLDFIEsl1 = bulk density of fine earth, measured at a depth of 0-5cm, BLDFIEsl6 = bulk density of fine earth, measured at a depth of 100 – 200cm. P-values and t-values for model covariates are also listed. Two asterisks next to a p-value indicates that value was a significant predictor.

Final Covariates: RT	Coefficient	t-value	p-value	Standard Error
Site Code	0.5793976	0.459	0.648	1.3164316
Diameter at Breast Height (cm)	-0.0005404	-0.194	0.847	0.0029024
Presence / Absence of WPBR	-0.0460732	-0.283	0.778	0.1680892
Aspect (8 categories)	0.0003844	1.176	0.243	0.146033
Relative Spring Humidity (%)	-0.0167181	-1.636	0.139	0.0107472
Bulk Density of Fine Earth (0-5cm depth)	0.0005687	0.781	0.438	0.0007646
Bulk Density of Fine Earth (100-200cm depth)	0.0003418	0.581	0.563	0.0006144

Final Covariates: RC	Coefficient	t-value	p-value	Standard Error
Site Code	1.7703420	0.476	0.637	2.389e+00
SWWP	0.3532516	0.968	0.362	3.002e-01
Diameter at Breast Height (cm)	0.0031307	0.498	0.620	6.261e-03
Presence / Absence of WPBR	-0.2698224	-0.816	0.435	2.737e-01
Aspect (8 categories)	-0.0009580	-1.257	0.212	3.118e-01
Bulk Density of Fine Earth (0-5cm depth)	-0.0009565	0.656	0.524	1.216e-03
Bulk Density of Fine Earth (100-200cm depth)	-0.0008505	-0.411	0.683	1.314e-03

Final Covariates: RS	Coefficient	t-value	p-value	Standard Error
Site Code	1.33560	21.47	<2e-16**	0.06221

Table 3.5, continued.

Final Covariates: W:D	Coefficient	t-value	p-value	Standard Error
Site Code	1.1170605	0.673	0.5041	1.722e+00
Diameter at Breast Height (cm)	-0.0007888	-0.171	0.8647	4.851e-03
Presence / Absence of WPBR	-0.2755058	-1.122	0.2769	2.586e-01
Aspect (8 categories)	-0.0004784	-0.888	0.3771	2.423e-01
Relative Spring Humidity (%)	-0.0355217	2.823	0.0272**	1.390e-02
Bulk Density of Fine Earth (100-200cm depth)	-0.0009292	-0.983	0.3286	1.004e-03

Table 3.6: Below, Bayesian Information Criterion (BIC) values are listed next to their corresponding linear mixed-effects models for RT (resistance), RC (recovery), RS (resistance), and W:D (wet:dry ratio).

BIC	Model
-44.81	RT
-22.43	RC
-9.49	RS
-43.53	W:D

DISCUSSION

HYBRID ANCESTRY AND DROUGHT RESPONSE

Key findings of this study from Q1 are statistically significant relationships between hybrid index and tree-ring sensitivity (R=0.78) and between monsoon index and tree-ring sensitivity (R=0.46), providing novel support for a linkage between hybrid inheritance and the influence of water availability on annual growth . This provides evidence in support of a connection between climate and genetics in PIST / PIFL hybrid sites and supports the idea that hybrid adaptation is driven by environmental gradients (Menon et. al. 2021). These key findings from our study are supported by a weak, positive relationship between W:D and hybrid index (Figure 3.6), as well as statistically significant correlations between hybrid index and four

climate variables related to patterns of regional water availability across the hybrid zone (Figure 3.5). The significance of MAT in our a priori models also supports our key findings, as increased temperature is likely to be a major driver of prolonged, severe drought in the future for the southwestern U.S. and potentially influence regional hybrid sensitivity. Sites that are more influenced by the monsoon cycle (DAV, for example) experience greater changes in growth, or exhibit higher sensitivity. We hypothesize that this is the case due to variation in time of the year when hybrids have access to precipitation. For example, it would make sense for a tree that received consistent precipitation throughout the water year to exhibit less sensitivity than a tree that only received a burst of precipitation during the monsoon season. As expected, based on prior research by Babushkina et. al. (2016) that failed to draw a strong connection between individual heterozygosity (similar to our hybrid index) and radial growth in Siberian larch *(Larix sibirica* Ledeb.), we failed to observe a strong relationship between hybrid index and the four drought indices (Rt, Rc, Rs, and W:D), the objective of Q2. In response to Q2, we observed weak, negative relationships between Rc/hybrid index and W:D/hybrid index (Figure 3.6). However, no relationships were observed between Rt/hybrid index or Rs/hybrid index. Correlation between W:D and the hybrid index supports our finding that tree-ring sensitivity varies with hybrid index and provides evidence for linkage between genetic inheritance and water availability within the hybrid zone. Our Pearson's correlation matrix also revealed a lack of correlation between SWWP (hybrid index) and any of the four drought indices (Rt, Rc, Rs, and W:D) (Figure 3.3).

In response to Q3, results of our a priori models indicated that hybrid index is not a significant predictor of growth response to drought conditions in hybrids (Table 3.4). Therefore, it was not surprising when that covariate also did not appear as a significant predictor in any of our four final models of growth response to drought (Table 3.5). Based on the results of the four final models that we developed, none of the indices of growth response to drought were successfully modelled, by hybrid index or any of the other covariates that were included in final models. One reason for this could be that the effect of drought conditions on growth response is lagged, meaning that changes in growth to not appear in the tree-ring record until years after drought. Past work has noted that putative hybrids from the San Francisco Mountains of Arizona may experience this type of lag after a stress event (Kane & Kolb 2014), a pattern that may be modelled more successfully using a Bayesian approach, which allows prior information (such as past drought and growth) to influence the outcome of a model (Peltier, Fell, & Ogle 2016). In addition, our small sample size (n=104) may not have been sufficiently large enough to elucidate a relationship between hybrid index and growth response to drought conditions. If replicated, this study would benefit from inclusion of more populations spread across a larger extent of the PIST / PIFL hybrid zone, especially populations from farther north that exhibit lower hybrid indices, as our samples generally came from trees with higher hybrid indices. Previous work has identified challenges in linking a broad measurement such as hybrid index, which averages the effects of many genes into two parental categories, with growth (Babushkina et. al. 2016). However, other studies have shown that using fine-scale genetic data such as SNPs from candidate genes in a genotype-phenotype association study along with tree-ring data is a successful technique for supporting relationships between candidate genes and tree response to

stress (Heer et al. 2018; Housset et. al. 2018). A hybrid SNP dataset related to water availability and cold hardiness are available (Menon et. al. 2021) and a future genotype-phenotype study using SNP data and the drought indices tested in this study (Rt, Rc, Rs, W:D) could help to disentangle the likely polygenic inheritance of drought adaptation in hybrids.

Although our four models were not successful at predicting hybrid drought response based on hybrid index alone or most of the covariates that we included in LASSO training, there are several model results that may provide beneficial information for future research endeavors. Despite a low proportion of sampled hybrids that were infected with white pine blister rust, our models revealed a potential effect of infection on drought response, warranting future research that considers the effects of rust infection on growth response to drought with a greater sampling of infected hybrids. In addition, percent relative spring humidity was identified as a significant predictor of the W:D ($p=0.03$), and had a positive relationship with W:D. This means that more dendrochronologically sensitive trees exhibiting high W:D may be benefitting from increased spring humidity. Humidity is a characteristic known to influence where PIST develops in Mexican populations, where PIST is often restricted to humid, cool gullies with deep, well-drained soils (Perry 1991; Garcia and Gonzalez 2003). It is also worth noting that bulk density of fine earth was included in 3 of 4 final models at the 100-200cm sampling depth, and in 2 of 4 final models at the 0-5cm sampling depth. Previous research has suggested that PIST is dependent on shallow water resources for success in the early growing season (Acosta-Hernández et. al. 2018). Therefore, future research groups seeking to understand PIST / PIFL and hybrids may consider focusing on the effects of soil water availability and groundwater dynamics at sites where those species already grow, as well as at locations where hybrids could be found in future circumstances under a northward migration scenario.

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CHAPTER FOUR: MANAGEMENT IMPLICATIONS

The future of *Pinus strobiformis* (PIST), *Pinus flexilis* (PIFL), and hybrids is at least partially dependent on managers' thorough understanding of the relationships between such species, changing climate, and drought for the purpose of developing strategic, well-informed management plans. The goals of this thesis, outlined in Chapter Three, were to (1) define relationships between hybrid index (percentage of PIST ancestry inherited by an individual), climate, and tree-ring sensitivity, (2) test for relationships between four tree-ring-based drought indices and hybrid index, and (3) develop multivariate models that could predict these four drought indices based on hybrid index and climate variables relevant to hybrid growth. We used broad scale (hybrid index) genetic data to identify relationships between hybrid index, climate variables relevant to regional water availability, and tree-ring sensitivity, or the average year-to-year percent change in tree-ring width for an individual. These relationships suggest that there may be a fine, underlying genetic architecture of drought-adaptive inheritance in hybrid populations, at the single-nucleotide polymorphism (SNP) level, for example.

Notable findings from Chapter Three are positive, statistically significant relationships between hybrid index and tree-ring sensitivity (R=0.78) and between monsoon index and tree-ring sensitivity (R=0.46) across the nine geographic sites from which we subsampled hybrids. Despite these two correlations, we did not observe a significant correlation between the W:D (wet:dry ratio) and hybrid index. Although the W:D and sensitivity are not identical (W:D measures growth from the most extreme wet and dry years in a population of trees, while sensitivity does not), they are both measurements that allow researchers to understand relative differences in growth responses to wet and dry periods among tree populations. It is still unknown whether monsoon index, along with other water availability gradients, or genetic

inheritance (i.e. hybrid index; SNP presence) have greater control over sensitivity. Given these results, managers should be conscious of how reforestation efforts that use seedlings transported from far distances across the hybrid zone will affect sensitivity to drought conditions. Proactive managers should identify monsoon indices for seedlings, both from provenances and at transplantation sites, prior to reforestation and consider transfer guidelines as outlined by Goodrich et. al. 2017 to ensure that transplanted seedlings will be able to grow successfully despite regional and seasonal differences in precipitation.

A less significant but still notable finding from Chapter Three was the inclusion of a particular covariate in 3 of 4 final multivariate models (despite statistical insignificance): the presence of white pine blister rust (WPBR) in a sampling area. The threat of white pine blister rust in the southwestern U.S. is acute due to low occurrence of major gene resistance to the disease in forest populations (Sniezko, Kegley, and Danchok 2008). Our sampling biased hybrid populations with greater PIST ancestry and did not include any infected populations with more PIFL than PIST ancestry (i.e. have a hybrid index higher than 0.5). To fully understand how compromised hybrid populations, such as those infected with forest disease, fare in drought conditions (something that was not considered in our study design), future research should emphasize an interaction between WPBR infection and drought response in sampled populations. Such sampled populations should ideally contain at least 25% WPBR-infected, PIST-dominant individuals and at least 25% WPBR-infected, PIFL-dominant individuals, rather than less than 10% of sampled individuals (which was the case for our study, where WPBR was not a focus).

Current management strategies for hybrids acknowledge that water-stressed conditions stimulate high differentiation for certain traits, and that this differentiation can be separated by northern and southern seedling provenances through non-metric multidimensional scaling

analyses (Goodrich et. al. 2016). Our results, which support the finding that hybrid inheritance varies latitudinally and with regional water availability, echo such observations of differentiation and indicate a need for greater consideration of how seedling transfer can affect drought response regionally within the hybrid zone. Isohydry plays a key role in acclimation of southwestern white pine to expected habitat drying and warming (Bucholz et. al. 2020). In addition, hybrid seedlings from northern provenances tend to exhibit lower water-use efficiency but longer survival in lethal drought experiments compared with seedlings from more southern populations (Goodrich et. al. 2016). If outplantings of WPBR-resistant hybrid seedlings are necessary to sustain hybrid, PIST, and PIFL populations, and genetic resistance is relatively rare, then it is unlikely that many outplanted seedlings will grow in places climatically similar to their provenances, meaning that reducing maladaptation to transfer is crucial (Goodrich et. al. 2016). Our multivariate models support seedling transfer to northern aspects, as well as planting on mesic sites, assuming that those sites can provide greater water availability and can help seedlings avoid tissue-moisture deficit (Goodrich et. al. 2017; Bucholz et. al. 2020). We also observed statistically significant correlations between hybrid index and several climate variables related to seasonal precipitation: relative spring humidity (R=0.24), winter precipitation received as snow (R=-0.56), and maximum temperature in autumn (R=0.36). Bucholz et. al. 2020 note that seasonal water availability is an important factor for understanding range-wide drought strategies in PIST, so given these correlations, it makes sense for managers to consider seasonal water availability during outplanting preparation and how that availability will affect drought behavior in genetically resistant seedlings across the hybrid zone.

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SUPPLEMENTAL TABLES AND FIGURES

Supplemental Table 1: Covariates used for each LASSO training for our four models of drought response.

| Covariate Code	Description	Mean	Coefficient of Variation
lat	Latitude	33.34026	0.06
long	Longitude	-107.75111	-0.02
ele	Elevation (m)	2546	0.10
SWWP	% ancestry contributed by PIST	0.66	0.44
Rs_avg	Average resilience across five drought years for an individual.	1.34	0.45
Rt_avg	Average resistance across five drought years for an individual.	0.93	0.33
Rc_avg	Average recovery across five drought years for an individual.	1.76	0.38
W:D	Ratio of 5 wettest to 5 driest water years for an individual.	1.53	0.35
DBH	Diameter at breast height (cm)	31.8	0.35
WPBR	Tree infected by white pine blister rust? (Y/N)	Yes (infected); No (not infected)	
aspect	Shape / alignment of slope where a tree is growing.	N; NE; E; SE; S; SW; W; NW	
BLDFIE_sl1	Bulk density of fine earth (kg/m^3) at 0-5cm.	1236	0.05
BLDFIE_sl6	Bulk density of fine earth (kg/m^3) at 100-200cm.	1546	0.02
CECSOL_sl1	Cation exchange capacity of soil (cmolc/kg) at 0-5cm.	26	0.09
CECSOL_sl6	Cation exchange capacity of soil (cmolc/kg) at 100-200cm.	22	0.14
ORCDRC_sl1	Soil organic carbon content (permille) at 0-5cm.	73	0.37
ORCDRC_sl6	Soil organic carbon content (permille) at 100-200cm.	7	0.50
PHIHOX_sl1	pH index measured in water at 0-5cm.	64	0.06
PHIHOX_sl6	pH index measured in water at 100-200cm.	66	0.05
PHIKCL_sl1	pH index measured in KCl solution at 0-5 cm.	53	0.05
PHIKCL_sl6	pH index measured in KCl solution at 100-200cm.	54	0.04

|			
Tmax_at	Average maximum autumn temperature (1981-2010) (°C)	16.4	0.11
DD_0_at	Number of autumn degree days below 0°C	19	0.63
DD5_sp	Number of spring degree days below 5°C	39	0.90
DD_18_sp	Number of spring degree days below 18°C.	13	1.47
DD18_at	Number of autumn degree days below 18°C.	23	0.73
PAS_wt	Winter precipitation as snow (mm)	37	0.72
RH_sp	Spring relative humidity (%)	56.3	0.11
MAT	Mean annual temperature (°C)	9.1	0.22
MWMT	Mean warmest month temperature (°C)	18.1	0.10
TD	Continentality(°C) – the difference between MWMT and MCMT	17.5	0.12
MAP	Mean annual precipitation (mm)	721	0.22
MSP	May – September precipitation (mm)	393	0.27
AHM	Annual heat-moisture index	28.9	0.26
SHM	Summer heat-moisture index	50.9	0.36
DD_0	Degree days below 0°C, or chilling degree days	224	0.53
DD5	Degree days above 5°C, or growing degree days	21	0.24
DD18	Degree days below 18°C, or heating degree days	34	0.73
NFFD	Number of frost-free days	221	0.18
bFFP	The day of the year on which the frost-free period begins	127	0.17
FFP	Frost-free period	158	0.23
PAS	Precipitation as snow (mm) between August in the previous year and July in the current year	54	0.78
EMT	Extreme minimum temperature over 30 years	-27	-0.18
EXT	Extreme maximum temperature over 30 years	35	0.05
Eref	Hargreaves reference evaporation (mm)	1027	0.12
CMD	Hargreaves climatic moisture deficit (mm)	436	0.30
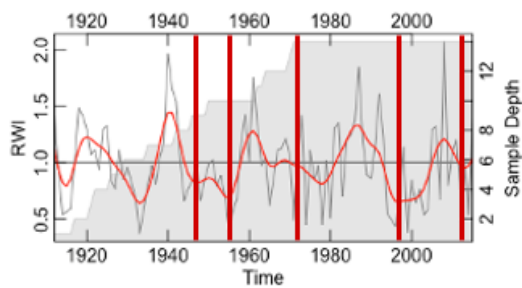
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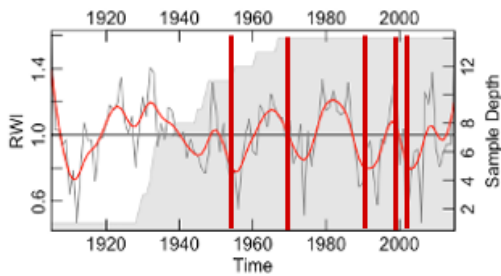
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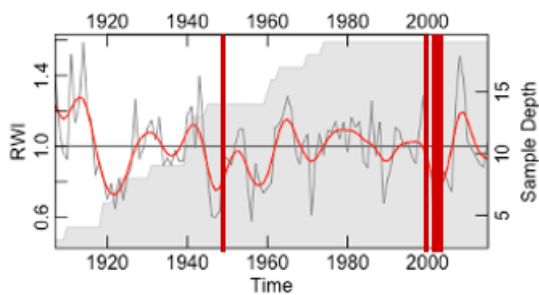
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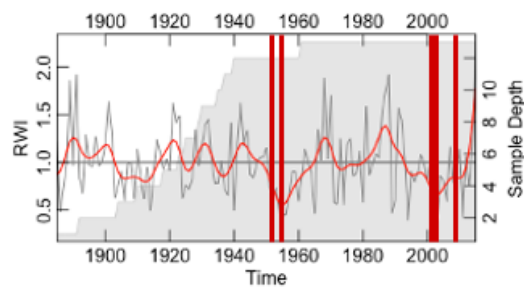
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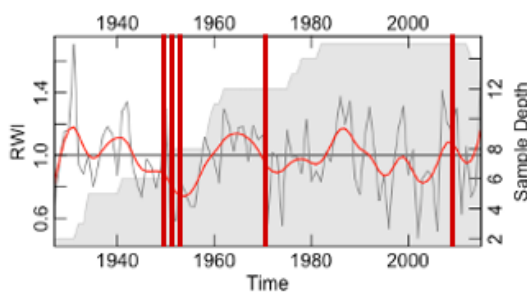
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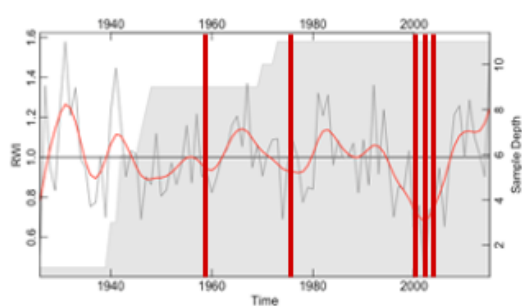
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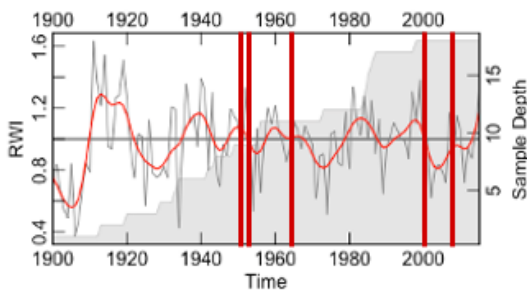
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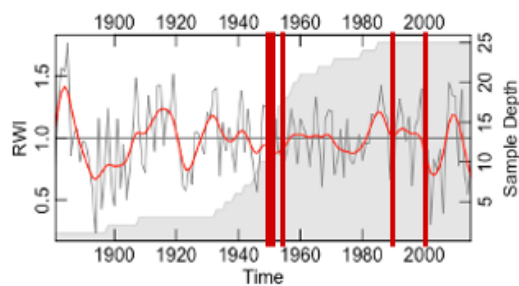
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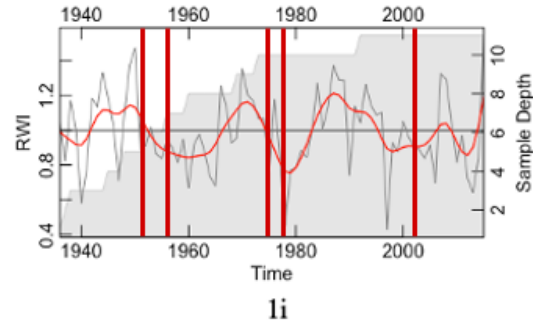
Supplemental Figure 1: RWI chronologies for nine geographic sites, (a) DAV (Davis Mountains, Texas), (b) CHI (Chiricahua Mountains, Arizona), (c) GRA (Mount Graham, Arizona), (d) GUMO (Guadalupe Mountains, New Mexico), (e) GILA (Gila National Forest, New Mexico), (f) MOG (Mogollon Rim, Arizona), (g) SAC (Sacramento Mountains, New Mexico), (h) SED (Zuni Mountains, New Mexico), and (i) COLO (San Juan Mountains, Colorado).
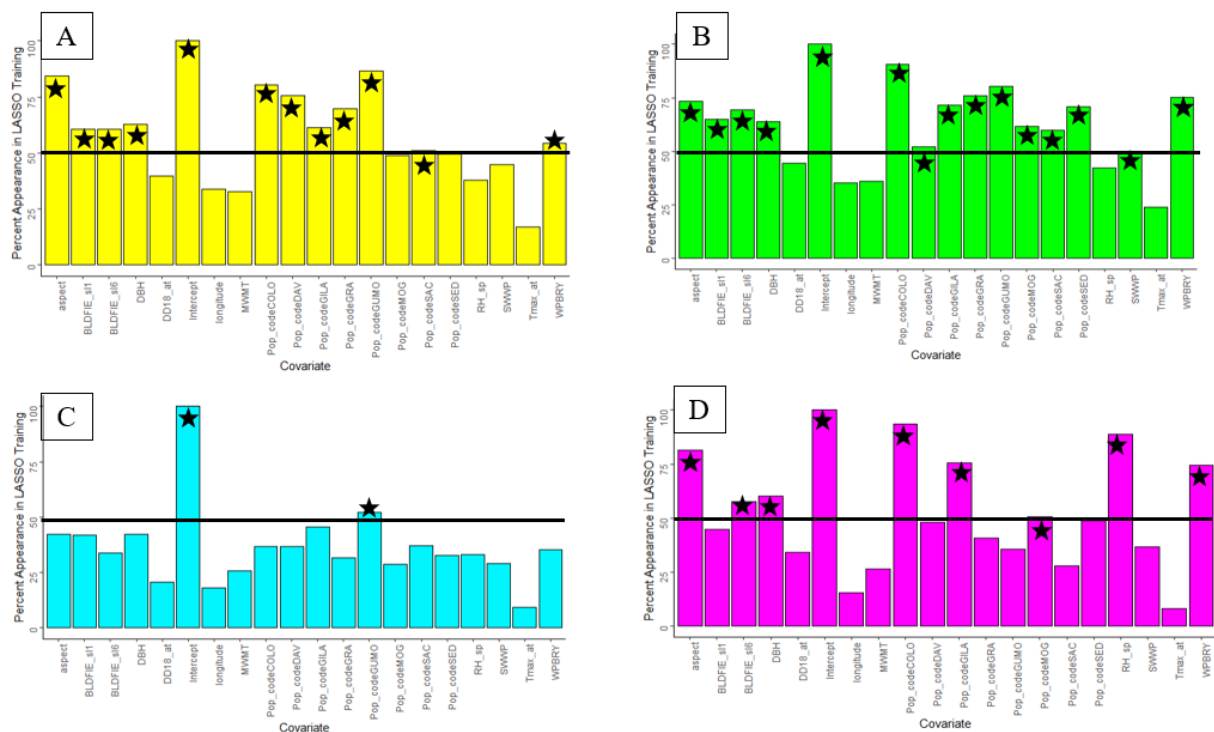
Supplemental Figure 2: Results of LASSO training for each of the four final multivariate models (Rt, Rc, Rs, and W:D). Covariates included in each training are listed, along with percentage of the time that LASSO selected each covariate for inclusion in 1000 training runs. The covariates that we included in our final multivariate models (starred) appeared more than 50 percent of the time in those training runs. Covariate abbreviations are defined in Supplemental Table 1.