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GROWING TINY PLANTS IN COMMON ENVIRONMENTS: ASSESSING PATTERNS AND MECHANISMS OF DROUGHT RESPONSE WITHIN SPECIES

By

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Growing Tiny Plants in Common Environments: Assessing Patterns and Mechanisms Of Drought Response Within Species

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As climate changes and drought frequency and intensity increases, understanding how plants respond will be critical both for predicting potential for adaptation to future climate and for implementing effective ecosystem conservation and management at a time of rapid change. However, gaps in knowledge about the extent to which species vary in key traits across their ranges and the evolutionary and physiological mechanisms which underlie this variation limits both theoretical understanding and effective management. The broad theme of this dissertation is to address within-species variation both to improve understanding of adaptation to future climate and inform ecosystem conservation and management. The three chapters in this dissertation contribute to a growing body of literature on genetic and plastic variation in key plant traits across environmental gradients, emphasizing the ecological and practical importance of plant trait variation both among and within provenances. Chapter I focused on identifying genetic variation among and within populations of the iconic tree *Araucaria araucana* (pewen) across its range in Chile to inform conservation and restoration efforts. Chapter II addressed whether within-species genetic and plastic variation in early plant phenotypes impacts drought survival for two Chilean forbs across a significant precipitation gradient. Chapter III synthesized patterns of within-species and across-species variation for a suite of drought response traits. The chapters in this dissertation are particularly timely as research and management efforts increasingly recognize that species are not a monolith and that characterizing the ecologically, genetically, and practically important variation within species is key to understanding adaptation to current and future climate and informing ecosystem management.

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INTRODUCTION

As climate changes, research on how plants respond will be critical both for understanding adaptation to future climate and for driving effective ecosystem conservation and management at a time of rapid change. However, gaps in knowledge relating to the extent to which species vary in key traits within and among populations across their ranges and the evolutionary and physiological mechanisms which underlie this variation limits both theoretical understanding of adaptation and effective management. The broad theme of this dissertation is to address within-species variation both in the context of understanding drought response and to inform conservation and management of species of conservation concern. The three chapters in this dissertation contribute to a growing body of literature which addresses variation in key plant traits across environmental gradients, emphasizing the ecological and practical importance of plant trait variation both among and within provenances.

Chapter I focused on identifying within-species variation in the iconic tree *Araucaria araucana* (pewen) across its range in Chile to inform conservation and restoration efforts. Given ambitious global commitments to restoration, science is needed to support capacity to achieve meaningful gains for both ecosystem integrity and human wellbeing. In Chile, identification and generation of appropriate plant material is a barrier to achieving major restoration goals under the United Nations (UN) Paris Climate Agreement and strategic plan of the UN Convention of Biological Diversity. Understanding genetic differentiation among plant populations is needed to maximize restoration success. For *Araucaria araucana*, a highly threatened iconic South American tree, this information is greatly needed to guide restoration and conservation efforts because this species occurs across a strong climate gradient. We grew seedlings from 12 populations of *A. araucana* across its range in Chile in a common garden to assess regional (coastal versus Andes mountain ranges) and population variation in key plant traits and relate this variation to environmental variables. We learned that pewen differs significantly in a suite of traits among and within regions and populations across its range in Chile and that this variation is at least partly explained by climate and soil variables. Temperature annual range, which explained the most trait variation, also explains genomic differentiation in this species. Thus, our results highlight the importance of conserving variation among and within regions, informing conservation strategies and seed sourcing guidelines for restoration.

Chapter II addressed patterns and mechanisms of within-species variation in drought response for two Chilean forbs across a significant precipitation gradient. Given rapidly changing climatic conditions, there is increasing focus on understanding how species will respond to disturbances such as drought. Understanding within-species variation in drought response is particularly important at the germination and seedling stages when plants are exceptionally vulnerable to drought mortality. However, little research addresses the relationship between key early plant traits, like germination and biomass allocation, and drought survival. To date, there is only limited understanding of the extent to which species vary across their ranges in key early plant traits, if these traits affect drought survival, and how local adaptation and phenotypic plasticity, alone or in concert, contribute to the ability of species to respond to drought. This information is of particular interest for widely distributed species, as provenances of these species that vary in water availability may respond differently to drought. Our study addressed if provenances from the wet and dry extremes of two Chilean forbs (*Acaena ovalifolia* and *Anenome multifida*) distributed across a large precipitation gradient differed in seed and germination traits and drought survival and if patterns of differentiation matched expectations for local adaptation and phenotypic plasticity. Additionally, we assessed the relationships between these traits and drought survival versus growth. For *A. ovalifolia*, provenances reached similar drought survival through two strategies: dry provenances were able to maintain a larger size via higher root:shoot ratios, while wet provenances remained small to survive drought longer. For *A. multifida*, wet provenances survived drought longer despite dry ecotypes showing a more resource conservative strategy, investing more in roots and growing slowly, while wet ecotypes invested more heavily in shoots and grew more quickly. Importantly, we showed that within- and among-provenance variation in early plant traits predicted drought survival, and that these traits sometimes had opposite relationships with growth. Our study highlights the importance of including early plant traits in studies of drought response– and important finding given that this stage is often not included.

Chapter III synthesized for a suite of drought response traits patterns of within-species and across-species genetic trait variation. As climate changes, the capacity of plants to adapt depends on genetic variation among and within populations of different species, the potential variation on which natural selection can act as selective pressures change. Characterizing genetic variation among populations in adaptive traits is, therefore, a key step in identifying the potential for local adaptation to current climate, for mismatch with future climate, and for gene flow of climate-adapted phenotypes to new areas. Climate change, and resultant increasing frequency and intensity of drought, is expected to exert intense and changing selective pressure on plants. However, our ability to make broad inference on a plant's capacity to adapt to drought is limited. One key limitation is that most studies that address plant responses to drought make inference at the *species* level, without consideration of *within-species* variation, despite increasing evidence that variation *among* and *within* populations is substantial and important. As a step towards understanding plant capacity to adapt to future drought, our synthesis characterized the relative genetic trait variation within and among populations for a suite of adaptive water relations traits (embolism resistance, including P12, P50, and P88, and water potential at turgor loss point) and co-varying plant economics traits (specific leaf area, root:shoot ratio, and wood density). Additionally, we tested if biological and ecological groupings commonly used in modeling and comparative studies which effectively differentiate species in respect to these traits were also effective in differentiating population-level genetic variation. Although there is a tendency to focus on drought responses at the species level, our results show that within- and among-population variation make up a substantial proportion of across-species genetic variation in the traits we studied, information that is needed to make inference on adaptation to future climate. Further, within-population variation was between 1.5 and 3 times greater than variation among populations, suggesting that populations may harbor substantial potential for adaptation to future climate. Additionally, we show that biological and ecological groupings, including life form, plant functional type leaf morphology, and leaf habit—which are commonly used in research and modeling and clearly differentiate species in respect to these traits—fail to capture key variation in water relations traits at the population level. Our study advances understanding of patterns of variation needed to predict adaptation to changing climate, but also highlights important gaps in information. For example, the pool of available studies that assessed genetic trait variation in drought response traits was heavily biased towards trees, northern hemisphere temperate ecosystems, and traits related to plant economics over water relations.

The chapters in this dissertation are particularly timely as research and management efforts increasingly recognize that species are not a monolith and characterize the ecologically, genetically, and practically important variation within species that contributes to understanding adaptation to future climate and informing management actions. Here, I emphasize the broader impacts of these chapters and discuss the efforts made to share these implications with the appropriate audiences. In Chapter I, we worked closely with Chilean research and management agencies (Instituto Forestal and Corporación Nacional Forestal) to identify questions relevant to the management of an iconic Chilean tree. As a result of this work, seed transfer zones, guidelines based on within-species variation to direct the movement of plant materials for restoration, are in development. Additionally, we are working with the Universidad de Concepción to create a Spanish language factsheet targeted towards managers to summarize key information and management implications from this chapter and additional recent research by collaborators at the Universidad de Concepción and the Instituto Forestal. For Chapter II, our results add to an extremely limited but growing body of knowledge on the importance of early life traits in drought survival, emphasizing the need for further research on this topic across and within species to better characterize broad patterns in how early plant phenotypes relate to drought response. Finally, in Chapter III, we provide strong evidence of the importance of within-species variation for a suite of plant traits related to drought response and indicate that common groupings including plant PFT should not be used for modeling or research on related to water relations traits. These findings provide important recommendations for future modeling and research efforts. Together, our work contributes to closing key knowledge gaps relating to the importance of plant variation within species, informing future research needs, and informing a body of science that drives ecosystem management.

CHAPTER I

Trait Variation Between and Within Andes and Coastal Mountain Ranges in the Iconic South American Tree *Araucaria araucana* in Chile

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Abstract

As global commitments to restoration are underway, science is needed to support capacity to achieve meaningful gains for ecosystems and human communities. In Chile, identification and generation of appropriate plant material is a barrier to achieving major restoration goals under the Paris Climate Agreement. Understanding genetic differentiation among plant populations is needed to maximize restoration success. For *Araucaria araucana*, a highly threatened iconic South American tree, this information is greatly needed to guide restoration and conservation efforts because this species occurs across a strong climate gradient. We grew seedlings from 12 populations of *A. araucana* across its range in Chile in a common garden to assess regional (coastal versus Andes mountain ranges) and population variation in key plant traits and relate this variation to environmental variables. We demonstrate that *A. araucana* is differentiated within regions and populations across its range in Chile by a suite of traits, particularly branch number and length (showing plant architectural differences) and needle width (showing leaf investment differences). We show that this variation is at least partly explained by climate and soil variables, with the most variation explained by differences between regions in temperature annual range. Thus, we recommend that restoration efforts focus on conserving genetic variation among and within regions and their populations and preventing the translocations of genotypes between coastal and Andes populations.

Introduction

As global ecosystems are increasingly affected by anthropogenetic degradation and climate change, ecological restoration is critically needed to repair ecosystems and support the human systems that depend on them. Towards that end, countries across the world are making ambitious restoration commitments. For instance, Chile aims to restore 1 million hectares of degraded land by 2050 as a part of its Nationally Determined Contribution under the Paris Climate Agreement (Gobierno de Chile 2020). One of the primary barriers to effective restoration is lack of understanding of appropriate plant materials (Gann et al. 2019; León-Lobos et al. 2020). To protect genetic diversity, avoid maladaptation to outplanting sites, and limit negative effects on adjacent populations, it is important to understand genetic differentiation among and within plant populations. (Lesica & Allendorf 1999; Kramer & Havens 2009; Breed et al. 2013). This information, however, is not yet available for many species of conservation concern in general, and specifically lacking in Chile, limiting restoration capacity (León-Lobos et al. 2020). We narrow this knowledge gap for the ancient and iconic South American conifer, *Araucaria araucana* (pewen), a tree of high cultural and ecological value in South America. Most genetic information for this threatened species addresses neutral genetic variation (e.g., Souza et al. 2008, Martín et al. 2014), thus we lack information on adaptive genetic variation (Bekessy et al.

2003). Here, we characterized among- and within-population variation in key plant traits across the range of pewen in Chile and related overall trait variation to climate and soil variables, which commonly drive large-scale patterns of differentiation in trees (Alberto et al. 2013). Our work provides the basis for both understanding patterns of genetic and phenotypic variation across the range of this species and improving management and restoration capacity.

As plants are rooted in place and cannot escape environments in which they germinate, they are often adapted to local conditions and thus genetically and phenotypically differentiated by environment across their ranges (Leimu & Fischer 2008; Anderson et al. 2011). As a result, population differentiation is extremely common in plants (Leimu & Fischer 2008) and occurs across spatial scales from meters (Lekberg et al. 2012) to hundreds of kilometers (Liepe et al. 2016; Supple et al. 2018). For instance, population differentiation has been found in 90% of forest trees studied (Alberto et al. 2013). It is not surprising that local adaptation is so common, as it has been shown to improve plant growth, reproduction, and survival at home sites (Joshi et al. 2001; Leimu & Fischer 2008). If plants are moved to foreign environments outside their range of local adaptation, population fitness may be low and deleterious effects may occur in adjacent populations (Lesica & Allendorf 1999; Hufford & Mazer 2003; McKay et al. 2005; Broadhurst et al. 2008). Thus, understanding genetic differentiation among and within populations of the same species in key fitness traits is critical to informing conservation and restoration across the species range (Hufford & Mazer 2003; Broadhurst et al. 2008; Breed et al. 2013; Gann et al. 2019).

Beyond characterizing patterns of population differentiation, there is considerable interest in identifying environmental variables that explain these patterns (Reich et al. 1997; Wright et al. 2005; Alberto et al. 2013; Aitken & Whitlock 2013; Anderegg et al. 2016, 2018). Climate gradients are often considered as drivers of plant population differences (Alberto et al. 2013; Bower et al. 2014), as plant distribution is strongly driven by climate (Webb 1986; Woodward 1987; Woodward et al. 2004). As a result of provenance studies which have been conducted for multiple centuries, within species, climate variably explains population differentiation depending on species, traits studied, and the magnitude of climate gradients (Alberto et al. 2013; Griffin-Nolan et al. 2018). Soil variables may play a role in driving population differentiation that is equal to or even greater than that of climate, despite soil variables varying at much smaller spatial scales (Macel et al. 2007; Lekberg et al. 2012; Siefert et al. 2014; Lajoie & Vellend 2015; Gibson et al. 2019). However, the relative contribution of these factors (and the scale of their variation) remains unresolved (but see Siefert et al. 2015). Here, we ask which climate and soil variables best explain multivariate genetic trait differentiation among populations, addressing large-scale climate versus small-scale soil heterogeneity as drivers of population differentiation.

There is increasing recognition of the importance of maintaining both genetic and phenotypic variation in species-specific conservation and restoration strategies, especially given anticipated rapid changes in climate (Kramer & Havens 2009; Breed et al. 2013; Havens et al. 2015; Gann et al. 2019). Understanding this genetic variation is valuable for managers as genetic variation can be both the result of previous natural selection and the raw material for future selection in response to environmental change (Kramer & Havens 2009; Kremer et al. 2012). Furthermore, understanding the extent to which within-species variation occurs within or among populations (population versus regional variation) may have implications for the appropriate sourcing of genetic material for restoration. For example, in a study of the threatened species *Eucalyptus melliodora* in Australia, most genetic variation occurred within versus among populations, and the authors concluded that seeds could be sourced broadly for restoration (Supple et al. 2018). Similarly, a high level of within-population variation was identified for a relatively small number of locally adapted populations of interior spruce complex *(Picea glauca, P. engelmannii*, and their hybrids) and lodgepole pine (*Pinus contorta)* across an area spanning British Columbia and Alberta (>1000 km in latitude and longitude) in Canada (Liepe et al. 2016). Meta-analysis supports these case studies to show that for trees (particularly those that are wind pollinated), this pattern of population differentiation across large spatial scales (on the order of hundreds to thousands of kilometers) and high within-population variation is common, even when gene flow is significant (Savolainen et al. 2007; Alberto et al. 2013; Liepe et al. 2016). However, the majority of this information is for temperate forest trees with large ranges (Alberto et al. 2013) and we don't yet know how species with restricted and fragmented ranges vary among and within populations.

Although understanding drivers and spatial patterns of genetic and phenotypic variation is generally important for ecosystem management, it is particularly important to have this information for pewen. There is considerable interest in restoration of this species across its range and restoration programs are in progress, but lack of information on genetically-based phenotypic variation (rather than neutral genetic variation, which has been largely resolved; see (Martín et al. 2014) limits understanding of genetically appropriate material for outplanting and ability to conserve genetic diversity (León-Lobos et al. 2020). Additionally, this species is experiencing drought-related mortality that varies among and within regions (Willhite 2019; Puchi et al. 2021), suggesting that climate and soil conditions may predict survival outcomes and adding urgency to the need for information on regional and population differentiation for this species.

We studied patterns of among- and within-population genetic variation of pewen across its range in Chile, in order to improve both ecological understanding and management and restoration of this unique species. Our study is one of only a handful that addresses within-species genetic variation in a suite of traits rangewide in South American conifers. For pewen, we build on previous phenotypic and genetic studies in this species that were limited in the number of sites and traits sampled to assess among- and within-population variation in a broad suite of traits and relate this variation to climate and soil variables. Specifically, we assessed:

Q1: Do plants from populations that experience different climate and soil conditions show trait variation among or within populations and regions (Andes vs. coastal mountain ranges)?

Q2: Which plant traits drive overall differences in phenotypes among and within populations and regions?
Q3: Which climate and soil variables drive overall differences in phenotypes among and within populations and regions?

Our findings contribute to the growing literature on among- and within- population variation in trees and uses common methods for developing seed transfer guidelines to lay the groundwork for developing these important resources for this species.

Methods

STUDY SYSTEM

Araucaria araucana (pewen) is native to the coastal and Andean cordilleras of central Chile (37° 31' to 39° 30') and Argentina (37 ° 45' to 40° 20') (Aagesen 1998; Figure 1). The range of pewen, although relatively small, spans substantial elevation (664-1227 m), precipitation (1100-2219 mm annual precipitation), and temperature (6.1-9.6 °C mean annual temperature) gradients (Table 1). *A. araucana* is a dioecious and wind pollinated masting species (Sanguinetti and Kitzberger 2008). This species is of cultural and spiritual importance to the Mapuche Pewenche (pewen people), and the sale and consumption of *ngülliw* (the large pinenut-like seeds of pewen) is important for subsistence (Herrmann 2006). Pewen has been listed as "Endangered" on the IUCN Red List since 2011 (Premoli 2015) due to historic deforestation (although it is now protected by the government of Chile), invasion by *Pinus contorta* (lodgepole pine), illegal harvest of seeds (legal for indigenous peoples only), and seed consumption by livestock (Cóbar-Carranza et al. 2014; Premoli 2015; Tella et al. 2016). Seed regeneration is poor, but vegetative reproduction may occur (Aagesen 1998). Because its significant climate gradient in Chile and its ecological and cultural importance, pewen is an excellent study system for addressing management-relevant questions about patterns and predictors of genetic variation among and within populations across a species' range.

STUDY POPULATIONS

We selected 12 sites (referred to hereafter as populations) throughout the range of pewen in both the Andean and coastal mountain ranges (regions) of Chile spanning altitude and climate gradients (Table 1, Figure 1). Populations were located within five genetic clusters (two coastal, three Andean) identified by Martín et al. (2014) using a landscape genetic approach.

SEED COLLECTION, SEEDLING GROWTH, AND TRAIT MEASUREMENTS

At each population, we collected seeds from trees that were at least 150 m apart and had available seeds in 2018 at the time of collection. Trees for seed collection were not chosen randomly, as they had to be producing seeds, and many were chosen nearby roads or trails because of convenient access (see *Limitations* in Discussion). We referred to seeds from a single tree at a given population as a family (specifically, they are half sibling families). At each population, we initially sampled 7-96 families per population depending on site size and availability; we randomly selected 20 families from each of the 12 populations for inclusion in the study (n=1 seedling per family), except in two populations (Lonquimay and Marimenuco), where n=7. Additionally, one individual was not measured by accident, reducing n to 19 for this population (Table 1).

After cold stratification at 4°C for two months, we cut the end of each seed and submerged them in water for 2 days at 4°C. Seeds were planted in plastic flats, germinated in a greenhouse in Yumbel, Chile (-37.098090, -72.562230), and then grown for one year. Seedlings experienced ambient light conditions and were well-watered (at least once and sometimes more than twice per day depending on temperature). We were unable to randomize the location of individual seedlings on benches because of the requirements of the commercial growing facility; however, we anecdotally noted that the effects of population and family on seedling traits were more prominent than greenhouse effects (see *Limitations* in Discussion). Germination rate was measured at 30 weeks, and seedling survival was measured after one year. Plants that were fully browned were considered dead.

In December of 2019, we measured a suite of traits to assess variation among and within regions and populations. Because no information exists on which traits are adaptive for this species, we selected traits related to seedling growth and biomass allocation, architecture, and leaf economics that are known to relate to resource use and stress-tolerance strategies (Table 2). We counted the number of whorls (opposite branches originating from a single point) and branches of each seedling and measured stem length, basal diameter, and the length of each branch (to calculate a mean branch length; if there were no branches, branch length was 0). Additionally, we measured the length and width of the three longest needles to calculate maximum needle lengths and widths (referred to as needle length and width throughout). We measured needle area using the app LeafByte (Getman-Pickering et al. 2020) and calculated needle mass per area. To measure needle density, we measured needle volume using the water displacement method (Hughes 2005) and divided volume by needle mass. Needle thickness was calculated by dividing needle volume by needle area. Additional descriptions of trait measurements and units are included in Table 2.

CLIMATE AND SOIL VARIABLES

We accessed climate and soil variables from WorldClim (Fick & Hijmans 2017), TerraClimate (Abatzoglou et al. 2018), and SoilGrids (Hengl et al. 2017) databases for the GPS coordinates of each family (see Table 3 for variables and units). WorldClim data were downloaded directly into R using the getData() function in the package raster (Hijmans & Van Etten 2021) in R Studio version 1.2.5042 (RStudio Team 2020). We extracted data for our coordinates using the extract() function in the package sp (Pebesma & Bivand 2005). For TerraClimate data, we used the getTerraClim() function in climateR (Johnson 2020) to download and extract data for our populations. Additionally, we accessed climate variables using regional climate models from the Center for Climate and Resilience Research at the Universidad de Chile (CR2; http://www.cr2.cl/) but they did not perform better than data from global models, so we excluded them from final analyses. For SoilGrids data, we used Google Earth Engine to access variables listed in Table 3.

STATISTICAL ANALYSIS

As our traits were measured in a common garden, which controls for most environmental variation, we assume trait differences are due to genetic differences rather than environment. To assess if individual traits varied among regions (coast vs. Andes) and populations (Q1), we used analysis of variance (ANOVA). For each trait, we ran nested models with region and population nested within region as factors to address the relative contribution of region and population and to identify traits which varied among populations and should be included in additional analysis (Supporting Information). Assumptions of ANOVA were checked using residuals plots and normal quantile plots. For count traits only (number of whorls, number of branches), we used a generalized linear model with a Poisson distribution instead of an ANOVA because these traits were not normally distributed (O'Hara & Kotze 2010). Traits that did not significantly vary among populations or regions ($p>0.05$) were not used for additional analyses (see Table 2 and Supporting Information for a list of the eliminated traits).

To address multivariate trait differences among regions and populations (Q1), we used principal component analysis (PCA) using Bray-Curtis distances with pairwise deletion of missing observations in the vegan package in R (Oksanen et al. 2019). We used multiple regressions with PC scores as response variables and traits as predictors to address which traits best explained overall differences in phenotypes regions and populations (Q2). A separate model was created for each of the first four PC axes (which explained 94% of variation). To select traits to include in our models, we used Spearman's r to identify the traits most correlated with each axis where $|r| \geq 0.20$ and $p \geq 0.05$ (Supporting Information). We then excluded traits that covaried with other traits using the cutoff of $|r|>0.60$ (Zuur et al. 2010), selecting traits with higher correlation with axes scores first and removing less highly correlated traits that covaried. We used backwards selection to remove additional traits that did not add predictive power to the model using the step() function in R (RStudio Team 2020).

To address which climate and soil variables best explained overall differences in phenotypes among regions and populations (Q3), we created separate multiple regression models for each PC axis using climate and soil variables as predictors. For each of the first three PC axes, we used Spearman's r to identify the traits most correlated with each axis where $|r| \geq 0.20$ and $p \geq 0.05$ (Supporting Information). We used backwards selection to remove additional traits that did not add predictive power to the model ($p > 0.05$). Overall contribution of climate variables in explaining trait variation across axes was assessed using PERMANOVA with the adonis() function in vegan with pairwise deletion of missing observations (Oksanen et al. 2019).

All analyses were conducted using RStudio version 1.2.5042 (RStudio Team 2020), and all figures except Figure 1 were made in R using ggplot2 (Wickham 2016). Figure 1 was made in ArcMap.

Results

PEWEN SEEDLINGS FROM ACROSS REGIONS AND POPULATIONS RANGE-WIDE DIFFER IN THEIR TRAITS (Q1)

Plants from different regions (coast and Andes) and populations varied significantly in their traits (Figure 2, Supporting Information). Across the 16 measured traits, 11 differed significantly to varying degrees among regions and populations (Figure 2A, Supporting Information). Across all traits, regions were highly distinct, with coastal populations differing from Andes populations (Figure 2B) both in PC1 (79.3% of overall variation) and PC2 (9.6% of overall variation). Populations within each region also varied significantly in their traits. For PC1, region accounted for 9.8% of variation in axis scores ($p<0.001$) and population accounted for 12.1% of variation ($p<0.001$) in a nested ANOVA model. For PC2, region accounted for 8.9% of variation ($p<0.001$), and population accounted for 1.9% of variation ($p<0.001$).

BRANCH ARCHITECTURE AND NEEDLE TRAITS EXPLAIN OVERALL REGION AND POPULATION TRAIT DIFFERENCES (Q2)

Branch architectural and needle traits explain overall trait differences between regions and among populations. For PC1, number of whorls, needle area, and needle succulence explained overall trait variation (Adjusted $R^2 = 0.83$, $p<0.001$; Table 4, Supporting Information). Our model initially included proportion of survival to 1 year; but it did not provide explanatory power beyond included variables (and was removed per our backwards selection method; $\Delta AIC=1.7$; Supporting Information). The number of branches and branch length were both highly correlated with the number of whorls (and thus not included in the model; Supplemental Information); and showed similar patterns among populations as number of whorls (shown in Figure 2C).

For PC2, needle width best explained overall trait variation (Adjusted $R^2=0.04$, $p=0.004$; Table 4, Supporting Information), although it explained relatively little variation. No other traits that were not collinear with needle width were correlated with this axis (where $|r|>0.20$). Needle area covaried with needle width and showed similar patterns across regions and populations as needle width (Figure 2D). PC3 (which explained 4.9% of overall trait variation) was best explained by needle succulence, needle width, and branch length (Adjusted $R^2=0.88$, $p<0.001$; Table 4, Supporting Information) after removal of survival percentage by backwards selection ($\Delta AIC=0.0$; Supporting Information). PC4 (which explained 1.8% of overall trait variation) was best explained by needle mass per area, needle length, and needle width (Adjusted $R^2=0.72$, $p<0.001$; Table 4, Supporting Information). No traits were removed from the full model.

The first two PC axes primarily differentiated Andes and coastal populations (regions) in their traits (Figure 2B). On average, compared to Andes populations, coastal populations tended to have more whorls (Figure 2C, Supporting Information) and branches (nearly twice as many) as well as branches that are on average 1.5x as long. Number of branches and branch length show similar patterns among regions and populations as number of whorls (shown in Figure 2C).

Significant variation is shown within the Andes region among populations as well as within populations in these traits. Additionally, coastal populations tended to have smaller and less succulent needles, with needle area and needle succulence showing similar patterns among regions and populations as needle width (Figure 2D). Needle trait effect sizes were smaller compared to branch architectural traits (Supporting Information).

TEMPERATURE ANNUAL RANGE BEST EXPLAINED OVERALL REGION AND POPULATION TRAIT DIFFERENCES (Q3)

Overall trait differences between regions and among populations were best explained by temperature annual range (TAR), the difference between maximum temperature in the warmest month and minimum temperature in the coldest month (Table 5). Additionally, mean vapor pressure deficit, soil organic carbon, and cation exchange capacity explained small amounts of variation in minor PC axes (Table 5). However, much trait variation remained unexplained by environmental variables.

For PC1, TAR and SOC together explained 10.6% of variation in PC1 scores ($p<0.001$, Table 5). Our initial model included SWE, OCD, and Silt, but these variables did not provide additional explanatory power beyond TAR and SOC ($\Delta AIC=0.0$; Supporting Information). Variation in PC2 was also best explained by TAR (although only 6.5% of overall variation was explained; Adjusted R^2, $p<.001$, Table 5). For PC2, our initial model included MDR (mean diurnal range) instead of TAR (as Spearman's r was slightly higher; Supporting Information), but it explained marginally more variation, so we ultimately used TAR for consistency with our model for PC1 ($\Delta AIC=-0.5$; Supporting Information). For PC3, 13% of variation was explained by mean vapor pressure deficit ($p<0.001$) and for PC4, 2.3% of variation was explained by CEC ($p<0.001$). For models for PC3 and PC4, no variables were removed from the full models.

Temperature annual range explains 12.0% of all trait variation ($p=0.001$) and varies significantly among regions and populations (Figure 3, Supporting Information). An additional suite of climate and soil variables covaried with TAR ($|r|>0.60$) and were thus not included in the multiple regression models (Supporting Information). Overall, coastal populations tend to have smaller temperature annual ranges than Andes populations (Figure 3). This is a result of both higher temperature minimums (-1.58 ± 2.25 vs. -8.00 ± 0.67 °C, $p < 0.01$) and lower temperature maximums (19.49 ± 2.12 vs. 23.38 ± 1.10 °C, $p < 0.001$).

Discussion

To identify ecotypes for effective restoration and conservation prioritization of threatened species, we must understand patterns of genetic variation in phenotypes across a species' range, especially in relation to climate and soil variables. Therefore, we asked how populations across the range of *pewen,* an iconic South American conifer species of restoration and conservation concern, varied in a suite of traits between regions and among populations and if this variation was related to climate and soil variables as expected from evidence in other tree species. Our results demonstrate that pewen differs significantly in a suite of traits among and within regions and populations across its range in Chile and that this variation is at least partly explained by climate and soil variables. Temperature annual range, which explained the most trait variation, also explains genomic differentiation in this species (Varas-Myrik et al. 2021). Thus, our results highlight the importance of conserving variation among and within regions, informing conservation strategies and seed sourcing guidelines for restoration.

PEWEN SHOWS DIFFERENTIATION BETWEEN REGIONS AND POPULATIONS, WITH HIGH WITHIN-POPULATION VARIATION

We found clear genetic differentiation in traits between regions. Coastal populations tended to have smaller, less succulent leaves and more branches, while Andes populations tended to have larger, more succulent leaves and fewer branches. Coast to Andes region differences were best explained by temperature annual range, with higher and lower temperature extremes occurring in the Andes region. Thus, we show significant variation in plant traits across the range of pewen in Chile*,* particularly between the coastal and Andes regions, suggesting that *regional* variation should be conserved. While some trait variation was explained by regional differences, significant variation was also explained by population differences. This suggests that coastal and Andean regions are not only differentiated from each other, but populations within regions are also differentiated from each other and *among-population* variance should be conserved. Additionally, we showed that a large proportion of variation was unexplained by region or population, suggesting that *within-population* variation should be considered as well.

REGIONAL DIFFERENTIATION AND HIGH WITHIN-POPULATION VARIATION IS CONSISTENT WITH PREVIOUS ASSESSMENTS OF PHENOTYPIC AND GENETIC VARIATION IN PEWEN

Our results are consistent with two previous assessments of trait and genetic variation in pewen*,* which also showed substantial differentiation between coastal and Andes regions and high within-population variation. A phenotypic study of concentrations of alkenes in foliar epicuticular wax, which may contribute to reducing cuticular water loss as an adaptation to drought, revealed differences between coastal and Andes populations (Rafii & Dodd 1998). Although only four populations were used, these authors additionally found high within-population variation in the studied trait. Additional work including nine populations across the coastal and Andes ranges and into pewen's range in Argentina found that 12% of variation in carbon isotope discrimination and 14% of variation in root:shoot ratio were explained by region (coast, Chilean Andes, Argentinian Andes; Bekessy et al. 2002). These patterns were also corroborated by a study of neutral genetic variation (rather than quantitative genetic variation in traits as assessed here), which found 16% of total variation explained by the region (coast vs. Chilean Andes; Martín et al. 2014). Two studies using fewer genetic markers and older technology did not detect these trends (Bekessy et al. 2003; Ruiz et al. 2007).

Consistent with other work on this species, we found strong evidence of differentiation among mountain ranges (regions). Regional differences in traits could be attributed to genetic isolation; Martín et al. (2014) attributed regional differentiation to geographic isolation among the ranges. The coastal range is thought to have originated long before the Andes range, and pewen is found on the western slope of the coastal range, a possible barrier to gene flow (as genetic material would have to travel over the coastal range to reach the Andes or vice versa). We also found significant variation that was unexplained by region or population (78% and 89% for PC1 and PC2, respectively; 69% to 95% depending on the trait). In other studies, unexplained trait variation is commonly assumed to be variation maintained within populations (see *Limitations* for further discussion). Within-population variation may be highly important given within-population variation in drought response and subsequent mortality seen in pewen (Puchi et al. 2021).

HIGH WITHIN-POPULATION VARIATION AND LARGE-SCALE REGIONAL DIFFERENTIATION ARE COMMON IN FOREST TREES

High within-population variation, maintained by gene flow (particularly in wind-pollinated species), is not uncommon for forest trees (Kremer et al. 2012; Alberto et al. 2013). For example, for a small section of the ranges of wind-pollinated ponderosa pine (*Pinus ponderosa)* and Douglas-fir (*Pseudotsuga menziesii)* in Oregon, United States across two mountain ranges with about twice the latitudinal gradient and the same longitudinal gradient as our study, *P. menziesii* but not *P. ponderosa* was differentiated between regions. However, both species showed significantly higher within-population variance compared to among-population variance (Sorensen & Weber 1994). In addition, similar patterns of low among-population variation and high within-population variation in a suite of morphological, phenological, and physiological traits was found in two Northern hemisphere spruces (*Picea glauca, P. engelmannii*) and lodgepole pine (*Pinus contorta)* (Liepe et al. 2016). We did not find additional studies assessing population differentiation in comparable plant traits for other conifers in South America, so we could not compare our results to other local species.

TEMPERATURE ANNUAL RANGE BEST EXPLAINS OVERALL TRAIT VARIATION

Although temperature annual range explained a limited amount of overall trait variation (12%), this is a substantial amount of variation for just a single climate variable. These findings are consistent with one other study on this species that addressed relationships with environmental variables, where TAR best explained genomic differentiation (Varas-Myrik et al. 2021). Usually, multiple environmental variables play a significant role in explaining multivariate trait variation across populations (Gibson et al. 2019). In our study, TAR primarily explained regional (coast vs. Andes) differences; the magnitude of these differences resulted from both increased minimum and decreased maximum temperatures in the coastal populations, although climate variables associated with temperature maximums tended to be more highly correlated with both PC axes. This suggests that temperature minimums and maximums are both important in shaping population variation in this species. Temperature minimums could explain regional genetic differences in branch traits, as Andes populations experiencing significant frosts (particularly those in the northern part of the range) may not be able to support many large branches due to loss by frost. Temperature maximums could explain regional differences in leaf succulence, with

Andes populations experiencing more severe drought having increased succulence (and leaf size) to store water under drought conditions.

Given previous observation of population differentiation in carbon isotope discrimination and cuticular wax alkenes, two traits related to adaptation to arid environments, it is a little surprising that our populations were not differentiated by precipitation or water availability variables. However, meta-analysis shows that most plant traits unrelated to water transport are generally unrelated to precipitation (Griffin-Nolan et al. 2018). Additionally, the lack of explanatory power of water availability variables could be explained by the relatively small precipitation range of this species. Further, additional studies in this species show that differential drought mortality may occur to a greater degree within versus among populations (Puchi et al. 2021).

LIMITATIONS

Our study has four potential limitations. First, selection of adult trees from which to collect seed was not randomized, as seed collection was limited due to availability of seed and ease of access. However, our minimum distance between trees used for seed collection (150m) was greater than that of other studies (50 and 100 m; Rafii and Dodd 1998, Bekessy et al. 2002) and our collection sites within populations varied considerably with respect to topography and microclimate . Thus, we do not feel that this limitation biased results. Second, seedlings were not randomized in the greenhouse, as they were grown in a commercial nursery and subject to procedures therein. However, we anecdotally note that we did not observe any greenhouse effects. Third, we did not replicate within families (trees) in our populations and, therefore, cannot differentiate between within-population variation and error (although it is a common practice in the literature to attribute variance unexplained by population to within-population variation; see Alberto et al. 2013a). If possible, future studies should further replicate within families to account for within-population variation. Finally, there is no information on which plant traits might be adaptive for this species, so we selected traits that have been observed to be important for other species. Thus, we cannot conclude that the variation we identified is adaptive. Future studies are needed to disentangle the traits that are in fact adaptive for this species. Additional work may also consider assessing response to light availability and other environmental factors (which could vary among populations as a result of differences in plant communities).

IMPLICATIONS FOR RESTORATION AND CONSERVATION OF PEWEN IN CHILE

As ecological restoration commitments ramp up in Chile and beyond, developing science-based resources to guide selection of plant materials is key to maximizing outcomes (Lesica & Allendorf 1999; McKay et al. 2005). In Chile, lack of genetically appropriate seed supply for restoration is a barrier to achieving restoration goals (León-Lobos et al. 2020), although efforts to strengthen seed systems are ongoing (Atkinson et al. 2021). Here, we provide valuable information to complement information on patterns of genomic differentiation (Varas-Myrik et al. 2021)(unpublished data, Ipinza et al. 2021) and assisted migration (Ipinza & Müller-Using

2021) being developed by colleagues to guide conservation prioritization for this species. Given that our data show patterns of variation among as well as within regions and populations, we recommend that restoration efforts aim to collect seed widely within populations across both coastal and Andes mountain ranges, collecting from as many trees within a population as possible to sample within-population diversity (to preserve genetic variation; Kramer and Havens 2009). Additionally, as other studies have concluded, we suggest that managers separate seeds by provenance, particularly avoiding mixing of coastal and Andes seed sources (to avoid maladaptation of seed sources to outplanting sites; Lesica and Allendorf 1999, Broadhurst et al. 2008). We emphasize that conservation of existing and future genetic variation (by widespread seed collection) is necessary to maximize adaptive potential under changing climate, as research indicates this species is at risk within parts of its range (Ipinza & Müller-Using 2021; Varas-Myrik et al. 2021).

Finally, our work sets the stage for the development of seed transfer zones, maps that identify putatively locally adapted ecotypes to guide seed sourcing for restoration (McKay et al. 2005). These resources are needed as provisional zones (which are not species-specific) are generally not sufficient (Gibson & Nelson 2017) and will directly build capacity for restoration in Chile, where collaborators in Chilean management agencies will immediately put them to use. This work, along with additional studies currently in progress by Chilean collaborators, will improve conservation and restoration outcomes for this living fossil species.

Literature Cited

Aagesen DL. 1998. On the Northern Fringe of the South American Temperate Forest: The History and Conservation of the Monkey-Puzzle Tree. Environmental History **3**:64. Available from https://academic.oup.com/envhis/article-lookup/doi/10.2307/3985427 (accessed April 12, 2019).

Abatzoglou JT, Dobrowski SZ, Parks SA, Hegewisch KC. 2018. TerraClimate, a high-resolution global dataset of monthly climate and climatic water balance from 1958-2015. Scientific Data **5**:1–12. Nature Publishing Groups.

Aitken SN, Whitlock MC. 2013, November 25. Assisted gene flow to facilitate local adaptation to climate change. Annual Reviews Inc. Available from https://www.annualreviews.org/doi/abs/10.1146/annurev-ecolsys-110512-135747 (accessed July 1, 2021).

Alberto FJ et al. 2013. Potential for evolutionary responses to climate change - evidence from tree populations. Global Change Biology **19**:1645–1661. John Wiley & Sons, Ltd. Available from http://doi.wiley.com/10.1111/gcb.12181 (accessed April 1, 2020).

Anderegg L, Berner LT, Law BE, L Anderegg LD, Badgley G, Sethi ML, HilleRisLambers J. 2018. Within-species patterns challenge our understanding of the leaf economics spectrum Tree range limits and climate change View project Within-species patterns challenge our understanding of the leaf economics spectrum. Available from https://www.researchgate.net/publication/323968054 (accessed March 31, 2020).

Anderegg WRL, Klein T, Bartlett M, Sack L, Pellegrini AFA, Choat B, Jansen S. 2016. Meta-analysis reveals that hydraulic traits explain cross-species patterns of drought-induced tree mortality across the globe. Proceedings of the National Academy of Sciences **113**:5024–5029. Available from http://www.pnas.org/content/113/18/5024.abstract.

Anderson JT, Willis JH, Mitchell-Olds T. 2011, July 1. Evolutionary genetics of plant adaptation. Elsevier Current Trends.

Atkinson RJ et al. 2021. Seeding resilient restoration: An indicator system for the analysis of tree seed systems. Diversity **13**:1–13.

Bekessy SA, Ennos RA, Burgman MA, Newton AC, Ades PK. 2003. Neutral DNA markers fail to detect genetic divergence in an ecologically important trait. Biological Conservation **110**:267–275.

Bekessy SA, Sleep D, Stott A, Menuccini M, Thomas P, Ennos RA, Burgman MA, Gardner MF, Newton AC. 2002. Adaptation of monkey puzzle to arid environments reflected by regional differences in stable carbon isotope ratio and allocation to root biomass. Page Forest Genetics. Available from https://www.unep-wcmc.org/system/dataset_file_fields/files/000/000/203/original/FG09-1_063-070.pdf?1399378443 (accessed July 9, 2019).

Bower AD, Clair JBS, Erickson V. 2014. Generalized provisional seed zones for native plants. Ecological Applications **24**:913–919. Available from http://www.jstor.org/stable/24432226.

Breed MF, Stead MG, Ottewell KM, Gardner MG, Lowe AJ. 2013. Which provenance and where? Seed sourcing strategies for revegetation in a changing environment. Conservation Genetics **14**:1–10. Kluwer Academic Publishers. Available from https://link.springer.com/article/10.1007/s10592-012-0425-z (accessed July 1, 2021).

Broadhurst LM, Lowe A, Coates DJ, Cunningham SA, McDonald M, Vesk PA, Yates C. 2008. Seed supply for broadscale restoration: maximizing evolutionary potential. Evolutionary Applications **1**:587–597. John Wiley & Sons, Ltd (10.1111). Available from http://doi.wiley.com/10.1111/j.1752-4571.2008.00045.x (accessed April 3, 2019).

Cóbar-Carranza AJ, García RA, Pauchard A, Peña E. 2014. Effect of Pinus contorta invasion on forest fuel properties and its potential implications on the fire regime of Araucaria araucana and Nothofagus antarctica forests. Biological Invasions **16**:2273–2291. Kluwer Academic Publishers.

Donohue K, Rubio de Casas R, Burghardt L, Kovach K, Willis CG. 2010. Germination, Postgermination Adaptation, and Species Ecological Ranges. Annual Review of Ecology, Evolution, and Systematics **41**:293–319. Annual Reviews. Available from http://www.annualreviews.org/doi/10.1146/annurev-ecolsys-102209-144715 (accessed April 2, 2020).

Fick SE, Hijmans RJ. 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. International Journal of Climatology **37**:4302–4315. John Wiley and Sons Ltd. Available from https://rmets.onlinelibrary.wiley.com/doi/full/10.1002/joc.5086 (accessed June 21, 2021).

Gann GD et al. 2019. International principles and standards for the practice of ecological restoration. Second edition. Restoration Ecology **27**:S1–S46. Blackwell Publishing Inc. Available from https://onlinelibrary.wiley.com/doi/10.1111/rec.13035 (accessed April 13, 2020).

Getman-Pickering ZL, Campbell A, Aflitto N, Grele A, Davis JK, Ugine TA. 2020. LeafByte: A mobile application that measures leaf area and herbivory quickly and accurately. Methods in Ecology and Evolution **11**:215–221. British Ecological Society. Available from https://besjournals.onlinelibrary.wiley.com/doi/full/10.1111/2041-210X.13340 (accessed June 21, 2021).

Gibson A, Nelson CR. 2017. Comparing provisional seed transfer zone strategies for a commonly seeded grass, pseudoroegneria spicata. Natural Areas Journal **37**:188–199.

Gibson A, Nelson CR, Rinehart S, Archer V, Eramian A. 2019. Importance of considering soils in seed transfer zone development: evidence from a study of the native *Bromus marginatus*. Ecological Applications **29**:e01835. Available from https://onlinelibrary.wiley.com/doi/abs/10.1002/eap.1835 (accessed April 18, 2019).

Gobierno de Chile. 2020. Chile's Nationally Determined Contribution.

Griffin-Nolan RJ et al. 2018. Trait selection and community weighting are key to understanding ecosystem responses to changing precipitation regimes. Functional Ecology **32**:1746–1756.

Grime JP. 1977. Evidence for the Existence of Three Primary Strategies in Plants and Its Relevance to Ecological and Evolutionary Theory. The American Naturalist **111**:1169–

1194. University of Chicago Press . Available from https://www.journals.uchicago.edu/doi/abs/10.1086/283244 (accessed September 10, 2021).

Havens K, Vitt P, Still S, Kramer AT, Fant JB, Schatz K. 2015. Seed Sourcing for Restoration in an Era of Climate Change. Natural Areas Journal **35**:122–133. Natural Areas Journal.

Hengl T et al. 2017. SoilGrids250m: Global gridded soil information based on machine learning. PLoS ONE **12**:e0169748. Public Library of Science. Available from http://www.isric.org/content/isric- (accessed June 21, 2021).

Herrmann TM. 2006. Indigenous Knowledge and Management of Araucaria Araucana Forest in the Chilean Andes: Implications for Native Forest Conservation. Biodiversity and Conservation **15**:647–662. Kluwer Academic Publishers. Available from http://link.springer.com/10.1007/s10531-005-2092-6 (accessed April 9, 2019).

Hijmans RJ, Van Etten J. 2021. raster: Geographic analysis and modeling with raster data.

Hufford KM, Mazer SJ. 2003. Plant ecotypes: genetic differentiation in the age of ecological restoration. Trends in Ecology & Evolution **18**:147–155. Available from http://www.sciencedirect.com/science/article/pii/S0169534703000028.

Hughes SW. 2005. Archimedes revisited: A faster, better, cheaper method of accurately measuring the volume of small objects. Physics Education **40**:468–474. IOP Publishing. Available from www.iop.org/journals/physed (accessed June 21, 2021).

Ipinza R, Müller-Using S. 2021. Migración asistida de Araucaria araucana. Santiago, Chile.

Johnson M. 2020. climateR: climateR.

Joshi J et al. 2001. Local adaptation enhances performance of common plant species. Ecology Letters **4**:536–544.

Kramer AT, Havens K. 2009, November. Plant conservation genetics in a changing world.

Kremer A et al. 2012. Long-distance gene flow and adaptation of forest trees to rapid climate change. Pages 378–392 Ecology Letters. Available from http://doi.wiley.com/10.1111/j.1461-0248.2012.01746.x (accessed March 15, 2019).

Lajoie G, Vellend M. 2015. Understanding context dependence in the contribution of intraspecific variation to community trait–environment matching. Ecology **96**:2912–2922. Ecological Society of America. Available from http://doi.wiley.com/10.1890/15-0156.1 (accessed April 1, 2020).

Leimu R, Fischer M. 2008. A Meta-Analysis of Local Adaptation in Plants. PLoS ONE **3**:e4010. Public Library of Science. Available from https://dx.plos.org/10.1371/journal.pone.0004010 (accessed March 31, 2020).

Lekberg Y, Roskilly B, Hendrick MF, Zabinski CA, Barr CM, Fishman L. 2012. Phenotypic and genetic differentiation among yellow monkeyflower populations from thermal and non-thermal soils in Yellowstone National Park. Oecologia **170**:111–122. Available from http://link.springer.com/10.1007/s00442-012-2297-9 (accessed July 13, 2020).

León-Lobos P et al. 2020. Lack of adequate seed supply is a major bottleneck for effective ecosystem restoration in Chile: friendly amendment to Bannister et al. (2018). Restoration Ecology **28**:277–281. Blackwell Publishing Inc. Available from https://onlinelibrary.wiley.com/doi/abs/10.1111/rec.13113 (accessed April 13, 2020).

Lesica P, Allendorf FW. 1999. Ecological Genetics and the Restoration of Plant Communities: Mix or Match? Restoration Ecology **7**:42–50. Available from http://onlinelibrary.wiley.com/doi/10.1046/j.1526-100X.1999.07105.x/abstract.

Liepe KJ, Hamann A, Smets P, Fitzpatrick CR, Aitken SN. 2016. Adaptation of lodgepole pine and interior spruce to climate: Implications for reforestation in a warming world. Evolutionary Applications **9**:409–419. Wiley-Blackwell. Available from http://tinyurl.com/ClimateWNA. (accessed June 30, 2021).

Lusk CH, Le-Quesne C. 2000. Branch whorls of juvenile Araucaria araucana (Molina) Koch: are they formed annually? Los verticilos de juveniles de Araucaria araucana (Molina) Koch: ¿son formados anualmente? Page Revista Chilena de Historia Natural.

Macel M et al. 2007. Climate vs. Soil Factors in Local Adaptation of Two Common Plant Species. Ecology **88**:424–433. Available from http://www.jstor.org/stable/27651115.

Mantovani A. 1999. A method to improve leaf succulence quantification. Brazilian Archives of Biology and Technology **42**:9–14. Instituto de Tecnologia do Parana.

Martín MA, Mattioni C, Lusini I, Molina JR, Cherubini M, Drake F, Herrera MA, Villani F, Martín LM. 2014. New insights into the genetic structure of Araucaria araucana forests based on molecular and historic evidences. Tree Genetics & Genomes **10**:839–851. Springer Berlin Heidelberg. Available from http://link.springer.com/10.1007/s11295-014-0725-1 (accessed April 12, 2019).

McKay JK, Christian CE, Harrison S, Rice KJ. 2005. “How Local Is Local?”-A Review of Practical and Conceptual Issues in the Genetics of Restoration. Restoration Ecology **13**:432–440.

Muñoz AA, Barichivich J, Christie DA, Dorigo W, Sauchyn D, González-Reyes Á, Villalba R, Lara A, Riquelme N, González ME. 2014. Patterns and drivers of *Araucaria araucana* forest growth along a biophysical gradient in the northern Patagonian Andes: Linking tree rings with satellite observations of soil moisture. Austral Ecology **39**:158–169. Available from http://doi.wiley.com/10.1111/aec.12054 (accessed November 19, 2019).

O’Hara RB, Kotze DJ. 2010. Do not log-transform count data. Methods in Ecology and Evolution **1**:118–122. Wiley. Available from http://doi.wiley.com/10.1111/j.2041-210X.2010.00021.x (accessed March 5, 2021).

Oksanen J et al. 2019. Vegan: Community Ecology Package.

Pebesma EJ, Bivand RS. 2005. Classes and methods for spatial data in R.

Premoli A. 2015. Araucaria araucana , Monkey Puzzle.

Puchi PF, Camarero JJ, Battipaglia G, Carrer M. 2021. Retrospective analysis of wood anatomical traits and tree-ring isotopes suggests site-specific mechanisms triggering Araucaria araucana drought-induced dieback. Global Change Biology:1–15.

Rafii ZA, Dodd RS. 1998. Genetic diversity among coastal and Andean natural populations of Araucaria araucana (Molina) K. Koch. Biochemical Systematics and Ecology **26**:441–451.

Reich PB, Walters MB, Ellsworth DS, Wang Y-P, Oleksyn J. 1997. From tropics to tundra:

global convergence in plant functioning. Proceedings of the National Academy of Sciences of the United States of America **94**:13730–4. National Academy of Sciences. Available from http://www.ncbi.nlm.nih.gov/pubmed/9391094 (accessed April 15, 2019).

RStudio Team. 2020. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA. Available from http://www.rstudio.com/.

Ruiz E, González F, Torres-Díaz C, Fuentes G, Mardones M, Stuessy T, Samuel R, Becerra J, Silva M. 2007. Genetic diversity and differentiation within and among Chilean populations of *Araucaria araucana (* Araucariaceae) based on allozyme variability. TAXON **56**:1221–1228. Available from http://doi.wiley.com/10.2307/25065913 (accessed April 12, 2019).

Sanguinetti J, Kitzberger T. 2008. Patterns and mechanisms of masting in the large-seeded southern hemisphere conifer Araucaria araucana. Austral Ecology **33**:78–87. John Wiley & Sons, Ltd (10.1111). Available from http://doi.wiley.com/10.1111/j.1442-9993.2007.01792.x (accessed April 9, 2019).

Savolainen O, Pyhäjärvi T, Knürr T. 2007. Gene Flow and Local Adaptation in Trees. Annual Review of Ecology, Evolution, and Systematics **38**:595–619. Annual Reviews. Available from http://www.annualreviews.org/doi/10.1146/annurev.ecolsys.38.091206.095646 (accessed April 1, 2020).

Siefert A et al. 2015. A global meta-analysis of the relative extent of intraspecific trait variation in plant communities. Ecology Letters **18**:1406–1419. John Wiley & Sons, Ltd (10.1111). Available from http://doi.wiley.com/10.1111/ele.12508 (accessed March 13, 2019).

Siefert A, Fridley JD, Ritchie ME. 2014. Community Functional Responses to Soil and Climate at Multiple Spatial Scales: When Does Intraspecific Variation Matter? Available from www.daymet.org (accessed March 2, 2020).

Sorensen FC, Weber JC. 1994. Genetic Variation and Seed Transfer Guidelines for Ponderosa Pine in the Ochoco and Malheur National Forests of Central Oregon.

Supple MA, Bragg JG, Broadhurst LM, Nicotra AB, Byrne M, Andrew RL, Widdup A, Aitken NC, Borevitz JO. 2018. Landscape genomic prediction for restoration of a Eucalyptus foundation species under climate change. eLife **7**. Available from https://elifesciences.org/articles/31835 (accessed April 16, 2019).

Tella JL, Lambertucci SA, Speziale KL, Hiraldo F. 2016. Large-scale impacts of multiple co-occurring invaders on monkey puzzle forest regeneration, native seed predators and their ecological interactions. Global Ecology and Conservation **6**:1–15. Elsevier. Available from https://www.sciencedirect.com/science/article/pii/S2351989415300275 (accessed April 12, 2019).

Varas-Myrik A, Sepúlveda-Espinoza F, Fajardo A, Alarcón D, Toro-Núñez Ó, Castro-Nallar E, Hasbún R. 2021. Predicting climate change-related genetic offset for the endangered southern South American conifer Araucaria araucana. Manuscript submitted for publication.

Webb T. 1986. Is vegetation in equilibrium with climate? How to interpret late-Quaternary pollen data. Vegetatio **67**:75–91.

Wickham H. 2016. ggplot2: Elegant Graphics for Data Analysis. Available from https://ggplot2.tidyverse.org.

Willhite E. 2019. Observations and Recommendations Regarding Araucaria araucana Branch and Observations and Recommendations Regarding Araucaria araucana Branch and Foliage Mortality (Daño Foliar de la Araucaria) in the National Parks of South-Central Chile.

Witkowski ETF, Lamont BB. 1991. Leaf specific mass confounds leaf density and thickness. Oecologia **88**:486–493. Springer-Verlag. Available from http://link.springer.com/10.1007/BF00317710 (accessed November 16, 2019).

Woodward FI. 1987. Climate and Plant Distribution. University of Cambridge Press, New York.

Woodward FI, Lomas MR, Kelly CK. 2004. Global climate and the distribution of plant biomes. Pages 1465–1476 Philosophical Transactions of the Royal Society B: Biological Sciences. Royal Society. Available from https://royalsocietypublishing.org/doi/abs/10.1098/rstb.2004.1525 (accessed June 22, 2021).

Wright IJ et al. 2004. The worldwide leaf economics spectrum. Available from www.nature.com/nature (accessed April 15, 2019).

Wright IJ et al. 2005. Assessing the generality of global leaf trait relationships. New Phytologist **166**:485–496. John Wiley & Sons, Ltd. Available from http://doi.wiley.com/10.1111/j.1469-8137.2005.01349.x (accessed July 6, 2020).

Zuur AF, Ieno EN, Elphick CS. 2010. A protocol for data exploration to avoid common statistical problems. Methods in Ecology and Evolution **1**:3–14.

Tables & Figures

Table 1. Collection sites covering the range of pewen in Chile vary in altitude and climate variables.

Population	Region[1]	Code[2]	Families[3]	Latitude	Longitude	Alt.[4]	MAP[5][6]	MAT[7]	TAR[8]
PN[9] Nahuelbuta	Coastal	NAH	20	-37.805609	-73.017985	1269	1604	6.1	19.9
Villas Araucarias	Coastal	ARA	20	-38.495328	-73.254247	664	1501	8.9	19.4
RN[10] Ralco	Andes	RAL	20	-37.939491	-71.334323	1239	1696	8.8	25.4
RN Las Nalcas	Andes	NAL	20	-38.269358	-71.489768	976	2219	9.6	24.7
RN Malalcahuello	Andes	MAL	20	-38.425844	-71.56517	1382	1765	7.7	24.3
Lonquimay (*)	Andes	LON	7	-38.426427	-71.421637	1376	1632	8.1	24.7
PN Conguillio	Andes	CON	19	-38.647372	-71.698783	1236	1860	7.9	23.6
PN Huequehue	Andes	HUE	20	-39.172097	-71.707628	1378	1464	6.7	23.0
Cruzaco	Andes	CRU	20	-38.800124	-71.235559	1424	1138	7.9	24.7
Icalma	Andes	ICA	20	-38.819732	-71.332654	1195	1355	8.7	24.4
Marimenuco (*)	Andes	MAR	7	-38.762456	-71.184824	1401	1110	8.2	24.7
PN Villarica	Andes	VIL	20	-39.569	-71.514951	1187	1145	7.5	23.0

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[1] Region refers to the mountain range from which the populations were selected (Andes vs. Coastal range, see Figure 1).

[2] Codes refers to populations throughout the manuscript.

[3] Families refer to sample size for each population.

[4] Alt. = altitude (m)

[5] Climate variable means were calculated by accessing WorldClim data for each family´s latitude and longitude coordinates (tree from which seeds were sampled) and calculating means at the population level.

[6] MAP = mean annual precipitation (mm)

[7] MAT = mean annual temperature (°C)

[8] TAR = temperature annual range (maximum temperature – minimum temperature; °C)

[9] PN = Parque Nacional

[10] RN = Reserva Nacional

Table 2. Traits measured in common garden seedlings.

Trait	Description	Significance and Citations
*Aboveground biomass**	Dry mass of aboveground tissue (g)	Measure of growth, resource allocation; indicative of growth versus stress tolerant strategies (Grime 1977)
*Belowground biomass**	Dry mass of belowground tissue (g)	Measure of growth, resource allocation; indicative of growth versus stress tolerant strategies (Grime 1977)
Number of whorls	Number of whorls of branches	Measure of plant architecture, resource allocation; indicative of growth versus stress tolerant strategies (Lusk & Le-Quesne 2000)
Number of branches	Number of branches	Measure of plant architecture, resource allocation; indicative of growth versus stress tolerant strategies (Lusk & Le-Quesne 2000)
*Stem length**	Length above/belowground tissue separation to top of apical bud (cm)	Measure of growth, resource allocation; indicative of growth versus stress tolerant strategies (Grime 1977)
*Basal diameter**	Diameter at above/belowground tissue separation (mm)	Measure of plant growth, biomass allocation; indicative of growth versus stress tolerant strategies (Lusk & Le-Quesne 2000)
Mean branch length	Average length of branches (cm)	Measure of plant growth, biomass allocation; indicative of growth versus stress tolerant strategies (Grime 1977)
Maximum needle length	Average length of longest 3 needles (cm)	Component of leaf size; indicative of leaf economic strategy (Wright et al. 2004)
Maximum needle width	Average width of longest 3 needles (mm)	Component of leaf size; indicative of leaf economic strategy (Wright et al. 2004)
Needle area	Needle area measured using Leaf Byte app (cm^2)	Component of leaf size; indicative of leaf economic strategy (Wright et al. 2004)
Needle mass per area	Needle dry weight / leaf area (mg/cm^2)	Leaf economics spectrum trait; indicative of leaf economic strategy (Wright et al. 2004)
Needle succulence	(Needle fresh weight - dry weight) / needle area (mg/cm^2)	Measure of leaf anatomy relating to water storage capacity (Mantovani 1999)
Needle thickness	Needle fresh volume / needle area (mm)	Component of leaf mass per area; indicative of leaf economic strategy (Wright et al. 200(Witkowski & Lamont 1991)4)
Proportion germination	Proportion of seeds that germinated	Measure of seed viability (Donohue et al. 2010)
Proportion survival (1 yr)	Proportion of seeds that survived to 1 year	Measure of survival under greenhouse conditions

Table 3. Bioclimatic and soil variables used for multiple regressions with trait PC scores (units in parentheses) extracted for the latitude and longitude coordinates of each tree from which seeds were sampled.

Variable	Description	Source
MAT	Mean annual temperature (˚C)	WorldClim
MDR	Mean Diurnal Range (Mean of monthly (max temp - min temp), °C)	WorldClim
ISO	Isothermality (BIO2/BIO7) (×100)	WorldClim
SEA	Temperature Seasonality (SD ×100, °C)	WorldClim
MTWM	Max Temperature of Warmest Month (°C)	WorldClim
MTCM	Min Temperature of Coldest Month (°C)	WorldClim
TAR	Temperature Annual Range (TAR; °C)	WorldClim
MTWQ	Mean Temp. of Wettest Quarter (°C)	WorldClim
MTDQ	Mean Temp. of Driest Quarter(°C)	WorldClim
MTWaQ	Mean Temp. of Warmest Quarter (°C)	WorldClim
MTCQ	Mean Temp. of Coldest Quarter (°C)	WorldClim
MAT	Annual Precipitation (mm)	WorldClim
PTM	Precipitation of Wettest Month (mm)	WorldClim
PDM	Precipitation of Driest Month (mm)	WorldClim
PSEA	Precipitation Seasonality (CV, mm)	WorldClim
PWQ	Precipitation of Wettest Quarter (mm)	WorldClim
PDQ	Precipitation of Driest Quarter (mm)	WorldClim
PWaQ	Precipitation of Warmest Quarter (mm)	WorldClim
PCQ	Precipitation of Coldest Quarter (mm)	WorldClim
AET	Actual evapotranspiration, derived using a one-dimensional soil water balance model (mm)	TerraClimate
DEF	Climate water deficit, derived using a one-dimensional soil water balance model (mm)	TerraClimate
PDSI	Palmer Drought Severity Index	TerraClimate
PET	Reference evapotranspiration (ASCE Penman-Montieth; mm)	TerraClimate
PR	Precipitation accumulation (mm)	TerraClimate
RO	Runoff, derived using a one-dimensional soil water balance model (mm)	TerraClimate
SoilM	Soil moisture, derived using a one-dimensional soil water balance model (mm)	TerraClimate
SRAD	Downward surface shortwave radiation (W/m^2)	TerraClimate
SWE	Snow water equivalent, derived using a one-dimensional soil water balance model (mm)	TerraClimate
TMMN	Minimum temperature (°C)	TerraClimate
TMMX	Maximum temperature (°C)	TerraClimate
VAP	Vapor pressure (kPa)	TerraClimate
VPD	Vapor pressure deficit (kPa)	TerraClimate
WS	Wind-speed at 10m (m/s)	TerraClimate
BDOD	Bulk density of the fine earth fraction (kg/dm^3)	SoilGrids
CEC	Cation Exchange Capacity (cmol(c)/kg)	SoilGrids
CFVO	Volumetric fraction of course fragments (%)	SoilGrids

Clay	Proportion of clay particles (<0.002 mm) in the fine earth fraction (%)	SoilGrids
Nitrogen	Total Nitrogen (g/kg)	SoilGrids
PHH20	Soil pH	SoilGrids
Sand	Proportion of sand particles (>0.5 mm) in the fine earth fraction (%)	SoilGrids
Silt	Proportion of silt particles (≥ 0.002, ≤ 0.05 mm) in the fine earth fraction (%)	SoilGrids
SOC	Soil organic carbon content in the fine earth fraction (g/kg)	SoilGrids
OCD	Organic carbon density (kg/m^3)	SoilGrids
OCS	Organic carbon stocks (kg/m^3)	SoilGrids

Table 4. Multiple regression models for traits that explain variation[15] in the first four PC axes.

	Coefficients	**Estimate**	**SE**[11]	**t**	**p**[12]	**Adj. R^2**[13]	**F (df)**	**p**[14]
PC1 (79.3%)	Intercept	0.8	2.8	0.3	0.8	0.83	318.7 (3, 194)	<0.001
	Number of whorls	-16.7	0.7	023.7	<0.001			
	Needle area	2.5	0.8	3.0	0.003			
	Needle succulence	1.4	0.7	2.2	0.03			
PC2 (9.6%)	Intercept	-4.7	1.5	-3.1	<0.01	0.04	8.6 (1, 202)	<0.01
	Needle width	0.7	0.2	2.9	<0.001			
PC3 (4.9%)	Intercept	22.3	0.6	38.4	<0.001	0.89	512.9 (3,192)	<0.001
	Needle succulence	-3.1	0.1	-24.7	<0.001			
	Needle width	-1.3	8.0x10^{-2}	-16.0	<0.001			
	Branch length	-0.5	2.0x10^{-2}	-23.6	<0.001			
PC4 (1.8%)	Intercept	-1.0	0.5	-1.9	0.07	0.72	172.6 (3, 193)	<0.001
	Needle length	-4.5	0.3	-17.4	<0.001			
	Needle width	0.7	7.5x10^{-2}	9.0	<0.001			

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[11] SE = standard error

[12] P-value for parameters

[13] Adjusted R^2

[14] P-value for model

[15] Additional traits correlated with these traits that were not included in the model are shown in Supporting Information. Preliminary full models before elimination of variables using backwards selection are shown in Supplemental Table 3.

Table 5. Multiple regression models for environmental variables that explain variation[16] in the first four PC axes.

	Coefficients	Estimate	SE	t	p[17]	Adj. R^2	F (df)	p[18]
PC1 (79.3%)	Intercept	-22.9	16.8	-1.4	0.17	0.11	13.4 (2, 207)	<0.001
	TAR[19]	0.17	$4.8x10^{-2}$	3.4	<0.001			
	SOC[20]	$-1.2x10^{-2}$	$5.9x10^{-3}$	-2.0	0.05			
PC2 (9.6%)	Intercept	-14.5	3.7	-4.0	<0.001	0.065	15.8 (1, 211)	<0.001
	TAR	$6. 2x10^{-2}$	$1.6x10^{-2}$	4.0	<0.001			
PC3 (4.9%)	Intercept	7.0	1.2	5.6	<0.001	0.13	32.51 (1, 211)	<0.001
	VPD[21]	-14.0	2.5	-5.7	<0.001			
PC4 (1.8%)	Intercept	2.4	1.1	2.4	0.02	0.023	5.9 (1, 208)	0.016
	CEC[22]	$-8.2x10^{-3}$	$3.3x10^{-3}$	-2.4	0.02			

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[16] Additional variables that are highly correlated with each axis but are collinear with variables included in the model are shown in Figure 2C. Preliminary full models before elimination of variables using backwards selection are shown in Supplemental Supporting Information.

[17] P-value for parameters

[18] P-value for models

[19] TAR = temperature annual range (°C)

[20] SOC = soil organic carbon (g/kg)

[21] VPD = vapor pressure deficit (kPa)

[22] CEC = cation exchange capacity (cmol(c)/kg)

Figure 1. Map of seed collection sites in Chile. The range of pewen is shown in brown. Study populations are shown by dots, with colors corresponding to populations as used in subsequent figures. The common garden site (Yumbel, Chile) is labeled with a triangle.

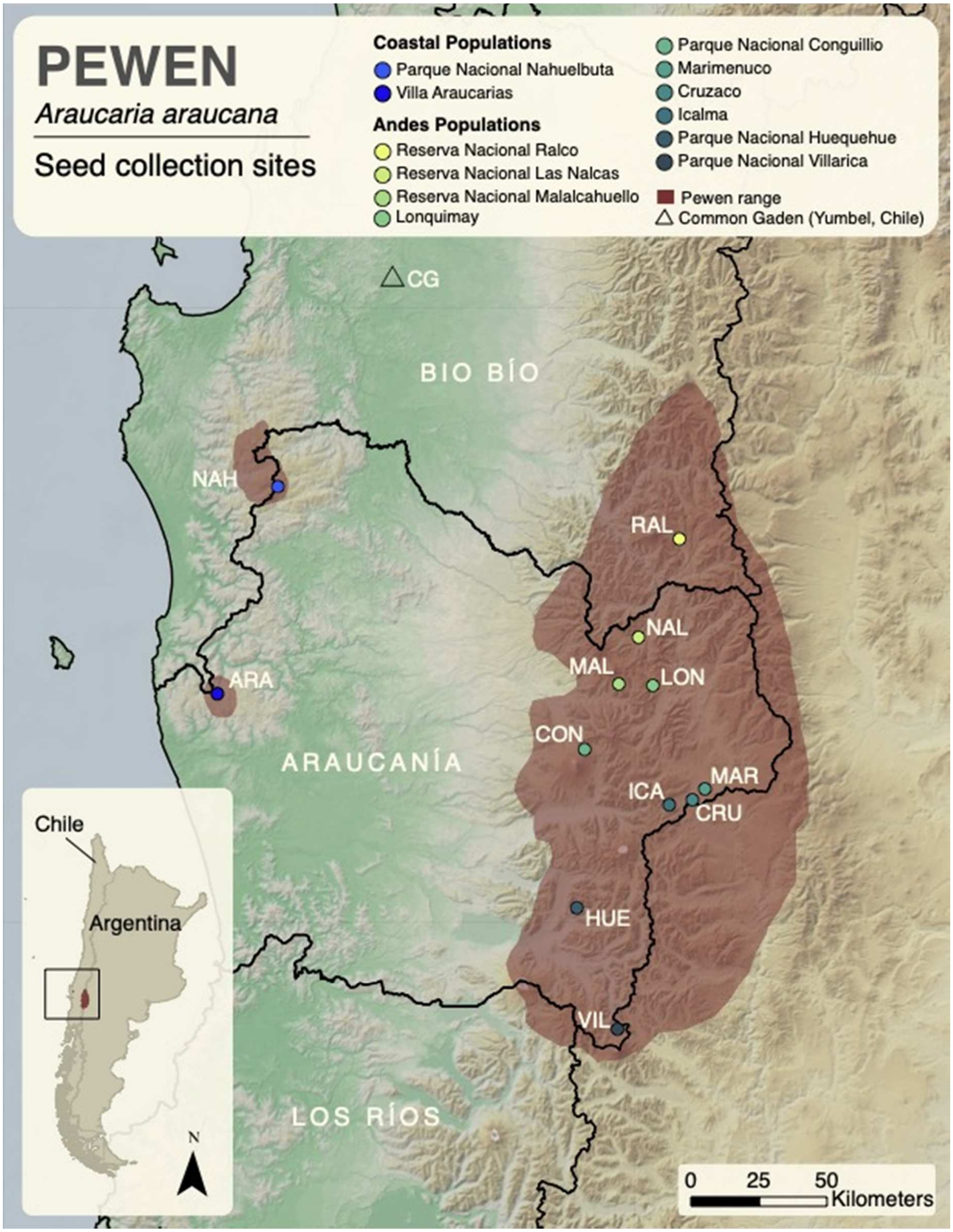


Figure 2. A) A suite of traits varies significantly among regions and populations across the range of pewen in Chile. Adjusted R^2 values (numbers) and p-values (symbols, see legend) for ANOVA models of plant traits by populations. Only traits for which $p<0.05$ are included (see Table 2 for excluded traits). See Table 2 for trait units. **B)** Traits of pewen vary by region and population. Mean PCA scores for PCA axes 1 and 2 (percent variation explained in parentheses) for each population (3-letter codes, see Table 1, Figure 1). Bars show standard errors. **C)** Branch and needle traits vary among and within regions and populations across the range of pewen in Chile. Bars show standard errors. Color categories correspond to region (green = Andes, blue = coast), with gradients by latitude from north (light) to south (dark). Population codes are printed (see Table 1 for more information on populations).

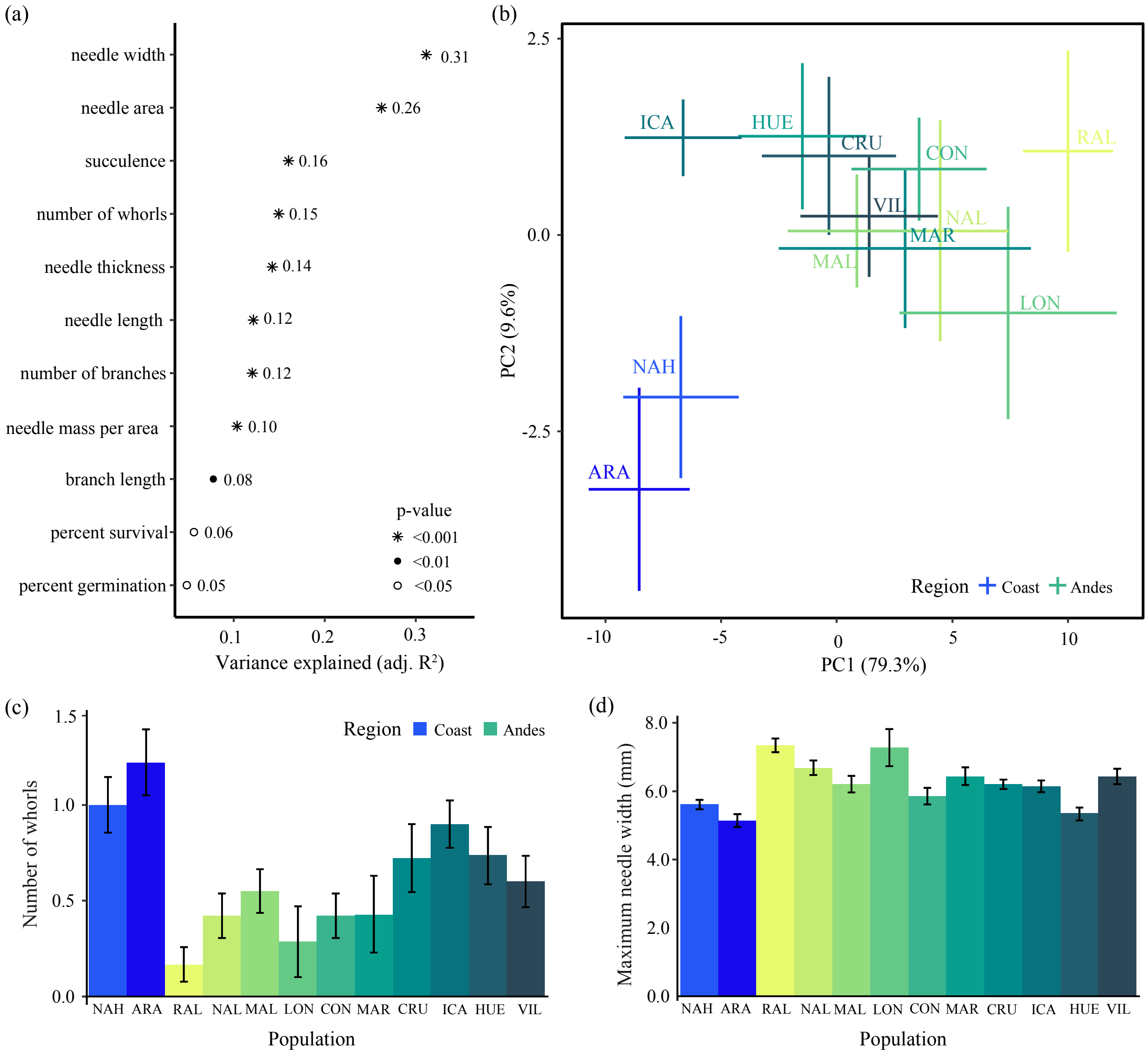
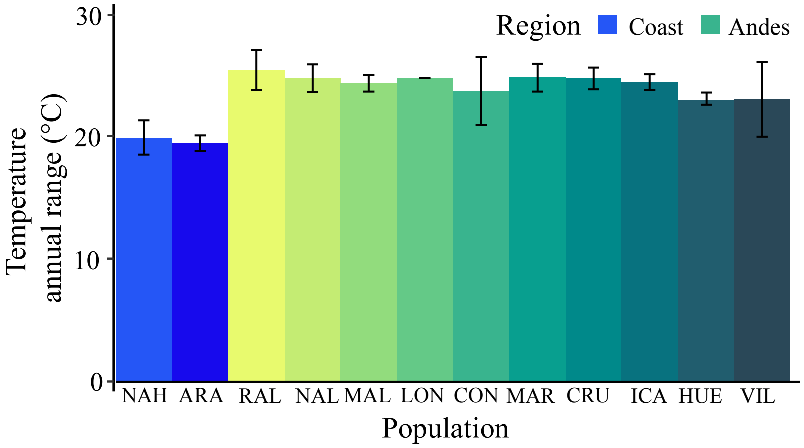


Figure 3. Temperature annual range (TAR, °C) varies significantly among regions and populations. Bars show standard errors. Color categories correspond to region (green = Andes, blue = coast), with gradients by latitude from north (light) to south (dark). Population codes are printed (see Table 1 for more information on populations).



CHAPTER II

Seed and Germinant Traits Predict Drought Survival in Two Widely Distributed Forbs Across Precipitation Gradient

Abstract

Given rapidly changing climatic conditions, there is increasing focus on understanding how species will respond to disturbances increasing in frequency such as drought. Specifically, information on genetic variation among and within populations informs potential for adaptation to increasing drought while information on phenotypic plasticity in response to drought informs potential to buffer environmental changes. For widely distributed species, provenances that vary in water availability may respond differently to drought, which varies spatially and temporally throughout the range of a species. Understanding within-species variation in drought response is particularly important at the germination and seedling stages where plants are exceptionally vulnerable to drought mortality. To date, there is only limited understanding of the extent to which widely distributed species vary across their ranges in key early plant traits, if these traits affect drought survival, and how local adaptation and phenotypic plasticity, alone or in concert, contribute to the ability of widely distributed species to respond to drought. We assessed if provenances of two species across a steep precipitation gradient in Patagonia, Chile (500-2500mm precipitation per year) differed in seed mass, germination rate and timing, biomass allocation, and drought survival in response to drought. We showed provenance differences for some, but not all, early life phenotypes and found patterns for both species that matched expectations for drought avoidance in dry provenances. However, contrary to expectations for local adaptation to water availability, dry provenances did not survive drought longer, meaning they were not necessarily better equipped to face drought. We found that early plant traits were important in predicting both drought survival and growth and that some traits had the opposite relationship with drought survival as with growth. Our results highlight the importance of multiple mechanisms—population differentiation, phenotypic plasticity, and within-population variation—in shaping drought response in plants. We emphasize the importance of conserving within- and among-provenance variation through conservation and restoration actions.

Introduction

As climate changes and drought becomes more frequent (Dai 2013), understanding how plant species and their populations will respond is critical for predicting adaptation to future climate and informing ecosystem management and restoration (Jump and Penuelas 2005; Gitlin *et al.* 2006; Isbell *et al.* 2011). Specifically, information on genetic variation among and within populations indicates potential for adaptation to increasing drought while information on phenotypic plasticity in response to drought informs potential to buffer rapid environmental changes through acclimation. For plants, identifying this information at the germinant and seedling level is particularly important, given that germination and establishment traits are the earliest phenotypes expressed and are particularly susceptible to climate- and drought-related mortality (Moles and Westoby 2004b; Leck *et al.* 2008) and that many restoration and revegetation efforts utilize seeds. However, for germinants and early seedlings, there is only limited information on the extent to which species vary genetically and plastically in key early plant traits and if these traits affect drought survival (Volaire 2018). Additionally, there is particular interest in how widely distributed species that already span significant climate gradients face drought, which occurs variably in time and space across their ranges (Sexton *et al.* 2017; Fajardo and Siefert 2019). Given that these species have a wide climatic gradient, provenances from the wet versus dry extremes of their range may exhibit differential genetic and plastic responses to drought due to local adaptation to water availability at their climate of origin (Volaire 2018). Here, we address if provenances from the wet and dry extremes of two Chilean forbs distributed across a large precipitation gradient differ in seed and germination traits and drought survival, if patterns of variation match expectations for local adaptation or phenotypic plasticity, and if these traits predict drought survival and growth.

To understand adaptation to drought in plants, it is important to assess early life stages. This is because germinants and seedlings are particularly vulnerable to drought (Moles and Westoby 2004b; Leck *et al.* 2008) and, therefore, selection pressures are particularly strong. Furthermore, these pressures may differ from those exerted on adults, as plant life stage may determine the resources required and the microenvironments experienced by plants (Cavender-Bares and Bazzaz 2000; Dayrell *et al.* 2018). Despite the importance of these early life stages, studies often do not link seed and germination traits to seedling fitness in the context of environmental stress (Moles and Westoby 2004a; Larson and Funk 2016; Saatkamp *et al.* 2019). Assessing these relationships is particularly important given that response to drought at the germinant stage and subsequent seedling mortality may determine both later drought response and shape population and community composition (Keddy 1992; Larson *et al.* 2015). Further, characterizing patterns and mechanisms of early plant drought response can inform restoration and revegetation efforts where seedling establishment is limiting due to drought (Kildisheva *et al.* 2018; Koutouan-Kontchoi *et al.* 2020).

Plant germination and establishment fundamentally requires water for cells to grow and develop (Larcher 2003). Therefore, early plant phenotypes, like seed mass, germination timing, and biomass allocation, at these life stages are expected to vary across precipitation gradients as a result of differential water availability. Seed mass, one of the most frequently measured early plant traits, varies across and within species with respect to precipitation and may have adaptive implications for plant germination and performance under drought. Large seeded species tend to be found in drier places (Jurado and Westoby 1992), and their seedlings tend to survive establishment better than those of smaller seeded species (Lloret *et al.* 1999; Moles and Westoby 2004a), suggesting large seed size may be advantageous under drought (Hallett *et al.* 2011). Within species, strong trends in populations across environmental gradients are well documented (Jurado and Westoby 1992, Völler et al. 2012), but meta-analysis shows that the magnitude and direction of trends are not consistent (Cochrane et al. 2015). Of the seven studies assessing seed mass in populations across water availability (aridity) gradients identified by Cochrane et al. (2015), low water availability was associated with lower seed mass for three studies, but higher seed mass for three other studies, and one study found no relationship. These results suggest variable strategies may exist within species relating to the role of seed mass in mediating drought response across their ranges.

If and when a plant germinates has enormous consequences for its likelihood of survival particularly in the context of seasonal water availability (Donohue *et al.* 2010). Germination rate and timing are both highly sensitive to environmental cues and often differ among populations (Baskin and Baskin 2000; Donohue *et al.* 2010). Plants germinate when water availability is sufficient for establishment (Bradford 1990), thus later germination and lower overall germination rates are common under drought (Donohue *et al.* 2010; Duncan *et al.* 2019). In contrast, earlier germination can increase seedling fitness because seedlings are larger when drought occurs (Verdú and Traveset 2005; Donohue *et al.* 2010; Warwell and Shaw 2019), but early-season mortality events (due to frost or drought) may favor late-germinating genotypes and maintain variation in germination strategies within populations (Donohue *et al.* 2010). Given these differential strategies, it is not surprising that Cochrane et al. (2015) identified inconsistent relationships among germination rate and timing and environmental gradients. Of the four studies identified that measured germination timing in populations across water availability (aridity) gradients, half had a positive relationship with water availability and half had a negative relationship. For percent germination, low water availability was associated with low germination in six studies, higher germination in one study, and one study found no relationship (JA Cochrane *et al.* 2015). Like seed mass, these results suggest that species may use different strategies with respect to germination in mediating drought response across precipitation gradients.

Finally, how seedlings allocate resources during establishment may result in differential fitness outcomes under drought. Root:shoot ratio, a measure of biomass allocation to roots relative to shoots, can elucidate strategies plants use for obtaining and using resources (Lloret *et al.* 1999; Poorter *et al.* 2012). High root:shoot ratio can increase capacity for water uptake, and plants may allocate greater biomass to roots when water is limiting growth (Larcher 2003; Poorter *et al.* 2012). Because deep roots may allow a seedling to access additional water resources and establish under lower water availability (Lloret *et al.* 1999; Padilla and Pugnaire 2007), increased root investment is extremely common under drought (Eziz *et al.* 2017). Further, when water availability is seasonally variable (e.g., dry provenances), plants may have greater plasticity in biomass allocation in response to changing water availability (Heschel *et al.* 2015; Carvajal *et al.* 2017). In contrast, in wet environments where water is not limiting, higher investment in shoots (low root:shoot ratio) may be advantageous to improve ability to compete for light and produce the carbon which is key for early growth (Kozlowski *et al.* 1991). Thus, we expect biomass allocation may play a role in mediating drought response across a precipitation gradient both through population differences and plasticity.

Beyond patterns of single trait variation across environmental gradients, it is critical to understand the extent to which these early plant phenotypes affect drought survival, how suites of traits come together to form strategies related to drought response (Volaire 2018), and the extent to which drought response strategies may tradeoff with growth-related strategies (Balachowski and Volaire 2018; Agrawal 2020). Although limited research connects key early plant traits to survival during drought, most studies do not make this connection (this gap is idenitifed in Moles and Westoby 2004a, Larson and Funk 2016, Saatkamp et al. 2019). Further, plants vary among and within populations in the strategies they use to respond to drought, and theory and some empirical evidence suggest traits that improve drought survival can be a detriment to growth under well-resourced conditions depending on the ecological scale (Grime 1977; Smith and Huston 1990; Orians and Solbrig 2015; Agrawal 2020). However, questions remain on when and how traits that contribute to drought survival might come at the expense of growth and vice versa (Grime 1977; Wiley and Helliker 2012; Pérez-Ramos *et al.* 2013; Lopez-Iglesias *et al.* 2014; Bongers *et al.* 2017). Assessing how seed and germination traits affect drought survival and growth depending on climate of origin (provenances, evolutionary history) and environment (drought treatment) may improve our understanding on tradeoffs associated with drought survival.

In addition to characterizing patterns of trait variation and relationships with drought survival and growth, studies of within-species variation can be leveraged to assess the mechanisms that generate this variation, which have implications for understanding the way species adapt to their environments and how to best conserve them. In response to variable conditions across their ranges, plants are often locally adapted, meaning they perform better in home versus away environments (Leimu and Fischer 2008; Anderson *et al.* 2011). Additionally, plants may respond plastically to changing environments by altering their traits within the lifespan of an individual (Bradshaw 1965; Nicotra *et al.* 2010; Lortie and Aarssen 2015). If populations are locally adapted to drought, populations from the dry extreme of the species range are expected to perform better under drought conditions compared to those from the wet extreme (Knight *et al.* 2006; Warwell and Shaw 2019; Blumenthal *et al.* 2020). Likewise, populations that experience greater variability in water availability (dry extremes) may also exhibit greater plasticity in drought response (Bradburd *et al.* 2013; Gianoli 2015; Carvajal *et al.* 2017). Beyond these strategies, plants may also harbor significant within-provenance variation in drought response strategies to "hedge their bets" when environment varies temporally (Bell 2010; Childs *et al.* 2010; Moran 2015). Local adaptation, phenotypic plasticity, and within-provenance variation can work independently or in concert to form drought response strategies across space and time (Ramírez-Valiente *et al.* 2010; Alberto *et al.* 2013; Franks *et al.* 2014; Cavender-Bares and Ramírez-Valiente 2017), but teasing apart which mechanisms underlie trait variation will have strong implications for conservation and restoration strategies (Alberto *et al.* 2013; Franks *et al.* 2014). For example, if local adaptation drives drought response, conserving ecotypic variation and ensuring appropriate selection of plant materials for restoration is critical (Lesica and Allendorf 1999; Joshi *et al.* 2001; Breed *et al.* 2013). In contrast, if phenotypic plasticity or within-provenance variation are high, maintaining within-provenance variation to allow for evolutionary response to changing climate and sourcing seeds more broadly may be higher priority (Liepe *et al.* 2016; Supple *et al.* 2018).

Our study is one of few that addresses provenance differences in early plant phenotypes across a precipitation gradient (see studies identified by Cochrane et al. 2015a) and one of the first to assess relationships between these early plant traits and both drought survival and growth (but see Hallett et al. 2011). For two Chilean forbs distributed across a large precipitation gradient, we conducted drought experiments in growth chambers to assess if wet and dry provenances differed in seed and germination traits and drought survival, if patterns of variation matched expectations for local adaptation or phenotypic plasticity, and if these traits predicted drought survival. Further, if within-species variation matches expectations for local adaptation and phenotypic plasticity in response to drought remains unknown. Specifically, our study asks:

Q1) Do provenances vary in seed, germination, growth, and biomass allocation traits? Do provenances differ in drought survival?
Q2) Do patterns of trait variation match expectations for local adaptation and / or phenotypic plasticity?
Q3) Which traits are associated with drought survival and do these traits have the same relationship with growth as they do with drought survival?

Our results provide important information on drought adaptation across environmental gradients for two forb species, helping to identify additional areas where research is needed to inform ecological theory and management of widely distributed species considering changing climate.

Methods

EXPERIMENTAL APPROACH

To address our questions, we selected two widely distributed forb species, *Acaena ovalifolia* and *Anemone multifida*, along a significant precipitation gradient in Southern Chile (600-2500 mm mean annual precipitation (MAP); Fig. 1, Table 1). For each of these two species, we collected seeds four sites each from provenances at the wet and dry extremes (wet and dry provenances) of each species range. We germinated and grew these seeds in growth chambers under control and drought treatments, and measured germination traits (timing and rate of germination), growth (biomass), and biomass allocation (root:shoot ratio). Then, we stopped watering seedlings and measured drought survival (days until fully browned) under dry-down conditions.

To answer Q1, we addressed differences between provenances in the measured traits in response to drought treatments. For Q2, we considered patterns of variation to match expectations for local adaptation to drought when measured traits showed *provenance differences in the direction predicted by adaptation to drought*. We predicted that dry provenances should show slower growth (lower biomass) and greater investment in roots over shoots (higher root:shoot ratios). We did not have predictions for seed and germination traits, as the literature showed variable patterns for these traits. We expected that dry provenances would have higher drought survival as a result of adaptation to drought. We considered patterns of variation to match expectations for plasticity in response to drought when measured traits showed *treatment differences* independently of predicted responses (e.g., plants increase or decrease root:shoot ratio in response to drought treatments). \Finally, we addressed which traits or trait values were associated with drought survival and growth (Q3). We expected low biomass and high root:shoot ratio would be associated with drought survival, while high biomass and low root:shoot ratio would be associated with growth. Again, we did not have expectations for seed and germination traits, as the literature showed variable patterns for these traits. We additionally expected that plants from both provenances would acclimate to drought, having higher drought survival when grown under drought conditions.

STUDY SYSTEM AND SEED COLLECTION

We selected the forb species *Acaena ovalifolia* (Rosaceae) and *Anemone multifida* (Ranunculaceae; non-native but naturalized) because they are widely distributed across steep precipitation gradients in southern Chile. Although the dry extremes of these species are similar (~599-734 mm MAP), *A. ovalifolia* can occupy wetter sites than *A. multifida* (~1396-2660 mm MAP vs. ~967-1198 mm MAP; Table 1). For each species, we selected four sites at each the wet and the dry extreme of each species range. Sites within each range extreme were generally >50 km apart km each other; some sites were ~10 km apart (Coyhaique Alto I and II, Villa Ortega I and II). From each site, seeds were collected in March 2019 from 10-14 healthy individuals without heavy herbivory that were fully sun exposed and were separated by >5 m along roadsides where these species typically occur. We refer to the four sites from each extreme together as wet or dry provenances from here on.

SEED TRAITS

After seed collection, seeds were cleaned, air-dried to remove humidity from the field, and cold treated at 4°C for approximately three months. We then sent seeds to the University of Montana (Missoula, Montana, USA) and stored seeds at room temperature for approximately 2 weeks while they were weighed before planting. To assess seed mass differences among provenances, we counted and weighed 50 seeds of each individual to calculate the average weight (mg) per seed (total weight / 50). We refer to seeds from the same individual as "families" from here on, as we germinated multiple seeds from the same mother (family) in a single pot in our germination experiments.

GERMINATION EXPERIMENTS AND TRAIT MEASUREMENTS

We used growth chambers at the University of Montana to implement drought treatments during germination. We planted 10 seeds of each family into one of two 4 cm pots (one pot per individual for each of two treatments) using standard unfertilized potting mix. One pot of each family was placed into one of two Percival Scientific PGC-40L2X chambers under 16-hour days (16:8 photoperiod) at 900 μmol/m^2, 50% relative humidity, and 20°C nighttime and 22°C daytime temperatures. One chamber received watering to field capacity every day (control) to maintain constant moisture and the other chamber received watering to field capacity every other day, allowing them to dry out between watering (drought treatment). Moisture differences were clearly visible between treatments. We recorded the date of germination for the first germinant in each pot. After germination stopped (approximately 3 months), we counted the number of seedlings in each pot to calculate germination rate per family.

If there was only one seedling per pot (family), it was transplanted into a larger pot 10x10 cm pot with the same potting mix for additional experiments and no biomass data was collected at the germination stage for that individual. If there were more than one seedling per pot (family), an average-sized seedling was transplanted, and the remaining seedlings were harvested for biomass. Harvested plants were washed, aboveground and belowground tissue was separated, dried at 70°C for 3 days, and weighed. For germinants from each family sampled (each pot), we calculated growth (average aboveground and belowground biomass per seedling: total biomass per pot / # seedlings) and biomass allocation (root:shoot ratio: average belowground biomass / average aboveground biomass). Thus, we calculated average germination timing and rate, growth (biomass), and biomass allocation (root:shoot ratio) *at the family level* (i.e., averaged within pots). As some families had no germinants in either drought or control treatments or both, sample sizes per site were sometimes lower than the original 10 (see Table 2 for sample sizes across experiments).

SEEDLING EXPERIMENTS AND TRAIT MEASUREMENTS

We implemented additional drought and dry-down experiments on singular individuals. For seedling drought experiments, we returned transplanted seedlings (one seedling per family per pot) to growth chambers using the same growth chamber conditions as above. For *A. multifida,* we began drought treatments immediately. For *A. ovalifolia,* which were quite small when transplanted them, we allowed plants to grow well-watered for two weeks before implementing drought treatments. We watered control treatments to field capacity every day and drought treatments every three days such that control treatments were constantly moist and drought treatments visibly dried out between waterings. After an additional 6 week of drought treatments, we began a dry-down experiment, ceasing watering completely. To determine drought survival, we monitored plants and recorded the number of days until they were fully brown and crispy (stems easily snapped when broken). We then separated aboveground and belowground biomass, dried the plants at 70°C for 3 days, and then weighed them to measure growth. We additionally calculated total biomass allocation (root:shoot ratio) for each individual (family).

STATISTICAL ANALYSIS

To address provenance and treatment comparisons, we used linear mixed models (West *et al.* 2006) including population, treatment, and their interactions (except for seed mass, which only included provenance as seeds were wild-collected) as fixed factors and site (nested within population) as a random factor. For models of germinant biomass and biomass allocation, we additionally included the number of seedlings in the pot as a random effect to control for differences in competition. For final models, we used backwards selection to remove factors that did not significantly improve the explanatory of our models (p>0.05). Models were implemented in R (RStudio Team 2020) using the lmerTest package (Kuznetsova *et al.* 2017). To address the significance of model parameters (and to obtain p-values), we used Satterthwaite's method, implemented in lmerTest (Kuznetsova *et al.* 2017).

To identify predictors of germinant and seedling growth and drought survival (Q3), we used linear mixed models (implemented as described above). To compare the magnitude of effects across factors, we listed model estimates and standard errors as generated by the glht() command in the multcomp package (Hothorn *et al.* 2008). To address the variation explained by each factor, we calculated marginal (fixed effects only) and conditional (fixed and random effects) R-squared using the r.squaredGLMM() command in the MuMln package (Nakagawa *et al.* 2017; Bartoń 2020).

All analyses were implemented in R Studio (RStudio Team 2020) and figures were made using ggplot2 (Wickham 2016).

Results

WET AND DRY PROVENANCES ARE DIFFERENT IN THEIR TRAITS AND DROUGHT RESPONSES

PROVENANCE DIFFERENCES

In *A. ovalifolia,* we found provenance differences in seed mass, biomass allocation in germinants and seedlings, and growth in seedlings. Wild collected seeds of dry provenances were nearly twice as heavy as seeds of wet provenances (p=0.04, Fig. 2A, Table 3). Germinants and seedlings from dry provenances had higher root:shoot ratios compared to wet provenances (p=0.04 and p=0.003, respectively; Figs. 2B&C, Table 3). Seedlings from dry provenances had about 50% more aboveground and nearly twice as much belowground biomass as did wet provenances (p=0.04 and 0.001, respectively; Fig. 2D&E; Table 3). We did not find provenance effects for germination rate or timing, or germinant total biomass.

In *A. multifida,* we found provenance differences in germinant and seedling growth and seedling biomass allocation. Germinants from dry provenances were about half as large as wet provenances (p<0.001; Fig. 2F, Table 3; trends were similar for aboveground and belowground biomass). Seedlings from dry provenances were about 25% smaller aboveground (p=0.07; Figs. 2G, Table 3) and had root:shoot ratios that were 15% greater (p<0.001; Fig. 2H, Table 3) when compared to wet provenances. We did not find provenance effects for seed mass, germination timing or rate, germinant biomass allocation, or seedling belowground biomass.

TREATMENT EFFECTS

In *A. ovalifolia,* we found treatment effects for germination timing and rate, germinant growth, and germinant and seedling biomass allocation. Under drought, germination took approximately twice as long (p<0.001; Fig. 2I, Table 4), and plants germinated at about half the rate of controls (p=0.005; Fig. 2J, Table 4). Germinants grown under drought conditions were about 40% smaller than those germinated under control conditions (p=0.03; Fig. 2K, Table 4). Seedlings grown under drought conditions had lower root:shoot ratios compared to those grown in control treatments (p=0.06; Fig. 2C, Table 3). We did not find treatment effects for seedling growth. For

A. multifida, treatment effects were seen for germination timing and seedling aboveground biomass only. Under drought, germination took ~30% longer ($p<0.001$; Fig. 2L, Table 4) and seedlings tended to be about 70% larger aboveground ($p=0.07$; Fig. 2G, Table 3). We did not find treatment effects for germination rate, germinant growth, seedling belowground biomass, or germinant or seedling biomass allocation.

We only found significant provenances x treatment interaction effects for germinant biomass allocation in *A. ovalifolia.* Dry provenances had 25% lower root:shoot ratios in drought treatments relative to control, while wet provenances had 15% higher root:shoot ratios ($p=0.02$; Fig. 2B, Table 3) in the drought treatments than in the well-watered control. We did not find significant provenances x treatment interaction effects for any other response variable for *A. ovalifolia* or for any variable for *A. multifida.*

DROUGHT SURVIVAL

For *A. ovalifolia*, despite significant differences in seed, germination, growth, and biomass allocation traits between provenances and treatments, drought survival did not vary between provenances or treatments ($p>0.05$; Fig. 3A). *A. multifida* also showed significant differences in seed germination, growth, and biomass allocation traits between provenances and treatments, and provenances varied in drought survival. However, contrary to expectations, dry provenances were significantly less drought tolerant than wet provenances ($p<0.001$; Fig. 3B, Table 5).

KEY EARLY PLANT TRAITS TEND TO HAVE OPPOSITE RELATIONSHIPS WITH DROUGHT SURVIVAL AND GROWTH

For *A. ovalifolia*, seed mass, germinant biomass allocation, and seedling growth were significant (but weak) predictors of drought survival. For each mg increase in seed mass, wet provenances (but not dry provenances) fully browned ~two days sooner (although this coefficient was only marginally different from zero, $p=0.08$; $p=0.03$ for provenances x seed mass interaction; model marginal $R^2=0.07$; Fig. 4A, Table 6). For both provenances, for each unit increase in germinant (but not seedling) root:shoot ratio, seedlings tolerated drought for nearly two additional days ($p=0.06$ for germinant biomass allocation effect; model marginal $R^2=0.06$; Fig. 4B, Table 6). Finally, for each gram increase in seedling biomass, seedlings fully browned about 0.5 days sooner ($p<0.001$; model marginal $R^2=0.07$; Fig. 4C, Table 6).

For *A. multifida,* seed mass, germination timing, germinant growth, and germinant biomass allocation were significant predictors of drought survival. For each mg increase in seed mass, *A. multifida* tolerated drought for about 1.25 fewer days ($p=0.02$ for seed mass effect; model marginal $R^2=0.06$; Figure 4D, Table 6). For each additional day to germinate, seedlings grown under drought conditions fully browned about 0.1 days sooner, while seedlings grown under control conditions fully browned about 0.1 days later ($p=0.004$ for treatment x germination timing interaction; model marginal $R^2=0.07$; Fig. 4E, Table 6). For each mg increase in germinant biomass, dry provenances grown under drought conditions fully browned about 0.2 days later, while dry provenances grown in well-watered control conditions fully browned 0.2

days sooner (p=0.05 for provenance x treatment x germinant biomass interaction; model R^2=0.08; Fig. 4F, Table 6). Finally, for each unit increase in germinant root:shoot ratio, dry provenances resisted drought for nearly two fewer days, while wet provenances resisted drought for nearly 3.5 additional days (p=0.002 for provenance x germinant biomass allocation interaction; model marginal R^2=0.21; Fig. 4G, Table 6).

For *A. ovalifolia,* seed mass, germinant growth, and germinant and seedling biomass allocation predicted seedling growth. For each mg increase in seed mass, dry provenance seedlings only were about 357 mg smaller (p=0.04 for provenance x seed mass interaction; model marginal R^2=0.16; Fig. 4H, Table 7). For each mg increase in germinant biomass*,* seedlings grown under drought conditions only were about 4 mg larger (Model R^2=0.36; p<0.001 for treatment x germinant biomass interaction; Fig. 4I, Table 7). For each unit increase in germinant root:shoot ratio, were about 800 mg smaller (p=0.02 for germinant biomass allocation effect; model marginal R^2=0.40; Fig. 4J, Table 7). However, for every unit increase in seedling root:shoot ratio, only wet provenance seedlings were smaller by about 3600 mg (p=0.002 for provenance x seedling root:shoot ratio; model marginal R^2=0.20; Fig. 4K, Table 7).

For *A. multifida,* seed mass, germinant growth, and seedling biomass allocation predicted seedling growth. For each mg increase in seed mass, seedling biomass increased by about 130 mg regardless of provenance (p=0.05 for seed mass effect; model marginal R^2=0.05; Fig. 4L, Table 7). For each mg increase in germinant biomass, we found that seedlings grown under control conditions (but not drought conditions) were about 8 mg larger (p=0.03 for treatment x germinant biomass interaction; model marginal R^2=0.16; Fig. 4M, Table 7). Finally, for each mg increase in seedling root:shoot ratio, seedlings were about 270 mg larger (p=0.005 for seedling biomass allocation effect; model R^2=0.08; Fig. 4N, Table 7).

Discussion

As drought becomes more frequent with changing climate, examining the response strategies of widely distributed species to variable climatic conditions across their ranges will advance our understanding of how plants may respond via adaptation and phenotypic plasticity to future climate scenarios. This understanding, in turn, will inform management and restoration of species with broad ranges. Our study addressed if provenances from the wet and dry extremes of two forbs distributed across a large precipitation gradient differed in seed and germination traits and drought survival, and if patterns of differentiation matched expectations for local adaptation and phenotypic plasticity. Additionally, we assessed the relationships between these traits and drought survival versus growth. For *A. ovalifolia*, provenances had similar rates of drought survival, but exhibited different survival strategies: individuals from dry provenances were able to grow larger via higher root:shoot ratios, while wet provenances remained small to survive drought. For *A. multifida*, contrary to expectations, wet provenances survived drought longer, even though dry provenances showed a more resource conservative strategy, investing more in roots and growing slowly, while wet provenances invested more heavily in shoots and grew more quickly. Our results show that, despite differences in resource conservation strategies, dry provenances do not necessarily survival drought longer. Finally, we showed that within-provenance variation in early plant traits predicted drought survival, and that these traits sometimes had opposite relationships with growth. Our study highlights the importance of early plant traits in drought response.

PROVENANCES VARY IN EARLY PLANT TRAITS BUT NOT NECESSARILY AS EXPECTED FOR LOCAL ADAPTATION. DROUGHT SURVIVAL WAS NOT GREATER FOR DRY PROVENANCES

We found evidence of provenance differences for both species, although they varied in which traits differed and if they matched expectations for local adaptation. Contrary to our expectations, dry provenances did not survive drought longer for either species. For *A. ovalifolia*, both provenances had similar drought survival, despite evidence of different drought response strategies. We saw differences among provenances in germinant and seedling biomass and biomass allocation, where dry provenances were larger (contrary to our expectations for local adaptation) but had higher investment in roots compared to shoots (consistent with our expectations for local adaptation). One possible explanation for our results for *A. ovalifolia* is that increased root investment could have allowed dry provenances to grow larger and still survive drought for as long as the smaller wet provenance seedlings did (Lloret *et al.* 1999; Poorter and Markesteijn 2008; Rose *et al.* 2009; Carvajal *et al.* 2017). In other words, if standardized by seedling size, dry provenances would survive drought longer per unit of mass. In the field, where soil does not dry as quickly as it does in pots and where roots can grow freely into deeper layers to access additional water, greater root investment as seen in dry provenances may in fact improve drought survival (Lloret *et al.* 1999; Larcher 2003; Padilla and Pugnaire 2007; Eziz *et al.* 2017) more so than in our growth chamber experiment. We therefore cannot rule out the possibility of differences in drought survival in the field between provenances for this species.

Additionally, wet and dry provenances of *A. ovalifolia* differed in seed mass. We did not have predictions for local adaptation to drought for this trait, as patterns in the literature are inconsistent (A Cochrane *et al.* 2015). Across species, seeds from drier locations often are larger, a pattern which is thought to buffer against desiccation in dry conditions and is associated with higher seedling survival (Jurado and Westoby 1992; Lloret *et al.* 1999). However, although dry provenances did have larger seeds, dry provenances did not survive drought longer, and within dry provenances, larger seeds were not associated with higher drought survival. Thus, our results do not provide evidence that differences in seed size are related to drought survival for *A. ovalifolia*.

A. multifida provenances differed strongly in drought survival; however, in contrast to our predictions for local adaptation, wet provenances had higher drought survival. These results are puzzling given that dry provenances had higher root:shoot ratios and lower growth as seedlings, consistent with a drought avoidance strategy and our expectations for local adaptation to water availability (Grime 1977; Larcher 2003). Our results do not provide a strong explanation for this pattern, but one that is possible is that under our experimental conditions dry provenances may not have the drought survival benefit of root investment when growing in pots.

PROVENANCES OF A. OVALIFOLIA AND A. MULTIFIDA SHOW PLASTICITY IN EARLY PLANT TRAITS IN RESPONSE TO DROUGHT TREATMENTS

We found treatment differences for both species, matching our expectations for phenotypic plasticity, although they again varied in which traits differed and the direction of those differences. *A. ovalifolia* showed later germination under drought consistent with plastic "bet-hedging" strategies, where seedlings wait for favorable condition to germinate (likely mediated by turgor thresholds; Bradford 1990), which is common under drought (Sharma 1973; Venable 2007; Donohue *et al.* 2010; Gremer and Venable 2014). *A. ovalifolia* germinants also had lower biomass under drought, which matches expectations of a conservative, drought avoidant slow growth strategy (Grime 1977; Larcher 2003). However, we additionally found plasticity in biomass allocation by germinants and seedlings of this species, but in the opposite direction we expected under drought. That plants increase root:shoot ratio under drought is a well-established pattern (Eziz *et al.* 2017), but we found the opposite: dry provenance germinants and seedlings *reduced* their root:shoot ratio, while the wet provenances increased their root:shoot ratio (for germinants only). These results are inconsistent with provenance differences in biomass allocation that could suggest a drought avoidance strategy for this species.

Our results for *A. multifida* indicate plasticity in response to drought in germination timing and seedling aboveground (but not belowground) biomass. We saw the same increase in days to germinate in *A. multifida* as in *A. ovalifolia,* and we suggest the same turgor-mediated strategy of waiting to germinate until water availability is sufficient is likely at play here (see discussion above). In response to drought, germinants regardless of provenance increased growth. This result is contrary to expectations for a more conservative growth strategy under drought but could possibly be explained by *A. multifida's* smaller hydric range on the wet extreme of its distribution. If well-watered control treatments were too wet for this species, growth could have been restricted, giving the appearance that drought increased growth.

SOME TRAITS HAVE OPPOSITE RELATIONSHIPS WITH GROWTH AND SURVIVAL

For both species, multiple early plant traits predicted drought survival. Further, some traits had differing relationships with drought survival versus growth (e.g., high root:shoot ratio was generally associated with drought survival, while low root:shoot ratio generally predicted growth), although these trends differed between species and among provenances. For *A. ovalifolia,* within-provenance variation in key traits may help to explain significant within-provenance and within-treatment variation in drought survival, although relationships are relatively weak for this species. For both provenances, plants that either had higher root:shoot ratios as germinants (but not seedlings) or were smaller had overall higher drought survival, as seen in other studies (Lloret *et al.* 1999; Larcher 2003; Rose *et al.* 2009; Taeger *et al.* 2013). Thus, for *A. ovalifolia,* there was a direct tradeoff between biomass and drought survival. As such, the traits that promoted growth were often opposite of those that promoted drought survival. For example, large seedlings had lower root:shoot ratios as germinants, in contrast to drought tolerant, small seedlings which had higher root:shoot ratio. That germinant biomass allocation, but not seedling biomass allocation, predicted drought survival suggests an important role for early germinant traits in predicting later plant performance under drought stress.

For *A. multifida*, some traits again had opposite relationships with drought survival and growth, although we did not observe a direct tradeoff between drought survival and growth (large seedlings were not necessarily less drought tolerant). Having a smaller seed was associated with increased drought survival for both provenances, and having a larger seed was associated with growth. Interestingly, root:shoot ratio predicted both drought survival and growth, but for different ontogenies. As germinants, dry provenances had higher drought survival if they had higher root:shoot ratios (as predicted for drought avoidant strategies; Poorter and Markesteijn 2008) and wet provenances had higher drought survival if they had lower root:shoot ratios. However, as seedlings, individuals of both provenances were larger if they had higher root:shoot ratios. Thus, our results indicate the importance of ontogeny in predicting both drought survival and growth, suggesting that beneficial strategies may depend on ontogeny and environment (Grime 1977; Cavender-Bares and Bazzaz 2000).

Notably, trait relationships with drought survival and growth did not consistently match the expectation that dry provenances would prioritize drought survival and wet provenances would prioritize growth. Although provenances sometimes plastically shifted to more drought-associated traits under drought (e.g., *A. ovalifolia* wet provenance germinants increased root:shoot ratio), much variation in drought survival and growth was found *within* provenances, more so for *A. ovalifolia* than *A. multifida*. One possible explanation for the maintenance of diverging growth and drought survival strategies within provenances is balancing selection. Balancing selection, selection which maintains genetic variation within populations, is often a result of environmental heterogeneity, allowing for the maintenance of plant strategies with variable fitness depending on environmental conditions (Hedrick 2006; Sthultz *et al.* 2009; Delph and Kelly 2014; Troth *et al.* 2018). Although this mechanism is more commonly invoked for short-lived annuals (e.g., Troth et al. 2018), it is also seen in perennial plants (García and Ingvarsson 2007; Wang *et al.* 2019; Carley *et al.* 2021). If multiple strategies for growth versus drought survival, or any other suite of strategies shaped by other stressors such as herbivory or cold tolerance which are likely present but not tested here, are in fact maintained within provenances of *A. ovalifolia* and *A. multifida* in Chile, they are available for selection to act on and may help this species to respond to changing climate.

LIMITATIONS

Our study highlights some challenges in identifying early plant drought strategies using growth chamber experiments which may help to improve future studies with this objective. We suggested that one possible explanation for dry provenances not surviving drought longer could be that plants with drought avoidance strategies mediated by root investment may not be able to reach additional water when growing in pots. If this is a major strategy for seedlings, as our results indicate, additional work growing plants in the field under drought and dry-down conditions is needed to determine if lack of local adaptation in drought survival is a result of experimental conditions. Although growth chamber and greenhouse experiments provide highly controlled conditions for which to address questions like in our study (and in many others; see references within Cochrane et al. 2015a), we encourage future studies to investigate these questions in the field or in large pots.

Another limitation of many common garden studies is maternal effects (Gibson *et al.* 2016), where the condition of an individual's mother contributes to its phenotype (Roach and Wulff 1987). Maternal effects may confound genetic variation in a common garden study because these two factors cannot be differentiated when seeds are wild collected. Progeny of mothers who experienced drought stress may be less well-provisioned, affecting seedling fitness variable depending on provenances (Riginos *et al.* 2019). Although seed mass is a commonly implemented method of controlling for maternal effects (Gibson *et al.* 2016), there is strong evidence that it is not effective (Bischoff and Müller-Schärer 2010). Thus, when using wild collected seeds like in this study, there is no effective method to control for maternal effects (without growing maternal material in a controlled environment for one generation prior to experimentation) and a large number of common garden studies (71%; see Gibson et al. 2016), like ours, share this limitation.

Conclusions

Our study provides novel information for two widely distributed forbs, *A. ovalifolia* and *A. multifida,* on genetic and plastic differentiation in key and understudied early plant phenotypes in response to drought in provenances distinguished by water availability. We contribute to a limited but growing body of literature (see references within Cochrane et al. 2015a) that demonstrates the importance of early plant traits, like seed mass, germination timing, biomass allocation and growth, in drought response. We further show that some early plant traits have opposite relationships with drought survival and growth, suggesting that tradeoffs may occur between stress tolerant and growth-oriented strategies. We also found that resource conservative strategies in dry provenances did not necessarily lead to longer drought survival. Finally, we highlight the importance of multiple mechanisms—population differentiation, phenotypic plasticity, and within-population variation—in shaping drought response in plants within species. As climate changes, maintaining trait variation within species will be critical drought response, as which trait combinations and strategies will be advantageous in future conditions is hard to predict. Therefore, we conclude that conservation and restoration actions that maintain genetic variation within species (among and within provenances) will allow natural selection to sort out which traits and strategies will perform best under future conditions.

Literature Cited

Agrawal AA. **2020**. A scale-dependent framework for trade-offs, syndromes, and specialization in organismal biology. *Ecology* **101**: 1–24.

Alberto FJ, Aitken SN, Alía R, *et al.* 2013. Potential for evolutionary responses to climate change - evidence from tree populations. *Global Change Biology* **19**: 1645–1661.

Anderson JT, Willis JH, Mitchell-Olds T. **2011**. Evolutionary genetics of plant adaptation. *Trends in Genetics* **27**: 258–266.

Balachowski JA, Volaire FA. **2018**. Implications of plant functional traits and drought survival strategies for ecological restoration. *Journal of Applied Ecology* **55**: 631–640.

Bartoń K. **2020**. MuMIn: Multi-Model Inference.

Baskin CC, Baskin JM. **2000**. *Seeds: Ecology, Biogeography, and, Evolution of Dormancy and Germination*. Academic Press.

Bell G. **2010**. Fluctuating selection: The perpetual renewal of adaptation in variable environments. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**: 87–97.

Bischoff A, Müller-Schärer H. **2010**. Testing population differentiation in plant species - how important are environmental maternal effects. *Oikos* **119**: 445–454.

Blumenthal DM, LeCain DR, Porensky LM, *et al.* **2020**. Local adaptation to precipitation in the perennial grass Elymus elymoides: Trade-offs between growth and drought resistance traits. *Evolutionary Applications* **14**: 1–12.

Bongers FJ, Olmo M, Lopez-Iglesias B, Anten NPR, Villar R. **2017**. Drought responses, phenotypic plasticity and survival of Mediterranean species in two different microclimatic sites. *Plant Biology* **19**: 386–395.

Bradburd GS, Ralph PL, Coop GM. **2013**. DISENTANGLING THE EFFECTS OF GEOGRAPHIC AND ECOLOGICAL ISOLATION ON GENETIC DIFFERENTIATION. *Evolution* **67**: 3258–3273.

Bradford KJ. **1990**. A Water Relations Analysis of Seed Germination Rates. *Plant Physiology* **94**: 840–849.

Bradshaw AD. **1965**. Evolutionary Significance of Phenotypic Plasticity in Plants. *Advances in Genetics* **13**: 115–155.

Breed MF, Stead MG, Ottewell KM, Gardner MG, Lowe AJ. **2013**. Which provenance and where? Seed sourcing strategies for revegetation in a changing environment. *Conservation Genetics* **14**: 1–10.

Carley LN, Mojica JP, Wang B, *et al.* **2021**. Ecological factors influence balancing selection on leaf chemical profiles of a wildflower. *Nature Ecology & Evolution 2021 5:8* **5**: 1135–1144.

Carvajal DE, Loayza AP, Rios RS, Gianoli E, Squeo FA. **2017**. Population variation in drought-resistance strategies in a desert shrub along an aridity gradient: Interplay between phenotypic plasticity and ecotypic differentiation. *Perspectives in Plant Ecology, Evolution and Systematics* **29**: 12–19.

Cavender-Bares J, Bazzaz FA. **2000**. Changes in drought response strategies with ontogeny in quercus rubra: Implications for scaling from seedlings to mature trees. *Oecologia* **124**: 8–18.

Cavender-Bares J, Ramírez-Valiente JA. **2017**. Physiological Evidence from Common Garden Experiments for Local Adaptation and Adaptive Plasticity to Climate in American Live Oaks (<Emphasis Type="Italic">Quercus</Emphasis> Section <Emphasis

Type="Italic">Virentes</Emphasis>): Implications for Conserva. *Liepe, K. J., A. Hamann, P. Smets, C. R. Fitzpatrick, and S. N. Aitken. 2016. Adaptation of lodgepole pine and interior spruce to climate: Implications for reforestation in a warming world. Evolutionary Applications 9:409–419.*: 107–135.

Childs DZ, Metcalf CJE, Rees M. **2010**. Evolutionary bet-hedging in the real world: empirical evidence and challenges revealed by plants. *Proceedings of the Royal Society B: Biological Sciences* **277**: 3055–3064.

Cochrane JA, Hoyle GL, Yates CJ, Wood J, Nicotra AB. **2015**. Climate warming delays and decreases seedling emergence in a Mediterranean ecosystem. *Oikos* **124**: 150–160.

Cochrane A, Yates CJ, Hoyle GL, Nicotra AB. **2015**. Will among-population variation in seed traits improve the chance of species persistence under climate change? *Global Ecology and Biogeography* **24**: 12–24.

Dai A. **2013**. Increasing drought under global warming in observations and models. *Nature Climate Change* **3**: 52–58.

Dayrell RLC, Arruda AJ, Pierce S, *et al.* **2018**. Ontogenetic shifts in plant ecological strategies. *Functional Ecology* **32**: 2730–2741.

Delph LF, Kelly JK. **2014**. On the importance of balancing selection in plants. *New Phytologist* **201**: 45–56.

Donohue K, Rubio de Casas R, Burghardt L, Kovach K, Willis CG. **2010**. Germination, Postgermination Adaptation, and Species Ecological Ranges. *Annual Review of Ecology, Evolution, and Systematics* **41**: 293–319.

Duncan C, Schultz NL, Good MK, Lewandrowski W, Cook S. **2019**. The risk-takers and-avoiders: germination sensitivity to water stress in an arid zone with unpredictable rainfall. *AoB PLANTS* **11**.

Eziz A, Yan Z, Tian D, Han W, Tang Z, Fang J. **2017**. Drought effect on plant biomass allocation: A meta-analysis. *Ecology and Evolution* **7**: 11002–11010.

Fajardo A, Siefert A. **2019**. The interplay among intraspecific leaf trait variation, niche breadth and species abundance along light and soil nutrient gradients. *Oikos* **128**: 881–891.

Franks SJ, Weber JJ, Aitken SN. **2014**. Evolutionary and plastic responses to climate change in terrestrial plant populations. *Evolutionary Applications* **7**: 123–139.

García M V, Ingvarsson PK. **2007**. An excess of nonsynonymous polymorphism and extensive haplotype structure at the PtABI1B locus in European aspen (Populus tremula): a case of balancing selection in an obligately outcrossing plant? *Heredity 2007 99:4* **99**: 381–388.

Gianoli E. **2015**. Plasticity of Traits and Correlations in Two Populations of Convolvulus arvensis (Convolvulaceae) Differing in Environmental Heterogeneity. *https://doi.org/10.1086/422050* **165**: 825–832.

Gibson AL, Espeland EK, Wagner V, Nelson CR. **2016**. Can local adaptation research in plants inform selection of native plant materials? An analysis of experimental methodologies. *Evolutionary Applications* **9**: 1219–1228.

Gitlin AR, Sthultz CM, Bowker MA, *et al.* **2006**. Mortality Gradients within and among

Dominant Plant Populations as Barometers of Ecosystem Change During Extreme Drought. *Conservation Biology* **20**: 1477–1486.

Gremer JR, Venable DL. **2014**. Bet hedging in desert winter annual plants: Optimal germination strategies in a variable environment. *Ecology Letters* **17**: 380–387.

Grime JP. **1977**. Evidence for the Existence of Three Primary Strategies in Plants and Its Relevance to Ecological and Evolutionary Theory. *The American Naturalist* **111**: 1169–1194.

Hallett LM, Standish RJ, Hobbs RJ. **2011**. Seed mass and summer drought survival in a Mediterranean-climate ecosystem. *Plant Ecology* **212**: 1479–1489.

Hedrick PW. **2006**. Genetic Polymorphism in Heterogeneous Environments: The Age of Genomics. *http://dx.doi.org/10.1146/annurev.ecolsys.37.091305.110132* **37**: 67–93.

Heschel MS, Sultan SE, Glover S, Sloan D. **2015**. Population Differentiation and Plastic Responses to Drought Stress in the Generalist Annual Polygonum persicaria. *https://doi.org/10.1086/421477* **165**: 817–824.

Hothorn T, Bretz F, Westfall P. **2008**. Simultaneous inference in general parametric models. *Biometrical journal. Biometrische Zeitschrift* **50**: 346–363.

Isbell F, Calcagno V, Hector A, *et al.* **2011**. High plant diversity is needed to maintain ecosystem services. *Nature 2011 477:7363* **477**: 199–202.

Joshi J, Schmid B, Caldeira MC, *et al.* **2001**. Local adaptation enhances performance of common plant species. *Ecology Letters* **4**: 536–544.

Jump AS, Penuelas J. **2005**. Running to stand still: adaptation and the response of plants to rapid climate change. *Ecology Letters* **8**: 1010–1020.

Jurado E, Westoby M. **1992**. Seedling Growth in Relation to Seed Size Among Species of Arid Australia. *The Journal of Ecology* **80**: 407.

Keddy PA. **1992**. Assembly and response rules: two goals for predictive community ecology. *Journal of Vegetation Science* **3**: 157–164.

Kildisheva OA, Erickson TE, Madsen MD, Dixon KW, Merritt DJ, Pritchard EH. **2018**. Seed germination and dormancy traits of forbs and shrubs important for restoration of North American dryland ecosystems.

Knight CA, Vogel H, Kroymann J, Shumate A, Witsenboer H, Mitchell-Olds T. **2006**. Expression profiling and local adaptation of Boechera holboellii populations for water use efficiency across a naturally occurring water stress gradient. *Molecular Ecology* **15**: 1229–1237.

Koutouan-Kontchoi MN, Phartyal SS, Rosbakh S, Kouassi EK, Poschlod P. **2020**. Seed dormancy and dormancy-breaking conditions of 12 West African woody species with high reforestation potential in the forest-savanna ecotone of Côte d'Ivoire. *Seed Science and Technology* **48**: 101–116.

Kozlowski TR, Kramer PJ, Pallardy SG. **1991**. *The Physiological Ecology of Woody Plants*. San Diego, California: Academic Press.

Kuznetsova A, Brockhoff PB, Christensen RHB. **2017**. lmerTest Package: Tests in Linear Mixed Effects Models . *Journal of Statistical Software* **82**: 1–26.

Larcher W. **2003**. *Physiological Plant Ecology*. Berlin: Springer-Verlag Berlin Heidelberg.

Larson JE, Funk JL. **2016**. Regeneration: an overlooked aspect of trait-based plant community assembly models (K Whitney, Ed.). *Journal of Ecology* **104**: 1284–1298.

Larson JE, Sheley RL, Hardegree SP, Doescher PS, James JJ. **2015**. Seed and seedling traits affecting critical life stage transitions and recruitment outcomes in dryland grasses. *Journal of Applied Ecology* **52**: 199–209.

Leck MA, Parker VT, Simpson RL, Simpson RS. **2008**. *Seedling Ecology and Evolution*. Cambridge University Press.

Leimu R, Fischer M. **2008**. A Meta-Analysis of Local Adaptation in Plants (A Buckling, Ed.). *PLoS ONE* **3**: e4010.

Lesica P, Allendorf FW. **1999**. Ecological Genetics and the Restoration of Plant Communities: Mix or Match? *Restoration Ecology* **7**: 42–50.

Liepe KJ, Hamann A, Smets P, Fitzpatrick CR, Aitken SN. **2016**. Adaptation of lodgepole pine and interior spruce to climate: Implications for reforestation in a warming world. *Evolutionary Applications* **9**: 409–419.

Lloret F, Casanovas C, Peñuelas J. **1999**. Seedling survival of Mediterranean shrubland species in relation to root:shoot ratio, seed size and water and nitrogen use. *Functional Ecology* **13**: 210–216.

Lopez-Iglesias B, Villar R, Poorter L. **2014**. Functional traits predict drought performance and distribution of Mediterranean woody species. *Acta Oecologica* **56**: 10–18.

Lortie CJ, Aarssen LW. **2015**. The Specialization Hypothesis for Phenotypic Plasticity in Plants. *https://doi.org/10.1086/297365* **157**: 484–487.

Moles AT, Westoby M. **2004a**. Seedling survival and seed size: a synthesis of the literature. *Journal of Ecology* **92**: 372–383.

Moles AT, Westoby M. **2004b**. What do seedlings die from and what are the implications for evolution of seed size? *Oikos* **106**: 193–199.

Moran NA. **2015**. The Evolutionary Maintenance of Alternative Phenotypes. *https://doi.org/10.1086/285369* **139**: 971–989.

Nakagawa S, Johnson PCD, Schielzeth H. **2017**. The coefficient of determination R2 and intra-class correlation coefficient from generalized linear mixed-effects models revisited and expanded. *Journal of The Royal Society Interface* **14**.

Nicotra AB, Atkin OK, Bonser SP, *et al.* **2010**. Plant phenotypic plasticity in a changing climate. *Trends in Plant Science* **15**: 684–692.

Orians GH, Solbrig OT. **2015**. A Cost-Income Model of Leaves and Roots with Special Reference to Arid and Semiarid Areas. *https://doi.org/10.1086/283199* **111**: 677–690.

Padilla FM, Pugnaire FI. **2007**. Rooting depth and soil moisture control Mediterranean woody seedling survival during drought. *Functional Ecology* **21**: 489–495.

Pérez-Ramos IM, Volaire F, Fattet M, Blanchard A, Roumet C. **2013**. Tradeoffs between functional strategies for resource-use and drought-survival in Mediterranean rangeland species. *Environmental and Experimental Botany* **87**: 126–136.

Poorter L, Markesteijn L. **2008**. Seedling Traits Determine Drought Tolerance of Tropical

Tree Species. *Biotropica* **40**: 321–331.

Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P, Mommer L. **2012**. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytologist* **193**: 30–50.

Ramírez-Valiente JA, Sánchez-Gómez D, Aranda I, Valladares F. **2010**. Phenotypic plasticity and local adaptation in leaf ecophysiological traits of 13 contrasting cork oak populations under different water availabilities. *Tree Physiology* **30**: 618–627.

Riginos C, Monaco TA, Veblen KE, *et al.* **2019**. Potential for post-fire recovery of Greater Sage-grouse habitat. *Ecosphere* **10**.

Roach DA, Wulff RD. **1987**. MATERNAL EFFECTS IN PLANTS. *Ann. Rev. Ecol. Svst* **18**: 209–244.

Rose L, Leuschner C, Köckemann B, Buschmann H. **2009**. Are marginal beech (Fagus sylvatica L.) provenances a source for drought tolerant ecotypes? *European Journal of Forest Research 2009 128:4* **128**: 335–343.

RStudio Team. **2020**. RStudio: Integrated Development for R.

Saatkamp A, Cochrane A, Commander L, *et al.* **2019**. A research agenda for seed□trait functional ecology. *New Phytologist* **221**: 1764–1775.

Sexton JP, Montiel J, Shay JE, Stephens MR, Slatyer RA. **2017**. Evolution of Ecological Niche Breadth. *https://doi.org/10.1146/annurev-ecolsys-110316-023003* **48**: 183–206.

Sharma ML. **1973**. Simulation of Drought and Its Effect on Germination of Five Pasture Species1. *Agronomy Journal* **65**: 982–987.

Smith T, Huston M. **1990**. A theory of the spatial and temporal dynamics of plant communities. *Progress in theoretical vegetation science*: 49–69.

Sthultz CM, Gehring CA, Witham TG. **2009**. Deadly combination of genes and drought: increased mortality of herbivore-resistant trees in a foundation species. *Global Change Biology* **15**: 1949–1961.

Supple MA, Bragg JG, Broadhurst LM, *et al.* **2018**. Landscape genomic prediction for restoration of a Eucalyptus foundation species under climate change. *eLife* **7**.

Taeger S, Zang C, Liesebach M, Schneck V, Menzel A. **2013**. Impact of climate and drought events on the growth of Scots pine (Pinus sylvestris L.) provenances. *Forest Ecology and Management* **307**: 30–42.

Troth A, Puzey JR, Kim RS, Willis JH, Kelly JK. **2018**. Selective trade-offs maintain alleles underpinning complex trait variation in plants. *Science (New York, N.Y.)* **361**: 475–478.

Venable DL. **2007**. BET HEDGING IN A GUILD OF DESERT ANNUALS. *Ecology* **88**: 1086–1090.

Verdú M, Traveset A. **2005**. EARLY EMERGENCE ENHANCES PLANT FITNESS: A PHYLOGENETICALLY CONTROLLED META-ANALYSIS. *Ecology* **86**: 1385–1394.

Volaire F. **2018**. A unified framework of plant adaptive strategies to drought: Crossing scales and disciplines. *Global Change Biology* **24**: 2929–2938.

Wang B, Mojica JP, Perera N, *et al.* **2019**. Ancient polymorphisms contribute to genome-wide variation by long-term balancing selection and divergent sorting in Boechera stricta. *Genome Biology 2019 20:1* **20**: 1–15.

Warwell M V., Shaw RG. **2019**. Phenotypic selection on ponderosa pine seed and seedling traits in the field under three experimentally manipulated drought treatments. *Evolutionary Applications* **12**: 159–174.

West BT, Welch KB, Galecki AT. **2006**. *Linear Mixed Models : A Practical Guide Using Statistical Software*. Chapman and Hall/CRC.

Wickham H. **2016**. *ggplot2: Elegant Graphics for Data Analysis*.

Wiley E, Helliker B. **2012**. A re-evaluation of carbon storage in trees lends greater support for carbon limitation to growth. *New Phytologist* **195**: 285–289.

Tables & Figures

Table 1. Geographic coordinates and climatic information for 13 seed collection sites for *A. ovalifolia* and *A. multifida*, with three dry sites shared by both species. **Species** indicates which species was collected at each site; and **Provenance** indicates whether the site was at the dry or wet extreme of the species range. **MAP** = mean annual precipitation (mm/year). Sites are ordered by MAP (least to greatest).

Site	**Species**	**Provenance**	**Latitude**	**Longitude**	**MAP (mm/year)**
Galera Chico III	*A. ovalifolia*	dry	-45.885419	-71.716042	599
Coyhaique Alto II	both	dry	-45.481669	-71.611146	673
Galera Chico II	both	dry	-45.851697	-71.777212	676
Galera Chico	both	dry	-45.850438	-71.840761	727
Coyhaique Alto	*A. multifida*	dry	-45.4960188	-71.686321	734
Reserva Nacional Coyhaique	*A. multifida*	wet	-45.533	-72.01	967
Villa Ortega II	*A. multifida*	wet	-45.423598	-72.006431	1055
Las Bandurrias II	*A. multifida*	wet	-45.559147	-71.97443	1132
Villa Ortega	*A. multifida*	wet	-45.373729	-71.993121	1198
Exploradores	*A. ovalifolia*	wet	-46.532914	-72.956224	1396
Rio Frio	*A. ovalifolia*	wet	-43.451043	-72.32393	2058
Parque Nacional Queulat	*A. ovalifolia*	wet	-44.4091	-72.572587	2313
Lago de los Palos	*A. ovalifolia*	wet	-45.337	-73.03	2660

Table 2. Sample sizes across experiments. Seeds were collected from 10 to 14 families per site and seed mass was measured for all families. Low germination resulted in lower sample sizes for germinants, although percent germination was calculated for all families. Families with <2 germinants were not transplanted, thus sample sizes for seedlings were further reduced.

			Life Stage		
Species	**Provenances**	**Treatment**	*Seed*	*Germinant*	*Seedling*
A. ovalifolia	Wet	Control	80	31	32
		Drought		22	19
	Dry	Control	80	23	21
		Drought		18	17
A. multifida	Wet	Control	93	35	32
		Drought		30	28
	Dry	Control	80	35	28
		Drought		30	29

Table 3. For each study species, output tables from separate linear mixed models showing significant provenance effects. Data are only shown for traits with significant provenance effects. In models, provenance was included as a fixed effect and site as a random effect. ANOVA table was generated using Satterthwaite's method. SSR=sum of squares. MSE=mean square error RSR=root:shoot ratio. AG=aboveground. BG=belowground. Bio=biomass. Significant p-values ($p<0.05$) are italicized.

Species	**Response variable**	**Coefficient**	**SSR**	**MSE**	**Df**	**F value**	**p value**
A. ovalifolia	Seed mass	Provenance	4.7	4.7	1	6.8	*0.04*
	Germinant RSR	Provenance	0.44	0.44	1	6.9	*0.04*
		Treatment	8.2×10^{-4}	8.2×10^{-4}	1	0.013	0.91
		Provenance x Treatment	0.38	0.38	1	6.0	*0.02*
	Seedling RSR	Provenance	2.5×10^{-1}	2.5×10^{-1}	1	4.8	*0.03*
		Treatment	1.9×10^{-1}	1.9×10^{-1}	1	3.7	*0.06*
	Seedling AG bio (mg)	Provenance	2.6×10^{6}	2.6×10^{6}	1	4.3	*0.04*
	Seedling BG bio (mg)	Provenance	2.8×10^{6}	2.8×10^{6}	1	11.2	*0.001*
A. multifida	Germinant bio (mg)	Provenance	6.1×10^{3}	6.1×10^{3}	1	13.5	*<0.001*
	Seedling AG bio (mg)	Provenance	8.4×10^{4}	8.4×10^{4}	1	5.0	0.07
		Treatment	5.5×10^{4}	5.5×10^{4}	1	3.3	0.07
	Seedling RSR	Provenance	4.7×10^{-1}	4.7×10^{-1}	1	4.8	*0.003*

Table 4. For each study species, output tables from separate linear mixed models showing significant treatment effects. Data are only shown for traits with significant treatment effects. Models for traits with both provenance and treatment effects are include in Table 3. In models, treatment is included as a fixed effect and site as a random effect. ANOVA table was generated using Satterthwaite's method. SSR=sum of squares. MSE=mean square error RSR=root:shoot ratio. AG=aboveground. BG=belowground. Bio=biomass. Significant p-values ($p<0.05$) are italicized.

Species	**Response variable**	**Coefficient**	**SSR**	**MSE**	**Df**	**F value**	**p value**
A. ovalifolia	Days to germinate	Treatment	$9.9\text{x}10^3$	$9.9\text{x}10^3$	1	67.2	*<0.001*
	Percent germination	Treatment	$2.5\text{x}10^3$	$2.5\text{x}10^3$	1	8.3	*0.005*
	Germinant bio (mg)	Treatment	$2.4\ \text{x}10^5$	$2.4\ \text{x}10^5$	1	4.8	*0.03*
A. multifida	Days to germinate	Treatment	$2.6\text{x}10^3$	$2.6\text{x}10^3$	1	22.3	*<0.001*

Table 5. Output table from linear mixed model for drought survival for *A. multifida* only showing significant provenance effects. In model, treatment is included as a fixed effect and site as a random effect. ANOVA table was generated using Satterthwaite's method. SSR=sum of squares. MSE=mean square error RSR=root:shoot ratio. Significant p-values (p<0.05) are italicized.

Species	**Response variable**	**Coefficient**	**SSR**	**MSE**	**Df**	**F value**	**p value**
A. multifida	*Drought survival*	Provenance	78.5	78.5	1	14.5	*<0.001*

Table 6. For each study species, output tables from separate linear mixed models showing significant predictors of drought survival. Predictors include seed mass (mg), days to germinate, germinant biomass (bio; mg), and germinant root:shoot ratio (RSR). In model, traits and provenances and / or treatment (if signficant) are included as fixed effects and site is included as a random effect. ANOVA table was generated using Satterthwaite's method. R^2 marg. = marginal R^2 (fixed effects only). R^2 cond. = conditional R^2 (fixed and random effects). SSR=sum of squares. MSE=mean square error. Significant p-values ($p<0.05$) are italicized.

Predictor: seed mass (mg)

Response Variable	**Species**	**Coefficient**	**SSR**	**MSE**	**Df**	**F value**	**p value**	**R^2 marg.**	**R^2 cond.**
Drought survival (days until fully browned)	*A. ovalifolia*	Provenance	10.0	10.0	1	2.8	0.11	0.07	0.46
		Seed mass (mg)	8.5	8.5	1	2.4	0.13		
		Provenance x seed mass (mg)	18.1	18.1	1	5.1	*0.027*		
	*A. multifida**	Seed mass (mg)	29.5	29.5	1	5.7	*0.019*	0.06	0.26

Predictor: days to germinate

Response variable	**Species**	**Coefficient**	**SSR**	**MSE**	**Df**	**F value**	**p value**	**R^2 marg.**	**R^2 cond.**
Drought survival (days until fully browned)	*A. multifida*	Treatment	37.8	37.8	1	7.3	*0.0081*	0.07	0.18
		Days to germinate	3.2	3.2	1	0.6	0.43		
		Treatment x days to germinate	45.3	45.3	1	8.7	*0.0039*		

Predictor: germinant growth (total biomass (mg))

Response variable	**Species**	**Coefficient**	**SSR**	**MSE**	**Df**	**F value**	**p value**	**R^2 marg.**	**R^2 cond.**
Drought survival (days until fully browned)	*A. multifida*	Treatment	13.5	13.5	1	2.6	0.11	0.08	0.24
		Germinant bio (mg)	32.0	32.0	1	6.1	*0.02*		
		Treatment x germ. bio (mg)	42.6	42.6	1	8.0	0.006		

Predictor: germinant biomass allocation (root:shoot ratio)

Response variable	**Species**	**Coefficient**	**SSR**	**MSE**	**Df**	**F value**	**p value**	**R^2 marg.**	**R^2 cond.**

Drought survival (days until fully browned)	*A. ovalifolia*	Germinant RSR	14.0	14.0	1	3.7	0.06	0.06	0.34
	*A. multifida**	Provenance	15.2	15.2	1	3.0	0.09	0.21	0.21
		Germinant RSR	1.1	1.1	1	2.1×10^{-1}	0.09		
Drought survival (days until fully browned)	*A. ovalifolia*	Seedling biomass	36.9	36.9	1	76.4	*<0.001*	0.10	0.43

Table 7. For each study species, output tables from separate linear mixed models showing significant predictors of growth (seedling total biomass in mg). Predictors include seed mass (mg), germinant biomass (bio; mg), germinant root:shoot ratio (RSR), and seedling root:shoot ratio (RSR). In model, traits and provenances and / or treatment (if signficant) are included as fixed effects and site is included as a random effect. ANOVA table was generated using Satterthwaite's method. R^2 marg. = marginal R^2 (fixed effects only). R^2 cond. = conditional R^2 (fixed and random effects). SSR=sum of squares. MSE=mean square error. Significant p-values ($p<0.05$) are italicized.

Predictor: seed mass (mg)

Response variable	Species	Coefficient	SSR	MSE	Df	F value	p value	R^2 marg.	R^2 cond.
Seedling total biomass (mg)	*A. ovalifolia**	Provenance	$1.7x10^{7}$	$1.7x10^{7}$	1	11.8	*<0.001*	0.16	0.16
		Seed mass	$1.1x10^{5}$	$1.1x10^{5}$	1	3.1	0.08		
		Provenance x seed mass	$6.0x10^{6}$	$6.0x10^{6}$	1	4.2	*0.04*		
	A. multifida	Seed mass	$3.5x10^{5}$	$3.5x10^{5}$	1	4.1	*0.05*	0.05	0.12

Predictor: germinant biomass (mg)

Response variable	Species	Coefficient	SSR	MSE	Df	F value	p value	R^2 marg.	R^2 cond.
Seedling total biomass (mg)	*A. ovalifolia*	Treatment	$6.1x10^{5}$	$6.1x10^{5}$	1	1.1	0.30	0.19	0.36
		Germinant bio (mg)	$5.1x10^{6}$	$5.1x10^{6}$	1	8.9	*0.004*		
		Treatment x germinant bio (mg)	$7.5x10^{6}$	$7.5x10^{6}$	1	13.2	*<0.001*		
	A. multifida	Treatment	$6.3x10^{5}$	$6.3x10^{5}$	1	7.5	*0.008*	0.16	0.17
		Germinant bio (mg)	$9.5x10^{5}$	$9.5x10^{5}$	1	11.3	*0.001*		
		Treatment x germinant bio (mg)	$4.4x10^{5}$	$4.4x10^{5}$	1	5.3	*0.03*		

Predictor: germinant root:shoot ratio

Response variable	Species	Coefficient	SSR	MSE	Df	F value	p value	R^2 marg.	R^2 cond.
Seedling total biomass (mg)	*A. ovalifolia*	Provenance	$5.6x10^{6}$	$5.6x10^{6}$	1	8.9	*0.02*	0.09	0.40
		Germinant RSR	$3.5x10^{6}$	$3.5x10^{6}$	1	5.6	*0.02*		

Predictor: seedling root:shoot ratio

Response variable	**Species**	**Coefficient**	**SSR**	**MSE**	**Df**	**F value**	**p value**	**R^2 marg.**	**R^2 cond.**
Seedling total biomass (mg)	*A. ovalifolia**	Provenance	4.3×10^6	4.3×10^6	1	3.2	0.08	0.20	0.20
		Seedling RSR	1.5×10^6	1.5×10^6	1	1.1	0.30		
		provenance x seedling RSR	1.4×10^7	1.4×10^7	1	10.6	*0.002*		
	A. multifida	Seedling RSR	6.6×10^5	6.6×10^5	1	8.5	*0.005*	0.08	0.14

Figure 1. Map of seed collection sites. For each species (shown by shapes), we collected seeds from four sites at the dry extreme (dry provenances) and four sites at the wet extreme (wet provenances) of each species range (shown by fill colors). Mean annual precipitation (mm/year) is shown by color gradient.

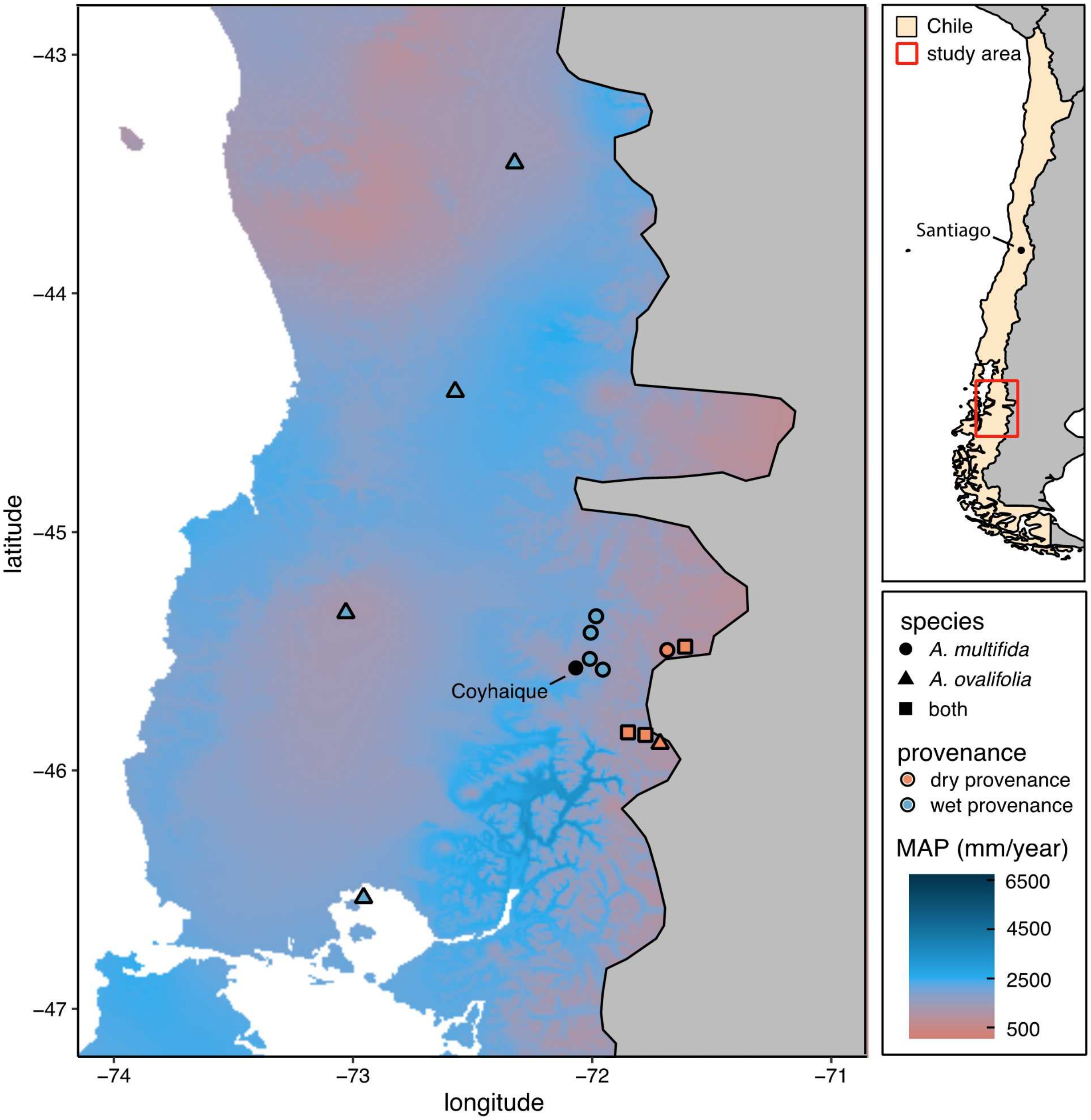


Figure 2. Significant provenance and drought treatment effects for *A. ovalifolia* and *A. multifida* for seed weight (mg; A), germinant root:shoot ratio (RSR; B), seedling root:shoot ratio (RSR; C, H), seedling aboveground biomass (AG bio; mg; C, G), seedling belowground biomass (BG bio; mg; E), germinant biomass (bio; mg; F, K), days to germinate (days; I, L), and percent germination (%; J). Color signifies wet (blue) and dry (orange) provenances. Dark circles around points indicate a significant provenance effect (p<0.05, unless noted). Solid lines connecting provenances across treatments indicate a significant treatment effect (p<0.05, unless noted). A star indicates a significant provenance x treatment interaction effect (p<0.05, unless noted).

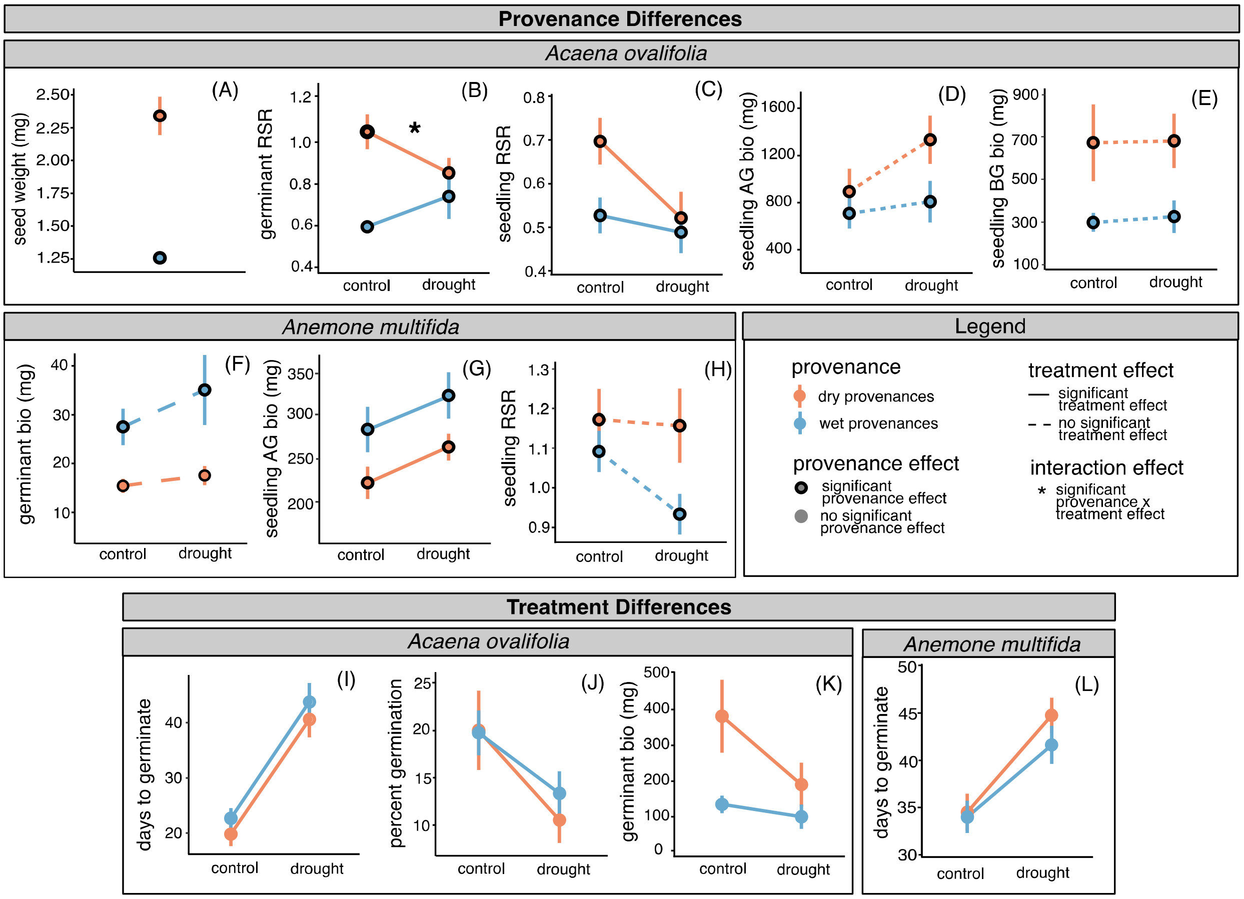


Figure 3. Provenance and drought treatment effects on drought survival (number of days to fully browned) for *A. ovalifolia* and *A. multifida*. Color signifies wet (blue) and dry (orange) provenances. Dark circles around points indicate a significant provenance effect (p<0.05). Solid lines connecting provenances across treatments indicate a significant treatment effect (p<0.05).

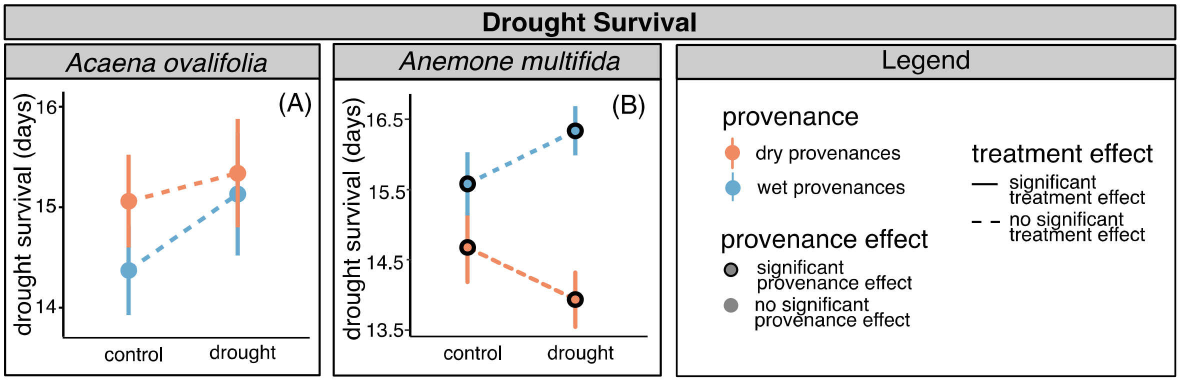
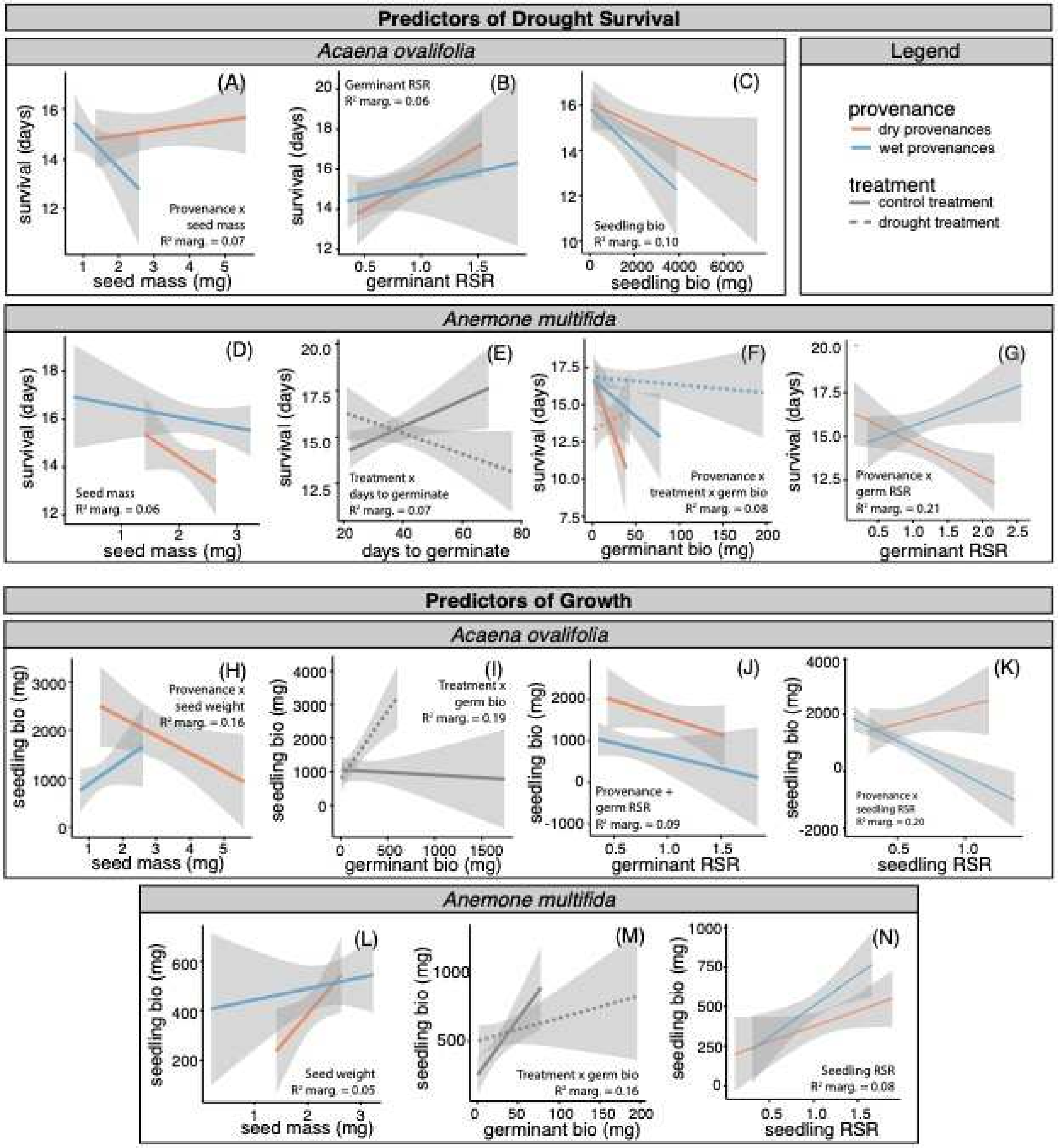


Figure 4. Predictors of drought survival (number of days to fully browned) (A-G) and growth (H-N) in control and drought treatments. Lines show generalized linear models. Predictors include seed mass (mg; A, D, H, L), germinant root:shoot ratio (RSR; B, G, J), seedling biomass (bio; mg; C), days to germinate (days; E), germinant biomass (bio; mg; F, I, M), and seedling root:shoot ratio (RSR; K, N). Color signifies wet (blue) and dry (orange) provenances. Grey shading shows 95% confidence interval for each coefficient. Line type signifies control (solid) and drought (dashed) treatments. Models and their mixed model marginal R^2 (for fixed effects only) are printed. Provenance and treatment effects are shown only when significant.



CHAPTER III

Predicting the Capacity of Plants to Adapt to Increasing Drought: the Ecological and Practical Importance of Within-Species Trait Variation

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Abstract

As climate changes, the capacity of plants to adapt depends on genetic variation among and within populations of different species, the potential variation on which natural selection can act as selective pressures change. Characterizing genetic variation among populations in adaptive traits is, therefore, a key step in identifying the potential for local adaptation to current climate, for mismatch with future climate, and for gene flow of climate-adapted phenotypes to new areas. Climate change, and resultant increasing frequency and intensity of drought, is expected to exert intense and changing selective pressure on plants. However, our ability to make broad inference on a plant's capacity to adapt to drought is limited. One key limitation is that most studies that address plant responses to drought make inference at the species level, without consideration of within-species variation, despite increasing evidence that variation among and within populations is substantial and important. We assessed the relative magnitude of genetic trait variation among and within populations for a suite of key water relations traits related to embolism resistance and maintenance of turgor (P12, P50, P88, and water potential at turgor loss point), along with covarying plant economics traits (specific leaf area, root shoot ratio, and wood density). We also assessed the efficacy of plant functional types (PFTs) and other biological and ecological groupings in capturing this variation. Our results show that both within- and among-population variation, but particularly within-population variation, are substantial compared to across-species variation (9-61% and 3-29% of across-species variation, respectively), with embolism resistance and wood density having the highest variation within species. For all traits assessed except SLA, common biological and ecological groupings (including PFTs) fail to capture genetic trait variation at the population level. Our research suggests that, if strong selection occurs as a result of changes in frequency and intensity of drought, plants may harbor sufficient genetic variation in drought response traits within and among their populations for adaptation to occur. Additionally, we recommend that research, modeling, and conservation efforts avoid the use of PFTs and other groupings to group plants or identify functional diversity with respect to plant drought response, particularly embolism resistance. We found key gaps in knowledge, however: most available information, particularly for water relations traits, is for northern hemisphere temperate tree species. To better inform understanding of drought response, there is a need for additional research addressing genetic variation in key drought response traits within and among populations for a broader suit of species than have been studied previously, and for additional work to identify new groupings that do successfully capture genetic variation in plant drought response at the population level.

Introduction

As climate changes, plants will adapt to new conditions within their home ranges, migrate, or die (Aitken et al. 2008). To predict the future of plants, we must understand their capacity to adapt to changing climate, as rates of migration likely will not keep up with the rate of climate change (Pearson 2006; Aitken & Bemmels 2016). Potential for adaptation to changing climate depends largely on genetic variation across organizational levels—from genes to species—in plant responses to climate (Namkoong 2011; Christmas et al. 2015). Therefore, understanding adaptation to changing climate requires looking below the species level, as variation among and within populations of a given species both drives local adaptation to current and future climates (Savolainen et al. 2007; Kremer et al. 2012) and is the raw material for natural selection to act on in response to changing climate (Christmas et al. 2015; Hamilton 2021). One increasing impact of climate change that has received considerable attention because of its impact on plant growth and survival is drought (Larcher 2003; McDowell et al. 2008; Dai 2013). Because plants vary substantially in their response to drought (Volaire 2018), there has been strong interest in identifying and characterizing plant traits that capture drought response and subsequent mortality risk. However, research on plant response to drought focuses primarily on differences among species without consideration of within-species variation (Choat et al. 2012; Bartlett et al. 2012) despite its magnitude, prevalence, and biological importance (Anderegg 2015; Siefert et al. 2015; Anderegg et al. 2018), limiting broad inference on adaptation to future drought in plants. Thus, for plant traits linked to drought response, synthesis of variation both across and within species is a critical step to understanding the capacity of plants to adapt to increasing drought under climate change.

As plants respond over evolutionary timescales to variation over space and time in their environments, adaptive genetic differences arise among and within populations (Clausen et al. 1940; Leimu & Fischer 2008; Hartmann et al. 2015; Agrawal 2020). Adaptive genetic differences among populations, which arise from local adaptation, improve plant performance under home environmental conditions and are found in over 70% of plants (Joshi et al. 2001; Leimu & Fischer 2008; Alberto et al. 2013). Local adaptation is often related to broad-scale climate variation (Clausen et al. 1940; Joshi et al. 2001; Kawecki & Ebert 2004). However, if climate changes more rapidly than plants can adapt, mismatch may occur between genotypes and their environments, meaning that once locally adapted populations will no longer perform well under new climate scenarios (Aitken & Bemmels 2016). If mismatch does occur, adaptation to new climate conditions may be possible as a result of either gene flow from populations locally adapted to similar historical climate conditions naturally or via assisted migration (see Aitken & Whitlock 2013), or the presence of genetic variation within populations on which natural selection can act (Jump & Penuelas 2005; Aitken & Whitlock 2013; Christmas et al. 2015). Thus, the extent to which populations are differentiated (variation *among populations*) in adaptive response to climate reflects adaptation to previous climate system, and thus potential for mismatch with future climate (Aitken & Bemmels 2016), while variation *within populations* indicates the potential for *in situ* adaptation to future climate (Christmas et al. 2015).

Understanding the relative magnitude of among- and within-population variation in adaptive plant traits is therefore important for predicting the capacity for plants to respond to changing climate.

One area where information on within-species variation is particularly needed is plant drought response. As drought is expected to increase in frequency and intensity due to changing climate (Dai 2013), there has been increasing interest in understanding plant vulnerability to drought and predicting impacts from populations to ecosystems (Maherali et al. 2004; Choat et al. 2012; Allen et al. 2015). Thus, a major interest in plant drought research has been identifying plant traits that provide insight into the physiological and evolutionary mechanisms by which plants face drought (Volaire 2018), linking environment and adaptation through a plant ecophysiological approach (Larcher 2003). To this end, researchers have utilized a variety of plant traits to explore drought vulnerability. Physiological traits directly related to water relations, a plant's ability to maintain favorable water balance that supports key physiological functions, have received the majority of focus in drought research because of their strong mechanistic underpinnings and predictive power (Maherali et al. 2004; Choat et al. 2012; Bartlett et al. 2012; Sapes & Sala 2021). For instance, vulnerability to embolism (P50) and water potential at turgor loss (π_{tlp}) provide insight into a plant's ability to supply water to its leaves and maintain turgor under water stress (Maherali et al. 2004; Bartlett et al. 2012), both of which are critical to survival and growth under drought (Choat et al. 2012; Allen et al. 2015). To take in CO_2 for photosynthesis (and thus essential functions, growth, and survival), plants lose water through their leaves, and thus must maintain a continuous water column to supply water from their roots to their leaves. Under drought, this water column comes under increasing tension (more negative water potential), causing interruptions in the water column and loss of capacity to conduct water if air bubbles enter the xylem, called embolisms (Tyree & Sperry 1989; Tyree & Zimmerman 2013). A plant's ability to endure higher tensions without suffering embolisms is referred to as embolism resistance, measured by the water potential at which 50% of conductivity loss occurs (P50; Maherali et al. 2004). P12 and P88, the tensions at which embolism begin to occur and at which irreversible xylem damage occurs, respectively, provide additional information on a plant's vulnerability to embolism (Urli et al. 2013). As a plant loses conductivity, it eventually closes its stomata to prevent further water loss. Wilting, or loss of cell turgor pressure, can occur when the plant is no longer able to supply water to its leaves and substantial water has been lost (Brodribb et al. 2003). The water potential at which turgor loss occurs, π_{tlp}, therefore indicates the water potential at which desiccation occurs, threatening a plant's ability to carry out photosynthesis and other critical cellular functions (Brodribb et al. 2003; Bartlett et al. 2012; Maréchaux et al. 2020). Thus, these water relations traits address two physiological components of plant water balance, embolism resistance, and capacity to avoid loss of cell turgor.

In addition to physiological traits, plant economics traits are often included in drought studies. They are more easily measured than physiological traits and have been used to provide additional explanatory power for drought vulnerability (Pratt et al. 2007; Markesteijn et al. 2011;

Greenwood et al. 2017). The plant economics spectrum uses key traits to identify strategies of plants along axes of resource availability, categorizing these strategies and the resource trade-offs that underlie them along a fast-slow resource use spectrum (Wright et al. 2004; Reich 2014). Plant economics theory suggests that plant resource use strategies are linked across resources (water, carbon, and nutrients) and plant organs (Reich 2014), thus it is not surprising that plant and leaf economics traits correlate with and improve predictive power of water relations traits. Specifically, specific leaf area (SLA), root:shoot ratio (RSR), and wood density (WD) are all predictive of drought mortality, particularly when considered in addition to water relations traits (Markesteijn et al. 2011; Greenwood et al. 2017). Together, because of their mechanistic links to drought response and resource economics in plants, water relations traits and covarying plant economics traits provide the opportunity to understand plant adaptation to drought, capturing variation in key physiological processes.

Despite the promise of water relations traits for predicting drought response, ability to make inference to adaptation to future climate is limited because research on drought response traits primarily focuses on species differences. We lack synthesis of genetic variation below the species level. Increasing evidence shows that within-species variation in drought response traits is both substantial in magnitude (Alberto et al. 2013; Anderegg et al. 2015; Siefert et al. 2015) and important for predicting drought mortality (Martínez-Vilalta & Piñol 2002; Suarez et al. 2004; Gaspar et al. 2013; Bolte et al. 2016; Garcia-Forner et al. 2016; Sapes & Sala 2021). For example, for P50, a previous meta-analysis showed that variation among populations within species was approximately one-third of the total variation within a genus (Anderegg 2015). However, this review did not differentiate between genetic variation and phenotypic plasticity. Thus, it is unknown if within-species variation arises from local adaptation (if the variation is largely genetically controlled and assuming P50 is adaptive, as shown by its link with drought mortality) or to buffer the effects of changing climate (if the variation in largely a result of phenotypic plasticity)(Anderegg 2015). Beyond this meta-analysis of P50, we are unaware of similar synthesis of either among- or within-population variation for other water relations traits, like P12, P88, or water potential at turgor loss. Furthermore, although there is a large body of research demonstrating rates of *among*-population variation in plant economics traits like SLA, RSR, and WD (Chave et al. 2006; Sánchez-Gómez et al. 2008; Siefert et al. 2014), these assessments do not usually distinguish genetic variation from the effects of environment (plasticity) we do not yet have synthetic information on genetic trait variation *within* populations, which is broadly known to be substantial for plants, particularly trees (Hamrick 2004).

Given that it is not possible to study responses of all species and their populations to drought, there is a tendency to use biological and ecological groupings of species to capture most variation in drought responses (e.g., Choat et al. 2012). Common groupings include life form (trees, shrubs, grasses, and forbs), plant functional types (PFT; Poulter et al. 2011), clade (gymnosperms, angiosperms), and leaf habit (evergreen, deciduous). At the species level, there is evidence that these types of groupings can have predictive power. For instance, leaf habit, clade, and plant functional types are predictive of *species* differences in SLA (Reich et al. 1997; Poorter et al. 2009; Shiklomanov et al. 2020). Similarly, leaf habit, clade, and functional groups differentiate *species* variation in P50 (Maherali et al. 2004). However, these studies use groupings to distinguish species means, ignoring variation *within species* which could wash out species differences and may contribute to the often poor performance of drought mortality models (Trugman et al. 2021). Thus, to test the reliability and utility of biological and ecological groupings in capturing within-species genetic variation, synthesis is needed.

Here, we synthesize genetic trait variation in key drought response traits across organizational levels (within populations to across species) and address the efficacy of biological and ecological groupings (life form, PFT, clade, and leaf habit) in differentiating within-species genetic trait variation. By focusing our analyses on research conducted in common gardens, we were able to distinguish genetic variation that may contribute to adaptation to future climate from environmental variation (plasticity). To our knowledge, this is the first synthesis of a full spectrum of genetic trait variation for these traits. Specifically, we addressed:

Q1: What is the relative magnitude of genetic trait variation within populations, among populations, and across species (as a benchmark to compare variation within and among populations) for P12, P50, P88, π_{tlp}, SLA, RSR, and WD?
Q2: To what extent do mean values of drought response traits differ among biological and ecological groupings (life form, PFT, clade, and leaf habit)?

Methods

DATA COLLECTION

To address the above questions, we identified publications that met all of the following criteria: 1) measured one or more of these traits; 2) included two or more wild populations; and 3) used a common garden experimental design (and thus measured genetic variation within and among populations in traits of interest; Clausen et al. 1940). We excluded studies on crop species.

We searched all databases in Web of Science (WoS) from 1900-2021 using the following keywords: ("common garden" OR "greenhouse" OR "provenance trial") AND ("intraspecific" OR "among-population*") AND (SLA OR "specific leaf area") OR ("WD") OR (P50 OR 50% loss of hydraulic conductivity OR "hydraulic safety margin") OR (P12 OR 12% loss of hydraulic conductivity) OR (P88 OR 88% loss of hydraulic conductivity) OR ("water potential at turgor loss" OR "water potential at turgor loss point" OR "turgor loss point") OR (root shoot ratio OR "root mass fraction" OR "shoot mass fraction" OR "biomass allocation"). This search identified 574 studies, but we eliminated those that: 1) did not meet all three of the criteria above; 2) were not primary research (i.e. we excluded reviews and meta-analyses); or 3) for which required data were not available (n=495 eliminated). In addition to the WoS search, we accessed additional studies (N=8) for P12, P50, and P88 in the Xylem Functional Trait Database (Hammond, unpublished data) that met our criteria for inclusion.

For the 79 articles that met all criteria, we extracted for each selected study trait the population-level mean, sample size, and at least one measure of variance (standard deviation and / or standard error), along with additional biological and ecological categorical information (see Table 1). These data were obtained from either the text, figures, or supplementary information; we contacted authors in cases where the data was not included in the publication or supplementary information. All included articles are in Supplemental Table 1 and all data collected are in Supplemental Table 2. Supplemental Figure 1 shows all species (and their life forms) included in the dataset by trait.

ANALYSIS

To address the geographic regions, ecosystems, and life forms for which there is existing information on genetic variation within species in drought response traits, we summarized for each trait the number of populations, species, and studies by geographic region (continent), ecosystem (Table 1A), and life form (Table 1B).

To quantify the relative magnitude of genetic trait variation within populations, among populations within species (hereafter "within species"), and across species (Q1), for each selected trait we calculated the coefficient of variation (CV, the standard deviation divided by the mean) for each of these organizational classification levels (Figure 1), to standardize the amount of variation *within* a given group (*sensu* Anderegg 2015). To account for differences in precision and sample size across studies, instead of using raw CVs we calculated weighted CVs for within-species and across-species CVs (for simplicity, in results and discussion we use "CVs" to refer to weighted CVs for these organizational levels), using means and SDs weighted by the inverse of standard error (*sensu* Anderegg 2015). To address variation within populations, we calculated weighted CV for each population for the relevant trait. To address variation among populations within species, we calculated species-level weighted CVs for each trait within each study, where each CV represents a species where populations were grown in the same environment (common garden) and traits were measured using the same method. That is, if multiple treatments (e.g., drought, nutrients), measurement methods, or common gardens were used, we calculated weighted CVs separately so that each CV reflected genetic variation in traits only. Finally, to address variation across all species, we calculated a global weighted CV using all species measured for each trait. For within-population CVs, we additionally analyzed CVs by species to identify if patterns were driven by a single or a few species. We removed species with large mean within-population CVs to determine if they affected overall mean within-species CVs. For across-species CVs, because water relations traits included only (or mostly) trees, we additionally constrained other traits to include trees only to see if overall variation was lower than when additional life forms were included and to facilitate better comparison across traits.

After calculating the weighted CVs, we statistically tested if they differed across phylogenic levels (within populations, within species, or among species), using linear mixed models (LMMs). To compare within-population and within-species weighted CVs, we used LMMs with study as a random factor. This method addressed issues of pseudo replication when multiple measurements (populations or species) were included from the same study. For within-population weighted CVs, this was always the case because studies necessarily measured more than two populations per the requirements for being included in our analysis. For within-species weighted CVs, this issue occurred when studies included multiple species or assessed the same species in multiple environments (e.g., the study included treatments or multiple common gardens).

Finally, we addressed if common biological and ecological groupings (life form, plant functional type, clade and leaf habit; Table 1) were effective at distinguishing among populations and species based on to the traits we assessed. To assess if groupings varied in population-level trait means, we used LMMs with population means as the response variable weighted by 1/SE to account for differences among populations in precision and sample size with study as a random effect and groupings (see Table 1) as fixed effects. If more than two groups were present, we used the emmeans() command in the emmeans package with Satterthwaite's degrees of freedom for post-hoc comparisons of 95% confidence intervals (Lenth 2022). To compare the amount of variation explained by each grouping factor and compare this to the amount of variation explained by species, we calculated marginal (fixed effects only) R^2 using the r.squaredGLMM() command in the MuMln package (Nakagawa et al. 2017; Bartoń 2020). We used PFTs as defined by the Community Land Model (CLM5) with the addition of a PFT for forbs, and did not differentiate functional types by ecosystem (as we did not have sufficient replication within additional PFT categories). We removed grouping levels where <3 species were represented. For example, if only two species were represented in a given PFT, it was removed. To assess groupings based on species means, we repeated the above procedures using species means instead of population means. Species means were calculated from population means (as described above for CVs).

Results

STUDIES ADDRESSING WITHIN-SPECIES VARIATION IN DROUGHT RESPONSE TRAITS BIASED TOWARDS TEMPERATE FORESTS AND TREES

We found substantial variation in the number of common garden studies that measured selected traits and the continents, ecosystems, and life forms that were studied (Figure 2). SLA and RSR were studied most frequently (39% and 24% of studies in our dataset, respectively), while P12, P50, P88, π_{tlp}, and WD were studied less frequently (11%, 18%, 11%, 6%, and 14% of studies, respectively; Figure 2A). For all traits, most studies were from North America and Europe (55%-80%) and, for some traits, we found no studies from South America (P12, P50, and P88), Australia (π_{tlp}), or Asia (P12, P88, π_{tlp}, and WD; Figure 2A). Additionally, for all traits except WD, most studies were conducted in temperate forest ecosystems (57%-100%; Figure 2B); WD was more often measured in tropical forest ecosystems. For vulnerability to embolism, all (P12, P88) or nearly all (P50) studies were conducted in temperate ecosystems. Finally, most information was limited to or highly biased towards trees, particularly for water relations traits. For P12, P50, and P88, only trees were studied. For $\pi_{tlp,}$ we found only one study out of five that looked beyond trees at the iconic Hawaiian forb *Argyroxiphium sandwicense*. Studies of non-tree life forms were more common for SLA, RSR, and WD, although across all traits, few studies considered grasses (one study of RSR and three studies of SLA) or shrubs (two studies of SLA; Figure 2B, Supplemental Figure 1).

VARIATION WITHIN POPULATIONS IS GREATER THAN VARIATION AMONG POPULATIONS, AND BOTH MAKE UP A SUBSTANTIAL PROPORTION OF ACROSS-SPECIES VARIATION

The magnitude of within-population variation varied approximately three-fold, with average CVs from 0.07 to 0.21. Although P50, P88, π_{tlp}, and WD had similar average variation within populations (with CVs from 0.07 to 0.09; Figure 3, Table 2), P12, SLA, and RSR had greater average variation within populations (average CVs = 0.19, 0.16, and 0.21, respectively; Table 2). Among-population variation was similar (average CVs between 0.04 and 0.05) for all traits except P12 (average CV = 0.08) and RSR (average CV = 0.12). Across species, water relations traits generally had about one-half to one-third the variation of plant economics traits (CVs for P12, P50, P88, and π_{tlp} were 0.31, 0.26, 0.25, and 0.31, respectively, while CVs for plant economics traits SLA and RSR were 0.77 and 1.39, respectively; Table 2). WD had the lowest variation across species, with a CV of 0.17. Because our dataset included mostly trees for water relations traits, we additionally constrained other traits to include trees only to identify if greater variation was due to greater representation of life forms. For SLA and RSR, the across-species CVs were reduced from 0.77 and 1.39 to 0.75 and 0.82, respectively.

Interestingly, all traits studied showed within-population variation significantly greater (1.5-3 x greater) than among-population variation (Figure 3; Tables 2&3; all $p<0.01$). For all traits, within-population variation made up a substantial but varying proportion of across-species variation (Figure 2, Table 4). For P12, P50, P88, and WD, within-population variation equaled a third or more (35% to 61%) of variation across species. Among-population variation was between 15% and 29% of variation across species (Table 4). For π_{tlp}, SLA, and RSR, within-population and among-population variation were smaller in proportion to across-species variation, approximately 9-21% and 3-11% of across-species variation, respectively (Table 4). To address if individual species drove high within-population variation, we plotted within-population CVs by species for each trait (Supplemental Figure 1) and identified a few outlier species for P12, SLA, and RSR. For P12, removing *Pinus canariensis* reduced the mean within-population CV from 0.19 to 0.16, but removing outliers did not substantially change mean within-population CV for SLA or RSR (Supplemental Table 3).

BIOLOGICAL AND ECOLOGICAL GROUPINGS FAIL TO CAPTURE VARIATION IN WATER RELATIONS TRAITS

When only genetic trait variation was considered for the traits assessed, the only trait for which biological and ecological groupings significantly differentiated population- and species-level means was SLA (Figure 4; see Table 1 for all groupings tested). These groupings did not differentiate population-level means of any other trait ($p>0.05$ for all models) and generally explained a low proportion of overall variation (Table 5, Table 6). Population means for SLA differed significantly by PFT and leaf habit ($p<0.05$) and marginally significantly for clade ($p=0.06$). For PFTs (Figure 4A), the weighted average SLA of broadleaf deciduous trees (BDT;

147.6 ± 8.3 cm^2/g) was about twice that of both needleleaf evergreen trees (NET; 73.4 ± 3.3 cm^2/g) and broadleaf evergreen trees (BET; 68.4 ± 2.6 cm^2/g), which did not significantly differ from each other. For clade (Figure 4B), the weighted average SLA of gymnosperms (74.1 ± 3.2 cm^2/g) was marginally lower than that of angiosperms (86.0 ± 3.6 cm^2/g). Finally, for leaf habit (Figure 4C), the weighted average SLA of evergreen trees (73.6 ± 2.0 cm^2/g) was about half that of deciduous trees (137.3 ± 5.1 cm^2/g). Similar patterns were seen for the groupings described above when species means, rather than population means, were used, where only species means varied significantly ($p<0.05$) by PFT and leaf habit and marginally significantly for clade (Table 6).

Regardless of model significance, biological and ecological groupings explained the most overall variation in population and species trait means for SLA, the least variation for water relations traits, and moderate variation for RSR and WD, and did not explain more variation than species for any of our traits (Table 5, 6). For SLA, PFT explained the most variation in population means of any grouping (marginal R^2=0.39), followed by leaf habit (marginal R^2=0.25), life form (marginal R^2=0.19), and clade (marginal R^2=0.18). The most variation in population means was explained by species (marginal R^2=0.40). For RSR, ecological groupings explained between <1.0% and 12% of variation in population means. PFT performed the best of all groupings tested (marginal R^2=0.12) but explained much less variation than did species (marginal R^2=0.60). For WD, PFT and clade (marginal R^2=0.13 for both) explained similar variance to species in population means (marginal R^2=0.15). For water relations traits, no grouping explained more than 9.0% of variation in population means, with most explaining <5.0% of variation (Table 5).

For species means, we saw similar patterns as when we used population means. For SLA, PFT explained greater variation than did species (marginal R^2=0.45 vs. 0.39), and all additional groupings explained 14-20% of variation (Table 6). For RSR, species explained by far the most variation (marginal R^2=0.75), followed by PFT (marginal R^2=19), and clade (marginal R^2=0.12). Life form and leaf habit explained <0.01% of variation (Table 6). For WD, species explained the most variation (marginal R^2=0.24), and PFT and clade both explained marginal variation (marginal R^2=0.21 and 0.15, respectively), while leaf habit explained <1% of variation (Table 6). PFT explained substantial variation in P88 (marginal R^2=0.23, but p=0.11). For π_{tlp}, we did not have sufficient replication within group levels to assess groupings other than species for populations or for species. Overall, when species means were included in place of population means, groupings had similar or slightly greater explanatory power.

Discussion

As drought increases globally under climate change, information on genetic variation below the species level—within and among populations—is critical to understanding the capacity of plants to adapt to future climate. As a step towards understanding plant capacity to adapt to future drought, our synthesis characterized the relative genetic trait variation within and among populations for a suite of adaptive water relations traits and co-varying plant economics traits.

Additionally, we tested if biological and ecological groupings commonly used in modeling and comparative studies which effectively differentiate *species* in respect to these traits were also effective in differentiating population-level genetic variation. Although there is a tendency to focus on drought responses at the species level, our results show that within-population and among-population variation make up a substantial proportion of across-species genetic variation in the traits we studied, information that is needed to make inference on adaptation to future climate. Further, within-population variation was between 1.5 and 3 times greater than variation among populations, suggesting that populations may harbor substantial potential for adaptation to future climate. Additionally, we show that biological and ecological groupings, including life form, PFT, clade, and leaf habit—which are commonly used in research and modeling and clearly differentiate species in respect to these traits—fail to capture key variation in water relations traits at the population and species level when genetic variation is separated. Our study advances understanding of patterns of variation needed to predict adaptation to changing climate, but also highlights important gaps in information. For example, the pool of available studies that assessed genetic trait variation in drought response traits was heavily biased towards trees, northern hemisphere temperate ecosystems, and traits related to plant economics over water relations.

HIGH WITHIN-POPULATION VARIATION, ESPECIALLY FOR WATER RELATIONS TRAITS, SUGGESTS POTENTIAL CAPACITY TO ADAPT TO CHANGING CLIMATE

This is the first synthesis that addresses the magnitude of genetic variation within and among populations in multiple water relations and drought response traits. Our results clearly show that both within-population and among population variation was a sizeable proportion of across-species variation, which we used as a benchmark for the magnitude of within-species variation. However, for all traits studied, within-population variation was greater than variation among populations, particularly for embolism resistance and WD. For embolism resistance, populations typically vary little, and this trend has been shown in studies assessing genetic variation both using neutral markers and phenotypes (for example, Lamy et al. 2011; Wortemann et al. 2011b; Hajek et al. 2016; Skelton et al. 2019). These results suggest that for embolism resistance, there is often little local adaptation in response to climate. However, species may harbor variation within populations with may be advantageous under future climate.

In contrast, for π_{tlp}, we found greater across-species variation than we did for embolism resistance, but remarkably little variation within or among populations (CVs=0.09 and 0.05, respectively). The pattern of high variation among species and limited variation within species suggest that this trait may be phylogenetically constrained and have little capacity for adaptation to future climate. Although we did not assess biome-level variation here, previous work has showed that this trait varies most by biome, and is strongly related to water availability within biomes (Bartlett et al. 2012). That we found low genetic variation within species could suggest that the results of Bartlett et al. (2012) are a result of species turnover or plasticity (see Bartlett et al. 2014) rather than within-species variation. However, given low sample sizes for this trait (n=5 studies), these results should be interpreted cautiously, and additional research should address if the relationship between π_{tlp} and water availability seen at the species level by Bartlett et al. (2012) holds up within and among species.

For plant economics traits other than WD, across species variation was generally 2-3x higher than that of water relations traits; for RSR, but not SLA, this was partially due to a greater diversity of life forms in our dataset, as compared to diversity of life forms for water relations traits. Even when we controlled for life form, however, we found much more substantial variation across species for these traits (CVs= 0.75 and 0.82 compared to <0.37), suggesting they may not be useful in addressing capacity for adaptation to changing climate, but may still be important in species-level responses (e.g., Greenwood et al. 2017). Contrary to SLA and RSR, WD had the lowest variation across species and the ratio of within-population to across-species variation. High within-population variation, ease of measurement, and strong predictive power for drought mortality across species (Greenwood et al. 2017) makes WD a good candidate (at least in trees) for tracking potential to adapt to future climate. Additional work is needed to assess its correlation with plant water relations within species.

Our results strongly indicate that research, modeling, or conservation approaches that treat species as homogeneous units with respect to the evaluated traits may not sufficiently capture functional variation in drought response. That within- and among-population variation is so substantial for water relations traits warrants careful consideration of sample size (which is often low and constrained by logistics, technology, and time for physiological studies; Pérez-Llorca et al. 2018) for studies aiming to characterize both populations and species with respect to these traits. For modeling efforts that use water relations traits, including within-species variation, where known, may improve predictive power, which is often low (Trugman et al. 2021). Conservation and restoration efforts that wish to incorporate information on drought vulnerability should prioritize genetic variation within and among populations in addition to species or functional diversity in order to maximize potential for adaptation to future climate (Jump & Penuelas 2005; Aitken & Whitlock 2013; Havens et al. 2015). For example, efforts to construct seed transfer zones, maps which identify genetically appropriate seed sources for restoration, generally include morphological traits only (but see Gibson et al. 2019) and focus on identifying among-population variation (e.g., Miller et al. 2011; Gibson et al. 2019), but not within-population variability (but see Höfner et al. 2021). Our results show that these practices could be improved by including physiological traits like embolism resistance when possible and focusing on conserving within-population variation in addition to among-population variation. For example, in lodgepole pine and the interior spruce complex in Canada, a study of neutral genetic variation across a suite of traits found that within-population was much higher than among-population variation and that the high number of seed transfer zones being used for these species were likely excessive (Liepe et al. 2016).

GENETIC VARIATION MAKES UP A SUBSTANTIAL PROPORTION OF WITHIN-SPECIES VARIATION IN EMBOLISM RESISTANCE

Within species, genetic variation made up a substantial proportion of overall (genetic and plastic) variation in P50. Comparable synthesis only existed for P50, so we were unable to compare our results for other traits. For P50, we found about half as much variation within species when only genetic variation was considered compared to when environmental variation was included (average CVs = 0.05 and 0.10, respectively; Anderegg 2015). This comparison provides an approximation for the magnitude of variation controlled by genetics versus plasticity, suggesting that both play an important role for embolism resistance within species. Across studies in which plasticity was studied, little to considerable plasticity was found (Wortemann et al. 2011; Corcuera et al. 2011; Lamy et al. 2014; Blackman et al. 2017), suggesting that responses may be population- or species-specific, but that in some cases, environmentally-mediated plasticity may help mitigate changes in climate as new seedlings acclimate. Acclimation within a tree's lifetime is slow (on the order of decades), as it depends on the turnover of sapwood, thus it is not likely a substantial strategy for facing drought. Acclimation could be quicker for non-tree species where tissue turnover is faster, but we are not aware of any research addressing temporal plasticity in P50 for non-tree species.

BIOLOGICAL AND ECOLOGICAL GROUPINGS FAIL TO CAPTURE WITHIN-SPECIES VARIATION IN WATER RELATIONS TRAITS

Given the limited number of species for which we have information about drought response traits, it would be useful to be able to make predictions about responses based on biological and ecological groupings like life form, PFT, leaf habit, and clade. For embolism resistance, RSR, and WD, none of the biological or ecological groupings we tested were significant in distinguishing trait population or species means when only genetic variation is considered. However, other studies that used groupings to distinguish species means have shown that species do vary by these groupings when both genetic and plastic variation is included (see Maherali et al. 2004; Bartlett et al. 2012; Anderegg 2015). There are multiple possible and potentially complementary explanations for these important results. For population means, because within-population and within-species variation is substantial for these traits, incorporating variation at these lower organizational levels may "wash out" differences seen when only species means are used. That population means for our traits were not significantly different among species (despite species often explaining substantial variation) provides support for this explanation. Second, the general PFTs that we used may not have categorized species into relevant functional types with respect to the traits we assessed, suggesting the need to better define functional type groupings for these traits. Finally, species mean comparisons in the literature did not distinguish genetic variation from environmental effects; thus, environment could be the driver of these differences among groupings rather than genetic trait variation either through phenotypic plasticity or sorting of groupings by environment. Further, for some traits (e.g., π_{tlp}), we do not yet have sufficient studies to address these groupings in addressing population or species variation.

Our results provide strong evidence that the commonly used groupings we tested fail to capture important variation in drought response traits when within-species genetic trait variation is considered. We found that that species-specific information generally provides the best (although limited) explanatory power and should be used over PFTs or other groupings in the likely absence of within-species information. We recommend that the groupings we tested should not be used in research and modeling efforts focusing on plant water relations or drought response, as they fail to capture genetic variation within and across species. Additionally, we caution conservationists and restorationists from using such groupings to identify functional diversity and recommend that they take a species-driven or (if possible) trait-based approach. We recognize that researchers and practitioners alike are constrained in their time and capacity, so methods to group plants with respect to their functions to reduce the information needed to make inference are greatly needed. To this end, we encourage additional research to develop useful and reliable groupings that reduce the need for information for (and within) each species, have substantial explanatory power, capture both environmental and genetic information, and hold when within-species variation is included.

INFORMATION IS LIMITED ACROSS MOST REGIONS, ECOSYSTEMS, AND LIFE FORMS

Our results clearly show that within-species information on genetic variation in the traits assessed is biased towards temperate tree species in North America and Europe. This is particularly true for water relations traits, where much interest has been on temperate trees (e.g., McDowell et al. 2008; Choat et al. 2012; Anderegg 2015). However, additional research that is inclusive of life forms beyond trees and biomes beyond temperate forests is needed, as increasing drought is important in biomes that are not tree-dominated, such as grasslands and shrublands (Doblas-Miranda et al. 2017; Jacobsen & Pratt 2018; Griffin-Nolan et al. 2019). Further, research shows that all forest biomes are equally vulnerable to hydraulic failure (Choat et al. 2012), therefore predicting drought-induced mortality in ecosystems beyond temperate forest trees is widely important. This will require understanding how variation is structured within and across populations and species for these ecosystems, which may differ from patterns seen in temperate systems. For example, in the tropics species differences could be substantially greater than within-species variation (Siefert et al. 2015) as a result of higher species turnover due to species ranges that are on average smaller and higher biodiversity (Stevens 2015). If this is the case, less within-species variation may inhibit adaptation to climate and more species may be lost due to changing climate and disturbances that reduce connectivity like deforestation. Thus, further research beyond temperate forest trees is needed to resolve these questions.

CONCLUSIONS

As climate changes and drought increases, our results show that plant populations may harbor substantial genetic variation for adaptation to future climate if natural selection for drought response is strong. We show that both within- and among-population variation, but particularly within-population variation, is substantial for key water relations and plant economics traits related to drought response. Our results demonstrate that commonly used groupings like PFTs are not effective in distinguishing population- and species-level genetic variation in these traits and thus should not be used for research, modeling, or management efforts where drought response is of interest. Given the importance of understanding potential for adaptation to increasing drought, future research should focus on expanding water relations research within and among populations across the ranges of species from a broader spectrum of biomes and life forms and innovating approaches to integrate within-species variation into drought mortality and vegetation modeling efforts, as well as conservation and restoration initiatives.

Literature Cited

Agrawal AA. 2020. A scale-dependent framework for trade-offs, syndromes, and specialization in organismal biology. Ecology **101**:1–24.

Aitken SN, Bemmels JB. 2016. Time to get moving: assisted gene flow of forest trees. Evolutionary Applications **9**:271–290. John Wiley & Sons, Ltd. Available from https://onlinelibrary.wiley.com/doi/full/10.1111/eva.12293 (accessed December 31, 2021).

Aitken SN, Whitlock MC. 2013, November 25. Assisted gene flow to facilitate local adaptation to climate change. Annual Reviews Inc. Available from https://www.annualreviews.org/doi/abs/10.1146/annurev-ecolsys-110512-135747 (accessed July 1, 2021).

Aitken SN, Yeaman S, Holliday JA, Wang T, Curtis-McLane S. 2008. Adaptation, migration or extirpation: climate change outcomes for tree populations. Evolutionary Applications **1**:95–111. John Wiley & Sons, Ltd. Available from https://onlinelibrary.wiley.com/doi/full/10.1111/j.1752-4571.2007.00013.x (accessed February 4, 2022).

Alberto FJ et al. 2013. Potential for evolutionary responses to climate change - evidence from tree populations. Global Change Biology **19**:1645–1661. John Wiley & Sons, Ltd. Available from http://doi.wiley.com/10.1111/gcb.12181 (accessed April 1, 2020).

Allen CD, Breshears DD, Mcdowell NG, Allen C:, Breshears DD, Mcdowell NG. 2015. On underestimation of global vulnerability to tree mortality and forest die-off from hotter drought in the Anthropocene. Ecosphere **6**:1–55. John Wiley & Sons, Ltd. Available from https://onlinelibrary-wiley-com.weblib.lib.umt.edu:2443/doi/full/10.1890/ES15-00203.1 (accessed December 31, 2021).

Anderegg L, Berner LT, Law BE, L Anderegg LD, Badgley G, Sethi ML, HilleRisLambers J. 2018. Within-species patterns challenge our understanding of the leaf economics spectrum Tree range limits and climate change View project Within-species patterns challenge our understanding of the leaf economics spectrum. Available from https://www.researchgate.net/publication/323968054 (accessed March 31, 2020).

Anderegg WRL. 2015, February. Spatial and temporal variation in plant hydraulic traits and their relevance for climate change impacts on vegetation. Available from https://onlinelibrary.wiley.com/doi/abs/10.1111/nph.12907.

Anderegg WRL et al. 2015. Tree mortality from drought, insects, and their interactions in a changing climate. New Phytologist **208**:674–683. John Wiley & Sons, Ltd. Available from https://nph.onlinelibrary.wiley.com/doi/full/10.1111/nph.13477%4010.1111/%28ISSN%291469-8137.Highlightsof2015 (accessed January 13, 2021).

Bartlett MK, Scoffoni C, Sack L. 2012. The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: a global meta-analysis. Ecology Letters

15:393–405. Available from http://doi.wiley.com/10.1111/j.1461-0248.2012.01751.x (accessed November 5, 2019).

Bartlett MK, Zhang Y, Kreidler N, Sun S, Ardy R, Cao K, Sack L. 2014. Global analysis of plasticity in turgor loss point, a key drought tolerance trait. Ecology Letters **17**:1580–1590. John Wiley & Sons, Ltd (10.1111). Available from http://doi.wiley.com/10.1111/ele.12374 (accessed April 15, 2019).

Bartoń K. 2020. MuMIn: Multi-Model Inference. Available from https://cran.r-project.org/package=MuMIn%0D.

Blackman CJ, Aspinwall MJ, Tissue DT, Rymer PD. 2017. Genetic adaptation and phenotypic plasticity contribute to greater leaf hydraulic tolerance in response to drought in warmer climates. Tree Physiology **37**:583–592. Oxford Academic. Available from https://academic.oup.com/treephys/article/37/5/583/2979206 (accessed February 8, 2022).

Bolte A et al. 2016. Desiccation and mortality dynamics in seedlings of different European beech (Fagus sylvatica L.) populations under extreme drought conditions. Frontiers in Plant Science **7**:751. Frontiers Research Foundation.

Brodribb TJ, Holbrook NM, Edwards EJ, Gutiérrez M V. 2003. Relations between stomatal closure, leaf turgor and xylem vulnerability in eight tropical dry forest trees. Plant, Cell & Environment **26**:443–450. John Wiley & Sons, Ltd. Available from https://onlinelibrary.wiley.com/doi/full/10.1046/j.1365-3040.2003.00975.x (accessed February 4, 2022).

Chave J, Muller-Landau HC, Baker TR, Toma´ T, Easdale TA, Steege H Ter, Campbell A, Webb O. 2006. REGIONAL AND PHYLOGENETIC VARIATION OF WOOD DENSITY ACROSS 2456 NEOTROPICAL TREE SPECIES. Ecological Applications **16**:2356–2367. Available from http://www.mobot.org/MOBOT/Research/APweb/i (accessed January 3, 2022).

Choat B et al. 2012. Global convergence in the vulnerability of forests to drought. Various articles **491**:752–755. Available from https://www.narcis.nl/publication/RecordID/oai:naturalis.nl:453830.

Christmas MJ, Breed MF, Lowe AJ. 2015. Constraints to and conservation implications for climate change adaptation in plants. Conservation Genetics 2015 17:2 **17**:305–320. Springer. Available from https://link.springer.com/article/10.1007/s10592-015-0782-5 (accessed February 4, 2022).

Clausen J, Keck DD, Heisey WM. 1940. Effect of Varied Environments on Western North American Plants. Page Experimental Studies on the Nature of Species. Carnegie Institute, Washington.

Corcuera L, Cochard H, Gil-Pelegrin E, Notivol E. 2011. Phenotypic plasticity in mesic populations of Pinus pinaster improves resistance to xylem embolism (P50) under severe drought. Trees - Structure and Function **25**:1033–1042. Springer. Available from https://link.springer.com/article/10.1007/s00468-011-0578-2 (accessed February 8, 2022).

Dai A. 2013. Increasing drought under global warming in observations and models. Nature

Climate Change **3**:52–58. Available from https://search.proquest.com/docview/1284370567.

Doblas-Miranda E et al. 2017. A review of the combination among global change factors in forests, shrublands and pastures of the Mediterranean Region: Beyond drought effects. Global and Planetary Change **148**:42–54. Elsevier.

Garcia-Forner N, Sala A, Biel C, Save R, Martínez-Vilalta J. 2016. Individual traits as determinants of time to death under extreme drought in Pinus sylvestris L. Tree Physiology **36**:1196–1209. Oxford Academic. Available from https://academic.oup.com/treephys/article/36/10/1196/2514646 (accessed February 4, 2022).

Gaspar MJ, Velasco T, Feito I, Alía R, Majada J. 2013. Genetic Variation of Drought Tolerance in Pinus pinaster at Three Hierarchical Levels: A Comparison of Induced Osmotic Stress and Field Testing. PLOS ONE **8**:e79094. Public Library of Science. Available from https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0079094 (accessed December 31, 2021).

Gibson A, Nelson CR, Rinehart S, Archer V, Eramian A. 2019. Importance of considering soils in seed transfer zone development: evidence from a study of the native *Bromus marginatus*. Ecological Applications **29**:e01835. Available from https://onlinelibrary.wiley.com/doi/abs/10.1002/eap.1835 (accessed April 18, 2019).

Greenwood S et al. 2017. Tree mortality across biomes is promoted by drought intensity, lower wood density and higher specific leaf area. Ecology Letters **20**:539–553. John Wiley & Sons, Ltd (10.1111). Available from http://doi.wiley.com/10.1111/ele.12748 (accessed April 15, 2019).

Griffin-Nolan RJ, Blumenthal DM, Collins SL, Farkas TE, Hoffman AM, Mueller KE, Ocheltree TW, Smith MD, Whitney KD, Knapp AK. 2019. Shifts in plant functional composition following long-term drought in grasslands. Journal of Ecology **107**:2133–2148. John Wiley & Sons, Ltd. Available from https://onlinelibrary.wiley.com/doi/full/10.1111/1365-2745.13252 (accessed December 31, 2021).

Hajek P, Kurjak D, Von Wühlisch G, Delzon S, Schuldt B. 2016. Intraspecific variation in wood anatomical, hydraulic, and foliar traits in ten European beech provenances differing in growth yield. Frontiers in Plant Science **7**:791. Frontiers Research Foundation.

Hamilton M. 2021. Population Genetics. John Wiley & Sons. Available from https://books.google.com/books?id=2lsXEAAAQBAJ&printsec=frontcover&source=gbs_ge_summary_r&cad=0#v=onepage&q&f=false (accessed February 4, 2022).

Hamrick JL. 2004. Response of forest trees to global environmental changes. Available from https://www.sciencedirect.com/science/article/pii/S0378112704003391.

Hartmann H, Adams HD, Anderegg WRL, Jansen S, Zeppel MJB. 2015. Research frontiers in drought-induced tree mortality: crossing scales and disciplines. New Phytologist **205**:965–969. John Wiley & Sons, Ltd. Available from https://onlinelibrary.wiley.com/doi/full/10.1111/nph.13246 (accessed December 31, 2021).

Havens K, Vitt P, Still S, Kramer AT, Fant JB, Schatz K. 2015. Seed Sourcing for Restoration in an Era of Climate Change. Natural Areas Journal **35**:122–133. Natural Areas Journal.

Höfner J, Klein-Raufhake T, Lampei C, Mudrak O, Bucharova A, Durka W. 2021. Populations restored using regional seed are genetically diverse and similar to natural populations in the region. Journal of Applied Ecology **00**:1–11. John Wiley & Sons, Ltd. Available from https://onlinelibrary.wiley.com/doi/full/10.1111/1365-2664.14067 (accessed February 7, 2022).

Jacobsen AL, Pratt RB. 2018. Extensive drought-associated plant mortality as an agent of type-conversion in chaparral shrublands. New Phytologist **219**:498–504. John Wiley & Sons, Ltd. Available from https://onlinelibrary.wiley.com/doi/full/10.1111/nph.15186 (accessed December 31, 2021).

Joshi J et al. 2001. Local adaptation enhances performance of common plant species. Ecology Letters **4**:536–544.

Jump AS, Penuelas J. 2005. Running to stand still: adaptation and the response of plants to rapid climate change. Ecology Letters **8**:1010–1020. John Wiley & Sons, Ltd. Available from http://doi.wiley.com/10.1111/j.1461-0248.2005.00796.x (accessed April 5, 2021).

Kawecki TJ, Ebert D. 2004. Conceptual issues in local adaptation. Ecology Letters.

Kremer A et al. 2012. Long-distance gene flow and adaptation of forest trees to rapid climate change. Pages 378–392 Ecology Letters. Available from http://doi.wiley.com/10.1111/j.1461-0248.2012.01746.x (accessed March 15, 2019).

Lamy J-B, Delzon S, Bouche PS, Alia R, Vendramin GG, Cochard H, Plomion C. 2014. Limited genetic variability and phenotypic plasticity detected for cavitation resistance in a Mediterranean pine. New Phytologist **201**:874–886. Available from https://www.jstor.org/stable/newphytologist.201.3.874.

Lamy JB, Bouffier L, Burlett R, Plomion C, Cochard H, Delzon S. 2011. Uniform Selection as a Primary Force Reducing Population Genetic Differentiation of Cavitation Resistance across a Species Range. PLOS ONE **6**:e23476. Public Library of Science. Available from https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0023476 (accessed February 4, 2022).

Larcher W. 2003. Physiological Plant Ecology. Springer-Verlag Berlin Heidelberg, Berlin. Available from https://books.google.com/books?hl=en&lr=&id=BgtzD4frr98C&oi=fnd&pg=PA1&ots=1oYULexb4R&sig=E-udYbJSrAZEYdkvxcCLbFGS420#v=onepage&q&f=false (accessed September 10, 2021).

Leimu R, Fischer M. 2008. A Meta-Analysis of Local Adaptation in Plants. PLoS ONE **3**:e4010. Public Library of Science. Available from https://dx.plos.org/10.1371/journal.pone.0004010 (accessed March 31, 2020).

Lenth R V. 2022. emmeans: Estimated Marginal Means, AKA Least-Squares Means. R package.

Liepe KJ, Hamann A, Smets P, Fitzpatrick CR, Aitken SN. 2016. Adaptation of lodgepole pine and interior spruce to climate: Implications for reforestation in a warming world.

Evolutionary Applications **9**:409–419. Wiley-Blackwell. Available from http://tinyurl.com/ClimateWNA. (accessed June 30, 2021).

Maherali H, Pockman WT, Jackson RB. 2004. Adaptive Variation in the Vulnerability of Woody Plants to Xylem Cavitation. Ecology **85**:2184–2199. Available from https://www.jstor.org/stable/3450284.

Maréchaux I, Saint-André L, Bartlett MK, Sack L, Chave J. 2020. Leaf drought tolerance cannot be inferred from classic leaf traits in a tropical rainforest. Journal of Ecology **108**:1030–1045.

Markesteijn L, Poorter L, Paz H, Sack L, Bongers F. 2011. Ecological differentiation in xylem cavitation resistance is associated with stem and leaf structural traits. Plant, Cell & Environment **34**:137–148. John Wiley & Sons, Ltd. Available from https://onlinelibrary.wiley.com/doi/full/10.1111/j.1365-3040.2010.02231.x (accessed December 31, 2021).

Martínez-Vilalta J, Piñol J. 2002. Drought-induced mortality and hydraulic architecture in pine populations of the NE Iberian Peninsula. Forest Ecology and Management **161**:247–256. Elsevier.

McDowell N et al. 2008. Mechanisms of Plant Survival and Mortality during Drought: Why Do Some Plants Survive while Others Succumb to Drought? The New Phytologist **178**:719–739. Available from https://www.jstor.org/stable/30149305.

Miller SA, Bartow A, Gisler M, Ward K, Young AS, Kaye TN. 2011. Can an Ecoregion Serve as a Seed Transfer Zone? Evidence from a Common Garden Study with Five Native Species. Restoration Ecology **19**:268–276. John Wiley & Sons, Ltd. Available from https://onlinelibrary.wiley.com/doi/full/10.1111/j.1526-100X.2010.00702.x (accessed February 7, 2022).

Nakagawa S, Johnson PCD, Schielzeth H. 2017. The coefficient of determination R2 and intra-class correlation coefficient from generalized linear mixed-effects models revisited and expanded. Journal of The Royal Society Interface **14**. The Royal Society. Available from https://royalsocietypublishing.org/doi/abs/10.1098/rsif.2017.0213 (accessed September 22, 2021).

Namkoong G. 2011. Forest genetics: pattern and complexity. https://doi.org/10.1139/x00-166 **31**:623–632. NRC Research Press Ottawa, Canada . Available from https://cdnsciencepub.com/doi/abs/10.1139/x00-166 (accessed February 4, 2022).

Pearson RG. 2006. Climate change and the migration capacity of species. Trends in Ecology & Evolution **21**:111–113. Elsevier Current Trends.

Pérez-Llorca M, Fenollosa E, Salguero-Gómez R, Munné-Bosch S. 2018. What Is the Minimal Optimal Sample Size for Plant Ecophysiological Studies? Plant Physiology **178**:953–955. Oxford Academic. Available from https://academic.oup.com/plphys/article/178/3/953/6116800 (accessed January 14, 2022).

Poorter H, Niinemets Ü, Poorter L, Wright IJ, Villar R. 2009. Causes and consequences of variation in leaf mass per area (LMA): a meta‐analysis. New Phytologist **182**:565–588.

John Wiley & Sons, Ltd (10.1111). Available from https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1469-8137.2009.02830.x (accessed April 15, 2019).

Poulter B, Ciais P, Hodson E, Lischke H, Maignan F, Plummer S, Zimmermann NE. 2011. Plant functional type mapping for earth system models. Geoscientific Model Development **4**:993–1010. Copernicus GmbH.

Pratt RB, Jacobsen AL, Ewers FW, Davis SD. 2007. Relationships among xylem transport, biomechanics and storage in stems and roots of nine Rhamnaceae species of the California chaparral. New Phytologist **174**:787–798. John Wiley & Sons, Ltd. Available from https://onlinelibrary.wiley.com/doi/full/10.1111/j.1469-8137.2007.02061.x (accessed December 31, 2021).

Reich PB. 2014. The world-wide 'fast–slow' plant economics spectrum: a traits manifesto. Journal of Ecology **102**:275–301. John Wiley & Sons, Ltd. Available from https://onlinelibrary.wiley.com/doi/full/10.1111/1365-2745.12211 (accessed February 11, 2022).

Reich PB, Walters MB, Ellsworth DS, Wang Y-P, Oleksyn J. 1997. From tropics to tundra: global convergence in plant functioning. Proceedings of the National Academy of Sciences of the United States of America **94**:13730–4. National Academy of Sciences. Available from http://www.ncbi.nlm.nih.gov/pubmed/9391094 (accessed April 15, 2019).

Sánchez-Gómez D, Zavala MA, Valladares F. 2008. Functional traits and plasticity linked to seedlings' performance under shade and drought in Mediterranean woody species. Annals of Forest Science **65**:311–311.

Sapes G, Sala A. 2021. Relative water content consistently predicts drought mortality risk in seedling populations with different morphology, physiology and times to death. Plant, Cell & Environment **44**:3322–3335. John Wiley & Sons, Ltd. Available from https://onlinelibrary-wiley-com.weblib.lib.umt.edu:2443/doi/full/10.1111/pce.14149 (accessed December 31, 2021).

Savolainen O, Pyhäjärvi T, Knürr T. 2007. Gene Flow and Local Adaptation in Trees. Annual Review of Ecology, Evolution, and Systematics **38**:595–619. Annual Reviews. Available from http://www.annualreviews.org/doi/10.1146/annurev.ecolsys.38.091206.095646 (accessed April 1, 2020).

Shiklomanov AN et al. 2020. Does the leaf economic spectrum hold within plant functional types? A Bayesian multivariate trait meta-analysis. Ecological Applications **30**:e02064. John Wiley & Sons, Ltd. Available from https://onlinelibrary.wiley.com/doi/full/10.1002/eap.2064 (accessed December 31, 2021).

Siefert A et al. 2015. A global meta-analysis of the relative extent of intraspecific trait variation in plant communities. Ecology Letters **18**:1406–1419. John Wiley & Sons, Ltd (10.1111). Available from http://doi.wiley.com/10.1111/ele.12508 (accessed March 13, 2019).

Siefert A, Fridley JD, Ritchie ME. 2014. Community Functional Responses to Soil and Climate at Multiple Spatial Scales: When Does Intraspecific Variation Matter? Available from www.daymet.org (accessed March 2, 2020).

Skelton RP, Anderegg LDL, Papper P, Reich E, Dawson TE, Kling M, Thompson SE, Diaz J, Ackerly DD. 2019. No local adaptation in leaf or stem xylem vulnerability to embolism, but consistent vulnerability segmentation in a North American oak. New Phytologist **223**:1296–1306. John Wiley & Sons, Ltd. Available from https://onlinelibrary.wiley.com/doi/full/10.1111/nph.15886 (accessed December 31, 2021).

Stevens GC. 2015. The Latitudinal Gradient in Geographical Range: How so Many Species Coexist in the Tropics. https://doi.org/10.1086/284913 **133**:240–256. University of Chicago Press . Available from https://www.journals.uchicago.edu/doi/abs/10.1086/284913 (accessed December 31, 2021).

Suarez ML, Ghermandi L, Kitzberger T. 2004. Factors predisposing episodic drought-induced tree mortality in Nothofagus– site, climatic sensitivity and growth trends. Journal of Ecology **92**:954–966. John Wiley & Sons, Ltd. Available from https://onlinelibrary.wiley.com/doi/full/10.1111/j.1365-2745.2004.00941.x (accessed December 31, 2021).

Trugman AT, Anderegg LDL, Anderegg WRL, Das AJ, Stephenson NL. 2021. Why is Tree Drought Mortality so Hard to Predict? Trends in Ecology & Evolution **36**:520–532. Elsevier Current Trends.

Tyree MT, Sperry JS. 1989. VULNERABILITY OF XYLEM TO CAVITATION AND EMBOLISM. Annu. Rev. Plant Phys. Mol. Bio **40**:19–38. Available from www.annualreviews.org (accessed February 4, 2022).

Tyree MT, Zimmerman MH. 2013. Xylem Structure and the Ascent of SapSecond Edition. Springer, New York. Available from https://books.google.com/books?hl=en&lr=&id=wnv1CAAAQBAJ&oi=fnd&pg=PA1&dq=%0AZimmermann,+M.+H.%0A1983.+%0AXylem+structure+and+the+ascent+of+sap.+Springer,+New+York,+New+York,+USA.%0A&ots=87Ji9vtVe2&sig=9BEbtx56gODTUyPzVvX1ht6uf2E#v=onepage&q=Zimmermann%2C M. H. 1983. Xylem structure and the ascent of sap. Springer%2C New York%2C New York%2C USA.&f=false (accessed February 4, 2022).

Urli M, Porté AJ, Cochard H, Guengant Y, Burlett R, Delzon S. 2013. Xylem embolism threshold for catastrophic hydraulic failure in angiosperm trees. Tree Physiology **33**:672–683. Oxford Academic. Available from https://academic.oup.com/treephys/article/33/7/672/1703733 (accessed January 10, 2022).

Volaire F. 2018. A unified framework of plant adaptive strategies to drought: Crossing scales and disciplines. Global Change Biology **24**:2929–2938.

Wortemann R, Herbette S, Barigah TS, Fumanal B, Alia R, Ducousso A, Gomory D, Roeckel-Drevet P, Cochard H, Abrams M. 2011. Genotypic variability and phenotypic plasticity of cavitation resistance in Fagus sylvatica L. across Europe. Tree Physiology **31**:1175–1182. Available from https://www.ncbi.nlm.nih.gov/pubmed/21989814.

Wright IJ et al. 2004. The worldwide leaf economics spectrum. Available from www.nature.com/nature (accessed April 15, 2019).

Tables & Figures

Tables & Figures

Table 1. Biological and ecological groupings used in analyses. Plant functional type (C) is referred to as PFT throughout. Clade (D) and leaf habit (E) refer only to trees.

(A) Ecosystem	(B) Life form	C) Plant functional type	(D) Clade	(E) Leaf habit
Temperate forest (TF)	Tree	Needleleaf evergreen tree (NET)	Gymnosperm	Evergreen
Boreal forest (BF)	Shrub	Needleleaf deciduous tree (NDT)	Angiosperm	Deciduous
Tropical forest (TRF)	Grass	Broadleaf evergreen tree (BET)		
Grassland (GL)	Forb	Broadleaf deciduous tree (BDT)		
Shrubland (SL)		Broadleaf evergreen shrub (BES)		
		C3 grass (C3G)		
		C4 grass (C4G)		
		Forb (FOR)		

Table 2. Mean weighted CV± SE and n (sample size for CV weighted mean) within populations, within species, and across species. *Within-species CVs were calculated for each species in one environment with the same measurement method, so multiple CVs may exist for one species. See methods for how we addressed this in our models.

Trait	**Level**	**Mean Weighted CV**	**n**
P12	*Within-population*	0.19 ± 0.01	75 populations
	Within-species	0.08 ± 0.01	15 species*
	Across-species	0.31	1 (8 species)
P50	*Within-population*	0.09 ± 0.00	94 populations
	Within-species	0.05 ± 0.01	18 species*
	Across-species	0.25	1 (11 species)
P88	*Within-population*	0.09 ± 0.01	74 populations
	Within-species	0.04 ± 0.01	13 species*
	Across-species	0.26	1 (7 species)
π_{tlp}	*Within-population*	0.07 ± 0.0	66 populations
	Within-species	0.04 ± 0.01	10 species*
	Across-species	0.37	1 (5 species)
SLA	*Within-population*	0.16 ± 0.01	431 populations
	Within-species	0.05 ± 0.01	40 species*
	Across-species	0.77	1 (22 species)
RSR	*Within-population*	0.21 ± 0.01	165 populations
	Within-species	0.12 ± 0.01	31 species*
	Across-species	1.39	1 (18 species)
WD	*Within-population*	0.08 ± 0.0	219 populations
	Within-species	0.05 ± 0.01	12 species*
	Across-species	0.17	1 (9 species)

Table 3. Linear mixed model output for tests of differences in CVs of traits across organizational levels. Models included trait CVs as response variables, organizational level as a fixed effect, and study as a random effect.

Trait	**SSR**	**MSE**	**Df**	**F value**	**p value**
P12	1.8	1.8	1	19.5	<0.001
P50	0.4	0.4	1	105.9	<0.001
P88	0.3	0.3	1	83.0	<0.001
π_{tlp}	0.3	0.3	1	10.3	<0.01
SLA	0.1	0.1	1	47.7	<0.001
RSR	8.4	8.4	1	31.7	<0.001
WD	0.0	0.0	1	53.0	<0.001

Table 4. Across-species variation and relative magnitude of variation within populations and species compared to across species. Proportions were calculated by dividing the mean CV for each trait within populations and species by the CV across all species.

		Proportion of Across-Species Variation	
Trait	**Across-Species Variation (CV)**	**Within-Population Variation**	**Within-Species Variation**
P12	0.31	0.61	0.26
P50	0.25	0.36	0.20
P88	0.26	0.35	0.15
π_{tlp}	0.37	0.19	0.11
SLA	0.77	0.21	0.06
RSR	1.39	0.15	0.09
WD	0.17	0.47	0.29

Table 5. Linear mixed model output for tests of differences in population means of traits among biological and groupings. Separate models included trait population means as response variables, groupings as a fixed effect, and study as a random effect. See Table 1 for levels of each grouping. Marginal R^2 indicates variation explained by the coefficient without variation explained by the random factor (study). NA indicates that there were not sufficient levels to assess a given grouping for that trait. Levels were included for all groupings if they were represented by two or more species.

Trait	**Coefficient**	**SSR**	**MSE**	**Df**	**F value**	**p value**	**Marginal R^2**
P12	Species	17.2	2.5	7	1.2	0.52	0.16
	PFT	5.2	2.6	2	1.3	0.32	0.05
	Life form	NA	NA	NA	NA	NA	NA
	Clade	4.9	4.9	1	7.7	2.50	0.05
	Leaf habit	0.7	0.7	1	0.4	0.57	0.01
P50	Species	21.1	2.1	10	2.0	0.32	0.40
	PFT	1.0	0.5	2	0.5	0.65	0.03
	Life form	NA	NA	NA	NA	NA	NA
	Clade	0.0	0.0	1	0.0	0.90	<0.01
	Leaf habit	0.6	0.6	1	0.6	0.36	0.02
P88	Species	11.2	1.9	2	1.1	0.53	0.16
	PFT	2.3	2.3	1	1.6	0.25	0.09
	Life form	NA	NA	NA	NA	NA	NA
	Clade	0.0	0.0	1	0.0	0.97	0.09
	Leaf habit	3.6	3.6	1	2.2	0.18	0.09
π_{tlp}	Species	0.3	0.1	4	0.0	1.00	0.01
	PFT	NA	NA	NA	NA	NA	NA
	Life form	NA	NA	NA	NA	NA	NA
	Clade	NA	NA	NA	NA	NA	NA
	Leaf habit	NA	NA	NA	NA	NA	NA
SLA	Species	3177.4	158.9	20	1.2	0.43	0.40
	PFT	1849.7	924.9	2	7.2	**0.01**	0.39
	Life form	961.6	320.5	3	2.4	0.09	0.19
	Clade	477.6	477.6	1	4.0	**0.06**	0.18
	Leaf habit	1061.6	1061.6	1	8.9	**<0.01**	0.25
RSR	Species	36.3	2.1	17	5.9	0.15	0.61
	PFT	1.9	0.9	2	2.6	0.11	0.12
	Life form	0.0	0.0	1	0.0	0.94	<0.01
	Clade	0.8	0.4	2	1.1	0.35	0.07
	Leaf habit	0.1	0.0	2	0.1	0.93	0.01

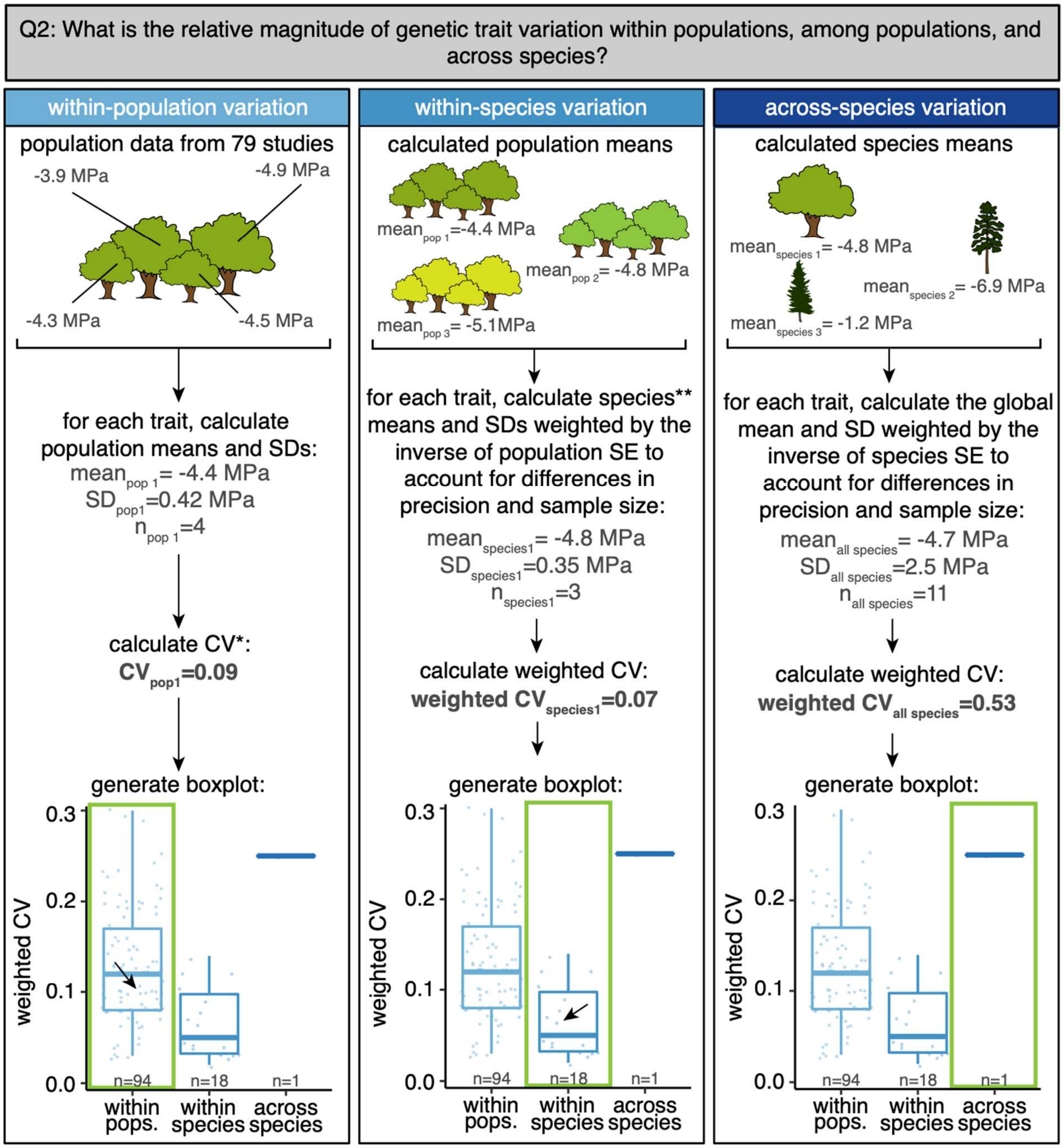
WD	Species	129.5	16.2	8	0.3	0.89	0.15
	PFT	106.2	53.1	2	1.1	0.39	0.13
	Life form	NA	NA	NA	NA	NA	NA
	Clade	79.7	79.7	1	1.7	0.24	0.13
	Leaf habit	0.0	0.0	1	0.0	0.99	<0.01

Table 6. Linear mixed model output for tests of differences in species means of traits among biological and groupings. Separate models included trait means as response variables, groupings as a fixed effect, and study as a random effect. See Table 1 for levels of each grouping. Marginal R^2 indicates variation explained by the coefficient without variation explained by the random factor (study). NA indicates that there were not sufficient levels to assess a given grouping for that trait. Levels were included for all groupings if they were represented by two or more species.

Trait	Coefficient	SSR	MSE	Df	F value	p value	Marginal R^2
P12	Species	68.8	9.8	7	1.0	0.60	0.05
	PFT	8.8	4.4	2	0.4	0.69	<0.01
	Life form	NA	NA	NA	NA	NA	NA
	Clade	5.5	5.5	1	0.5	0.49	<0.01
	Leaf habit	0.2	0.2	1	<0.1	0.9	<0.01
P50	Species	45.9	4.9	10	1.5	0.39	0.23
	PFT	3.3	1.7	2	0.4	0.67	0.02
	Life form	NA	NA	NA	NA	NA	NA
	Clade	0.2	0.2	1	<0.1	0.84	<0.01
	Leaf habit	2.0	2.0	1	0.5	0.49	0.01
P88	Species	15.1	2.5	6	0.9	0.60	0.23
	PFT	15.5	7.8	2	3.1	0.11	0.23
	Life form	NA	NA	NA	NA	NA	NA
	Clade	0.1	0.1	1	0.0	0.85	<0.01
	Leaf habit	5.3	5.3	1	1.8	0.22	0.09
π_{tlp}	Species	<0.1	<0.1	4	0.4	0.82	0.18
	PFT	NA	NA	NA	NA	NA	NA
	Life form	NA	NA	NA	NA	NA	NA
	Clade	NA	NA	NA	NA	NA	NA
	Leaf habit	NA	NA	NA	NA	NA	NA
SLA	Species	1580.0	79.0	20	0.86	0.63	0.39
	PFT	1525.2	508.4	3	5.8	**0.01**	0.45
	Life form	600.8	200.3	3	2.3	0.11	0.20
	Clade	320.03	320.03	1	3.4	0.08	0.14
	Leaf habit	423.8	423.8	1	4.5	**0.05**	0.19
RSR	Species	57.3	3.4	17	10.3	0.09	0.75
	PFT	1.5	0.5	3	1.4	0.28	0.19
	Life form	0.1	<0.1	2	0.1	0.91	<0.01
	Clade	1.0	1.0	1	2.5	0.14	0.12
	Leaf habit	0.1	0.1	1	0.2	0.69	<0.01
WD	Species	473.2	59.2	8	0.33	0.89	0.24
	PFT	313.5	156.8	2	1.0	0.40	0.21

Life form	NA	NA	NA	NA	NA	NA
Clade	197.0	197.0	1	1.4	0.27	0.15
Leaf habit	5.4	5.4	1	<0.1	0.85	<0.01

Figure 1. Overview of methods for calculating CVs (coefficient of variation) within and across organizational levels, using example data for the trait P50. All data are for example purposes only.



*all individuals in a population were weighted equally

** we calculated species means separately for each study and environment to address genetic variation in traits only

Figure 2. (A) Distribution of studies across regions for each trait studied, including the number of populations (pops), species, and studies by region, with darker colors indicating more information. **(B)** Distribution of studies across ecosystems and life forms. On the x-axis, bars show ecosystems (see legend for codes); widths indicate the number of populations. On the y-axis, bar heights correspond to the proportion of populations represented by each life form (indicated by color) for each ecosystem. For SLA, the final two unlabeled bars represent shrublands and boreal forests, respectively.

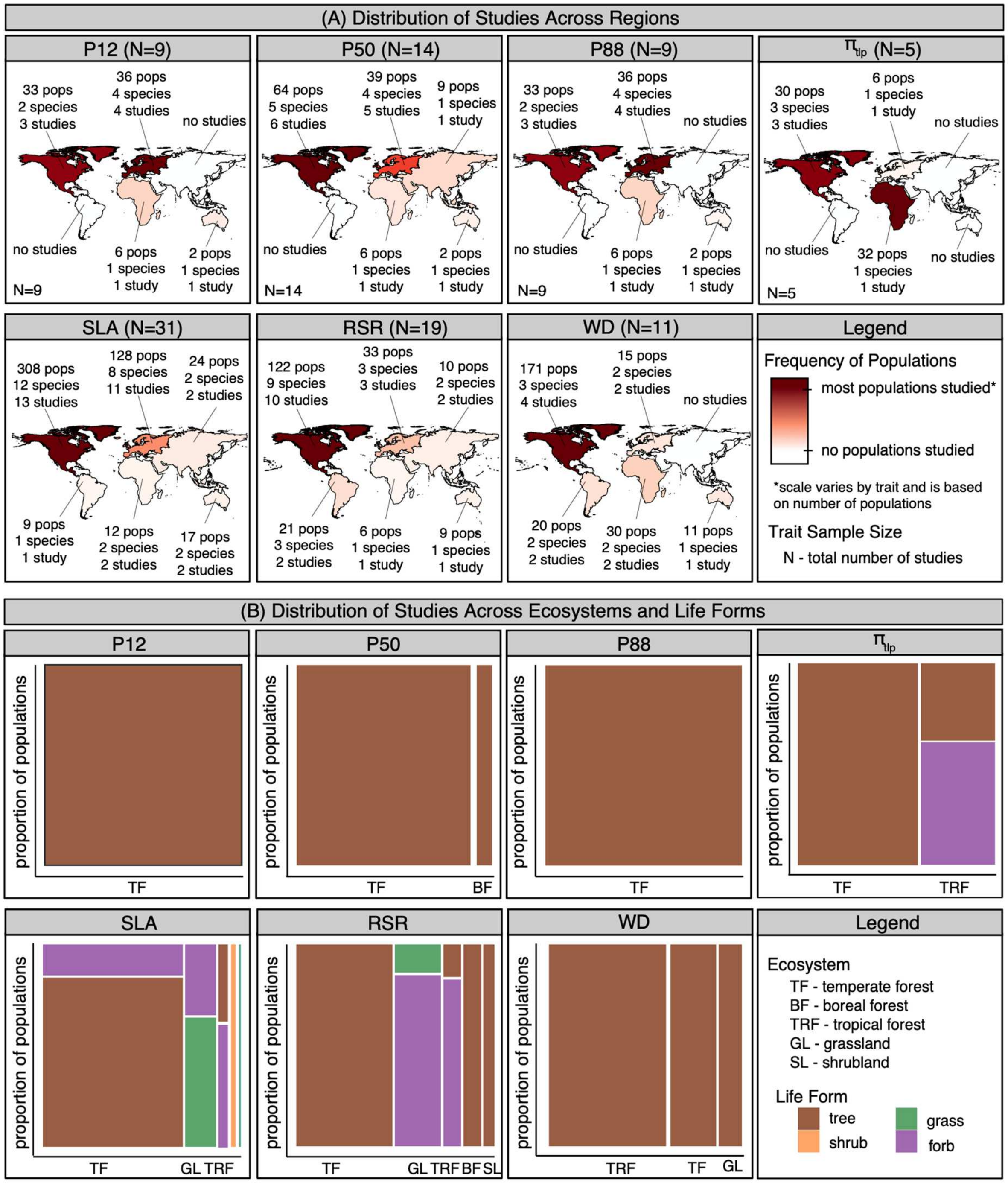


Figure 3. Magnitude of variation across organizational levels. Boxplots show coefficient of variation (CV) within populations, among populations, and across all species studied. Organizational levels are indicated by colors (see legend). Boxes show median, 25th and 75th percentiles, with lines extending to 1.5* inter-quantile range (IQR), and outliers (beyond 1.5*IQR) plotted as open circles. Sample sizes for CVs are printed below boxes. Stars indicate significance of within population vs within species comparison.

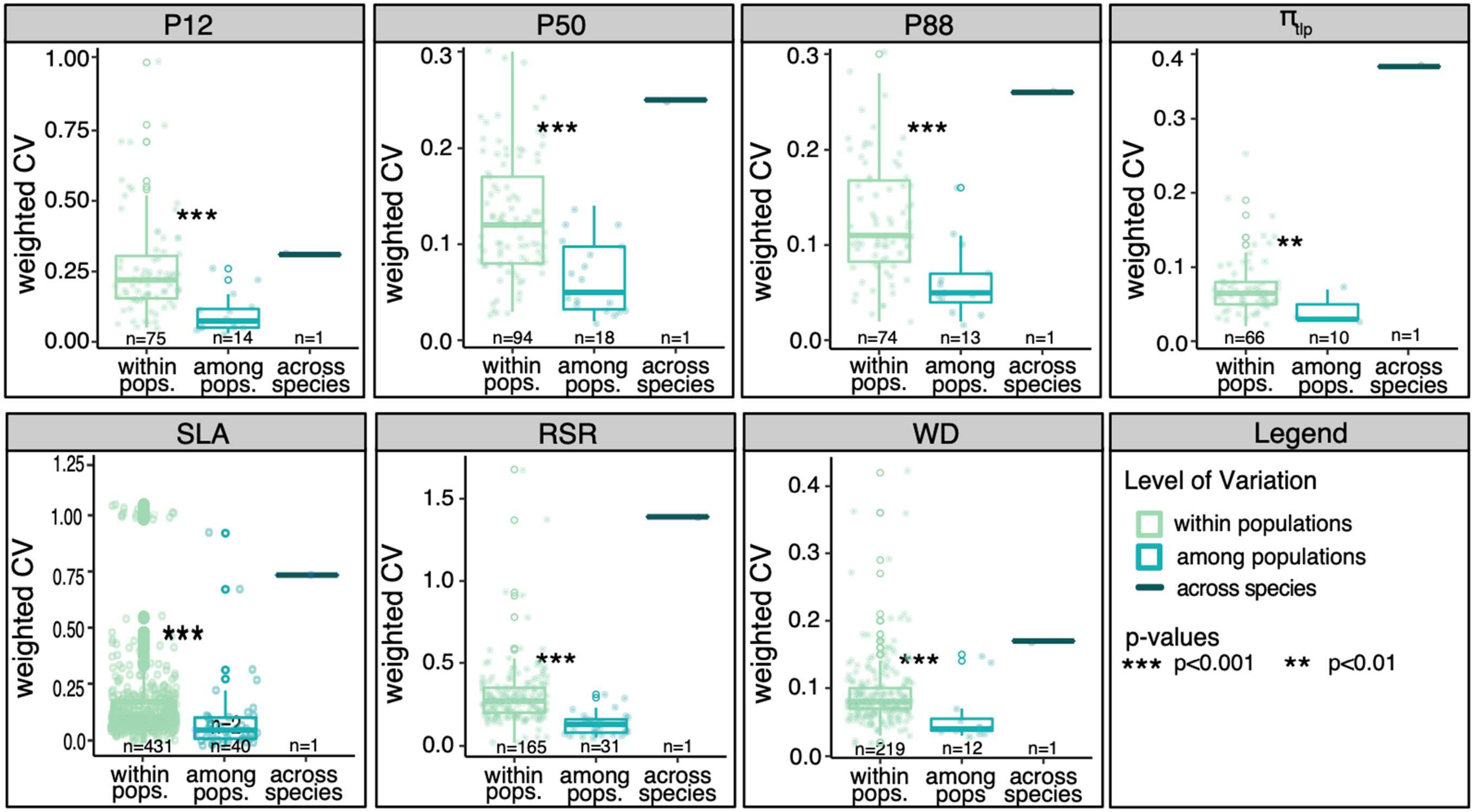
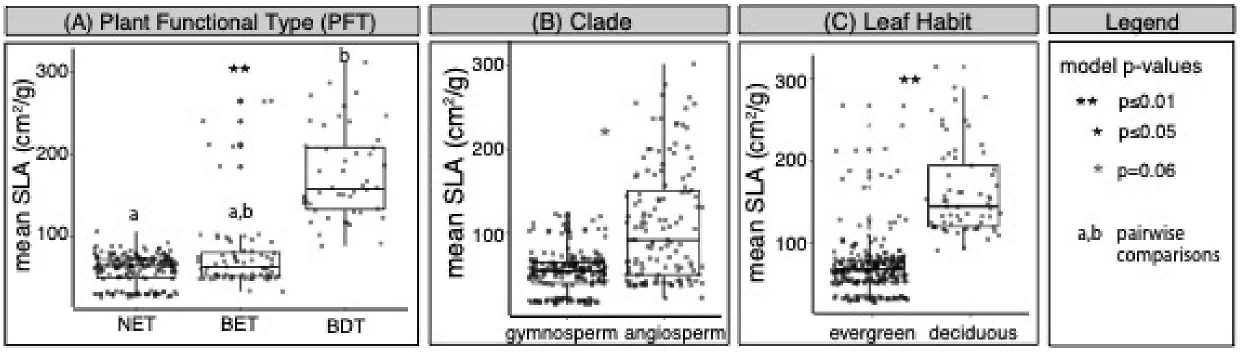


Figure 4. Population means across biological groupings. (A) Plant functional type (PFT); see Table 1 for abbreviations. (B) Clade. (C) Leaf habit. For each trait and grouping, we show only groupings that were significant in our models. Boxes show median, 25th and 75th percentiles, with lines extending to 1.5*IQR, and outliers (beyond 1.5*IQR) plotted as open circles. Stars indicate significance of models with only two groups, while letters indicate post-hoc significant differences between groups.



SUPPLEMENTAL INFORMATION

Chapter I

ANOVA models for all traits with region and population as nested factors (Appendix S1), PC score-trait and -environment correlations (Appendix S2), a figure showing PC score-trait correlations (Appendix S3), full multiple regression models for traits (Appendix S4), means and SEs for traits differentiated by region (Appendix S5), full multiple regression models for environmental variables (Appendix S6), ANOVA models for temperature annual range with region and population as nested factors (Appendix S7), and a figure showing PC score-environmental variable correlations (Appendix S7) are included in the supplemental information.

Appendix 1. ANOVA tables for traits that varied significantly among regions or populations and were included in PCA (top) and traits that did not vary significantly among regions or populations and were not included in PCA (bottom).

Traits included in Multiple Regression Models

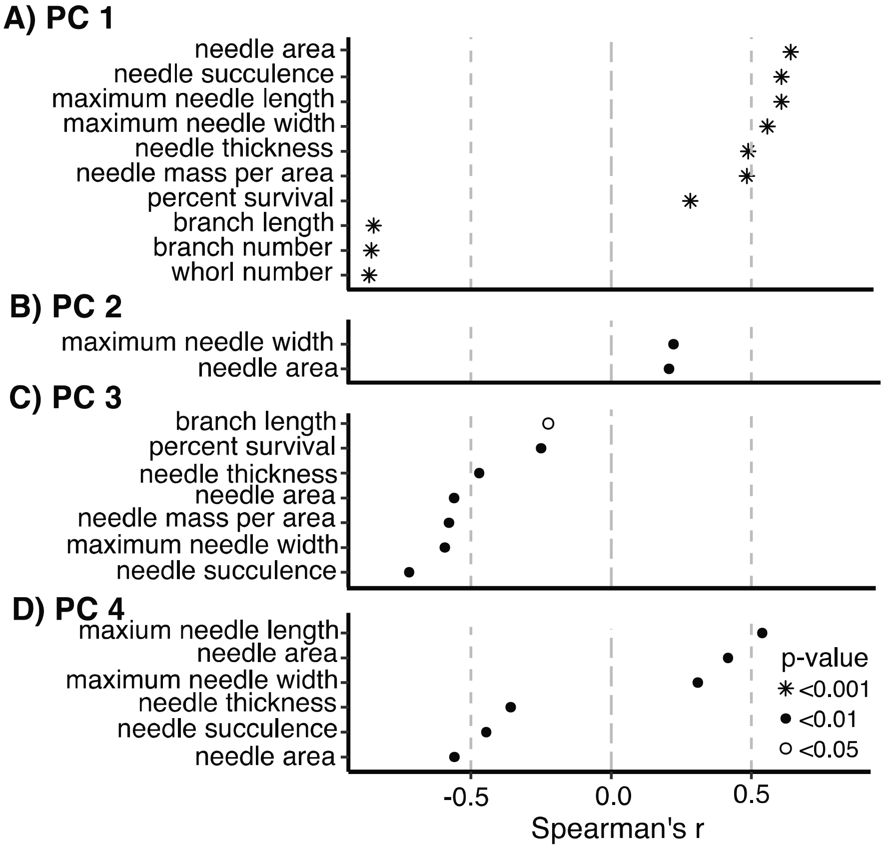
		df	SS	MSE	F	p	F (df)	Adj. R^2	p
Needle width	Region	1	26.7	26.7	34.5	<0.001	9.3 (11,192)	0.31	<0.001
	Population	10	52.7	5.3	6.8	<0.001			
	Residuals	192	148.5	0.8					
Needle area	Region	1	15.6	15.6	69.5	<0.001	7.6 (11, 194)	0.26	<0.001
	Population	10	3.2	3.2	1.4	0.2			
	Residuals	194	43.6	0.2					
Needle succulence	Region	1	11.0	11.0	28.1	<0.001	4.6 (11, 194)	0.16	<0.001
	Population	10	8.6	0.9	2.2	0.02			
	Residuals	194	76.0	0.4					
Number of whorls	Region	1	9.5	9.5	27.5	<0.001	4.6 (11, 193)	0.16	<0.001
	Population	10	8.0	0.8	2.3	0.01			
	Residuals	193	66.9	0.3					
Needle thickness	Region	1	0.5	0.5	26.3	<0.001	5.3 (11, 194)	0.19	<0.001
	Population	10	0.6	0.1	3.2	0.001			
	Residuals	194	3.7	1.9x10^{-2}					
Needle length	Region	1	14.9	14.9	35.3	<0.001	3.9 (11, 192)	0.13	<0.001
	Population	10	3.1	0.3	0.7	0.7			
	Residuals	192	81.2	0.4					
Number of branches	Region	1	24.7	24.7	20.0	<0.001	3.8 (11, 193)	0.13	<0.001
	Population	10	26.7	2.7	2.2	0.02			
	Residuals	193	237.5	1.23					
Needle mass per area	Region	1	0.6	0.6	8.0	<0.01	3.2 (11, 194)	0.10	<0.001
	Population	10	2.1	0.21	2.7	<0.01			
	Residuals	194	15.3	7.9x10^{-2}					
Branch length	Region	1	133.2	133.2	11.3	<0.001	2.7 (11, 192)	0.09	0.002
	Population	10	222.4	22.2	1.9	0.05			
	Residuals	192	2268.7	11.8					
Percent survival	Region	1	0.2	0.2	14.0	<0.001	2.2 (11, 201)	0.06	0.014
	Population	10	0.1	1.5x10^{-2}	1.1	0.40			
	Residuals	201	2.8	0.01					
Percent germination	Region	1	2.1x10^{-2}	2.1x10^{-2}	1.7	0.2	2.0 (11, 201)	0.05	0.03
	Population	10	0.3	2.510^{-2}	2.0	0.03			
	Residuals	192	2.4	1.2x10^{-2}					

Traits not Included in Multiple Regressions

		df	**SS**	**MSE**	**F**	**p**			**df**
Aboveground biomass	Region	1	1.4	1.4	0.5	0.5	*Aboveground biomass*	Region	1
	Population	10	11.5	1.2	0.5	0.9		Population	10
	Residuals	178	457.4	2.6				Residuals	178
Belowground biomass	Region	1	1.0x10^{-2}	7.5 x10^{-3}	2.0 x10^{-2}	0.9	*Belowground biomass*	Region	1
	Population	10	1.81	0.2	0.6	0.8		Population	10
	Residuals	178	56.3	0.3				Residuals	178
Stem length	Region	1	3.4	3.4	0.4	0.5	*Stem length*	Region	1
	Population	10	50.7	5.1	0.5	0.9		Population	10
	Residuals	189	1783.3	9.4				Residuals	189
Basal diameter	Region	1	1.8	1.8	1.5	0.2	*Basal diameter*	Region	1
	Population	10	16.2	1.6	1.4	0.2		Population	10
	Residuals	186	215.0	1.2				Residuals	186

Appendix 2. Correlations between PC scores and traits or environmental variables. Provided as a separate Excel file.

Appendix 3. A suite of traits are correlated with PC axis scores, contributing to explaining the largest proportion of overall trait variation among populations across the range of pewen in Chile. Spearman's r correlations among A) PC1 axis scores, B) PC2 axis scores, C) PC3 axis scores, D) PC4 axis scores and traits, where |r|>0.20. Large dashed gray line denotes r=0 and small dashed grey lines are where |r|=0.50. Symbols denote p-values for Spearman's correlations (see legend). Trait units are shown in Table 2.



Appendix 4. ANOVA outputs from full PC score-trait models before nonsignificant traits were excluded. Reduced models which were used in this manuscript and associated ΔAIC values are included in Table 4. For PC2 and PC4, full models are used (Table 4). Traits which were included in final models are italicized.

	Coefficients	Estimate	SE	t	p	Adj. R^2	F (df)	p	AIC (Δ AIC)
PC1 (79.3%)	Intercept	2.0	3.6	0.6	0.6	0.83	238 (4, 193)	<0.001	1237.1 (1.7)
	Number of whorls	*-16.7*	*0.7*	*-23.6*	*<0.001*				
	Needle area	*2.6*	*0.8*	*3.0*	*0.003*				
	Needle succulence	*1.5*	*0.7*	*2.2*	*0.03*				
	Percent survival	-1.7	3.3	-0.5	0.6				
PC3 (4.9%)	Intercept	22.8	0.7	32.6	<0.001	0.89	387 (4, 191)	<0.001	572.6 (0.0)
	Needle succulence	-3.1	0.1	-24.2	<0.001				
	Needle width	-1.3	0.1	-15.8	<0.001				
	Branch length	-0.5	2.2×10^{-2}	-23.7	<0.001				
	Percent survival	-0.9	0.6	-1.4	0.2				

Appendix 5. Means ± standard errors for key branch and needle traits that are differentiated regionally.

Trait	**Coastal populations**	**Andes populations**
Number of whorls	1.1 ± 0.11	0.6 ± 0.05
Number of branches	1.9 ± 0.23	0.98 ± 0.08
Branch length (cm)	5.3 ± 0.23	3.3 ± 0.08
Needle width (mm)	5.4 ± 0.12	6.3 ± 0.08
Needle succulence (mg/cm^2)	3.50 ± 0.07	4.1 ± 0.05

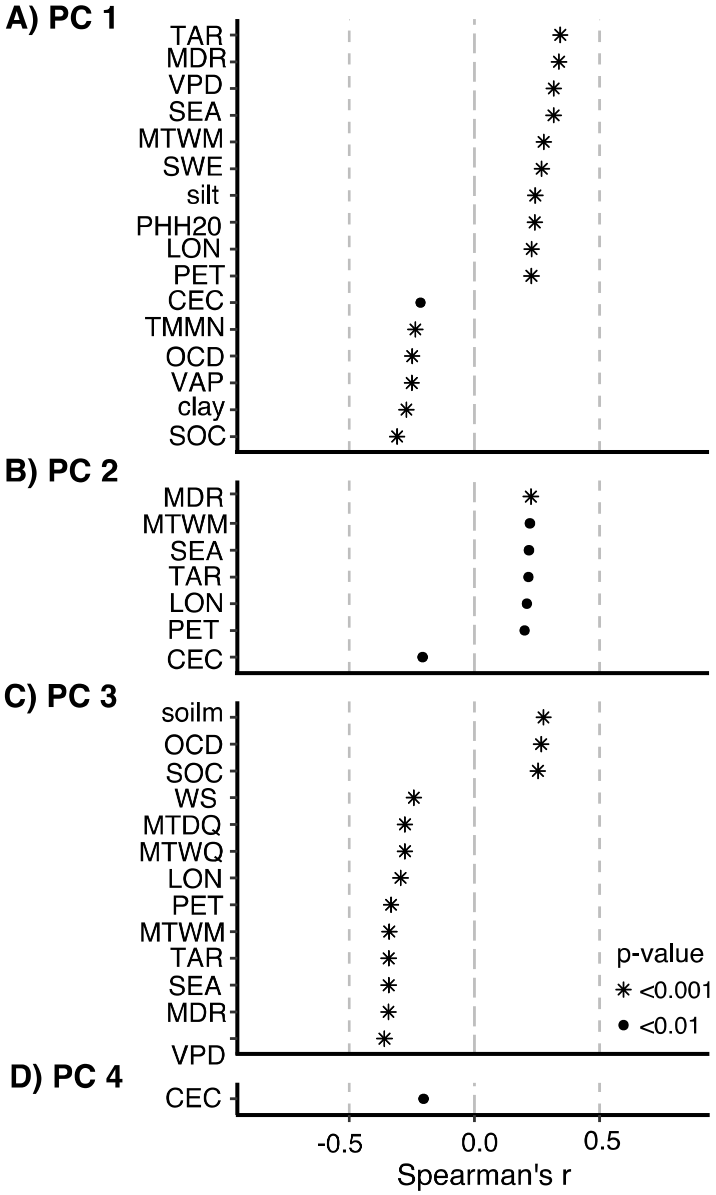
Appendix 6. ANOVA outputs from full PC score-environmental variable models before nonsignificant traits were excluded. Reduced models which were used in this manuscript and associated ΔAIC values are included in Table 4. For PC3 and PC4, full models are used (Table 4). Traits which were included in final models are italicized.

	Coefficients	**Estimate**	**SE**	**t**	**p**	**Adj. R^{2}**	**F (df)**	**p**	**AIC (Δ AIC)**
PC1 (79.3%)	Intercept	30.1	45.9	0.7	0.5	0.09	4.3 (7, 202)	<0.001	1653.9 (0.0)
	TAR	-4.6x10^{-4}	0.1	-3.6x10^{-1}	1.0				
	SWE	-3.3x10^{-2}	7.3x10^{-2}	-0.4	0.7				
	Silt	3.5x10-^{2}	2.4x10^{-2}	1.5	0.1				
	SOC	-1.2x10^{-2}	6.2x10^{-3}	-1.9	0.05				
	Clay	-1.7x10^{-2}	2.8x10^{-2}	-0.6	0.5				
	VAP	-24.93	23.7	-1.1	0.3				
	OCD	-5.9x10^{-3}	2.6x10-2	-0.2	0.8				
PC2 (9.6%)	Intercept	-14.0	3.6	-3.9	<0.001	0.06	15.28 (1, 211)	<0.001	1237.2 (-0.52)
	MDR	0.1	2.7x10-^{2}	3.9	<0.001				

Appendix 7. ANOVA model outputs for differentiation in temperature annual range (TAR, °C) by region and population (nested within region).

		df	**SS**	**MSE**	**F**	**p**	**Adj. R^2**	**p**
TAR	Region	1	66900	66900	28271	<0.001	0.99	<0.001
	Population	10	11003	1100	465	<0.001		
	Residual	201	476	2				

Appendix 8. A suite of environmental variables are correlated with PC axis scores, explaining the largest proportion of overall trait variation among regions and populations across the range of pewen in Chile. **A)** Spearman's r correlations among PC1 axis scores and environmental variables where |r|>0.20. **B)** Spearman's r correlations among PC2 axis scores and environmental variables, where |r|>0.20. Large dashed gray line denotes r=0 and small dashed grey lines are where |r|=0.50. Symbols denote p-values for Spearman's correlations (see legend). Full environmental variable names and units are included in Table 3).



Chapter III

Supplemental Table 1. Metadata for all studies included in this synthesis.
(Included as an Excel file)

Supplemental Table 2. Full dataset and metadata for the analysis included in this synthesis.
(Included as an Excel file)

Supplemental Table 3. Changes in mean within-population CVs when outlier species (see Supplemental Figure 1) are removed.

Trait	**Species Removed**	**Mean Within-Species Weighted CV**
P12	*With* Pinus canariensis	0.19 ± 0.01
	Without Pinus canariensis	0.16 ± 0.01
SLA	*With* Fagus sylvatica	0.16 ± 0.01
	Without Fagus sylvatica	0.15 ± 0.00
RSR	*With* Quercus suber	0.21 ± 0.01
	Without Quercus suber	0.21 ± 0.01

Supplemental Figure 1. For all traits studied, within-population CVs separated by species. Life forms are indicated by colors (see legend). Boxes show median, 25th and 75th percentiles, with lines extending to 1.5* inter-quantile range (IQR), and outliers (beyond 1.5*IQR) plotted as open circles. Study sample sizes for species are printed below boxes.