INVESTIGATION INTO CLIMATIC EFFECTS ON THE GROWTH AND GENETIC STRUCTURE OF SKY ISLAND PONDEROSA PINE

Bу

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A DISSERTATION

Submitted to Michigan State University in partial fulfillment of the requirements for the degree of

Plant Biology–Doctor of Philosophy

ABSTRACT

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In the desert southwest, significant variations in moisture and temperature occur along steep altitudinal gradients. Ponderosa pine forests in the Santa Catalina Mountains, southeast Arizona, USA, consist of two partially sympatric species, the variable needle Pinus ponderosa var. brachyptera (3- and mixed-needle) that prefers cool and moist conditions, and the 5-needle *P. arizonica* that prefers hot and dry conditions. The objective of this research program is to determine how disturbance-that is variations in climate–contributes to shifts in population structure. Data was collected along two south-facing slopes (of similar elevation and aspect) for average needle number per fascicle, tree-ring widths, and gene frequencies, to examine limiting factors to growth, climatic shifts in growth, and population genetic structure. Our data show that cooccurring ponderosa pine needle-types (3-, mixed-, and 5-needle) have filled different ecological niches; their growth is limited by seasonal water availability, which also controls the length of the growing season. Three distinct genetic groups are present in the Santa Catalina Mountains. Genetic variability is reduced for the 5-needle type suggesting a possible bottleneck or founding event. Results from this study about moisture limitations will help land managers to determine range limits for seed planting zones.

This dissertation is dedicated to my husband, Jerry Rayala, for all your support.

ACKNOWLEDGMENTS

I thank my major professor, Frank Telewski, and other committee members Kim Scribner, Jim Smith and Nate Swenson for mentoring and guidance on the project, and for their helpful comments on the final versions of the manuscripts. The USDA Forest Service, and the Paul Taylor Fellowship, Department of Plant Biology and Beal Botanical Garden, both of Michigan State University, supported the project. Departmental staff from Plant Biology (Michigan State University) and Northern Research Station (United States Forest Service) aided with purchasing and logistics. The Coronado National Forest provided access to samples and the soils data.

I thank the following people for help on the project: J. Rayala, J. Grimes, G. Friedlander, J. Kilgore, and B. Epperson assisted with field data collection. A. Foss, R. Williging, J. Lund, M. Meisenheimer, H. Jenson, H. Stricker, and S. Lietz, provided lab, data processing, or GIS support. J. Stanovick provided statistical support. S. Chinn supplied expertise in the use and interpretation of dendrochronology software. J. and L. Griffith, and A. and T. Harlan (both deceased) provided logistic support for the field research. R. Cronn contributed the DNA sequences for the microsatellite marker development project. D. McKenney provided the spatial climate datasets for the Santa Catalina Mountains. B. Sturtevant provided constructive feedback on the project. D. Donner, J. Smith, and K. Scribner reviewed papers. L. Rayala edited papers.

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CHAPTER 1

MONITORING FOREST RESPONSES TO CHANGING CLIMATE ABSTRACT

Contemporary climate change has unprecedented potential to alter forest structure, productivity, disturbance patterns, and community composition. To meet the challenge of managing forests with climate change, new interdisciplinary tools need to be developed to monitor forest responses. An important area of research is employed that uses tree-ring widths and population genetic structure to model inter-annual climatic response, which is explained in terms of niche tradeoffs, physiological responses to climate, and adaptation by speciation or hybridization. The distribution of plant species in the Santa Catalina Mountains, Arizona, USA, and significance of the study will also be discussed. The main conclusion of the study is that two closely related ponderosa pine species have different ecological requirements for growth at their zone of sympatry. The resource most limiting to growth is seasonal water availability, which also controls the length of the growing season—a topic of great interest to land managers in determining range limits for seed planting zones.

INTRODUCTION

Long-term climatic changes will have profound effects on forest structure, productivity, disturbance patterns, and community composition. Increases in air pollution from the combustion of fossil fuels, deforestation, and land use change elevates the concentrations of greenhouse gases in the atmosphere, which correlates with increases in the mean global temperature. Significant warming has occurred over the past fifty years with increased occurrences of extreme weather events such as drought (IPCC, 2007). Plant responses to changing climate can be persistence (if the change is tolerable), range shifts to more favorable areas, adaptation, or extinction (Davis et al., 2005). The range shifts can be elevational as well as geographic range shifts, and these elevational clines should be predictive of geographic clines.

The Pleistocene saw dramatic change in climate 2 Myr (Table 1.1). Plant and animal species retreated to higher elevations to escape warming temperatures and drought of the advancing desert in the American southwest and persisted in isolated mountaintop refuges called the Sky Islands (Rehfeldt 1999). The refuges are scattered throughout the southwestern United States and northern Mexico and are surrounded by deserts that act as barriers to migration. These mountain islands are ideal for studying changes in forest structure as significant variation in moisture and temperature along steep elevational gradients permits the coexistence of species filling different ecological niches over a highly compressed spatial scale. Elevational gradients are useful for studying constraints on plant growth by climate (Adams and Kolb 2004). These studies can be enhanced by considering water availability simultaneously given variability in water supply in high elevation forests with changing climate. For example, as

concentrations of atmospheric carbon dioxide and mean global temperatures rise, snow cover is predicted to decline, and growing seasons lengthen (IPCC, 2007). Potential outcomes of the projected change in environmental conditions due to reduced water availability are drier soils and forests. Furthermore, changes in the water and carbon cycle will impose additional water stress on plants leading to decreased growth.

Gymnosperms existing prior to dramatic shifts in climate would have accumulated species during times of climatic stability. The species that survived changes in temperature may have done so because of wide physiological tolerances to climate (Coomes and Grubb, 1996), biological interactions resulting in niche tradeoffs (Tilman, 2004), or neutral forces such as stochastic dispersal and other population level processes (Kraft et al., 2008). Alternatively, changing climatic conditions could have been the impetus for speciation. Young pine species that originated during the Early Tertiary 7 Myr (Table 1.1) through a period of changing climate that brought dramatic changes in vegetation (Millar 1993) were a recently diverged fraction that coexisted with old species after radiation. Subsequently, periods of cooling may have repeatedly caused the pines to retreat, followed by independent evolution until drier periods reunited the pines. The repeated warming and cooling cycles could have increased hybridization between closely related species, leading to greater diversity. Thus, adaptation by speciation or hybridization would have been governed by harsh climate. Environmental factors can also have a strong influence on the location and maintenance of interspecific hybrid zones (Grant 1981), especially on more xeric sites where water availability would be a driving factor. (Swenson et al. 2008). Molecular markers are used to monitor hybrid zones in forest trees (Bennuah et al. 2004), which

have been reported to be narrow on southern slopes (Ito et al. 2008), or undetected (Epperson et al. 2009).

The Santa Catalina Mountains are located north of Tucson in southeastern Arizona, USA, and are one of eleven "sky islands" described by Rehfeldt (1999). The plant species assemblage changes abruptly along a steep elevational gradient, 760 m near Tucson, to more than 2740 m at the summit. The vegetation varies from Sonoran Desert Scrub on the lower slopes to Pine-Oak Woodlands at mid-slope, and subalpine forest near the summits. The mountain range is influenced strongly by Mexican and Latin America flora creating a remarkable gradient of plants along its southern slope. Species richness increases along a moisture gradient from higher to lower desert elevations (Whittaker and Neiring, 1965). Other major ranges in the area that share similar patterns in vegetation are the Pinaleno, Chirricahua, Santa Rita, Huachuca, and Rincon. Although similar in species composition, forest types may be reduced or absent at higher elevations, and ranges further eastward lack the desert communities present at the base of the Santa Catalina mountains (Whittaker and Neiring, 1965).

The southwest high elevation ponderosa pine forests predominately consist of two partially sympatric taxa-the well-characterized *Pinus arizonica* and *P. ponderosa* var. *brachyptera* (Willyard et al. 2017). On the south facing slopes of Mt. Lemmon (Santa Catalina Mountains), the transition between these species is quite dramatic over 100 meters horizontal distance and is often complete (Epperson et al. 2001; Epperson et al. 2009). *P. arizonica* is more successful at the warmer and drier, lower elevations, and extends its range far south into central Mexico. Whereas, *P. ponderosa* var. *brachyptera* survives exclusively on the highest mountaintops and cold air drainages; at

the colder and wetter, higher elevations. The sharp elevational transitions observed between these species' distributions are inconsistent with pine dispersal suggesting there are strong differences in fitness between the two species in response to environmental selection pressures. (Fig. 1.1a; Housset et al. 2018). Southern slopes have difficult growing conditions, which are hot and dry, and *P. arizonica* is most likely more heat and drought resistant than *P. ponderosa* var. *brachyptera*.

The goal of this work is to determine how changing climate variables are affecting the growth and genetic makeup of these ponderosa pine forests, as expressed through easy to identify traits used to monitor these changes. The project aims to determine how pine species are generally responding to changing climate factors to predict of how pines will respond genetically and physiologically to increasing temperatures. The main questions of this study are: 1) can we identify the environmental variable for determining the P. ponderosa var. brachyptera – P. arizonica distributions (Chapters 2-3), and 2) can we resolve the genetic relationships between the two species (Chapter 4). To address these questions we will ascertain carbon sequestration by quantifying the growth and genetic responses of natural pine forests to increasing precipitation, temperature, and drought. The main objectives of the study are to: 1) correlate annual patterns of growth for two ponderosa pine species with seasonal climate variables (Chapters 2-3), 2) examine the temporal stability of the climate-growth correlations (Chapter 3), and 3) evaluate the population genetic structure of two pine species (Chapter 4).

Five hypotheses tested the growth response of ponderosa pine forests to climate variables: 1) *P. ponderosa* var. *brachyptera* growing at its lower-warm elevational limit

will be more sensitive to dry conditions and changes in temperature than *P. arizonica* growing near its upper cool-moist elevational limit (Chapter 2; limits hypothesis), 2) *P. ponderosa* var. *brachyptera* (originating from moist climate) will be more susceptible to moisture stress than *P. arizonica* (originating from dry climate; Chapter 2; niche tradeoff hypothesis), 3) the seasonality of limiting factors will change over time because rising temperatures will influence weather patterns and drought in the desert southwest (Chapter 3; shifts hypothesis), 4) the climate gradient may be a morphological response of *P. ponderosa* var. *brachyptera* and *P. arizonica* to changing environmental conditions (Chapters 3-4; morphological hypothesis), or 5) the climate gradient may reflect the presence of hybridizing populations of *P. arizonica* and *P. ponderosa* var. *brachyptera* (Chapter 4; hybridization hypothesis).

RATIONALE AND SIGNIFICANCE

A great need exists for collecting fine scale data as part of a forest indicator and monitoring program to validate models that predict climate change effects on individual forest stands (Marsh et. al. 2009). Integrating a physiological and genetics approach (Cavender-Bares and Pahlich 2009) will address landscape heterogeneity and uncertainty about growth responses by maximizing the common growth signal among intraspecific trees at a site (Babst et al. 2018). Examined will be individuals sampled from southern aspects where the distributions of two species of ponderosa pine overlap and are potentially hybridizing in the Santa Catalina Mountains. Dendrochronology is an ideal approach for monitoring growth trends over decades and centuries because the approach has been used to addresses interspecies relationships for pine species

growing along climate gradients where water stress is known to be growth limiting (Marquardt et al. 2018). Also, climate models predict more variable climate patterns, which should affect the timing of pollen shed and female cone receptivity (Tauer et al. 2012). Consequently, changing environmental conditions are expected to play a role in the timing of reproduction, hybridization, and the formation of population genetic structure.

Our discussion about collecting fine scale tree-ring data emphasized the need for field sampling to precisely capture species identification. We used average needle counts per fascicle to rapidly identify species in the field (Epperson et al 2001), a phenotypic measure with high heritability (Rehfeldt 1993). To confirm our initial classifications, more robust genetic analyses are required; although, experimental data is often lacking for studying genetic structure because adequate molecular markers are not always available (e.g. we developed 8 new microsatellite markers for ponderosa pine). These knowledge gaps are especially true for non-model conifer species like ponderosa pine with large and complex genomes (Zimin et al. 2014). Current developments in sequencing technologies are rapidly advancing and improving our ability to study neutral and adaptive genetic variation in wild species (Segelbacher et al. 2010). Such developments allow new possibilities for basic research on plant responses to environmental stress including growth and hybridization. Therefore, basic information was developed because the problem cannot be attacked through straightforward approaches using readily available, clear-cut methods. The lack of sufficient basic knowledge resolving genetic patterns with the growth responses of trees is the primary factor accountable for the knowledge gap about the causes of

interspecific differences in annual growth in response to changing climate. Resolving tree-ring growth with genetic patterns requires a detailed analysis of morphological, tree-ring, and genetic characters to distinguish groups of individuals with different requirements for seasonal precipitation and habitat suitability. Varying site conditions limit the applicability of generalized guidelines; therefore, one goal was to determine how individual species will establish across sites on a local scale to overcome this limitation (Fig. 1.1a). Thus, the results of this study that integrates physiology and genetics will provide a more comprehensive ability to predict the effects of climate change on tree growth and population genetic diversity, which will provide new information for forest management guidelines (Fig. 1.1b).

Ponderosa pine, a widespread North American tree species, has high economical, ecological, and historical value for wood production, erosion control and wildlife habitat, and ethnobotanical uses (USDA NRCS National Plants Data Center). Incorporating climate gradients while studying the growth, physiological, and population genetics responses of ponderosa pine will improve our understanding of the role climate tolerances play in driving annual growth and diversity patterns, as temperatures increase and water becomes more limiting. We expand on previous work by Kilgore (2007) and Epperson et al. (2009) by characterizing replicate transition zones of two closely related taxa (*P. arizonica* and *P. ponderosa* var. *brachyptera*) for differences in sensitivity to climatic variability and genetic structure. The project is of value to physiologists and geneticists studying the stress response of closely related plant species. The study results combine estimates of resource use and plant growth, with assessments of genetic diversity, allowing differences in intra- and inter-specific plant

water relations to be coupled to climate. In addition, the conclusions are useful to land managers because the study of quantified differences in tree-ring widths and the genetic diversity of two species of ponderosa pine in the Santa Catalina Mountains will help to determine range limits for seed planting zones. Such an approach allows recommendations to be made that provide viable options for long-term improvement of reforestation efforts and sustainable management of natural areas. Moreover, a better understanding of the response of these two species to climate change will provide comparisons for *P. ponderosa* var. *scopulorum*, a dominant pine in the ponderosa pine ecosystem.

METHODS

The study sites are located within the Santa Catalina Mountains, near Tucson, Arizona, USA. Climate variables were collected at three contact zones (of similar elevation, slope and southern aspect) that differ in soil water availability, and where sympatric morphotypes are potentially hybridizing. The average available water holding capacities are 3.8%, 4,7% and 9.2% for Mt. Bigelow (BIG), Palisades (PAL), and Mt. Lemmon (MTL), respectively (Marquardt et al. 2018). The small scale of the study minimizes micro-environmental effects from (soil, climate, water, light, elevation) on the analysis of annual growth. The three populations have been undisturbed except for fire, with MTL and PAL more heavily impacted by fire than BIG in recent years. Identification of species was based on the average needle number per fascicle (Haller 1965). *P. ponderosa* var. *brachyptera* contained two needle types identified as mixed-needle (MN; $3.2 \le$ mean ≤ 4.6 needles per fascicle), and 3-needle (3N; < 3.2 needles per fascicle;

Rehfeldt 1993, 1999). Trees that average > 4.6 needles per fascicle were designated *P*. *arizonica* (5N; Peloquin 1984). From each species, 30 continuous mature trees were sampled from MTL and BIG for tree rings at each site for genetic analysis. One tree was sample every 10m along a 5x9 grid. The exception was at the center of the transition zone, where one *P. ponderosa* var. *brachyptera* and one *P. arizonica* were sampled at the three innermost transects. The average series length (years \pm SD) for BIG and MTL combined is 75 \pm 32 years (Marquardt et al. 2018). Additional trees were sampled for genetic evaluation at six-locus haplotypes (chloroplast) collected from populations located higher in elevation (MTL) and lower in elevation [Green Mountain (GRN), Rose Canyon (ROS)] than the transition zones. Seedlings located near the transition zones, and above the transition zones were also sampled for genetic analysis at MTL and PAL.

CONCLUSIONS AND FUTURE WORK

Because of changing climate, gaining knowledge about climate-growth relationships of closely related species is vital for forest management. Precipitation is known to be a major factor limiting growth of ponderosa pine in high elevation forests such as the Santa Catalina Sky Islands, but the mechanisms have not been thoroughly understood. Therefore, we focused on changes in the annual growth of trees and standardized the growth response in stands (a relative measure) to account for variation introduced by microsite differences in environmental conditions. This allowed us to focus on the growth response to climate stress when comparing sites in contrasting environments.

Currently, ponderosa pine is managed as one distinct species under the jurisdiction of the Coronado National Forest. Although proper forest management needs accurate knowledge about the structure of genetic variation with respect to environmental conditions, the population genetic structure of ponderosa pine in the Santa Catalina Mountains has yet to be fully determined. Therefore, we also conducted a preliminary study of population genetic structure using distance and frequency based genetic measures to determine the number of operational taxonomic units.

In our study, we analyzed the differences in annual growth, climate sensitivity and genetic structure between P. arizonica and P. ponderosa var. brachyptera (both as two species and as three needle-types) at three transition zones in southeast USA, near Tucson, Arizona. Moisture availability is the major factor controlling growth through the length of growing season for these conifers, especially for cool season correlations during winter and spring. The major conclusion from this study are that two closely related ponderosa pine species have different ecological requirements for growth and habitat suitability, which resulted in three distinct taxa occupying different elevational niches. Statistical analyses show subtle and significant ecological divergence in climate sensitivities and genetic structure, implying that climate change may be a major factor in the differentiation of *P. arizonica and P. ponderosa* var. brachyptera. The four most important points of the first tree ring study (Chapter 2) are that 1) sympatric pine species growing at high elevation have seasonal differences in precipitation requirements for growth, 2) the most consistent pattern of differences in climatic sensitivity between species is the variability in soil moisture availability, 3) the variation in water availability between sites is further influenced by disturbance-especially at the less dry site-where

growth reductions are larger, and 4) the variability in local site conditions helps to resolve the differences in the species' response to climate. In the second tree-ring study (Chapter 3) a dramatic shift of seasonal climate-growth relationships from summer to spring is reported, suggesting 1) water limitations control the length of the growing season, 2) a redirection of resources from maximum biomass production (summer water) towards reproductive structures (spring water), and 3) warming trends may be impacting the stability of climate growth relationships, which has implications for conservation management and climate reconstruction. Also, cambial plasticity (a response to variable winter precipitation) may provide opportunity for hybridization. Lastly, the genetic study of haplotype frequencies (Chapter 4) show 1), genetic variability is reduced for the 5-needle pine 2), higher divergence was observed for distant (i.e. pure) than sympatric populations, and 3) gene flow is high among all populations.

Moving forward, we will complete a detailed genetic analysis of the study populations with six-nuclear microsatellite markers. To date we have developed eight new microsatellite markers for ponderosa pine (two to be used in this study), verified the motifs by Sanger sequencing, obtained genotypes and complied the allele frequency data.

In addition, few studies have analyzed tree-ring and genetic data jointly in natural populations (Pluess and Weber 2012; Cole et al. 2010; Babuskina et al. 2016; Heer et al. 2018), and only Heer et al. (2018) found a strong signal between growth and a genetic trait – 15 genes linked tree-ring phenotypes to photosynthesis and drought stress. The lack of significant results in the other studies could be attributed to too few

genetic data (i.e. lack of power). Therefore, to facilitate the integration of tree-ring and genetic approaches, we propose future work that scales tree data to local conditions by integrating individual chloroplast haplotypes to assess climate response in natural populations. This would be accomplished by rebuilding composite tree-ring chronologies based on haplotypes and conducting correlations with climate to determine which haplotypes are most sensitive to drought and precipitation. We acknowledge that the tree-ring sample size will be a concern and power of the test would be monitored as the chronologies were being constructed.

APPENDICES

APPENDIX A

Manuscripts

This dissertation is the foundation for three peer reviewed papers, which are referred to

below by their chapter number in the dissertation:

- Marquardt, PE, Miranda, BR, Jennings, S, Ginger, T and Telewski, FW (2018) Variable Climate Response Differentiates the Growth of Sky Island Ponderosa Pines. TREES DOI: 10.1007/s00468-018-1778-9
- 3. Marquardt, PE, Miranda, BR, and Telewski, FW. Shifts in Climate-Growth Relationships of the Sky Island Pines. *Manuscript*
- Marquardt, PE, Willyard, A., Miranda, BR, and Telewski, FW. Genetic Differentiation among Sky Island Populations of Ponderosa Pine. – *Manuscript*

APPENDIX B

Tables and Figures

Era	Period	Epoch	Myr ¹
Cenozoic	Quaternary	Holocene	0.01
		Pleistocene	2.25
	Tertiary	Pliocene	7
		Miocene	26
	Paleogene	Oligocene	34
		Eocene	54
		Paleocene	65

Table 1.1 Geologic time scale modified from White et al. (2007; Table 1)

¹Million years ago

(A) Costs of site heterogeneity

(B) Benefits of basic and applied research



Figure 1.1 Establishing the links between tree-ring traits, genotypes, and the climatic constraints limiting to tree growth. (a) Possible consequences of environmental heterogeneity on fitness, population structure, and distribution of taxa. (b) Benefits of linking basic ecology to applied research through the study of population structure

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CHAPTER 2

VARIABLE CLIMATE RESPONSE DIFFERENTIATES THE GROWTH OF SKY ISLAND PONDEROSA PINES

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Acknowledgments: This paper is part of a dissertation submitted to Michigan State University in partial fulfillment of requirements for a Doctor of Philosophy degree. The Institute for Applied Ecosystem Studies, USDA Forest Service, and the Paul Taylor Fellowship, Dept. of Plant Biology and Beal Botanical Garden, both of Michigan State University, supported the project. We thank three anonymous Reviewers and a Communicating Editor for constructive comments that improved the manuscript, and the following people for help on the project: J. Rayala, J. Grimes, G. Friedlander, J. Kilgore, and B. Epperson assisted with field data collection. A. Foss and S. Lietz provided lab, data processing, and GIS support. J. and L. Griffith, and A. and T. Harlan (both deceased) provided logistic support for the field research. The Coronado National Forest provided access to samples and the soils data. A. Willyard and B. Sturtevant provided constructive feedback. J. Smith reviewed the paper. L. Rayala edited the paper.

ABSTRACT

Key message: The seasonally cool and moist conditions of spring improved the growth of two co-occurring ponderosa pine species, which displayed different seasonal climatic responses and length of correlations to drought.

Abstract: We examined the climatic sensitivity of two partially sympatric pine species growing at their transition zone in the Santa Catalina Mountains, AZ, USA. Pinus arizonica is found at lower elevations compared to P. ponderosa var. brachyptera. Ring widths were measured in trees at two sites and correlated with precipitation, temperature, and Palmer Drought Severity Index to assess the influence of climate on growth. The two species were analyzed within and between sites, which have similar elevation, aspect, and species composition, although soils at the two sites have different water-holding capacities. Response function analyses of *P. arizonica* [sampled near its upper (and wetter) elevation limit], and *P. ponderosa* var. *brachyptera* [sampled near its lower (and drier) elevation limit] indicated that annual growth correlated positively and strongly with spring precipitation at both study locations. Local site conditions had a major impact on tree growth and variability in site conditions helped resolve the differences in species' response to climate. For example, at the less dry site, growth of the lower elevation pine (*P. arizonica*) responded to early-winter precipitation, while *P.* ponderosa var. brachyptera did not. Also, correlation analysis indicated that P. arizonica's growth was more sensitive to drought for longer periods than P. ponderosa var. brachyptera. Finally, partial temperature-growth correlations of P. arizonica and P. ponderosa var. brachyptera indicated growth was limited by increased growing season and winter respiration, respectively. Rising night-time temperatures during spring

significantly reduced growth of *P. arizonica* at Mt. Lemmon. These findings demonstrate subtle yet meaningful interspecies differences in sensitivity to seasonal moisture stress and use of carbon resources.

Keywords: Dendroecology; drought stress; Pinaceae; response function; tree ring; *Ponderosae*

INTRODUCTION

The Santa Catalina Mountains, near Tucson, Arizona, USA, are among the bestknown and well-studied Madrean Sky Islands, which are high elevation mountains defined by pine-oak forests (Shreve 1915, 1917, 1919; Whittaker and Niering 1965, 1975; Whittaker et al. 1968; Bezy 2016). Two partially sympatric pine species grow at the higher elevations: *Pinus arizonica* Engelm. and *P. ponderosa* var. *brachyptera* (Engelm.) Lemmon, a closely related variety of *P. ponderosa* [and previously misidentified as *P. ponderosa* var. *scopulorum* (Engelm.)], also known as Taxon X (Rehfeldt 1999; Epperson et al. 2009; Willyard et al. 2017).

P. arizonica and *P. ponderosa* var. *brachyptera*, Diploxylon pines of the subsection *Ponderosae*, are large trees that grow throughout the mountainous and semiarid regions of the southwestern United States (Perry 1991; Farjon and Styles 1997; Price et al. 1998). *P. ponderosa* var. *brachyptera*, comprising three-needle and mixed-needle morphotypes, survive exclusively at the highest elevations (2300-3000 m) and in cold air drainages (Rehfeldt 1999; Epperson et al. 2001), while the five-needle *P. arizonica* is found at lower elevations (1800-2600 m), and is considered part of the ponderosa pine complex. As mean annual precipitation increases with elevation from

orographic processes (Sheppard et al. 2002), *P. arizonica* is more successful at surviving in warmer and drier habitats, whereas *P. ponderosa* var. *brachyptera* survives in cooler and wetter habitats. *P. arizonica* extends from southeastern Arizona and southwestern New Mexico to the Sierra Madre of northern Mexico (Perry 1991). In comparison, *P. ponderosa* var. *brachyptera* ranges from northern Nevada to southern Texas (USDA, NRCS 2017). On south-facing slopes of the Santa Catalina Mountains, shallow soils are mostly homogeneous lithosols of low water-holding capacity that could promote moisture limitations on annual growth (Shreve 1915, 1919; Whittaker and Niering 1965; Whittaker et al. 1968; Bezy 2016). The transitions in taxa on south slopes are quite dramatic occurring over 100 meters horizontal distance (Epperson et al. 2001).

Evaluating geographic variation in locally adapted populations helped determine differences in species' growth responses to climate throughout their range (Norris et al. 2006; Shinneman et al. 2016; McCullough et al. 2017). Although previous studies indicate North American pine growth is influenced primarily by seasonal precipitation (Dodge 1963; Fritts et al. 1965, Fritts 1976; Norris et al. 2006; Griffin et al. 2013; Dannenberg and Wise 2016; Shinneman et al. 2016; Gonza lez-Ca sares et al. 2017), few ecological studies have compared morphologically distinct taxa to identify interspecific differences (Haller 1965; Peloquin 1984; Rehfeldt 1993; Epperson et al. 2001).

Our study design allowed us to identify individual taxa in the field and evaluate differential sensitivity to climate and water stress. This was accomplished by correlating seasonal climate data with widths of annual tree rings collected from transition zones where both taxa were present. Taxonomic relationships were determined by estimating

(from a small number of branchlets) the frequency of needles per fascicle, an easy way to identify traits in the field and useful for characterizing populations (Epperson et al. 2001). We sampled ring widths to provide information on the climatic factors limiting growth from trees sampled under environmental stress, which occurs near the lower and upper limits of species distributions (Lamarche and Stockton 1974). The growth of trees in this semiarid region is primarily limited by water stress rather than temperature (Fritts 1976). Under low moisture conditions, individual trees produce rings that are very narrow.

Adapted to different ecological conditions, *P. arizonica* and *P. ponderosa* var. *brachyptera* are high-elevation tree species that express dissimilar tolerances to water shortage through their growth responses. Projected climate warming in these semiarid mountainous regions could reduce tree growth as increasing temperatures and decreasing precipitation elevate moisture stress (Barichivich et al. 2014). We expect water stress to be greatest at a tree's lower elevational limit where soil moisture available for growth is lowest (Sheppard et al. 2002). Thus, at the transition zone where the two species co-occur, our first hypothesis predicts *P. ponderosa* var. *brachyptera* growing at its lower-warm elevational limit to be more sensitive to dry conditions and changes in temperature than *P. arizonica* growing near its upper cool-moist elevational limit (Lamarche and Stockton 1974; Adams and Kolb 2005). Palmer Drought Severity Index (PDSI; a measure of soil moisture availability), was evaluated in a second hypothesis predicting that positive growth-PDSI correlations would differ between taxa. The premise is that trees originating from moist (*P. ponderosa* var. *brachyptera*)

compared to dry (*P. arizonica*) environments would be more susceptible to moisture stress.

METHODS

Study area and Sampling

The study areas of Mt. Lemmon (MTL; 32.443°, -110.788°) and Mt. Bigelow (BIG; 32.414°, -110.715°) are located within the Santa Catalina Mountains (2,500 m a.s.l.) of the Coronado National Forest, approximately 28 kilometers straight-line distance northeast of Tucson, Arizona, Pima County, USA (Fig. 2.1). The ponderosa pine species were sampled from steep slopes [average gradient of 45% (MTL) and 37% (BIG); data not shown], of Lithic Haplustolls (Buol 1966, Brown 1968) derived from granite and gneiss, gravelly to rocky in texture and shallow in depth (*c.* 50 to 140 cm; data not shown); a frigid complex with average available water-holding capacity (AWHC) of 9.2% for MTL and 3.8% for BIG (Suppl. Fig. 2.S1). The mixed conifer forest comprises *P. arizonica, P. ponderosa* var. *brachyptera, P. strobiformis, Pseudotsuga menziesii, Quercus hypoleucoides, Q. gambelii, Q. reticulata* (Brown 1968), and rarely *Abies concolor.* Half of the annual precipitation falls during the summer monsoon (July through September; JAS). Winter storms (November through March: NDJFM) provide an additional third or more of the annual rainfall (Table 2.2; Sheppard et al. 2002).

Ponderosa pine species were identified in the field by conducting average needle counts ($c. \ge 10$ fascicles). Trees with average needle numbers per fascicle < 4.6 and \ge 4.6 were designated *P. ponderosa* var. *brachyptera* and *P. arizonica*, respectively (Haller 1965; Peloquin 1984). While avoiding ridges and cold air drainages, we

sampled transition zones within the lower and upper moisture availability limits, respectively, for *P. ponderosa* var. brachyptera, and *P. arizonica*. Nine horizontal transects were established at each study area (MTL and BIG) on southern aspects of similar slope. The nine transects were equivalent to a 5 x 9 grid with 10-m spacing. The nearest tree was sampled to each point, except for the middle of the transition zone (i.e. the three innermost transects), where one P. ponderosa var. brachyptera and one P. arizonica were sampled (Table 2.1). From 2010 to 2012 all healthy trees > 8 cm diameter at breast height (DBH) were selected and sampled. Individual sample trees were tagged and mapped using North American Datum of 1983 (NAD83) geographic coordinates obtained through global positional system (GPS) data collected at the field plots (n = 120; i.e. 2 sites x 2 species x 30 trees). Two or more tree-core samples were collected from each tree using a 5.5-mm increment borer, dried and glued in wood core mounts, and sanded to 600 grit to visualize clear ring boundaries. We visually crossdated all samples using the skeleton-plot technique (Stokes and Smiley, 1996). Tree-ring widths were measured to the nearest 0.01 mm using scanned images (2400 dpi resolution) and CooRecorder[™] software (Cybis Elektronik, 2010). Accurate calendar dates were assigned to each ring in the time sequence with CDendro[™] software (Cybis Elektronik, 2010). Sample dating was statistically verified with the program COFECHA (Holmes et al. 1986).

Climate data

Specific climate data sets for MTL and BIG were developed with 1-km resolution using the ANUSPLIN package (Hutchinson and Xu 2013) by creating 1925-2009

estimates of the temperature and precipitation variables (McKenney et al. 2011). Similarly, the 4-km gridded PDSI values obtained from the West Wide Drought Tracker website (Abatzoglou et al., 2017) were used to create estimates of the local PDSI variable (model and website development by the University of Idaho and the Desert Research Institute, Reno, Nevada). The composite PDSI index is based on variation in temperature, precipitation, and the local AWHC of the soil, and quantifies longer-term departures from normal moisture patterns; more negative values indicate drought stress, ranging from \leq -4 for extremely dry conditions to \geq +4 for extremely wet conditions (Palmer 1965). Climatic variables were summarized as monthly values of average temperature (TAVG), average minimum temperature (TMIN), average maximum temperature (TMAX), total precipitation (PCP), and average PDSI. To validate the quality of climate data, we conducted correlation analysis using cor function in R (R core Team 2016) with local datasets and displayed a matrix of correlation coefficients created using the corrplot package (Wei and Simko 2017). Correlation was also used to examine the interrelationship between climate variables at both sites (Appendix A).

Trend and Climate-growth analysis

Juvenile growth trends were removed from the average ring-width series of individual trees (i.e. de-trended) by fitting a modified negative exponential curve to create standardized indices using the ARSTAN program (Cook 1985). Autocorrelation arises from the persistence of climatic effects on tree growth, which was removed by autoregressive modeling (Cook 1985). The final master ARSTAN chronology was

created using a bi-weight robust mean to account for climatic variance and endogenous disturbance (Cook 1985). We calculated the descriptive statistics of running RBar, expressed population signal (EPS), signal-to-noise ratio (SNR), mean sensitivity (MS), Gini coefficient (G), standard deviation (SD), and autocorrelation (AC) using the dpIR package (Bunn 2008, 2010) for R, which was also used to plot the ARSTAN chronologies. Smoothing splines were applied to the chronologies with defaults in dpIR. All other tree-ring statistics were obtained directly from the ARSTAN DOS output files (Cook 1985). The running RBar [i.e. between-tree correlation (Rbt)] is the average correlation coefficient between all possible pairs of indexed series drawn from different trees (Wigley et al. 1984; Briffa and Jones 1990); we chose a 40-year moving window with 20-year overlap. The coefficient of variation (CV) of Rbt was expressed as the SD of the mean for all species and sites combined. EPS measures the common variability of a chronology when series are averaged, ranging from 0 to 1, and a value greater than 0.85 indicates a strong common signal of the chronology (Wigley et al. 1984; Briffa and Jones 1990, Speer 2010). SNR in de-trended tree-ring series is the ratio of two variances (common climate signal and random error). Mean sensitivity measures the relative change in ring widths from year-to-year (Fritts 1976). It theoretically ranges from 0 (all rings have same width) to 2 (locally absent ring every other year), but in practice varies from 0.1 to 0.6 (Biondi and Qeadan 2008). Gini coefficient ranges from 0 to 1, measures diversity in tree-ring chronologies regardless of the degree of autocorrelation, and improves on intra-annual variability comparisons among species and sites (Bunn et al. 2013; DeRose et al. 2015).

Because monthly climate variables are often highly inter-correlated, tree-ring analyses were conducted using both partial correlations (Meko et al. 2011) and regression involving principal components (Fritts 1974). Partial correlations address collinearity of the primary and secondary climate variables prior to summarizing the seasonal climate signal in the tree-ring data. Partial correlations were used to compare each residual plot chronology to monthly climatic variables to test the null hypothesis of no effects (r = 0; $\alpha < 0.05$). Pearson correlation coefficients were calculated for the primary climate variable (PCP summed; or PDSI averaged), and partial correlations were computed for the secondary climate variable (TAVG), independent of the variance related to the primary variable. Climate variables (PCP, TAVG, and PDSI) were tested over a 14-month climate window from the preceding August to September of the current growth year, to account for the influence of prior environmental conditions on the current season's growth. Subsequently, maximum climate-growth correlations were determined across season lengths of 1, 3 and 6 months reflecting seasonal fluctuations of PCP (primary) and TAVG (secondary) climate variables. Season lengths of 1, 3, 6, 12, and 20 months determined the maximum influence of PDSI (primary) and TAVG (secondary) variables on climate-growth correlations over a time window from the previous October to current August. All seasonal correlations were completed with the treeclim package (Zang and Biondi 2015) for R, using the 'seascorr' function. Finally, we quantified the effect size of PDSI using Pearson correlation coefficients (r) for single months using cor function in R.

We investigated significant variables (p \leq 0.05) identified by simple correlation analysis (treeclim package; Zang and Biondi 2015), which were consistent in sign and

magnitude, as potential predictors to include in response function analysis. Fritts (1974) introduced regression involving principal components to address multicollinearity by transforming predictor climate variables to produce a set of uncorrelated data points. The calculation of the response function regresses tree-ring data against transformed monthly climatic variables to select the parameters that influence tree growth (Fritts 1974, 1976). Each principal component variable explains partial variance in the data set. Relationships between climatic variables and ring-width indices were examined using multivariate estimates obtained from the principal component regression model. The analysis computed bootstrapped response functions using the treeclim package (Zang and Biondi 2015). To obtain robust parameter estimates, we used bootstrapping to test regression coefficients and the stability of estimates (Guiot 1991). Twenty-eight monthly climatic variables (i.e. 14 PCP + 14 TMIN) were analyzed from July through December of the previous year (excluding the transition month of October), and January through September of the current year. Climate variables were partitioned into seasons to quantify water balance (i.e. PDSI), precipitation, and temperature effects during key periods in our study plots (winter, spring and summer). Seasonal variables were derived by combining the monthly data into two rainy seasons consisting of three and five months respectively: summer (JAS) and winter (NDJFM) separated by three months of arid spring (AMJ). Summer season spanned the months from previous July to previous September, and current July to current September; winter season spanned the months from previous November to the current March, and arid spring spanned the months from current April to current June. Climate was also divided into four seasons of three months each, which partitioned winter into fall (OND) and winter (JFM). Mean

chronologies and correlation bar graphs were plotted using plotting functions in the dpIR package; all other plots were created using standard R plotting functions. Seasonal variables were tested for departure from normality with the Kolmogorov-Smirnoff test (α = 0.05). Normality tests, and distributions of single variables were analyzed using JMP Pro, v 13.0 (Copyright © 2016 SAS Institute Inc., Cary, NC), or Sigma Plot, v 13.0 (Copyright © 2014 Systat Software, Inc., San Jose, CA).

RESULTS

Annual and Monthly climate

Climate data of the two sites were averaged to obtain a summary record for the Santa Catalina Mountains. Over the 84-year study period (1925-2009), average yearly PCP is 681.1 \pm 163.9 mm and average yearly TAVG is 10.9 \pm 0.5 °C (Table 2.2). Multicollinearity between PCP and TAVG is low, but significant, with negative correlations of *r* = -0.3 (Prob > IpI = 0.005; Zar 2010). The average PDSI broadly ranges between \pm 4.0 with a few values from 5.0 to 7.0 (Suppl. Fig. 2.S3). We identified four dry periods (PDSI < -2.0) at both sites of two or more year's duration: 1936-1939; 1947-1948; 2002-2003; 2006-2007.

Seasonal climate

PCP was evenly distributed between two rainy seasons: summer (JAS; average 299 \pm 73.3 mm) and winter (NDJFM; average 299.2 \pm 155.0 mm), with August being the wettest month (Table 2.2; Suppl. Fig. 2.S4). The driest season was spring (AMJ; average 48.3 \pm 29.4 mm), with May being the driest month (median < 10 mm). Winter's

median monthly TAVG remained below 6.5 °C, with January being the coldest month (2.7 °C; Table 2.2; Suppl. Fig. 2.S4). Summer remained above the median monthly TAVG of 18.5 °C, with July being the hottest month (19.5 °C). Spring (the driest season) saw the steepest increase in median TAVG from 9 °C in April, to 18.5 °C in June (a rise of 9.5 °C; Suppl. Fig. 2.S4B).

Series length, Chronology statistics, and Growth patterns

Sawtimber trees > 25 cm diameter DBH were the predominant size class sampled at MTL with some pole-sized trees (10 to 25 cm); mean DBH (\pm SD) is 37.1 \pm 14.4 cm, ranging from 15.5 to 85.3 cm. At BIG the sampled trees were smaller, including pole-sized and two saplings (5 to 10 cm); mean DBH (\pm SD) is 27.7 \pm 13.2 cm, ranging from 7.7 to 61.2 cm. Although there were differences in average DBH between sites, the average series length (years \pm SD) and percentage of locally absent rings were similar between sites. Observed mean series length are 73 \pm 23 years ranging from 35 to 148 years, and 76 \pm 41 years ranging from 30 to 162 years, respectively for MTL and BIG. Locally absent rings accounted for 0.32% (MTL) and 0.24% (BIG) of the total rings in all series, combined.

The Gini coefficient and mean sensitivity index quantified moderate year-to-year differences that varied little between taxa or site (Table 2.3). In comparison, the indexed ring widths at MTL are significantly narrower for *P. arizonica* than *P. ponderosa* var. *brachyptera*, and *P. ponderosa* var. *brachyptera* exhibits the highest correlation in growth among trees (Rbt), higher SNR than *P. arizonica* at both sites, and significantly higher SD (14%) than *P. arizonica* at BIG, (p < 0.05; Table 2.3). Considering Rbt², the

coefficient of variation between species is 18% ($\overline{x} = 0.14$; SD = 0.02; Table 2.3). Firstorder autocorrelation values for the standard chronology are higher for BIG (0.41) on average than for MTL (0.22; Table 2.3).

Growth was reduced from the late 1930s to the late 1940s for *P. arizonica* and *P. ponderosa* var. *brachyptera* at BIG (Fig. 2.2C-D). A similar reduction in growth for the 1930s-1940s was not evident at MTL (Fig. 2.2A-B). The average growth of the two species was calculated to obtain indexed ring widths for BIG (0.70 ± 0.12), and MTL (0.91 ± 0.08) during seven drought years. The seven years of decreased growth corresponded to low PDSI values recorded for 1936-39 and 1946-48 (Suppl. Fig. 2.S3). Average annual PDSI for the period was greater (wetter) at BIG (-2.15) than at MTL (-2.45). In contrast, the combined average index of annual rings measured at MTL (0.76 ± 0.23) during the latter eight-year dry period (2002-09) is less than for BIG (0.90 ± 0.28). Average annual PDSI values were similar between sites for the 2002-09 period (MTL, -2.39; BIG, -2.42).

Correlations

Simple correlation and Response function

Correlation analysis (Suppl. Fig. 2.S5) identified seasonal variables for further response function analysis. Fig. 2.3A shows significant positive growth responses with spring PCP (AMJ; p < 0.05) for both taxa, and the correlations were greater at MTL than at BIG. Figs. 2.3A-B also show significant positive PCP-growth correlations for *P. arizonica* at MTL during winter (ndJFM; p < 0.05), and early winter (ond; p < 0.05), and

P. arizonica's growth is negatively correlated with spring TMIN (AMJ; Fig. 2.3A; p < 0.05).

PCP correlation and TAVG partial correlation

Tree-ring index regressions with precipitation approach linearity, indicating they were not controlled by outliers (Suppl. Fig. 2.S6A-D; p < 0.05). Correlations between annual growth and single-month PCP for both species are positive and significant, increasing in strength when summing over multiple months (Fig. 2.4). The maximum positive PCP-growth correlations for *P. arizonica* occur for the three-month periods ending in December, May, and June ($r \approx 0.4$) at MTL (Fig. 2.4A), and in June and July (r \approx 0.4) at BIG (Fig. 2.4B). For *P. ponderosa* var. *brachyptera*, maximum correlations occur for the three-month periods ending in December ($r \approx 0.35$) and June ($r \approx 0.4$) at MTL (Fig 2.4C), and in June and July at BIG ($r \approx 0.35$, 0.4, respectively; Fig 2.4D). Partial correlations between annual growth and single-month TAVG at MTL for P. arizonica and P. ponderosa var. brachyptera are negative and significant, increasing in strength when averaging over multiple months (Fig. 2.4A; 2.4C). The strongest partial correlations between growth and TAVG for *P. arizonica* and *P. ponderosa* var. *brachyptera* occur for the three-month period ending in July ($r \approx -0.30, -0.25$, respectively). Although the trend was largely negative, there are no significant negative partial correlations for *P. arizonica* at BIG (Fig. 2.4B), and the trend for *P. ponderosa* var. brachyptera is significant for the three-month period ending in July ($r \approx -0.25$; Fig. 2.4D).

PDSI correlation and TAVG partial correlation

Correlations between annual growth and single-month PDSI are positive and significant, with maximum values reached in July at both sites for *P. arizonica* [0.7 (MTL); 0.4 (BIG); Table 2.4A] and *P. ponderosa* var. *brachyptera* [0.5 (MTL); 0.4 (BIG); Table 2.4B]. Table 5 shows July's PDSI average effect size of 0.6 at MTL and 0.4 at BIG. Partial correlations between annual growth and single-month TAVG are negative but non-significant for both species at MTL (data not shown), increasing in strength, and becoming significant when averaging over multiple months (Fig. 2.5A, 2.5C). There are no significant temperature correlations for either species at BIG (Fig. 2.5B, 2.5D).

Correlations between annual growth and seasonal PDSI at MTL were of longer duration and stronger for *P. arizonica* (Table 2.4A) than *P. ponderosa* var. *brachyptera* (Table 2.4B). Significant positive correlations (*r*) range from $r \approx 0.3$ to 0.7 for season lengths of 1, 6, 12, and 20 months for *P. arizonica*, and from $r \approx 0.2$ to 0.5 for season lengths of 1, 6, and 12 months for *P. ponderosa* var. *brachyptera*. In contrast, at BIG there were no observed differences between the two species, the significant correlations only occur for season lengths of 1 month and 6 months, and effect sizes were generally lower ($r \approx 0.3$ -0.4; Table 2.4 A-B).

DISCUSSION

Series length, Chronology statistics, and Growth patterns

The average age of the sampled trees was *c*. 74.5 years. This conservative estimate was obtained from the increment cores, not adjusting for pith. Similar aged cohorts (between sites) were sampled to rule out age-related differences. Sympatric

areas (within a site) were sampled to rule out disturbance-related differences, such as insect infestation or fire that could influence the ring-width growth for just one species.

High mean sensitivity and Gini values indicate variability in annual ring widths known as a sensitive growth response to climate. Variability measured as mean sensitivity (MS) is similar to values reported by Fritts (1974) for *P. ponderosa* var. *brachyptera* (average MS = 0.35) and *P. arizonica* (MS = 0.35) populations sampled near our study site at Mt. Bigelow in the early 1960s, which indicate long-term sensitivity to climate and extends the formative work of Fritts. *P. ponderosa* var. *brachyptera* expressed the highest common growth signal based on Rbt (Table 2.3), which suggests the species is responding to strong external climate signals. The dendroclimatic response varies modestly between species and sites, as quantified by the coefficient of variation, which suggests the two closely related ponderosa pine species have different ecological requirements for growth when sympatric.

Autocorrelation is expected to be higher on extreme sites because stressed trees take a year or more to recover following a harsh growing season, thereby conveying persistent physiological effects (Fritts et al. 1965; Monserud and Marshall 2001). Autocorrelation was significantly higher at Mt. Bigelow than Mt. Lemmon, suggesting the former site is more extreme. Large values of autocorrelation are often explained by the storage of carbohydrates in parenchyma tissue or as the result of temporal autocorrelation found in the precipitation variable (e.g. Matalas 1962; Esper et al. 2015). It is unlikely that annual rainfall is the source of the observed persistence in tree-ring indices because total precipitation between sites is highly correlated. Therefore, the

higher value of autocorrelation at Mt. Bigelow is likely caused by biological properties rather than temporal variation in rainfall.

One possible explanation for differences in environmental conditions between sites affecting autocorrelation is the dissimilarity in elevation between the two mountains. The volume of water released after the melt of snowpack is expected to be greater on Mt. Lemmon than Mt. Bigelow as the former is *c*. 250 meters higher in elevation with higher snowfall. Also, the AWHC of the soil at Mt. Lemmon (9.2) is more than two times greater than at Mt. Bigelow (3.8). The combined effect of these features is to increase the storage capacity of the winter snowmelt and prolong the moist conditions of the soil into the growing season when the water demand is highest (Barnett et al. 2005). Thus, the growing environment at Mt. Lemmon may be more favorable for tree species adapted to moister growing conditions.

The two ponderosa pine species experienced recurrent periods of reduced growth. Narrow rings formed during the years 1934, 1936-39, and 1947-48 correspond to low annual PDSI values, and past reports of reduced streamflow (1932-36) and drought (1942-64) in Arizona (McNab and Karl 1991). In both instances, the drought signals of narrow rings were more pronounced at Mt. Bigelow than Mt. Lemmon, a possible result of rockier soils with lower water-holding capacity contributing to lower annual tree-growth at Mt. Bigelow (Candel-Pérez et al. 2012). More recently, growth was reduced during 2002-09 reflecting severe drought impacting the entire Southwest region (Woodhouse et al. 2010). Interestingly, the strongest drought signal for the decade is at Mt. Lemmon.

Fire explains the reversal in site responses observed for the 1930-40s drought compared to the 2002-09 drought. The change in growth pattern may have resulted from wildfire decreasing the available soil moisture and amount of photosynthetic foliar tissue, thereby reducing annual growth at Mt. Lemmon. In fact, throughout the early 21st century, the Santa Catalina Mountains were impacted by several large wildfires. These included the Oracle (2002), Bullock (2002), and Aspen (2003) fires. During the peak growing season of 2003, the Aspen wildfire burned the south slope of Mt. Lemmon, including our study site, but left the Mt. Bigelow site relatively undisturbed. The heat of the fire resulted in foliar injury (Telewski, personal observation), which likely decreased cambial growth through a reduction in foliage and photosynthetic capacity. The formation of a hydrophobic soil layer during wildfire may increase runoff. Dyrness (1976) reported that following a large wildfire in the High Cascades of Oregon, USA (c. 3116 ha), which burned lodgepole pine stands, precipitation runoff increased and recovery (of the soils) was imperceptible until 3-5 years after the fire. The phenomenon of increased runoff would occur even during a drought. Therefore, moisture stress induced by fires in 2003 at Mt. Lemmon would have further reduced observed growth for several more years. Additionally, the PDSI values modeled at Mt. Lemmon would not have captured the effect of increased runoff due to fire because the soil attributes are assumed to be stable.

Response Function Analysis

Response function analysis indicates strong direct positive correlations between precipitation and annual tree growth, which is consistent with previous reports for

ponderosa pine species and moisture limitations in the Southwest (Fritts et al. 1965; Brown 1968; Fritts 1974) and Pacific Northwest (Dannenberg and Wise 2016). Our study agrees with Brown's (1968) work on ponderosa pine species growing near the summit of Mt. Bigelow, which determined soil moisture is often more limiting to photosynthesis (hence cambial growth) than low air temperatures for semiarid conifers, even in winter. There are seasonal influences and our results show that cool springtime conditions increased the inter-annual growth for both taxa (April-June) and demonstrate seasonal variation for the lower elevation species (P. arizonica). P. arizonica is also sensitive to winter moisture at Mt. Lemmon, which implies growing season use of winter water extracted from deep soil for growth (previous November-March). Response function analysis indicates early winter precipitation (previous October-previous December) was influencing the growth of *P. arizonica* as opposed to late winter (January-March), which suggests that snow melt was not a major factor in the species response to winter climate. Although responses to temperature were weaker than to precipitation, Fig. 2.3 shows a growth reduction occurred for *P. arizonica* when minimum spring temperature (April-June) rose above a critical level at Mt. Lemmon. The observed reduction in correlations between tree rings and minimum temperature has been reported in other southwestern ponderosa pine studies and is explained by increased respiration or drought stress (Fritts 1974, 1976; Adams and Kolb, 2005), which negatively affected the growth of *P. arizonica* but not *P. ponderosa* var. brachyptera.

Correlations to Climate

Analysis of seasonal response functions of ponderosa pine indicated that high precipitation during the cool-moist conditions of winter (November-March), and spring (April-June) are most important for tree growth. Our results broadly agree with González-Cásares (2017) who reported maximum positive correlation for the 9-month period ending in June (October-June) for the annual growth of *P. arizonica* in northwestern Mexico.

Further analysis of the climate-growth relationships with correlation analyses indicated that the annual growth of the ponderosa pine species was under significant moisture stress in spring. The strong positive correlation to spring precipitation and negative partial correlation to spring temperature indicates that the semiarid local climate of the Santa Catalina Mountains has negatively impacted the growth of *P. ponderosa* var. *brachyptera* growing at its lower elevational limit. In comparison, the growth of *P. arizonica* growing at its upper elevational limit was only impacted at Mt. Lemmon. A consistent temperature signal is lacking for the tree-ring indices at Mt. Bigelow, demonstrating differences in habitat suitability between sites for ponderosa pine growth.

These results correspond to the conceptual models of Fritts (1974, 1976) that describe the relationships leading to moisture stress prior to or during the growing season, resulting in the formation of narrow rings. High temperature and low precipitation are primarily responsible for decreased growth of semiarid site conifers. With these models in mind, we considered drought conditions in spring, which can lead

to high evapotranspirational demand and the formation of narrow rings during the growing season.

Sensitivity to PDSI

P. arizonica and *P. ponderosa* var. *brachyptera* are drought-sensitive species (Peltier et al. 2016), which show strong positive PDSI-growth correlations, although the climate-growth relationships vary between species at Mt. Lemmon. The PDSI-growth correlations did not support our hypothesis that *P. ponderosa* var. *brachyptera* would be more sensitive to soil moisture availability. *P. arizonica* has a stronger long-term relationship, while *P. ponderosa* var. *brachyptera*'s correlations were for a shorter duration and weaker. There are differences between sites; correlation to drought lasts longer at Mt. Lemmon than Mt. Bigelow, regardless of species.

A multi-species synthesis of ring-width variance has shown that *P. ponderosa* has low resistance and slow recovery to drought (Peltier et al. 2016). Similarly, the observed extended correlation of growth with PDSI in this study implies that *P. arizonica* and *P. ponderosa* var. *brachyptera* may be vulnerable to changing climate (Gonzalez-Casares 2017). Thus, warmer conditions may have a more negative effect on *P. arizonica*, as periods with low PDSI become longer or more common. Other studies have noted differences in growth sensitivity to climate for the *Pinus* species of Mexican pine forests (Bickford et al. 2011; Gonzalez-Casares et al. 2017). These dissimilarities have been partially credited to different drought tolerances of the species under study. Although more work is required to build the appropriate models, the length of correlation to PDSI could be one factor useful in determining tolerance to moisture stress.

It is interesting that tree growth correlated to PDSI for the longest period at Mt. Lemmon, the more favorable site. Although not as extreme as the crevice sites in the Western USA described by Fritts (1976), the ponderosa pine species of Mt. Bigelow also grow in an extreme environment with shallow, rocky soils, and restricted root space. Though counterintuitive, Fritts' (1976) description of crevice sites in Arizona describes dry soils as constraining root and crown growth for longer periods of time, and trees acclimate to these conditions so that growth responses to precipitation will be less vigorous than trees growing on more favorable sites. Thus, trees growing on more favorable sites will suffer more stress from drought than trees growing on drier sites, which have acclimated to low water availability. Therefore, one reason for the longer drought influence at Mt. Lemmon is the greater loss of green needle tissue related to prolonged water stress. This hypothesis of reduction in photosynthetic capacity is supported by Galiano et al. (2011) who determined that carbon reserves are key to tree recovery and forest resilience following periods of drought; consequently, respiration will influence carbon reserves and tolerance to moisture stress.

Respiration

Temperatures on south-facing slopes may exceed the optimum range for plant processes during the growing season or winter months (Fritts 1974). Therefore, slope aspect greatly affects the correlation of ring widths with climate, and annual ring widths are expected to correlate negatively with variations in monthly temperature. The conceptual models of Fritts (1974, 1976) describe the pathways leading to increased respiration, and the formation of narrow rings. These negative temperature effects on

growth are apparent during the growing season due to high temperature and respiration reducing the plant photosynthetic processes (Fig. 1 of Fritts 1974). Negative growth effects are also expected from elevated temperatures during the winter months (i.e. prior to the growing season) due to the direct effects of warm air temperature on respiration resulting in increased carbon consumption and decreased carbon allocation; consequently, cold hardiness is reduced (Fig. 2 of Fritts 1974; Ögren et al. Sundbald 1997). Our results support Fritts' model of growing season respiration and winter respiration. The correlation analysis indicated growth of *P. arizonica* was limited by spring and summer drought stress (+, PDSI) and temperature (-, TAVG) at Mt. Lemmon, an indication of increased respiration during the growing season. In comparison, P. ponderosa var. brachyptera was limited by moisture stress (+, PDSI) and temperature (-, TAVG) during Mt. Lemmon's winter season. Thus, the two species are responding differently to water balance requirements and carbon limitations. The most common cause of increased evapotranspirational demands is high air temperature at low elevations, which may cause increased moisture stress in spring and summer (P. arizonica) and decreased cold tolerance in winter (P. ponderosa var. brachyptera).

CONCLUSIONS

The growth of the two species responded positively to the cool-wet conditions of spring, and winter precipitation also improved the growing conditions for *P. arizonica*. We did not find support for higher climate sensitivity in *P. ponderosa* var. *brachyptera* (at its lower elevational limit) above that observed in *P. arizonica* (at its upper elevational limit). However, we report small differences in ring-width variances between

P. ponderosa var. brachyptera and P. arizonica, and that both species are sensitive to the negative effects of respiration on annual growth in different seasons, winter and summer, respectively. Notably, rising night-time temperatures during springtime reduced growth for *P. arizonica* but not *P. ponderosa* var. brachyptera at Mt. Lemmon, most likely from increased respiration or moisture stress. Tree rings correlated to PDSI at different temporal scales, which suggests that *P. arizonica* is sensitive to drought for longer periods. Although our data did not support our hypothesis that *P. ponderosa* var. *brachyptera* would be more sensitive to soil moisture availability, the temporal differences in drought sensitivities distinguish the two taxa by their varying tolerances to water shortages. Both species exhibit a stronger climatic response at Mt. Lemmon (the less dry site) than Mt. Bigelow, and moisture availability between sites can be further influenced by disturbance. In summary, the growth of *P. ponderosa* var. brachyptera and *P. arizonica* at their transition zone demonstrated subtle, biologically meaningful differences in seasonal precipitation requirements for growth, sensitivity to moisture stress and tolerance to cold temperatures.

Data availability: The tree-ring datasets generated and analyzed during the current study are available from the International Tree-Ring Data Bank (ITRDB) repository, https://www.ncdc.noaa.gov/data-access/paleoclimatology-data/datasets/tree-ring. These datasets include the raw ring widths for 90 trees (204 radii) and 4 composite chronologies listed in "Methods" (see Tree Growth Data).

Electronic supplementary material: The online version of this article (https://doi.org/10.1007/s0046 8-018-1778-9) contains supplementary material, which is available to authorized users. The supplementary materials are also included in

Appendix D, which comprise Suppl. Fig. 2.S1: Figures of soil AWHC, Suppl. Fig. 2.S2: Correlations of modeled and locally collected climate data, Suppl. Fig. 2.S3: Plots of annual PDSI values, Suppl. Fig. 2.S4: Climate graphs, Suppl. Fig. 2.S5: Correlation coefficients of seasonal climate variables with tree-ring chronologies, Suppl. Fig. 2.S6: Scatterplots of mean tree-ring index against the average primary precipitation variable.

Author contributions: P.M. conceived the study; P.M. and F.T. designed the experiments and collected samples in the field; P.M. led and S.J. and G.T. assisted with preparing the samples; P.M. led and B.M assisted with data analysis; P.M. wrote the paper; and B.M, F.T., S.J., and G.T. edited the paper.

Conflicts of interest: The authors declare no conflicts of interest.

APPENDICES

APPENDIX C

Validation of Climatic Data

Validation of Climatic Data

Soils data used in the West Wide Drought Tracker PDSI calculation were obtained from Penn State University (Abatzoglou et al. 2017). The applicability of the 4km resolution PDSI data was assessed by comparing the AWHC values of the specific soil units in the calculation (Abatzoglou et al. 2017) to those represented in the local soils plots near the research sites (n= 180; 60 trees x 3 sites; Top panel; Suppl. Fig. 2.S1). The soils data used in the PDSI calculation indicate AWHC of 6.0% for the area encompassing the study sites. The modeled PDSI data were validated by interpolating AWHC values from soil pedons sampled from the Coronado National Forest (n = 14; Lower Panel; Suppl. Fig. 2.S1) and averaging the interpolated values for sampled tree locations (n = 120; 60 trees x 2 sites; AWHC = 5.9%). Precipitation and TAVG data were validated by correlating the modelled site data with locally collected weather data that were accurate but too short for tree-ring analysis. Palisades' 17 years of PCP data (1965 to 1981) collected at 2425 m a.s.l. within 0.7 km of the study sites (Vose et al. 2014), were used to validate the monsoonal patterns (Suppl. Fig. 2.S2). Kitt Peak's 56 years of climate data (1960 to 2015; Vose et al. 2014) within c. 95 km and similar in elevation (2070m) to the study sites, were used to validate winter PCP and TAVG (Suppl. Fig. 2.S2B-C). Pearson correlations were applied to validate the gridded climate dataset (McKenney et al. 2011). Correlations were consistently highest for the McKenney / NOAA PAL associations. Same month-month correlations are positive and range from 0.8 (June-June) for the TAVG association (Suppl. Fig. 2.S2C), to 1.0 (e.g. Feb.-Feb.) for the PCP associations (Suppl. Fig. 2.S2).

Validation of Climatic Data (continued)

The strength of the collinear correlation is weakest for June-June PCP for the McKenney Mt. Lemmon / Kitt Peak dataset (0.52; Suppl. Fig. 2.S2) because summer monsoon strongly influences Mt. Lemmon's climate but not Kitt Peak's.

APPENDIX D

Supplementary Figures

Supplementary figures



Figure 2.S1 Figures of soil AWHC (%) used to validate PDSI data analyzed in study.

Figure 2.S1 (continued)

The upper map depicts an AWHC of 6.0% (the modeled data set). The lower map was generated by values interpolated from 14 soil samples taken from three locations within the Coronado National Forest: MTL (Blue), Palisades (PAL; Green), and BIG (Yellow). The average AWHC 5.9% among study plots (n = 180) was adjusted for depth to bedrock and for rock content (AWHC for MTL, PAL and BIG are 9.2, 4.7, and 3.8% respectively). The lower map used a conventional weighted average of the soil horizon available water capacity (AWC)
	Jan	Feb	Mar	Apr	May	ηη	٦٢	Aug	Sep	Oct	Nov	Dec	
Jan	0.99	0.3		0.24	-0.14	-0.42	0.67	-0.26	-0.13	-0.44	0.49	0.2	- 0.9
Feb	0.26	1	0.28	0	0.41	-0.04	0.07	0.1	-0.44	-0.54	0.34	0.01	- 0.8
Mar	-0.09	0.26	1	0.35	0.42	-0.36	0.47	-0.66	-0.18	-0.62	-0.12	-0.29	- 0.7
Anr	0.16	0.02	0.31	0.00	0.33	0.45	0.5	0.44	0.24	.0.4	0.26	0.52	- 0.5
	0.10	0.02	0.51	0.99	-0.55	-0.45	0.5	-0.44	-0.24	-0.4	0.20	0.52	- 0.4
Мау	-0.12	0.47	0.53	-0.32	0.99	0.11	0.1	-0.27	-0.56	-0.21	0.25	-0.38	- 0.2
Jun	-0.37	-0.13	-0.45	-0.48	0.01	0.98	-0.66	0.2	0.09	0.6	-0.14	-0.08	- 0.1
Jul	0.47	0.24	0.62	0.31	0.35	-0.54	0.89	-0.61	-0.07	-0.47			0.1
Aug	-0.25	0.24	-0.61	-0.46	-0.11	0.25	-0.67	0.96	0.25	0.17			0.2
Sep	-0.07	-0.42	-0.22	-0.39	-0.47	-0.01	-0.13	0.35	0.98	0.17	-0.72	-0.36	0.4
Oct	-0.36	-0.59	-0.6	-0.42	-0.19	0.59	-0.44	0.21	0.16	1	-0.36	-0.03	0.5
Nov	0.51	0.39	-0.09	0.26	0.27	-0.2	0.22	-0.11	-0.65	-0.42	0 99	0.56	0.7
_	0.01	0.59	-0.03	0.20	0.21	-0.2	0.22	-0.11	-0.00	-0.42	0.99	0.00	0.8
Dec	0.15	-0.01	-0.34	0.54	-0.34				-0.2		0.55	1	-0.9

Figure 2.S2 Correlations of modeled and locally collected climate data are collinear and validate the accuracy of the gridded climate dataset (i.e. same month correlations with high positive *r*-value, e.g. May-May). The McKenney et al. (2011) modelled variables are on the y-axis and corresponding local NOAA variables are on the x-axis. (A)Total PCP: modeled PAL (y) to local PAL(x)

Figure 2.S2 (continued)

	Jan	Feb	Mar	Apr	May	ηυΓ	٦٢	Aug	Sep	oct O	Nov	Dec	
Jan	0.85	0.18	0.13	-0.27	0.14		-0.15		-0.16				- 0.
Feb	0.28	0.77	0.32	-0.06	0.23	-0.2	-0.19	0.16	-0.03	-0.14	0.36	0.13	- 0.
Mar	0.16	0.32	0.88	-0.04	0.33	-0 14	0.07	-0.23	-0.03	-0.04	0.09	-0.03	- 0.
	0.10	0.02	0.00	0.04	0.00	0.14	0.01	0.20	0.00	0.04	0.00	0.00	- 0.
Apr	-0.11	-0.09	-0.11	0.79	-0.18	-0.11	0.35	-0.14	0	-0.12	0.17	0.34	- 0.
Мау	0.26	0.18	0.23	-0.13	0.71	0.12	-0.28	0.04	-0.16	-0.01	0.16	0.27	- 0.
Jun	-0.14	-0.3	-0.12		0.1	0.52	0.36			0.14		0.26	- 0.
Jul	-0.15		-0.03	0.24	-0.14		0.79	0.13	0.15			-0.13	0
Aug	-0.01	0.12	-0.08	-0.1	-0.02	-0.05	0.1	0.77	0.31	0.11	0.02	0.08	-0
Sep	-0.24	-0.25	0.08	0.17	-0.26	-0.09	0.36	0.04	0.77	-0.01	-0.08	0.08	0
Oct	-0.07	-0.21	-0.05	-0.04	-0.17	0.39	-0.16	0.2	0.2	0.93	0.14	-0.02	0
Nov	0.05	0.02	0.11	-0.03	0.2	-0.06	-0.07	-0.07	0.12	0.19	0.79	0.25	0
_	0.00	0.02	0.11	0.00		0.00	0.01	0.07	V.12	0.10		0.20	0
Dec		-0.11	-0.01	0.16	0.21	-0.1	-0.13	-0.12			0.46	0.92	-0

(B) Total PCP: modeled MTL (y) to local Kitt Peak (x)

Figure 2.S2 (continued)

	Jan	Бeb	Mar	Apr	May	ηη	٦٢	Aug	Sep	Oct	Nov	Dec	
Jan	0.91	0.43	0.1	0.09	0.22	0.06	0.15	0.15	-0.05	-0.18	-0.02	0.08	- 0.9
Feb	0.14	0.92	0.04	0.16	0.1	-0.01	0.01	-0.26	-0.03	-0.05	0.11	0.01	- 0.8
Mar	0.05	0.08	0.92	0.34	0.28	0.28	0.09	-0.04	0.07	-0.19	-0.05	-0.39	- 0.6
Apr	-0.05	0.26	0.2	0.91	0.23	0.38	0.2	0.09	0.03	-0.05	-0.09	0.06	- 0.5
Мау	0.09	0.26	0.28	0.31	0.87	0.2	0.07	-0.04	0.06	-0.18	0.05	-0.11	- 0.3
Jun	0.01	0.2	0.21	0.49	0.19	0.8	0.07	0.05	-0.14	-0.08	0.03	0.04	- 0.2
Jul	-0.08	0.05	0.01	0.17	0.07	0.09	0.81	0.03	0.22	0.02	-0.13	0.23	- 0
Aua	0.15	-0.01	0	0.3	-0.01	0.19	0.06	0.86	0.26	0.1	0.22	0.19	-0.1
Sen	.0.14	0.06	0.04	0.11	0.07	0.04	0.31	0.10	0.90	0.26	0.03	0.26	0.3
Oct	0.06	0.00	0.04	0.05	0.01	0.42	0.02	0.10	0.40	0.20	0.00	0.02	0.5
Neu	-0.20	-0.08	-0.20	-0.05	-0.10	-0.15	0.02	-0.02	0.19	0.94	0.22	0.05	0.6 0.7
NON	0.03	0.28	-0.07	0.02	0.1	0.04	-0.07	0.17	0.11	0.24	0.94	0.15	0.8
Dec		0.1	-0.43				0.22		0.14			0.91	-0.9

(C) TAVG: modeled MTL (y) to local Kitt Peak (x)



Figure 2.S3 Plots of average annual PDSI values estimated for two study plots MTL (top) and BIG (bottom), for the reference period 1925-2009. Yearly values are depicted along the x-axis and average PDSI index along the y-axis. Negative values denote dry conditions



Figure 2.S4 Climate graphs of mean monthly climate data analyzed for BIG (Mt. Bigelow) research site (-110.71495, 32.41378; 2535 m elevation) for the reference period of 1925-2009 (McKenney et al., 2011).

Figure 2.S4 (continued)

Climate variables are total precipitation PCP (A) and mean average temperature TAVG (B). The box shows location of the middle quartile of monthly observations, the median is marked by the horizontal line in the center of the box, and the whiskers extend to the most extreme values not considered outliers. An outlier is marked by a closed circle and is defined as any data value more or less than 1.5d beyond the edges of the box, where *d* is the interquartile range



Figure 2.S5 Correlation analysis for *P. arizonica* (white), *P. ponderosa* var. *brachyptera* (grey) growing at two transition zones within the Santa Catalina Mountains: MTL (hatched bars) and BIG (non-hatched bars).

Figure 2.S5 (continued)

Analyzed were climate variables of precipitation (PCP) and minimum temperature (TMIN) with seasons: Previous (Pr) Summer (Jul-Sep), Winter (Nov-Mar), Spring (Apr-Jun), and Summer (Jul-Sep) (A); and climate variables of PCP and maximum temperature (TMAX with 3-month seasons: Pr Summer, Early Winter (Oct-Dec), Late Winter (Jan-Mar), Spring, and Summer (B), which were related to the composite chronologies for the period of 1925 - 2009. The standard index was used in the growth analysis of *P. arizonica* on MTL, and autoregressive modeling was applied to all other tree-ring time series to remove autocorrelation from past-year's indices



Figure 2.S6 Scatterplots of the mean tree-ring index (ARSTAN or Standard chronology) against the average primary PCP variable (summing across months) for the most highly correlated periods in the Seascorr analyses (Meko et al. 2011). These periods are defined as the three season-lengths with the highest correlation.

(A) Plot of Mt. Lemmon *P. arizonica* tree-ring index (y) by PCP (x); all p-values are highly significant

Figure 2.S6 (continued)



(B) Plot of Mt. Lemmon *P. ponderosa* var. *brachyptera* tree-ring index (y) by PCP (x); all p-values are highly significant



(C) Plot of Mt. Bigelow P. arizonica tree-ring index (y) by PCP (x); p-values are sig.



(D) Plot of Mt. Bigelow *P. ponderosa* var. *brachyptera* tree-ring index (y) by PCP (x), all p-values are highly significant

APPENDIX E Tables and Figures Table 2.1 Sampling design used for two south-facing slopes, Santa Catalina range

PPB	PPB	PPB	PPB	PPB
PPB	PPB	PPB	PPB	PPB
PPB	PPB	PPB	PPB	PPB
PPB/	PPB/	PPB/	PPB/	PPB/
PAZ	PAZ	PAZ	PAZ	PAZ
PPB/	PPB/	PPB/	PPB/	PPB/
PAZ	PAZ	PAZ	PAZ	PAZ
PPB/	PPB/	PPB/	PPB/	PPB/
PAZ	PAZ	PAZ	PAZ	PAZ
PAZ	PAZ	PAZ	PAZ	PAZ
PAZ	PAZ	PAZ	PAZ	PAZ
PAZ	PAZ	PAZ	PAZ	PAZ

(sites MTL and BIG)

Elevation increases from the bottom to top of schematic. Each box represents 1 sample

point on a 5 x 9 grid. One tree was sampled c. every 10 m except for the center of the

transition zone, where 1 PPB and 1 PAZ each were sampled

N = 30 trees collected per species

PAZ P. arizonica, PPB P. ponderosa var. brachyptera

Table 2.2 Summaries for climate data obtained from gridded data sets (McKenney et al. 2011) for two study sites: MTL and BIG

	MTL	BIG
Geographic Coordinates, WGS84	32.43, -110.79	32.41, -110.71
(Latitude, Longitude)		
Elevation (m)	2577	2534
Years spanned	1925-2009	1925-2009
Years of record	84	84
Yearly TAVG (mean ⁰ C <u>+</u> SD)	11.0 <u>+</u> 0.6	10.8 <u>+</u> 0.5
January TAVG (mean ⁰ C <u>+</u> SD)	2.8 <u>+</u> 1.7	2.6 <u>+</u> 1.8
July TAVG (mean ⁰ C <u>+</u> SD)	19.5 <u>+</u> 1.0	19.4 <u>+</u> 0.8
Yearly PCP (mean mm <u>+</u> SD)	678.8 <u>+</u> 164.1	685.0 <u>+</u> 163.8
Winter PCP (mean mm <u>+</u> SD)	298.7 <u>+</u> 154.5	299.7 <u>+</u> 155.4
Arid spring PCP (mean mm <u>+</u> SD)	47.8 <u>+</u> 29.2	48.8 <u>+</u> 29.7
Summer PCP (mean mm <u>+</u> SD)	296.9 <u>+</u> 72.9	301.1 <u>+</u> 73.7

Temperature year considers coldest and hottest months: January and July.

Precipitation year is divided into three seasons: winter (previous November - current

March), arid spring (April – June), and summer (July – September)

Table 2.3 General statistics for the common interval (1925-2009) of the de-trended chronologies for *P. arizonica* and *P. ponderosa* var. *brachyptera* from transition zones at two sites (MTL and BIG)

	M	ITL	BIG			
	P. arizonica	brachyptera	P. arizonica	brachyptera		
No. trees (No. radii)	25 (58)	24 (64)	24 (40)	20 (40)		
Ave No. <u>+</u> yrs. SD	67 <u>+</u> 10	63 <u>+</u> 12	63 <u>+</u> 20	59 <u>+</u> 22		
RWI	0.96 a	1.00 b	0.96	0.97		
SD	0.38	0.36	0.40 a	0.46 b		
MS	0.35	0.34	0.36	0.36		
Gini	0.21	0.20	0.22	0.25		
Rbt	0.35	0.39	0.33	0.40		
Rbt ²	0.12	0.15	0.11	0.16		
EPS	0.94	0.94	0.90	0.92		
SNR	14.93	17.09	9.44	12.08		
Autoregressive model	0	2	2	2		
Variance due to Autoregression	n.a.	16.4	23.4	28.7		
AC1 ^a	0.13	0.32	0.38	0.45		

Statistically significant differences are indicated by lowercase letters (p<0.05)

RWI ring width index; SD standard deviation; MS mean sensitivity; Gini Gini coefficient;

Rbt between tree correlations; EPS expressed population signal; SNR signal-to-noise

ratio; AC1 first-order autocorrelation

^a Standard Chronology

(4A)	PAZ												
SITE	MTH	LEN	COEF	SIG									
MTL	J	1	0.44	*	6	0.33	*	12	0.29	*	20	0.26	
MTL	F	1	0.45	*	6	0.38	*	12	0.31	*	20	0.29	*
MTL	М	1	0.49	*	6	0.44	*	12	0.34	*	20	0.31	*
MTL	А	1	0.53	*	6	0.48	*	12	0.37	*	20	0.33	*
MTL	М	1	0.59	*	6	0.52	*	12	0.41	*	20	0.36	*
MTL	J	1	0.61	*	6	0.55	*	12	0.45	*	20	0.38	*
MTL	J	1	0.67	*	6	0.58	*	12	0.49	*	20	0.42	*
MTL	А	1	0.58	*	6	0.61	*	12	0.53	*	20	0.44	*
MTL	S	1	0.53	*	6	0.62	*	12	0.56	*	20	0.46	*
BIG	М	1	0.27	*	6	0.21		12	0.15				
BIG	J	1	0.29	*	6	0.23		12	0.17				
BIG	J	1	0.36	*	6	0.26	*	12	0.19				
BIG	А	1	0.31	*	6	0.29	*	12	0.22				
BIG	S	1	0.29	*	6	0.31	*	12	0.24				

Table 2.4 Correlation coefficients of seasonal PDSI with tree-ring chronologies

	Table 2.4	(continued)
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(4B) PPB	
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Site	Μ	LEN	COEF	SIG	LEN	COEF	SIG	LEN	COEF	SIG	LEN	COEF	SIG
MTL	J	1	0.23	*	6	0.06		12	-0.01		20	-0.06	
MTL	F	1	0.24	*	6	0.13		12	0.02		20	-0.03	
MTL	Μ	1	0.28	*	6	0.20		12	0.05		20	-0.01	
MTL	А	1	0.33	*	6	0.25	*	12	0.09		20	0.02	
MTL	Μ	1	0.40	*	6	0.30	*	12	0.14		20	0.05	
MTL	J	1	0.43	*	6	0.33	*	12	0.19		20	0.08	
MTL	J	1	0.48	*	6	0.38	*	12	0.24	*	20	0.12	
MTL	А	1	0.40	*	6	0.40	*	12	0.29	*	20	0.15	
MTL	S	1	0.35	*	6	0.42	*	12	0.32	*	20	0.17	
BIG	М	1	0.31	*	6	0.25		12	0.22				
BIG	J	1	0.32	*	6	0.27		12	0.24				
BIG	J	1	0.39	*	6	0.30	*	12	0.26				
BIG	А	1	0.35	*	6	0.32	*	12	0.28				
BIG	S	1	0.33	*	6	0.34	*	12	0.30				

(A) PAZ P. arizonica

(B) *PPB P. ponderosa* var. *brachyptera, MTH* month, *LEN* length of season in months. One-, 6-, and 12-month seasons were analyzed at both sites; 20-month increment is also analyzed at MTL, significance level of 0.05. Partial correlation coefficients for the secondary climate variable (TAVG) are not shown

		Strongest	Month		Strength
Site	Таха	Score	highest <i>r</i>	r	effect
		Single			
MTL	P. arizonica	month	July	0.67	Large
		Single			
MTL	P. pond. var. brachyptera	month	July	0.45	Medium
		Single			
BIG	P. arizonica	month	July	0.36	Medium
		Single			
BIG	P. pond. var. brachyptera	month	July	0.39	Medium

Table 2.5 Effect sizes (*r*) of PDSI as a measure of drought sensitivity ($\alpha = 0.05$)

All p-values are highly significant (p < 0.001)



Fig. 2.1 Locations of two study sites sampled for tree-ring analysis: MTL (Mt. Lemmon) and BIG (Mt. Bigelow). Black triangles mark the study sites northeast of Tucson, AZ USA



Inset shows the study area located in southeast AZ, USA (red circle)



Fig. 2.2 ARSTAN or Standard chronologies of *P. arizonica* (A,C) and *P. ponderosa* var. *brachyptera* (B,D) when Expressed Population Signal (EPS) is greater than 0.85, and sample depth is > 14, for the reference period of 1925–2009. Two sites were evaluated: MTL (A,B) and BIG (C,D). The corresponding sample depth (number of tree cores) is indicated by shading





Figure 2.3 (continued)

Climate variables of precipitation (PCP) and minimum temperature (TMIN) with seasons: Previous (Pr) Summer (July-September), Winter (November-March), Spring (April-June), and Summer (July-September) (A); and climate variables of PCP and maximum temperature (TMAX with 3-month seasons: Pr Summer, Early Winter (October-December), Late Winter (January-March), Spring, and Summer (B), which were related to the composite chronologies for the period of 1925-2009. The standard index was used in the growth analysis of *P. arizonica* on MTL, and autoregressive modeling was applied to all other tree-ring time series to remove autocorrelation from past-year's indices



Figure 2.4 Climate-growth associations obtained by relating total precipitation (PCP) as primary climate variable (top in each panel) and mean average temperature (TAVG) as secondary climate variable (bottom in each panel) to the ARSTAN ring-width chronologies of *P. arizonica* (A,B) and *P. ponderosa* var. *brachyptera* (C,D) at two sites MTL (A,C) and BIG (B,D) [Note: Standard chronology for (A)]. PCP was summed and TAVG was averaged for a period of three months whose ending months are shown on the x-axes (previous August to current September). Significant correlations and partial correlations ($p \le 0.05$) are shown by dark bars



Figure 2.5 Climate-growth associations obtained by relating average PDSI as primary climate variable (top in each panel) and mean average temperature (TAVG) as secondary climate variable (bottom in each panel) to the ARSTAN ring-width chronologies of *P. arizonica* (A,B) and *P. ponderosa* var. *brachyptera* (C,D) at two sites MTL (A,C) and BIG (B,D) [Note: Standard chronology for (A)]. PDSI was summed and TAVG was averaged for a period of three month whose ending months are shown on the x-axes (previous August to current September). Significant correlations and partial correlations ($p \le 0.05$) are shown by dark bars

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CHAPTER 3

SHIFTS IN CLIMATE-GROWTH RELATIONSHIPS OF THE SKY ISLAND PONDERSOA PINES

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Acknowledgments: This paper is part of a dissertation submitted to Michigan State University in partial fulfillment of requirements for a Doctor of Philosophy degree. The USDA Forest Service, and the Dept. of Plant Biology, Michigan State University provided financial support for the project. We thank the following people for help on the project: M. Munro and C. Baisan supplied H. Fritts' chronology samples. F. Telewski helped with experimental design. B. Miranda provided processing support. J. Stanovick provided statistical support. H. Jenson provided GIS support. D. Donner reviewed the paper. We also thank the USDA Coronado National Forest for providing permission to sample trees in the Santa Catalina Mountains.

ABSTRACT

Abstract: Steep climate gradients permit the coexistence of species filling different ecological niches. We compared the sensitivity and temporal stability of climate-growth relationships across two contact zones in southeastern, Arizona, USA to quantify the growth responses of sympatric morphotypes (3-, mixed-, 5-needle) of ponderosa pine to changing climate. Positive climate-growth correlations in semiarid forests indicated a seasonal shift from summer- to spring-dominant precipitation since 1950, impacting tree growth and reproduction. Mixed- and 5-needle responded to winter precipitation, a possible hybrid condition. Furthermore, opposing trends in response to temperature were observed at the less dry site, which can dilute the climate signal when data are combined into a single chronology. Our results highlight important functional differences among morphotypes in response to climate. Tree phenology and sensitivity of growth functions were impacted by climatic shifts; therefore, a climate trend that increases local moisture stress may impact hybridity and the stability of climate-growth relationships.

Keywords: dendrochronology; ecology; Pinaceae; *Pinus;* Ponderosae; response function; tree rings

INTRODUCTION

The Madrean Sky Islands are rugged mountain ranges isolated by desert that signify natural ecological laboratories heavily influenced by the last glacial period 10-20,000 years ago (Warshall 1995). The steep rocky terrain offers opportunities to study biological populations across varied microclimate gradients and landscapes. Tree growth at the elevational limits of gradients are particularly susceptible to varying climate because small changes in the environment may have a large impact on annual growth (Jump et al. 2006; Tegel et al. 2014). In the Western United States, the consequences of rising temperatures and seasonal shifts toward earlier onset of spring and reduced snowpack are being observed at high elevation, and these trends in shifting climate are expected to increase the length of hydrological drought by the end of the century (USGCRP 2017).

The Sky Islands of Southeastern Arizona include the well-known Santa Catalina Mountains, which contain habitat suitable for stands of ponderosa pine that include three morphological variants representing two distinct species (Rehfeldt 1999; Epperson et al. 2009). At high elevation two species co-occur, *Pinus arizonica* Engelm. and the closely related *P. ponderosa* Lawson & Lawson var. *brachyptera* (Engelm.) Lemmon. The latter species is also known as Taxon X and was previously misidentified as *P. ponderosa* Lawson & Lawson var. *scopulorum* Engelm. (Rehfeldt 1999; Epperson et al. 2009; Willyard et al. 2017), even though the two are clearly distinguishable by needle traits with high heritability (Rehfeldt 1992). *P. ponderosa* var. *brachyptera* exists in two forms, a nearly pure 3-needle type that survives at the highest altitudes on southern slopes (2300 - 3000 m), northern slopes and cold air drainages, and a mixed-needle

type that is interspersed with the 3-needle type at transition zones. *P. arizonica* is a 5needle type found at lower elevations (below 2600 m). Thus, the 5-needle type is more successful at warmer and drier, lower elevations; whereas, the 3-needle type survives at colder and wetter, higher elevations. On steep southern slopes the sharp transition of species occurs over just *c*.130 meters slope distance (Epperson et al. 2001, 2009).

We combined our dataset with Hal Fritts' Mt. Bigelow chronologies (Dodge 1963; Fritts 1963, 1974), part of an earlier meta-analysis which consisted of the three cooccurring needle types (3-, mixed- and 5-needle) sampled across a narrow climate gradient. We sampled the same population *c*. 50-years later for a comparative analysis of similar aged cohorts, and also sampled the less dry Mt. Lemmon location to determine the effect of site conditions on growth responses. Our goal was to determine whether the climate-growth relationships have changed over the last century. Shifting seasonality or relationships with limiting factors would have implications for reliably predicting the vulnerability of tree species and needle types to climate change, conservation management, and climate reconstruction.

Previously, we reported on the two species of ponderosa pine displaying different growth responses to moisture stress that varied based on the microsite environment (Marquardt et al. 2018b). *P. arizonica's* growth was reduced for longer periods by drought than *P. ponderosa* var. *brachyptera*, and the climatic response was greater at the site with higher soil moisture content. Since 1906, average global temperatures have been steadily increasing (0.74 °C \pm 0.18 °C; IPCC 2007). Because rising temperatures and predicted shifts in the monsoon season will influence weather patterns and drought in the Southwestern USA (Pascale et al. 2017), we hypothesized
that the seasonality of limiting factors will change over time. The objective of the study was to compare the correlations of ring widths with temperature and precipitation to assess shifts in the seasonality of climate-growth responses.

METHODS

Study area and Sampling

Tree-ring cores were sampled from two rugged southern slopes in the Santa Catalina Mountains located 28 km northeast of Tucson, Arizona, USA: Mt. Lemmon (MTL) and Mt. Bigelow (BIG; Fig. 3.1). BIG (32.41, -110.71, 2534 m a.s.l.) is drier than MTL (32.43, -110.79, 2577 m a.s.l.), with average available water holding capacities of 3.8% and 9.2%, respectively. The climate of the desert southwest is semiarid and warm with two rainy seasons, the summer monsoons (July through September) and winter cyclones (November through March) each deliver up to half of the annual precipitation to the region (Sheppard et al. 2002). P. arizonica, and P. pond. var. brachyptera are dominant species in the mixed conifer forest. We sampled transition zones where all needle types grew together while avoiding cold-air drainages, which included the lower and upper moisture availability limits for *P. ponderosa* var. brachyptera and *P. arizonica*, respectively. Because the objective of this study is to compare our tree-ring data with the analysis conducted c. 50 years ago by Fritts (1963, 1974), we analyzed a minimum of six trees of each needle type selected from a larger population of trees sampled from our earlier study (Marquardt et al. 2018b), which provides full site and sampling details. Trees that average > 4.6 needles per fascicle were designated *P*. arizonica (Peloguin 1984), and P. ponderosa var. brachyptera contained two needle types identified as a

mixed-needle tree ($3.2 \le \text{mean} \le 4.6$ needles per fascicle), and a 3-needle tree (< 3.2 needles per fascicle; Rehfeldt 1993, 1999; Epperson et al. 2001).

Climate data

The local meteorological stations provided only short but accurate climate records; so, specific high-resolution gridded climate (1km) datasets were developed using the ANUSPLIN package (McKenney et al. 2011; Hutchinson and Xu 2013). Climate dataset development and validation were described earlier (Marquardt et al. 2018b). Climatic variables were summarized to monthly values of average temperature (TAVG), average minimum temperature (TMIN), and total precipitation (PCP) because they were previously found to be significant drivers of growth responses (Marquardt et al. 2018b).

Tree growth chronologies

We obtained tree-ring widths for *P. arizonica* [chronology (crn) 651000], P. ponderosa var. brachyptera (3-needle type; crn 631000, and mixed-needle type; crn 641000), located at 2500 meters in elevation on south facing slopes, from the Laboratory of Tree-Ring Research (LTRR, Tucson, AZ; Suppl. Table S2; Suppl. Fig 3.S1). These early period (E) data (1881-1960) were acquired for BIG as three composite chronologies that had been standardized with a negative exponential curve and converted to unit-less ring-width indices (RWI; Dodge 1963; Fritts 1963; 1974).

In addition, we developed six new chronologies (3 from BIG, 3 from MTL) from raw ring-widths archived with the International Tree-Ring Data Bank (ITRDB; Suppl.

Table 3.S1, Suppl. Figs 3.S1-S2; Marquardt et al. 2018a) from trees characterized by averaged needle number per fascicle (Table 3.1). Consistent with the early period data, the recent period (R; 1950-2007) raw chronologies were de-trended using a modified negative exponential curve to remove juvenile and geometric age trends, then converted to standardized ring-width indices using the ARSTAN program (Cook 1985). The mean chronologies (computed by ARSTAN or acquired from the LTRR) were visualized by using plotting functions in the dpIR package (Bunn 2008; 2010) for R (R Core team 2016).

Climate-growth relationships

Our first analysis of climatic influence on tree-ring growth examined the correlations between climatic variables and ring-width index values using multivariate estimates obtained from the principal component regression model (Fritts 1974). We used the relationship between local climatic variables and inter-annual growth to determine the differences in seasonal growth responses. Full methods of the response function correlations can be found in Marquardt et al. (2018b), with modifications: standard chronologies were analyzed rather than ARSTAN chronologies; fewer climate variables were analyzed in this study; needle type was the subject of analysis rather than species; and two time periods were considered: early period and recent period. Briefly, the analysis computed bootstrapped response functions using the TREECLIM package (Zang and Biondi 2015), at three site x period combinations (BIG-E, BIG-R, MTL-R). Eight seasonal climatic variables (4 PCP and 4 TMIN) were partitioned to quantify precipitation and temperature effects during peak times in our study areas.

Seasonal variables were derived by combining the monthly data into two rainy seasons consisting of three and five months respectively: summer (JAS) and winter (NDJFM) separated by three months of arid spring (AMJ). For specific seasons with significant response function values (p < 0.05), those values lying above the axis show a positive response between tree-ring width and climatic variables, and values lying below the axis show a negative response. To examine the impact (on growth) of the climate reversal in 1961 from dry winter precipitation to normal precipitation in 1962, we used the percent difference to quantify the growth variability between years with contrasting moisture conditions.

To evaluate the temporal stability of climate-growth relationships, we compared climate data and composite chronologies using a univariate moving window correlation analysis within the TREECLIM package for R using the 'dcc' function (Zang and Biondi 2015). The 18-month dendroclimatic window was set from September (current year) to April (previous year) with 30-year moving interval and 5-year offset. Two climate variables were considered separately for analysis. Total precipitation (PCP) and average temperature (TAVG) were divided into four seasons of three months each: winter (JFM), spring (AMJ), summer (JAS), and fall (OND). The previous summer and fall seasons were also considered, which increased the number of seasons analyzed to six. Temporal instability of the moving correlation functions was tested with G-test to determine which time series fluctuations were significantly different from those of a random time series (Zang and Biondi 2015). The TREECLIM results of the moving window analyses were plotted using functions in the 'corrplot' package (Wei and Simko 2017) for R.

RESULTS

Seasonal climate-growth relationships

Winter precipitation was averaged between sites for two years with contrasting moisture patterns. The winter of 1961 was one of the driest on record (137.2mm), but the following year (1962) was of normal moisture conditions (319.43mm). Considering mean ring-width indices, growth for 5-needle type is 28% greater on average in 1962 than 1961 (Table 2). A smaller increase in growth occurred for the 3-needle type (20%) and mixed-needle type (16%). Current spring PCP was the strongest predictor of tree growth for all needle types for BIG-R (average $r = 0.27 \pm 0.04$) and MTL-R (average r =0.36 + 0.04) after 1950. The strongest growth responses were positive, producing significant correlations with spring PCP for all needle types at both sites for the recent period (Fig 3.2). In contrast, significant positive PCP-growth correlations were observed in summer rather than spring for BIG-E for all needle-types (aver $r = 0.30 \pm 0.02$; Fig. 3.2). The response function analysis (independent of period and location) indicated that 44% (4 out of 9) of mixed- and 5-needle type populations recorded a significant climate signal during winter prior to the growing season (Fig. 3.2). For the two periods, significant positive PCP-growth correlations in winter were observed only for BIG-E (average r = 0.25 + 0.03) and at MTL-R (average r = 0.33 + 0.03) for mixed- and 5needle types.

Temporal stability of climate-growth relationships

The PCP-growth relationships were stable; the G-tests for PCP are nonsignificant (p > 0.05) for all needle types analyzed across sites and periods (BIG-E; BIG-

R; MTL-R; data not shown). Because these correlation results supported the response function analysis reported above, we have only shown data for the PCP response function analysis.

The TAVG-growth relationship was stable from 1896-1960 for BIG-E across needle types (i.e., G-test p values > 0.05; Fig. 3.3A). The season with the largest number of individual significant negative correlations (c. < -0.4; $\alpha = 0.05$) was PrevSummer with 5-7 correlations (out of 8), followed by Summer with 4-5 correlations (out of 8). In contrast, the strong stable relationship between TAVG and growth became unstable during the recent period from 1958 to 2007 (p < 0.05; Fig. 3.3B-C). This instability was indicated by significant G-tests, and changes in sign for seasonal TAVGgrowth correlations, from negative to positive for PrevSummer for BIG-R (Fig. 3.3B) and from positive to negative for PrevFall at MTL-R (Fig. 3.3C) across all three needle types. For BIG-R, the Fall season had the greatest frequency of significant positive correlations (c. > 0.4; $\alpha = 0.05$) with 4-5 correlations (out of 5). In comparison, the growth of MTL-R trees experienced mostly negative correlations with TAVG. The season with the largest number of significant negative correlations (c. < -0.4; α = 0.05) was Winter with 2-3 correlations (out of 5) for all needle types, followed by Spring with 2 correlations (out of 5) for mixed- and 5-needle types.

DISCUSSION

Seasonal climate-growth relationships

Our chronology data highlighted a dramatic shift in annual growth in response to climatic changes since the late 1950's when CO₂ levels began rising steadily (Kienast

and Luxmoore 1988). Mt. Bigelow ring widths (early period) correlated most strongly with summer precipitation, indicating moisture conditions were sufficient for stomatal conductance and annual growth to occur during the entire summer season (Levesque et al. 2014); thus, maximizing biomass production. In comparison, recent climate-growth relationships were cool season correlations that occur when trees restrict their wood production to early spring while conditions are favorable for growth at dry sites (Fritts 1974; Levesque et al. 2014). Thus, our recent season data indicated spring precipitation was the most important variable for tree growth, a crucial time for the formation of male and female reproductive structures. Therefore, these results support our hypothesis that the seasonality of climate variables important to annual growth has shifted over time in response to changing climate. We also found that winter precipitation improved the growth of mixed- and 5-needle types but not the 3-needle type, suggesting hybridization occurred between the pure needle types on southern slopes (Dodge 1963; Fritts 1963; Rehfeldt 1999; Epperson et al. 2009).

We report important fundamental differences within and between species in seasonal response to climate suggesting that adaptation of tree species to length of growing season may have occurred during the last glacial period. These results support the influence of historical precipitation patterns and climate restrictions for different taxa (Norris et al. 2006; Kilgore 2007). Ziaco et al (2018) also determined the length of growing season of *P. ponderosa* in Nevada was controlled entirely by moisture conditions and not temperature by monitoring the cambial activity for two consecutive years with contrasting moisture, one wet winter and one dry spring. Summer water was not used by the trees unless the start of the growing season was delayed by dry winter

and/ or spring conditions. Because moisture availability determines the length of growing season for all needle types of ponderosa pine studied in the Santa Catalina Mountains (Marquardt et al. 2018b), we hypothesize that one needle type may tolerate a shorter or longer growing season (5-needle, 3-needle, respectively; Dodge 1963), and a third needle type may grow best under a variable growing season (mixed-needle or hybrid; unpublished data; i.e., dry cool-season hypothesis).

In support of this dry cool-season hypothesis, Dodge (1963) measured cambial activity during the dry 1961 growing season (average PDSI c. -3.0; Marquardt et al. 2018b), and found that *P. ponderosa* var. *brachyptera* (the 3- and mixed-needle types) started cambial expansion under dry winter conditions in February, followed by the 5needle type (P. arizonica) six weeks later in April. Cambial growth occurred for all needle types during the summer, and cessation of growth coincided in September. All needle types co-existed at the Mt. Bigelow transition zone (2500 m a.s.l.) near our study site including the trees that Fritts (1963) analyzed for climatic response. Although Dodge (1963) did not determine the initiation of radial growth for the following normal moisture year (average PDSI c. -0.5; Marguardt et al. 2018b), we can predict the growth response of 5-needle type to wet winter conditions from the recent literature on moisture driven cambial activity described above (Ziaco et al. 2018). Because 5-needle type responds positively to winter precipitation, the taxon should initiate growth earlier. If the mixed-needle tree is a hybrid possessing new adaptive gene complexes, then the mixed trees in this system may have inherited flexibility in using winter moisture (from 5-needle type) and cold tolerance (from 3-needle type; Kilgore 2007), providing increased fitness under variable growing season conditions. Further support was provided by our

chronology data, which shows that growth for 5-needle type is robust when winter moisture conditions are normal (compared to dry winter). Also, an 8-12% increase in growth for 5-needle type was observed above that for the 3- and mixed-needle types, respectively, for a normal moisture winter. This increase in growth is suggestive that 5- needle starts cambial expansion earlier in wet springs than dry springs. Therefore, our study results reinforce that moisture availability determines the length of growing season of ponderosa pine, and that some needle types may display variability in the timing of xylogenesis (Ziaco et al. 2018).

Temporal stability of climate-growth relationships

We found opposing temperature trends in response to temperature after 1950 in the tree-ring data collected from the less dry Mt. Lemmon site, which can dilute the climate signal when data are combined into a single chronology. Wilmking et al. (2004) first reported opposing temperature-growth trends in white spruce stands occurring at tree-line in two mountain ranges sampled across Alaska, and temperature explained more variability in annual tree growth post-1950. These regional forests are composed of individual trees growing in heterogeneous environments where temperature thresholds operate, influencing tree biology and the final chronology response to limiting factors. Our report extends these tree-line studies to semiarid regions primarily controlled by precipitation rather than temperature as the factor most limiting to tree annual growth. We also observed a significant shift from positive to negative growth correlations with temperature under less dry conditions for 5-and mixed- needle types during cool seasons, which is indicative of temperature-induced drought stress (Wilmking et al.

2004), or growing season respiration (Marquardt et al. 2018b) limiting the ponderosa pine annual growth. In comparison, at the drier site we observed a shift from negative to positive temperature correlations during Fall season post-1950, indicating reduced water stress. By extending our analysis to include the Fall season, we observed that growth in individual trees is positively and significantly correlated to temperature. These results suggest that all needle types may respond positively to a range of temperature increases late in the season at the contact zone of dry habitats. However, an extended growing season most likely will be determined by moisture availability and not rising temperatures, as recently described for *P. ponderosa*, a closely related species (Ziaco et al. 2018).

CONCLUSIONS

A temporal shift in limiting factors correlated with a shift in climate, indicating spring is the most important growing season for recent period; thus, the three taxa that established in summer wet habitats pre-1950 are now responding to spring wet habitats post-1950, influencing reproduction. In comparison, 5- and mixed-needle types have similar winter responses suggesting soil moisture was controlling the length of growing season. The 3-needle type did not respond to winter moisture, which may be suggestive of a hybrid condition. Although phenological plasticity plays a role in the formation of annual rings, our data suggest a genetic component is also acting on wood formation. In addition to the hybrid index developed using leaf morphology defined by the average needle number per fascicle, a full genetic analysis of the tree populations is required to provide further insight about hybrid formation and wood phenology under

increased evaporative demand, which determines length of growing season. Finally, we found significant fluctuations in temperature-growth correlations during recent period for all needle types, with the effects more variable at the less dry Mt. Lemmon site. Thus, shifts in climate that impact the growth sensitivity of trees, such as warming trends that increase local moisture stress, may also impact the stability of climate-growth relationships.

Supplementary materials: Supplementary data include Suppl. Table 3.S1: Ponderosa pine tree-ring data archives, Suppl. Fig. 3.S1: Figures of average yearly chronologies for 3-, mixed-, and 5- needle types, Mt. Bigelow (Fritts 1963), Suppl. Fig. 3.S2: Figures of average yearly chronologies for 3-, mixed-, and 5- needle types, Mt. Bigelow (new data), Suppl. Fig. 3.S3: Figures of average yearly chronologies for 3-, mixed-, and 5- needle types, Mt. Bigelow (new data), Suppl. Fig. 3.S3: Figures of average yearly chronologies for 3-, mixed-, and 5- needle types, Mt. Bigelow (new data), Suppl. Fig. 3.S3: Figures of average yearly chronologies for 3-, mixed-, and 5- needle types, Mt. Bigelow (new data).

Author contributions: P.M. conceived the study, designed the experiments, and analyzed data; B.M assisted with data analysis; P.M and F.T. collected samples in the field; F.T. contacted the LTRR to obtain historical tree-ring data files; P.M. wrote the paper; F.T. and B.M. edited the paper.

Conflicts of interest: The authors declare no conflicts of interest.

APPENDICES

APPENDIX F

Supplementary Tables and Figures

Table 3.S1 Ponderosa pine tree-ring data obtained from the ITRDB, except where noted. Three needle types were analyzed [5-, 3, and mixed (M)] at two sites: BIG and MTL. N = the number of trees sampled. Fritts' trees were sampled from an earlier period (pre-1950) than Marquardt et al. (xxxxx chronologies will be deposited with ITRDB within 1 yr.)

Site	Туре	ID	Lat	Long	Elev (m)	N	Scholar	Source
BIG	5	65100	32°22′	110° 43′	2500	6	Fritts	LTRR, Tucson, AZ; citation doi.org/10.2307/1942448
BIG	3	63100	32°22′	110° 43′	2500	6	Fritts	LTRR, Tucson, AZ; citation doi.org/10.2307/1942448
BIG	М	64100	32°22′	110° 43′	2500	6	Fritts	LTRR, Tucson, AZ; citation doi.org/10.2307/1942448
BIG	5	XXXXX	32.414	-110.715	2534	7	Marquardt et al.	www.ncdc.noaa.gov/paleo/study/xxxxx
BIG	3	XXXXX	32.414	-110.715	2534	7	Marquardt et al.	www.ncdc.noaa.gov/paleo/study/xxxxx
BIG	Μ	XXXXX	32.414	-110.715	2534	6	Marquardt et al.	www.ncdc.noaa.gov/paleo/study /xxxxx
MTL	5	XXXXX	32.434	-110.790	2577	6	Marquardt et al.	www.ncdc.noaa.gov/paleo/study /xxxxx
MTL	3	XXXXX	32.434	-110.790	2577	7	Marquardt et al.	www.ncdc.noaa.gov/paleo/study/xxxxx
MTL	Μ	XXXXX	32.434	-110.790	2577	7	Marquardt et al.	www.ncdc.noaa.gov/paleo/study/xxxxx



Figure 3.S1 Average yearly standard chronology for 3-, mixed-, and 5- needle types in the early BIG tree-ring study (Dodge 1963; Fritts 1963). Each chronology represents the mean of 12 cores from 6 trees for the reference period of 1881-1960. A = 3-needle pine, B = mixed-needle pine, and C = 5-needle pine.



Figure 3.S2 Average yearly standard chronologies for 3-, mixed-, and 5- needle types in the recent BIG tree-ring study. Each chronology represents an EPS \geq 0.82 and sample depth \geq 12, for the reference period of 1950–2007. A = 3-needle type, B = mixed-needle type, and C = 5-needle type



Figure 3.S3 Average yearly standard chronologies for 3-, mixed-, and 5- needle types in the recent MTL tree-ring study. Each chronology represents an EPS \geq 0.84 and sample depth \geq 15, for the reference period of 1950–2007. A = 3-needle type, B = mixed-needle type, and C = 5-needle type

APPENDIX G

Tables and Figures

Table 3.1 Average needle counts for individual trees sampled for tree-ring correlation analyses at two sites: MTL and BIG

Site	Туре	Ave (#)	Range (#)	SD (#)	Ν
BIG	3	3.1	3.0 - 3.1	0.1	7
MTL	3	3.1	3.0 - 3.2	0.1	7
BIG	М	3.7	3.2 - 4.6	0.6	6
MTL	М	3.8	3.4 - 4.6	0.5	7
BIG	5	4.9	4.8 - 5.0	0.1	7
MTL	5	4.9	4.9 - 5.0	0.0	6

The needle counting method is described in Marquardt et al. (2018b)

Type needle type, *Ave* (#) grand mean of average needle number per fascicle, *SD* standard deviation, *N* number of trees sampled. Three needle types were sampled: *P. ponderosa* var. *brachyptera* (3-needle type), *P. ponderosa* var. *brachyptera* [mixed (M) needle type], and *P. arizonica* (5-needle type)

Table 3.2 Percent difference (% DIFF) in growth between one dry winter (1961) and one normal winter (1962) for 3-, mixed-, and 5-needle types

3-needle type								
Year	BIG-R	MTL-R	AVE	SD	% DIFF			
1961	730	769	749.5	027.6	20			
1962	998	886	942.0	079.2				
Mixed-needle type								
Year	BIG-R	MTL-R	AVE	SD	% DIFF			
1961	832	673	752.5	112.4	16			
1962	967	828	897.5	098.3				
5-needle type								
Year	BIG-R	MTL-R	AVE	SD	% DIFF			
1961	716	627	671.5	062.9	28			
1962	966	898	932.0	048.1				

Growth data for each needle type were obtained from standardized chronologies

developed for recent period tree-ring correlation analyses at two sites: BIG-R and

MTL-R



Figure 3.1 Locations of the two populations sampled for growth analysis: Mt. Lemmon (MTL) and Mt. Bigelow (BIG). Black symbols locate study plots northeast of Tucson, AZ where 3-, mixed-, and 5-needle pines are sympatric



Figure 3.2 Total PCP-growth relationships were determined by principal components multiple regression for three needle types [3-needle = white, mixed-needle = blue, 5-needle = grey] growing at two transition zones within the Santa Catalina Mountains. Within a plotted color, standard index composite chronologies analyzed were BIG-E (double hatched bars) for the early period of 1881–1960, and BIG-R (hatched bars) and MTL-R (non-hatched bars) for the recent period 1950–2007. Climate variables analyzed were seasonal PCP: PrSummer (Jul-Sep), Winter (Nov-Mar), Spring (Apr-Jun), Summer (Jul-Sep)]



Figure 3.3 TAVG-growth relationships were determined by moving window correlations. From top to bottom are correlations for 3-, mixed and 5-needle types. A = BIG-E data, B = BIG-R data, and C = MTL-R data. Color scale = correlation coefficients; significant correlations ($p \le 0.05$) are denoted by *. Intervals during which a variable was not significantly correlated to tree growth are shaded pale red or pale blue (*c*. <u>+</u> 0.2). Figure 3.3 (continued)

X axis = time periods and a significant G-test denoted by #, which means TAVG displays a change in correlation sign with tree growth over time (i.e. a sign of temporal instability). Y axis = seasons, which are defined as PrevSummer = (April-June), PrevFall = (July-September), Winter = (October-December), Spring = (January-March), Summer = (April-June), and Fall = (July-September). The standard index was used in the growth analyses REFERENCES

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CHAPTER 4

GENETIC DIFFERENTIATON AMONG SKY ISLAND POPULATIONS OF PONDERSOA PINE

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Acknowledgments: This paper is part of a dissertation submitted to Michigan State University in partial fulfillment of requirements for a Doctor of Philosophy degree. The Northern Research Station, USDA Forest Service, and the Dept. of Plant Biology, Michigan State University provided financial support for the project. We thank the following people for help on the project: F. Telewski helped conceptualize research questions. F. Telewski, J. Rayala, J. Grimes, G. Friedlander, and B. Epperson assisted with field data collection. B. Miranda, A. Willyard, H. Stricker, A. Foss, H. Jensen, S. Lietz, J. Lund, B. Prom and M. Meisenheimer provided lab, data processing, or GIS support. J. and L. Griffith, and A. and T. Harlan (both deceased) provided logistic support for the field research. K. Scribner reviewed the paper. We also thank the Coronado National Forest for providing permission to sample trees in the Santa Catalina Mountains.

ABSTRACT

Discontinuous distributions limit gene flow and promote population differentiation through selective and stochastic processes. Ponderosa pine individuals were identified based on needle type (3-, 5-, and mixed), which easily distinguished between ecotypes of two closely related ponderosa pine species sampled from three contact zones in southeastern Arizona, USA. We collected similar data for P. arizonica and P. ponderosa var. brachyptera at lower and higher elevations; respectively, to describe pure-needle types. Subsequently, the needle type identities were confirmed with haplotype frequencies representative of each phenotypic population sampled. Mixed needle is more closely related to P. ponderosa var. brachyptera than P. arizonica based on paternal lineage. Genetic analyses of chloroplast microsatellites indicated that variability is lower for the 5-needle *P. arizonica* compared to 3- and mixed-needle type. Higher divergence in haplotypes was observed in areas with distant groups (3- and 5needle types) than sympatry (3-, mixed, and 5-needle types). Finally, integrating morphologic characters and genetic data is an effective way of identifying ponderosa pine taxa and quantifying population genetic structure, which can be used to classify genetically differentiated populations that could be considered separately in management plans.

Keywords: edge effect; genetic structure; microsatellites; plastids; Pinaceae; *Ponderosae*

INTRODUCTION

Genetic divergence among populations is often described as caused by geographic barriers or isolation by distance (Wright 1946; Slatkin 1987) despite high dispersal. The southwestern USA high elevation ponderosa pine forest is one system where species transitions occur over short distances. Marquardt et al. (2018) recently showed that two closely related ponderosa pine species growing sympatrically have different ecological requirements for growth, primarily based on water availability.

Ponderosa pine (*Pinus* subsection *Ponderosae*) are large trees that grow throughout the mountainous and semi-arid regions of western North America, broadly distributed from British Columbia to northern Mexico. From the wide distribution of the *Ponderosae* (Conkle and Critchfield 1988) evolved regionally adapted ecotypes that are collectively referred to as subspecies or varieties within a ponderosa pine complex, or as separate species (see Appendix Table 4.S1 for glossary of terms). The coastal *P. ponderosa* var. *ponderosa*, and the interior *P. ponderosa* var. *scopulorum*, are two commonly accepted varieties with a sharp transition at their Montana contact zone (Potter et al. 2013; 2015; Shinneman et al 2016), corresponding to a climatic boundary (Norris et al. 2016). A recent biogeographic study of ponderosa pine described four distinct species (*P. benthamiana* Hartw., *P. brachyptera* Engelm., *P. ponderosa* Laws. and *P. scopulorum* Engelm. (Willyard et al. 2017). Two of these species also co-occur in the southwestern United States (*P. brachyptera* and *P. scopulorum*; refer to Appendix J for taxonomy of *P. brachyptera*).

In the Santa Catalina Mountains, *P. ponderosa* var. *brachyptera* is a spatial mix of two needle types (3- and mixed-needle), which is synonymous with Taxon X (Table

4.A1). P. ponderosa var. brachyptera grows along with the well characterized P. arizonica at several transition zones on the mountain range (Epperson et al 2001, 2009), a possible glacial refuge for ponderosa pine during the Wisconsin epoch (Betancourt et al. 1990). P. arizonica is formally known as P. arizonica Engelm. And sometimes has been recognized as a subspecies or variety of *P. ponderosa* (Table 4.A2). These high elevation forests of the Santa Catalina Mountains are of interest because they are dominated by the two ponderosa pine species that vary with elevation, and the differences in elevation are repeated throughout their ranges (Peloguin 1984). Not only do ecological differences exist between the two species from climate limitations (Marquardt et al. 2018), but P. arizonica is also often confused with P. ponderosa var. brachyptera south of the Mogollon Rim, where the ranges of the two species overlap, with possible hybridization and introgression (Farjon and Styles 1997), as a mixed-needle phenotype. Moreover, at transition zones in the Santa Catalina Mountains, similarity in climate-growth responses between *P. arizonica* and the mixedneedle phenotype is suggestive of a hybrid condition (Marquardt et al. 2018).

An elevational gradient in average needle number per fascicle is shown to decrease from the lower to upper elevational limits of two ponderosa pine species sampled on Mt. Lemmon (Dodge 1963; Epperson et al. 2001). This gradient may be a morphological response of ponderosa pine to changing environmental conditions (Morphology hypothesis), or it may reflect the presence of hybridizing populations of *P. arizonica* and *P. ponderosa* var. *brachyptera* (Hybridization hypothesis). The use of microsatellite DNA markers should allow discrimination between these two hypotheses presented.

In this study we analyzed the genetic structure of *P. arizonica*, *P. ponderosa* var. *brachyptera*, and a mixed-needle ponderosa pine with chloroplast microsatellite markers, which are also useful for detecting admixture as fewer haplotype frequency changes are required for differentiation of the admixed and reference populations (Haasl and Payseur 2011). We report preliminary results of tests for chloroplast microsatellites sampled from three transition zones (Mt. Lemmon, Mt. Bigelow, Palisades) and adjacent areas above and below the zone of contact. We examined evidence for genetic differentiation among ponderosa pine by considering habitat suitability for moisture availably. Our goal is to determine whether genetic differentiation can be attributed to the transition zone between *P. arizonica* and *P. ponderosa* var. *brachyptera*. Future work for hypothesis testing of the chloroplast haplotypes will consider shifts in haplotype nucleotide frequencies. We will also analyze nuclear microsatellite markers to examine hybridization and gene flow in the two species.

METHODS

Study site

Gene frequencies were estimated from trees on three southern slopes in the Santa Catalina Mountains located 28 km northeast of Tucson, Arizona, USA: Mt. Lemmon (MTL), Mt. Bigelow, (BIG), and Palisades (PAL; Fig 4.1; Table 4.1). A buildup of fuel from wildfire suppression in the Santa Catalina Mountains resulted in destructively hot wildfires: Bullock (2002) and Aspen (2003) fires burned nearly 114,000 acres (Bezy 2016). The Mt. Bigelow's study area was lightly burned, while a heavy burn impacted the Mt. Lemmon and Palisades study areas, allowing regeneration to occur.

The semiarid climate of the desert southwest is warm with the summer monsoons (July through September) and winter cyclones (November through March) each delivering up to half of the annual precipitation to the region (Sheppard et al. 2002; Fig 4.1). P. arizonica and P. pond. Var. brachyptera dominate the canopy of the mixed conifer forest. P. ponderosa var. brachyptera contained two needle types, a mixed-needle tree (3.2 < mean < 4.6 needles per fascicle), and a 3-needle tree (< 3.2 needles per fascicle; Rehfeldt 1993, 1999; Epperson et al. 2001). Trees that average > 4.6 needles per fascicle were designated *P. arizonica* (Peloquin 1984). We sampled from transition zones of steep altitudinal gradients where the distributions of taxa overlap and are potentially hybridizing, while avoiding cold-air drainages, which include the upper and lower moisture availability limits, respectively, for *P. arizonica* and *P. ponderosa* var. brachyptera. We also sampled pure populations in adjacent areas above and below the zone of contact. Sampled were three x 3-needled sites above the elevation limit for P. arizonica (c. 2700 m) near the summit of MTL (MLA, MLN, MLS; Fig. 4.1; Table 4.1), and two x 5-needled populations below the elevation limit for *P. ponderosa* var. brachyptera (c. 2200 meters) near Rose Canyon (ROS) and Green Mountain (GRN). Full site details for MTL and BIG are provided elsewhere (Marguardt et al. 2018).

Sampling and Marker analysis

Needle tissue was collected for DNA analysis on a 5 x 9 grid with 10-m spacing. From 60 to 75 over story ponderosa pine (*P. arizonica, P.ponderosa* var. *brachyptera*) at each of three sites using a pole pruner during May and June, 2010–2012 (Mt. Lemmon, Mt. Bigelow, Palisades). In total, 195 adult trees were sampled at transition

zones. Eighty regenerated seedlings c.10-60 cm in height were harvested on a 2 x 10 grid with 10-m spacing at two transition zones during May 2011–2012 (Mt. Lemmon, Palisades). During 2012-13, 20-40 seedlings per site were harvested at higher elevations near pure 3-needle adults (Mt. Lemmon, Palisades). In total, 140 entire seedlings including roots were harvested by hand. In addition, sixty pure 3-needle trees (P. ponderosa var. brachyptera) were sampled on a 6 x 10 grid from three highelevation Mt. Lemmon sites (MLA, MLN, MTS) and fifty-four pure 5-needle trees (P. arizonica) were sampled from two low-elevation sites (Rose Canyon, Green Mountain). [Note: MLA was sampled along a horizontal transect, 1 tree every 30 meters]. In total, 114 pure-needle trees were sampled. All sample locations (n = 449) were mapped using North American Datum of 1983 (UTM-NAD83) through global positioning system (GPS) data gathered at the field sites. Community base station (Trimble Navigation Ltd., Sunnyvale, California) was used to differentially correct the GPS data for accuracy to 10 m (Adults only for MTL and BIG). Individual adult trees were tagged with aluminum tags. Fresh needle tissue was transported on dry ice, then freeze dried and stored at -20C (2010), or dried and transported on silica desiccant, then stored at room temperature (2012-2013) until DNA extractions were completed.

Total DNA was extracted from 20 mg dried needle tissue using Plant Dneasy Mini Kits (QIAGEN Inc., Germantown, Maryland, USA) with following modifications to manufacturers protocol: needles were broken then ground dry for 1 minute using the Mini Beadbeader (BioSpec Products, Bartlesville, OK) with 0.25-inch ceramic bead and 0.14g garnet sand in 2-ml Fastprep extraction tubes. Dry ground needles were homogenized in AP1 buffer and Rnase for 1 minute. Eluted DNA eluted was quantified
using the Qubit fluorimeter (Life Techonologies, Carlsbad, CA, USA). Stock DNA solutions were diluted to 4.0 ng/ul in T10E1 (10 mmol/L Tris-Cl pH 8.0, 1 mmol/L EDTA) for amplification by the polymerase chain reaction (PCR).

We surveyed variation in 6-chloroplast microsatellite markers [Pt71936 (cp13), Pt100783 (cp15), Pt87268 (cp14), Pc10 (cp10), PcG2R1 (cp9), Pc2T1 (cp8); Vendramin et al. 1996, Stoehr and Newton 2002] that were multiplexed as a set of six mononucleotide loci with four color genotyping (Wofford et al. 2014) using three-primer fluorescent labeling during PCR (Culley et al. 2008, 2013). The reverse primer was unlabeled, one of four unique sequences was added to the 5' end of the forward primers, and the third primer contained the same unique tagged sequence with a fluorescent label attached to the 5' end (6-Fam, NED, VIC or PET). Forward primers with tags (Suppl. Table 4.S2) were purchased from Integrated DNA Technologies, Coralville, Iowa, and the fluorescent primers from Life Technologies, Carlsbad, California, USA. Qiagen's Multiplex PCR kit (QIAGEN Inc.) was used for amplifications with the following modification; a 1um primer master mix with six forward, six reverse, and four labeled primers in a 1:4:4 (forward: reverse: labeled) volume ratio to limit forward primers (0.25 μ) to $\frac{1}{4}$ of the reverse and labelled primers (1 μ); Culley et al. 2008, 2013). Reaction mixtures contained 1ng/ul DNA, 1uM primers, and 3Mm MgCl in 10-ul reaction volumes consisting of 5 ul (2x) Multiplex Master Mix, 2 ul (5x) primer mix, and 3 ul dH20. DNA was dried in wells prior to amplification. Cycling conditions were modified from Wofford (2014) by decreasing the number of cycles to thirty using Biorad Icyclers (Biorad Inc, Hercules, CA): 95C for 15 min, followed by 30 cycles each of 94C for 30 sec., 58C for 90 sec, 72C for 90 sec, and final extension at 72C for 10 min.

A subsample of each PCR was evaluated for correct size product and quantified by agarose gel electrophoresis (2 ul of reaction mixture on 2% gel). PCR products were diluted 1:20 for fragment analysis. Samples were run on an ABI 3730xl DNA analyzer (Life Technologies), with the GS600 LIZ as the internal size standard at the Purdue Genomics Core Facility, and fragments visualized and recorded using GeneMarker genotype analysis software (Softgenetics, State College, PA). Haplotypes were confirmed by independent scoring by two trained laboratory personal. Thirteen-percent of multiplexed haplotypes were randomly selected for retesting in pcr and fragment analysis to assess scoring error (< 0.07%).

Diversity and Population structure

We defined a population as a discrete group of individuals of the same species, and samples collected from a population can be readily identified by a common morphological characteristic that is easily recorded and analyzed. Genetic profiles were developed from nearly pure 3- and 5- needle pine populations selected from sampled trees removed from the contact zone. Haplotype diversity was calculated separately for each population and locus. Firstly, we analyzed the plastid genetic structure of 21 populations of ponderosa pine (Table 4.1) by principal coordinate analysis (PCoA) to resolve clusters based on a distance matrix among all pairs of populations using GenAIEx6 (Huff et al.1993; Peakall and Smouse 2012). Genetic PCoA (an ordination technique) visually characterizes population structure by delineating species and populations within contact zones (Mallet 1995). Samples clustered closer together have smaller genetic distance values than samples clustered further apart. Secondly, convex

polygons were applied to more easily visualize genetic structure. Thirdly, we evaluated whether haplotype frequencies of mixed-needle populations disproportionately reflect frequencies of one pure population (suggesting genetic relatedness) by plotting a histogram of the first axis of PCoA. Fourthly, we assessed whether genetic differentiation between species in distant populations exceeds that in sympatry, as expected with hybridization and introgression (pairwise population PhiPT values, Peakall and Smouse 2012). Lastly, genetic differentiation (using a distance matrix among haplotypes) was analyzed by analysis of molecular variance (AMOVA) using Poppr function (Kamvar et al. 2014) in R (ade4 package; Bougeard and Dray 2018; R Core Team 2016). Within and among region phiRT and pairwise phiRT was calculated with GenAlEx6. Populations were divided into three regions based on the results of the PCoA (i.e. the three needle types: 3-, 5- and mixed-needle). Histograms, polygons, and PCoA's were plotted using functions in the 'ggplot2' package (Wickham 2010) for R.

RESULTS

Identification of groups

In our study, all tree populations had average needle counts that ranged from 3.0 to 4.9 (Table 4.1). The PCoA based on chloroplast haplotype frequencies showed that all 21 populations grouped with the three needle types (Figs. 4.3, 4.4) except for one mixed-needle and one 5-needle population near 0.05 on the x axis (Fig. 4.4). There is a gap between the mixed-needle populations in the center of the distribution (between 0 and 0.05 on the x axis). Mixed needle populations varied in number of needles formed from year to year, allowing them to be distinguished from pure needle populations based on

the SD of average needle number per fascicle (NF; Table 4.1). Pines retain needles, and growth is counted and averaged over 3-5 years that needles are retained. We set SD \geq 3.0 as the limit for mixed-type; subsequently, five populations were selected as mixed-needle (BGM, LSI2, MLM, PLM, PSI2). The three sigma rule allows for 99.7% of values to fall within three standard deviations of the mean (for normal distributions), indicating a significant result. Two more populations were selected as mixed-needle (bringing the total to 7) based on > 3.2 average needle count per fascicle (LSI2; NF=3.32; Rehfeldt 1999) or SD > 2 (PSI1; SD=.21). Two sigma rule allows for 95% of the population to lie within two standard deviations of the mean.

Genetic variation among haplotypes and populations

The chloroplast locus was polymorphic in all populations and diversity was high (Table 4.2). Among the populations, a total of 42 multi-locus haplotypes were detected in 427 samples based on a 6-locus chloroplast haplotype, the most common haplotype is 12 (Table 4.S3). Referring to Table 4.2, the number of haplotypes ranged from 4 to 16 per population. The adult 3-needled populations (11.5 ± 2.8 ; n=6) had more haplotypes on average \pm SD than the adult 5-needled populations (6.2 ± 1.6 ; n=5). Haplotype 12 is present in all populations but MLN, ranging in frequency from 0.053-0.741 (Table 4.S4). All population samples showed moderate to high unbiased haplotype diversity, which ranged from 0.486 to 0.94. In comparison, 5-needle (n=5) had the lowest diversity (0.453-0.600), contrasted to 3-needle (n=9) with the highest diversity (0.914-0.943). Diversity of mixed needle was more varied, ranging from 0.638-0.911 (n=7). The number of private haplotypes was low and ranged from 0 to 2 per population, the

majority occurring in reference populations: 2 private haplotypes occurred in GRN (5needle), 2 in MLA (3-needle), and 2 in MLS (3-needle). Few private haplotypes occurred at the transition zones: 1 each in 5-needle populations BGZ and PLZ, 1 in 3needle population MLB, and 1 in mixed needle population PLM.

Population structure

The AMOVA results indicated that 13% and 87% of the haplotype variation can be attributed to variation among regions (needle types) and among individuals within populations, respectively (p < 0.01; Table 4.3). There was no variation among populations within needle types.

The average pairwise PhiPT values (Table 4.4) were large between distant populations and lowest between populations in sympatry. The distant populations represent the pure needle reference populations (3-needle vs 5-needle) and the populations in sympatry represent the populations in contact at the transition zone (3-, 5- and mixed-needle). The PhiPT pairwise values are consistently highest between populations of 5- and mixed-needle pines (e.g. MLZ-BGB; Table 4.5). In comparison, 3and mixed-needle pines are most similar in genetic structure (e.g. BGB-MLB; Table 4.5).

PCoA revealed three groups of ponderosa pine populations (Fig. 4.3). Axes 1 and 2 accounted for 81.58% and 8.53% of the total variation. The 3-needle populations group in the left quadrants of the first principle coordinate (e.g. MLN, MLS, PSU1), and the 5-needle populations group in the right upper quadrant (e.g. PLZ, ROS, MLZ).

Mixed needle populations group between 3- and 5- needle populations of the first principle coordinate (e.g. MLM, PSI2, LSI2).

DISCUSSION

We analyzed the spatial population genetic structure of *P. arizonica* and *P. ponderosa* var. *brachyptera* on the Santa Catalina Mountains and found evidence for three distinct genetic groups; genetic variability is reduced for the low elevation speciees compared to the high elevation species. Below we discuss the relevance of our results with respect to the management of the Sky Island pine forest.

Population structure and Differentiation

The ponderosa pine populations from the Santa Catalina Mountains cluster genetically into three distinct groups based on chloroplast haplotype frequency. The mixed-needle populations clustered between the 3- and 5- needle populations (Figs 4.3; 4.4). The gaps in the center of the histogram plot (between 0 and 0.05 on the x axis) could be indicative of distant populations being differentiated from transition zone populations (Adams 1982). The groups corresponded to the environmental boundary of high and low elevation slopes, which separates cool-moist habitat from dry-hot habitat (Fig. 4.2).

Geographically, *P. arizonica* is located at lower elevations, *P. ponderosa* var. *brachyptera* is located at higher elevations, and mixed-needle type is located between populations of the two species. Consistent with a hypothesis about climatic barriers to gene flow, the mixed-needle populations were closest in genetic structure to 3-needle

populations. Divergence was lowest in the transition zone indicating higher levels of gene flow than between distant populations.

An earlier study of *P. arizonica and P. ponderosa* var. brachyptera on Mt. Lemmon used two of the six chloroplast loci in our study (Epperson et al. 2009). The four loci used in the 2009 study revealed similar haplotype diversity (mean h = 0.85) to our study and approximately half the differentiation among needle types ($\theta = 0.07$). Whereas the average value of θ was based on two groups (*P. arizonica* and *P.* ponderosa var. brachyptera), our measure (Phi) was based on three groups (5-, 3-, mixed). Both studies found significant differences in genetic structure for the two ponderosa pine species. A third study using chloroplast haplotypes to analyze ponderosa pine genetic structure did not resolve P. ponderosa var. brachyptera from P. arizonica (Willyard et al. 2017), which suggests these two species are more closely related to each other than they are to western ponderosa pine species or to P. ponderosa var. scopulorum. Like our study, the authors reported reduced diversity for *P. arizonica* as compared to other ponderosa pine species sampled in this range-wide study. For our study, we found that 5- needle type encompassed c. 40% lower levels of haplotype diversity than did 3-needle type, which is consistent with *P. arizonica* being near its northern range boundary and having experienced a bottleneck during range expansion (Eckert et al. 2008). In comparison, a range wide study of ponderosa pine with nuclear microsatellite markers and isozymes did not find reduced genetic diversity near edges of species ranges (Potter et al. 2015).

Epperson et al. (2009) conducted spatial autocorrelation analysis of 3-, 3- plus mixed-, and 5- needle types separately, indicating randomly mating populations. A

fourth analysis was of the entire transect (all needle types were grouped together), which indicated strong autocorrelation at short distances. Although the significance of this result was not discussed by the authors, the pattern of fine scale genetic structure within populations (of the needle types combined) may decrease the chances of hybridization thru reduced gene flow. Our study results differ from Epperson et al. (2009) who reported no correlation between needle number and haplotypes for *P. ponderosa* var. *brachyptera* (3- plus mixed- needle types). Our research found a strong relationship between the two factors in our principle coordinate analysis, whether we grouped mixed-needle with 3-needle or analyzed them separately. One main difference between the two studies is the type of analyses conducted. Epperson et al. (2009) analyzed chloroplast alleles as independent observations in a spatial analysis. Our study used PCoA looked for a relationship between haplotype genetic distance and needle type, an easy to measure trait useful for identifying genetic structure.

CONCLUSIONS

Two competing hypotheses explain the climate gradient in average needle number observed in southeast Arizona, the greatest expression of which is found in the Santa Catalina Mountains (Dodge 1963). This gradient may be a response to environmental conditions or it may reflect the presence of hybridizing populations of *P. arizonica* and *P. ponderosa* var. *brachyptera*.

The morphology hypothesis states that the gradient in average needle number is a habitat response, indicated by a reduction in needle number that is expressed in trees more suited to cold, moist environments, i.e. the 3-needle type is more tolerant of cold

than 5-needle type (Kilgore 2007). In comparison, an increase in average needlenumber will be expressed in trees that show greater effects of cold damage on cold sites (Haller 1962, 1965), or adaptation with water loss on dry sites (this thesis). Two separate taxa occur on the Santa Catalina Mountains; *P. arizonica,* a 5-needle pine and *P ponderosa* var. *brachyptera,* a putative spatial mix of 3- and mixed-needle types (Epperson et al. 2009). Mixed needle type contains 3-4-5 needles per fascicle, which occurs only at the contact zones of the two species, and the gradient in average needle number increases from high to low elevation. If the mixed-needle trait is a result of habitat response to arid conditions the previous fall (unpublished data), then variance in needle number possibly modulates inter-annual water stress, increasing carbon gain.

The gradient could also be the result of hybridizing populations of *P. arizonica* and *P. ponderosa* var. *brachyptera* that were established in a colder and drier environment, where pure individuals outperformed the hybrids (or there was no opportunity to hybridize). Then when changes in phenology occurred because growing seasons became longer and/ or more variable based on water availability (Ziaco et al. 2018), interbreeding also had opportunity to occur. Hybrids that formed may have outcompeted in the warmer climate with increased evaporative demand, and if they were fertile, could have interbred with parents.

The ponderosa pine populations of the Santa Catalina Mountains may be in jeopardy because high elevation species not only occupy a small but important niche, they are also more vulnerable to changing environmental conditions. We found high levels of genetic variation for 3- and mixed-needle types; levels are reduced by *c*. 40% for *P. arizonica* compared to *P. ponderosa* var. *brachyptera*. These results highlight the

dissimilar genetic structure of the two species, suggesting they should be considered differently in management plans in areas where they coexist. *P. arizonica* populations are near their northern range boundaries, have reduced diversity and are most at risk from climate change because they may be less fit due to environmental stress; therefore, conservation efforts may in want to represent these peripheral populations. The three taxa form three distinct genetic groups reflective of elevational niches (3-, mixed, and 5- needle); the upper and lower elevation divisions contribute most to the observed genetic variation with divergence greatest among distant populations.

APPENDICES

APPENDIX H

Supplementary Tables and Figures

Table 4.S1 Glossary of genetic terms

Admixture: Two or more previously isolated populations begin interbreeding, introducing new genetic lineages into a population.

Conspecific: Belonging to the same species. For example, it describes the interactions between two or more individuals of the same species.

Ecotype: a genetically distinct group that is adapted to its local environment. An ecotype is a variant in which the phenotypic differences are too few or too subtle to warrant being classified as a subspecies.

Hybridization: the movement of genes from one species into the gene pool of another resulting in an even mix of two parental species in the first generation.

Hybridity: The offspring of genetically dissimilar parents produced by breeding plants or animals of different varieties, species, or races.

Infraspecific: variation or taxonomic division within a species, e.g. subspecies or variety.

Introgression: the incorporation of alleles from one species into the gene pool of a second species, usually through hybridization and repeated backcrossing resulting in a complex mixture of parental genes.

Morphology: the study of the form and structure of organisms and their evolutionary history.

Morphotype: in taxonomy, an alternate form or phenotype to illustrate variation within a population (of a single species).

Population: a locally discrete group of individuals of the same species where individuals can be readily identified by a common morphological characteristic.

Population structure: populations are subdivided in some way, which allows them to diversify and evolve. Such populations have deviations from Hardy-Weinberg proportions, for example when there is inbreeding, selection, migration, or genetic drift.

Species: a genetically distinct group that forms a single cluster from a genetic sample if all individuals belong to a single species, and multiple clusters if there are two or more species present.

Subspecies: a taxonomic group that is a division of a species, and usually occurs from migration barriers causing isolation within a species.

Table 4.S1 (continued)

Variety: a unique group that displays genetic divergence that is not considered high enough to be a different subspecies. The origin of a new variety may reflect neutral mutation without changes in fitness and would not be a different ecotype. Also, the variety may be the result of adaptation by natural selection producing an ecotype.

			Fluorescent	length				
Source ²	Function	Sequence (5' to 3')	label ¹	(bp)				
M13(-21)	Unique Tag	TGTAAAACGACGGCCAGT	6-FAM	18				
M13(-21)A	Unique Tag	TACGAGTGCAGCAAGCAT	NED	18				
M13(-21)B	Unique Tag	CACTGCTTAGAGCGATGC	PET	18				
Т7	Unique Tag	CTAGTTATTGCTCAGCGGT	VIC	19				
¹ The 5' end of each unique sequence is labeled with a fluorescent tag								
² The unique sequences are M13(-21), two modifications of M13(-21), and T7 (Culley et al 2008								

Table 4.S2 Fluorescently labeled unique sequences used in 3-primer PCR

Primers amplify mononucleotide microsatellite repeats in the chloroplast genome (Vendramin et al. 1996; Stoehr and Newton 2002). Refer to Wofford et al. 2014 (Table 3) for repeat information and locus characteristics. In 3-primer PCR (Culley et al. 2008), the forward primer incorporates a unique tailed sequence, the reverse primer is unlabeled, and the third primer incorporates the same tailed sequence that is attached to a fluorescent label

Haplotype	Count	1	2	3	4	5	6	Ex. Sample	Ex. Pop
1	1	277	104	206	163	176	125	PALS001	PSI1
2	1	277	104	206	163	177	125	GRN008	GRN
3	1	277	104	207	163	177	125	PALS051	PSU1
4	1	277	104	207	163	178	125	PAL006	PLZ
5	1	277	104	207	164	178	124	BIG043	BGZ
6	1	277	104	207	164	179	124	MTLA011	MLA
7	1	277	105	206	161	177	125	MTLS034	LSI2
8	3	277	105	206	162	176	125	ROS079	ROS
9	3	277	105	206	162	177	125	PAL004	PLZ
10	8	277	105	206	162	177	126	MTL277	MLZ
11	18	277	105	206	163	176	125	ROS094	ROS
12	166	277	105	206	163	177	125	ROS093	ROS
13	3	277	105	206	163	178	125	PAL002	PLZ
14	1	277	105	206	163	179	126	GRN009	GRN
15	12	277	105	206	164	177	125	PALS049	PSI2
16	17	277	105	206	164	178	124	PALS090	PSU2
17	15	277	105	206	164	179	124	PALS018	PSI1
18	4	277	105	206	164	180	124	PALS087	PSU2
19	2	277	105	206	164	181	124	MTL050	MLM
20	1	277	105	206	165	179	124	MTLA004	MLA
21	1	277	105	207	162	177	125	PAL071	PLM
22	5	277	105	207	162	179	124	PALS069	PSU1
23	28	277	105	207	163	177	125	ROS083	ROS
24	5	277	105	207	163	177	126	MTLA027	MLA
25	9	277	105	207	163	178	124	PALS091	PSU2
26	2	277	105	207	163	178	125	PAL039	PLB
27	3	277	105	207	164	177	124	PALS015	PSI1
28	1	277	105	207	164	177	125	MTL239	MLS
29	32	277	105	207	164	178	124	PALS089	PSU2
30	33	277	105	207	164	179	124	PALS088	PSU2
31	1	277	105	208	162	179	124	MTL236	MLS
32	3	277	105	208	163	177	125	PALS084	PSU2
33	11	277	105	208	164	177	124	PALS016	PSI1
34	1	277	105	208	164	177	125	PALS035	PSI2
35	15	277	105	208	164	178	124	PALS083	PSU2
36	3	277	105	208	164	179	124	PALS057	PSU1
37	6	277	105	208	165	178	124	PALS063	PSU1

Table 4.S3 Summary information for the 42 chloroplast haplotypes analyzed

Haplotype	Count	1	2	3	4	5	6	Ex. Sample	Ex. Pop
38	1	277	105	209	164	179	124	MTLS065	LSU
39	2	277	105	209	164	180	124	MTLS050	LSI2
40	1	277	106	207	164	178	124	MTL249	MLB
41	1	278	105	207	164	178	124	PALS075	PSU2
42	3	278	105	207	164	179	124	MTL123	MLN

Count = number of haplotypes, 1-6 = mononucleotide loci, Ex. = Example

	BGZ	GRN	MLZ	ROS	PLZ	BGM	LSI1	LSI2	MLM	PLM	PSI1	PSI2
Ν	28	30	27	24	30	10	15	22	11	9	23	14
1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.043	0.000
2	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
4	0.000	0.000	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000
5	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
7	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.045	0.000	0.000	0.000	0.000
8	0.000	0.000	0.000	0.042	0.000	0.000	0.133	0.000	0.000	0.000	0.000	0.000
9	0.000	0.000	0.000	0.000	0.033	0.100	0.067	0.000	0.000	0.000	0.000	0.000
10	0.000	0.000	0.074	0.000	0.000	0.000	0.133	0.182	0.000	0.000	0.000	0.000
11	0.036	0.067	0.074	0.125	0.033	0.000	0.067	0.000	0.182	0.000	0.000	0.286
12	0.679	0.700	0.741	0.708	0.600	0.300	0.600	0.500	0.273	0.444	0.348	0.429
13	0.036	0.000	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000
14	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
15	0.107	0.067	0.037	0.000	0.033	0.000	0.000	0.045	0.000	0.000	0.043	0.071
16	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.043	0.000
17	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.091	0.000	0.087	0.000
18	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
19	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.091	0.000	0.000	0.000
20	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
21	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.111	0.000	0.000
22	0.000	0.000	0.000	0.000	0.000	0.100	0.000	0.000	0.000	0.000	0.043	0.000
23	0.071	0.033	0.037	0.125	0.233	0.100	0.000	0.045	0.091	0.222	0.000	0.143
24	0.036	0.033	0.000	0.000	0.000	0.100	0.000	0.000	0.000	0.000	0.000	0.000
25	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.111	0.000	0.000
26	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 4.S4 Haplotype frequencies (42 haplotypes; 1 locus; 427 samples; N, number of individuals per 21 populations)

Table 4.S4 (continued)

	BGZ	GRN	MLZ	ROS	PLZ	BGM	LSI1	LSI2	MLM	PLM	PSI1	PSI2
Ν	28	30	27	24	30	10	15	22	11	9	23	14
27	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.043	0.000
28	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
29	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.182	0.000	0.087	0.000
30	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.091	0.000	0.000	0.174	0.000
31	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
32	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
33	0.000	0.000	0.000	0.000	0.000	0.100	0.000	0.000	0.091	0.000	0.043	0.000
34	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.071
35	0.000	0.000	0.037	0.000	0.000	0.200	0.000	0.000	0.000	0.000	0.043	0.000
36	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
37	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.111	0.000	0.000
38	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
39	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.091	0.000	0.000	0.000	0.000
40	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
41	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
42	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 4.S4 (continued)

	BGB	LSU	MLA	MLN	MLS	MLB	PLB	PSU1	PSU2
Ν	21	20	30	14	15	21	23	21	19
1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.048	0.000
4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000
7	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
8	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
9	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
10	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
11	0.000	0.000	0.000	0.000	0.000	0.000	0.043	0.000	0.053
12	0.143	0.200	0.100	0.000	0.200	0.095	0.217	0.286	0.053
13	0.000	0.000	0.000	0.000	0.000	0.048	0.000	0.000	0.000
14	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
15	0.048	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000
16	0.000	0.050	0.100	0.286	0.200	0.095	0.043	0.000	0.105
17	0.095	0.150	0.067	0.214	0.000	0.048	0.043	0.000	0.000
18	0.095	0.000	0.000	0.000	0.000	0.000	0.043	0.000	0.053
19	0.048	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
20	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000
21	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
22	0.000	0.000	0.033	0.000	0.000	0.000	0.043	0.048	0.000
23	0.000	0.050	0.033	0.071	0.067	0.143	0.000	0.000	0.000
24	0.048	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000
25	0.000	0.050	0.067	0.071	0.000	0.000	0.000	0.095	0.105
26	0.000	0.000	0.000	0.000	0.000	0.000	0.043	0.000	0.000

Table 4.S4 (continued)

	BGB	LSU	MLA	MLN	MLS	MLB	PLB	PSU1	PSU2
Ν	21	20	30	14	15	21	23	21	19
27	0.000	0.000	0.000	0.071	0.000	0.048	0.000	0.000	0.000
28	0.000	0.000	0.000	0.000	0.067	0.000	0.000	0.000	0.000
29	0.095	0.300	0.200	0.143	0.200	0.048	0.174	0.048	0.158
30	0.190	0.050	0.067	0.071	0.067	0.143	0.217	0.286	0.211
31	0.000	0.000	0.000	0.000	0.067	0.000	0.000	0.000	0.000
32	0.000	0.000	0.000	0.000	0.000	0.000	0.043	0.000	0.105
33	0.048	0.050	0.067	0.000	0.067	0.143	0.000	0.000	0.000
34	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
35	0.095	0.050	0.067	0.000	0.000	0.095	0.043	0.048	0.105
36	0.048	0.000	0.000	0.000	0.000	0.000	0.043	0.048	0.000
37	0.000	0.000	0.033	0.000	0.067	0.048	0.000	0.095	0.000
38	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000
39	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
40	0.000	0.000	0.000	0.000	0.000	0.048	0.000	0.000	0.000
41	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.053
42	0.048	0.000	0.033	0.071	0.000	0.000	0.000	0.000	0.000

Populations are grouped by needle-type: five 5-needle (e.g. BGZ, GRN, MLZ, ROS, PLZ),

seven mixed-needle populations, and nine 3-needle populations

APPENDIX I

Tables and Figures

SITE	POP	Ν	TYPE	NF	SD	SPECIES	COHORT	ELEV
PAL	PLB	23	3N	3.05	0.07	brachyptera	adult	2501
BIG	BGB	21	ЗN	3.05	0.06	brachyptera	adult	2510
MTL	MLB	21	3N	3.08	0.08	brachyptera	adult	2568
MTL	MLA	30	3N	3.11	0.17	brachyptera	adult	2756
MTL	MLS	15	3N	3.03	0.06	brachyptera	adult	2761
MTL	MLN	14	3N	3.01	0.02	brachyptera	adult	2768
PAL	PSU2	19	3N	3.00	0.09	brachyptera	seedling	2553
PAL	PSU1	21	3N	3.03	0.16	brachyptera	seedling	2566
MTL	LSU	20	3N	2.94	0.08	brachyptera	seedling	2741
GRN	GRN	30	5N	4.87	0.11	P. arizonica	adult	2155
ROS	ROS	24	5N	4.80	0.22	P. arizonica	adult	2172
PAL	PLZ	30	5N	4.91	0.08	P. arizonica	adult	2457
BIG	BGZ	28	5N	4.90	0.08	P. arizonica	adult	2498
MTL	MLZ	27	5N	4.88	0.09	P. arizonica	adult	2529
PAL	PLM	9	MN	3.82	0.59	brachyptera	adult	2488
BIG	BGM	10	MN	3.85	0.60	brachyptera	adult	2514
MTL	MLM	11	MN	3.98	0.49	brachyptera	adult	2557
MTL	LSI1	15	MN	3.32	0.13	brachyptera	seedling	2453
MTL	LSI2	22	MN	4.14	0.36	brachyptera	seedling	2454
PAL	PSI2	14	MN	4.12	0.29	brachyptera	seedling	2470
PAL	PSI1	23	MN	3.14	0.21	brachyptera	seedling	2471

Table 4.1 Summary information for the 21 pine populations analyzed in this study

Five study sites are Mt. Lemmon (MTL), Mt. Bigelow (BIG), Palisades (PAL), Rose Canyon (ROS), and Green Mountain (GRN) which comprise 21 ponderosa pine populations that were sampled for genetic analysis. POP is population, NF is average number of needles per fascicle, *brachyptera* is *P. pondersoa* var. *brachyptera*, and mean elevation of the area sampled for each population is reported in meters

Table 4.1 (continued)

The study areas included three transition zones (where two species of ponderosa pine grow together) located on Mt. Lemmon (MLB, MLZ), Palisades (PLB, PLZ), and Mt. Bigelow (BGB, BGZ). The "B" and "Z" of the site code (third digit) designate *P. ponderosa* var. *brachyptera* and *P. arizonica*, respectively. Also sampled were reference groups of three pure 3-needle sites located at high elevation on North Slope (MLN), MTLA (MLA), and South slope (MLS), and two pure 5-needle sites sampled at low elevation on Rose Canyon (ROS) and Green Mountain (GRN). In addition to these adult populations, seven groups of ponderosa pine seedlings were sampled: three at Mt. Lemmon and four at Palisades. Mt. Lemmon's seedlings comprised one upper elevation 3-needle group (LSU) and two intermediate elevation 3-needle groups (PSU1; PSU2) and two intermediate elevation mixed-needle groups (PSU1; PSU2) and two intermediate elevation mixed-needle groups (PSU1; PSI2)

Site	Туре	Рор	n	ph	Na	h	uh
BIG	5	BGZ	28	1	7	0.512	0.537
GRN	5	GRN	30	2	8	0.496	0.513
MTL	5	MLZ	27	0	6	0.436	0.453
PAL	5	PLZ	30	1	7	0.580	0.600
ROS	5	ROS	24	0	4	0.465	0.486
BIG	М	BGM	10	0	7	0.820	0.911
MTL	Μ	LSI1	15	0	5	0.596	0.638
MTL	Μ	LSI2	22	2	7	0.694	0.727
MTL	Μ	MLM	11	0	7	0.826	0.909
PAL	Μ	PLM	9	1	5	0.716	0.806
PAL	Μ	PSI1	23	1	11	0.820	0.858
PAL	Μ	PSI2	14	1	5	0.704	0.758
BIG	3	BGB	21	0	12	0.893	0.938
MTL	3	LSU	20	1	10	0.830	0.874
MTL	3	MLA	30	2	16	0.909	0.940
MTL	3	MLN	14	0	8	0.827	0.890
MTL	3	MLS	15	2	9	0.853	0.914
MTL	3	MLB	21	1	12	0.898	0.943
PAL	3	PLB	23	0	12	0.858	0.897
PAL	3	PSU1	21	1	9	0.807	0.848
PAL	3	PSU2	19	1	10	0.875	0.924
		mean	20.3	0.8	8.4	0.734	0.779
		SD	6.6	0.7	3.0	0.157	0.170

Table 4.2 Sites, needle type, populations, sample sizes, and haplotype polymorphism characterized for 21 populations of ponderosa pine at 5 sites

n, number of individuals; ph, number of private haplotypes; Na, number of different haplotypes; h, haplotype diversity = 1 - Sum pi^2; uh, unbiased diversity = (N / (N-1))* h; where pi is the frequency of the ith allele for the population and Sum pi^2 is the sum of the squared population haplotype frequencies

Table 4.3 Analysis of molecular variance of plastid SSRs for 9 populations of ponderosa pine at contact zones in

southwest Arizona, USA

Hierarchical level*	df	SS	MS	Est Variance	Var (%)	Stat	Phi	р
Among needle types (3, 5, M)	2	13.404	6.702	0.1069	12.8	PhiRT	0.129	0.0001
Among populations within needle types	6	4.480	0.747	0.0008	0.1	PhiPR	0.001	0.5243
Within populations	171	124.771	0.730	0.7296	87.1	PhiPT	0.128	0.0001
Total	179	142.655	0.797	0.8374	100.0			

*3 regions (needle types), 9 populations, 180 trees, 28 haplotypes;

Hierarchical level, source; df, degrees of freedom; SS, sum of squared observations;

MS, mean of squared observations; Est. Variance, Estimated Variance; Var(%), percentage variance;

Phi, value of variation; p, p-value;

PhRT, proportion of the total genetic variation that are between region;

PhiPR, proportion of the total variation that are among populations within a region;

PhiPT, proportion of the total genetic variance that are among individuals within a population

Table 4.4 Pairwise PhiPT values between sympatric and distant populations of ponderosa pine

Sympatry	BLB	BGZ	BGM	Distant	GRN	ROS
MLB	0.000	0.208	0.000	MLA	0.215	0.221
MLZ	0.228	0.000	0.148	MLN	0.325	0.329
MLM	0.002	0.123	0.000	MLS	0.190	0.194
Average	0.077	0.110	0.049	Average	0.244	0.248
SD	0.131	0.104	0.086	SD	0.07	0.07

Twenty-one populations were evaluated for genetic distance. Shown are comparisons between populations sampled at two zones of contact (Sympatry) and populations located far from zones of contact (Distant) Table 4.5. Pairwise PhiPT values between populations of ponderosa pine from contact zones in the Santa Catalina

Mountains

	BGB	MLB	PLB	BGZ	MLZ	PLZ	BGM	MLM	PLM	
BGB	0.000	0.510	0.380	0.010	0.010	0.010	0.380	0.490	0.070	BGB
MLB	0.000	0.000	0.180	0.010	0.010	0.010	0.460	0.360	0.120	MLB
PLB	0.000	0.010	0.000	0.010	0.010	0.010	0.270	0.460	0.070	PLB
BGZ	0.185	0.208	0.162	0.000	0.360	0.280	0.030	0.040	0.220	BGZ
MLZ	0.228	0.253	0.196	0.000	0.000	0.100	0.030	0.010	0.060	MLZ
PLZ	0.165	0.158	0.143	0.003	0.030	0.000	0.070	0.060	0.380	PLZ
BGM	0.003	0.000	0.020	0.105	0.148	0.067	0.000	0.450	0.410	BGM
MLM	0.002	0.009	0.000	0.123	0.163	0.086	0.000	0.000	0.360	MLM
PLM	0.063	0.044	0.053	0.035	0.080	0.000	0.000	0.000	0.000	PLM
	BGB	MLB	PLB	BGZ	MLZ	PLZ	BGM	MLM	PLM	

Nine populations were evaluated for genetic distance. Shown are comparisons between populations sampled for regional

comparisons of needle types



Figure 4.1 Black circles mark the locations of five study sites comprising 21 populations that were sampled for genetic analysis.

Figure 4.1 (continued)

These include three transition zones (where two species of ponderosa pine grow together) located on Mt. Lemmon, Palisades, and Mt. Bigelow. Highlighted are 3-needle reference plots (1 site, red oval), transition zones (3 sites, blue oval), and 5-needle reference plots (2 sites, green oval). Insets mark the location of individual trees and seedlings on Mt. Lemmon and Palisades, and mature trees only on Mt. Bigelow, for three transition zones (blue oval). These data reflect the locations of individuals from 13 populations: Mt. Bigelow (BGM, BGB, BGZ); Mt. Lemmon (MLM, MLB, MLZ, LSI1, LSI2); and Palisades (PLM, PLB, PLZ, PSI1, PSI2). Not shown on the map are pure 3-needle individuals from Mt. Lemmon (MLA, MLN, MLS; red oval); and pure 5-needle individuals from Green Mountain (GRN; green oval) and Rose Canyon (ROS; green oval), which range in elevation from 2685-2780m (3-needle) and 2140-2171m (5-needle). Locations for individuals from two additional high elevation seedling sites (3-needle type) are also not shown (PSU1, PSU2; 2542-2576m elevation). Refer to Table 4.1 for full population and site information



Figure 4.2 Climate diagram for the Santa Catalina Mountains, for the 84 year reference period spanning 1925 - 2009 for (a) average annual temperature (°C; red line) and (b) total annual precipitation (mm; blue line), respectively. The average temperature increased by 1.3°C over the course of the study (from 10.6°C to 11.9°C). Site specific climate data sets (MTL, BIG) were generated using the methods of McKenney et al. (2011) and averaged together to construct this diagram. The dotted lines are the linear trend lines. See Marquardt et al. (2018) for details



Figure 4.3 Convex polygons of principal coordinates analysis of 21 a priori populations of ponderosa pine collected from five sites. Axis1 explained 81.58% of the variance, and Axis2 explained 8.53% of the variance. PCoA analysis was based on pairwise genetic distance estimated using haplotype frequencies. The three needle types are well resolved. Refer to Table 4.1 for population and site information



Figure 4.4 Histogram plot of the first axis from PCoA with 21 a priori populations of ponderosa pine plotted on the X axis. Y axis is the number of populations counted in each stack. The mixed-needle types are well resolved except for one mixed-needle population and one 5-needle population

APPENDIX J

Taxonomy of ponderosa pine

Taxonomy of ponderosa pine

P. brachyptera (Southwestern ponderosa pine) has been regarded as a species, a single taxon within *P. ponderosa* (*P. ponderosa* var. *brachyptera*), or the accepted name *P. ponderosa* var. *scopulorum* (Rocky Mountain ponderosa pine; Table 4.A1). Thus, *P. ponderosa* var. *scopulorum* refers to two major varieties, the Southwestern ponderosa pine (*P. ponderosa* var. *brachyptera*) and the Rocky Mountain ponderosa pine (*P. ponderosa* var. *brachyptera*) and the Rocky Mountain ponderosa pine (*P. ponderosa* var. *brachyptera*) and the Rocky Mountain ponderosa pine (*P. ponderosa* var. *scopulorum*), causing taxonomic confusion because the two taxa are clearly distinct. Characteristics that distinguish the Southwestern form are its open foliage and lack of 2-needle fascicles, which contrasts with the typical short and stiff, 2-needle fascicles of the Rocky Mountain form (Conkle and Critchfield 1998). In contrast to the narrow contact zone of coastal and interior varieties, the transition zone between the Southwestern and Rocky Mountain forms is broad across Nevada's Great Basin into central Colorado (Conkel and Critchfield 1998), possibly indicating different stages of speciation (Kindler et al. 2017). We will refer to the Southwestern form as *P. ponderosa* var. *brachyptera*

Table 4.A1 Authorities for three taxa of the genus *Pinus* growing on Mountain Islands in southwestern USA: Sky Island⁷;

P. arizonica and P. brachyptera were obtained from The Plant List (2013)

Species	Infraspecific		Authority	Year Pub.	Citation	The Plant List Status	Ref. ^{1,2,4,5}
arizonica			Engelm.	1879	i.	accepted	4
ponderosa	subsp.	arizonica	(Engelm.) A.E.Murray	1982	ii.	synonym	
ponderosa	var.	arizonica	(Engelm.) Shaw	1909	iii.	synonym	
brachyptera			Engelm.	1848	iv	synonym	7
ponderosa	subsp.	brachyptera	(Engelm.) Silba	2011	v.	synonym	
ponderosa	var.	brachyptera	(Engelm.) Lemmon	1888	vi.	synonym	
ponderosa	var.	scopulorum	Engelm.	1880	vii.	accepted	
ponderosa		Sky Island		2017		synonym	7
ponderosa		Taxon X		1999		synonym	3,6
Table 4.A1 (continued)

Citation refers to the place and date of original publication of name of taxa. Provided also are the accepted name for the taxonomic unit, and the synonyms, which are alternative names used to refer to the taxa that are not currently accepted. Ref. are additional references which review accepted species names and synonyms (1,2,4,5), *P. arizonica* and *P. brachyptera* as species unique from *P. ponderosa* (5,7), and a proposed new species (3,6,7)

i. Rep. U.S. Geogr. Surv., Wheeler 6(Bot):260.1878. (1879).

ii. Kalmia 12: 23. (1982).

iii. Publ. Arnold Arbor. 1: 24. (1909).

iv. Wisl. Mem.Tour N Mexico [Wislizenus] 89. (1848).

v. J. Int. Conifer Preserv. Soc. 18: 16. (2011).

vi. Bienn. Rep. Calif. State Board Forest. 2: 73. (1888).

vii. S.Watson [W.H.Brewer] Bot. California 2: 126 (1880).

- 1. Callaham 2013
- 2. Conkle and Critchfield 1988
- 3. Epperson et al. 2001, 2009
- 4. Farjon and Styles 1997
- 5. Lauria 1996
- 6. Rehfeldt et al. 1996, Rehfeldt 1999
- 7. Willyard et al. 2017

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