RESTORATION ECOLOGY
The Journal of the Society for Ecological Restoration
SER SOCIETY FOR ECOLOGICAL RESTORATION

RESEARCH ARTICLE

Trait variation between and within Andes and coastal mountain ranges in the iconic South American tree *Araucaria araucana* in Chile

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A major barrier globally to achieving restoration goals is the identification and production of appropriate plant materials. To produce high-quality plant materials for restoration success, prevent negative consequences to plant populations, and conserve genetic diversity, it is critical to understand ecotypic variation among plant populations, especially in relation to environmental conditions. For *Araucaria araucana*, a highly threatened iconic South American tree that grows across a substantial climate gradient, this information is urgently needed to guide restoration and conservation efforts. We conducted a common garden experiment using seedlings from 12 populations of *A. araucana* across its range in Chile. This approach allowed us to assess regional (coastal versus Andes mountain ranges) and population-level variation in plant traits and relate this variation to environmental variables. Our results demonstrate that *A. araucana* exhibits differentiation at both regional and population levels, particularly in traits such as branch number and length (indicative of plant architectural differences) and needle width (reflecting variation in leaf investment). This variation is at least partly explained by climate variables, with the most significant differences attributed to regional variations in temperature annual range and mean vapor pressure deficit. Based on these findings, we recommend restoration efforts prioritize conserving genetic variation both among and within regions and their populations, while avoiding translocations of genotypes between coastal and Andes populations.

Key words: climate adaptation, ecological restoration, monkey puzzle tree, plant material selection, population-level trait differentiation

Implications for Practice

- In *Araucaria araucana*, coastal and Andean regions are differentiated from each other in putatively adaptive traits.
- Populations within regions also showed genetic differentiation in traits, suggesting that when selecting seed sources for restoration of *A. araucana*, collecting seeds from multiple populations and regions may maximize variation.
- A large proportion of trait variation was unexplained by region or population, suggesting that within-population variation may be substantial.
- Overall, restoration efforts should consider sourcing seeds 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.

Introduction

As anthropogenetic degradation and climate change affect ecosystems, ecological restoration is increasingly needed for repair. Toward this end, countries worldwide have made ambitious restoration commitments. 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). However, successful restoration requires careful selection of site-appropriate plant materials (seeds or plants that are planted in a restoration site), a factor often overlooked (Gann et al. 2019; León-Lobos et al. 2020). To protect genetic diversity, prevent maladaptation, and minimize negative effects on adjacent populations (such as founder effects, genetic swamping, outbreeding depression, or any other deleterious effects of introducing plant materials that are not adapted to their

Author contributions: MM, JG-C, RI, MAB-S, RH, CRN contributed to conceptual development of this study; MM, CRN determined study design; JG-C, RI selected seed collection sites and facilitated seedling germination and growth with a commercial greenhouse; MM, PD, OT-S, collected data; MM analyzed the data and wrote the manuscript; all authors reviewed the manuscript.

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doi: 10.1111/rec.70268
Supporting information at:
http://onlinelibrary.wiley.com/doi/10.1111/rec.70268/suppinfo

environments), understanding genetic differentiation and ecotypic variation among and within plant populations is crucial (Lesica & Allendorf 1999; Kramer & Havens 2009; Breed et al. 2013). This information is lacking for many species of conservation concern, and in Chile, it is limiting restoration capacity (León-Lobos et al. 2020).

In this study, we address this knowledge gap for the iconic South American conifer, *Araucaria araucana* (referred to throughout as pewen). This is a species of profound cultural significance—sacred to the Mapuche Pewenche people and legally protected in Chile—and plays a vital ecological role in high-elevation forests. As a long-lived, fire-tolerant, and structurally dominant conifer, it contributes to ecosystem integrity through soil stabilization, carbon sequestration, and provision of habitat for endemic fauna (Shepherd & Ditgen 2013; Canale & Ladio 2020; Puchi et al. 2021). Seed harvest remains central to the cultural identity and seasonal practices of Pewenche communities (Canale & Ladio 2020). Ecological functions include promoting faunal interactions, sustaining forest regeneration, and enhancing drought resilience in montane ecosystems (Puchi et al. 2021). Most genetic information for this species focuses on neutral genetic variation (e.g. Martín et al. 2014); there is limited understanding of adaptive genetic variation. While molecular markers have been used to estimate adaptive variation, these findings remain unconfirmed through trait analysis (Bekessy et al. 2003; Varas-Myrik et al. 2022). Here, we characterized among- and within-population variation in putatively adaptive plant traits across the range of pewen in Chile and related variation to climate and soil variables, which often drive large-scale patterns of differentiation in trees (Alberto et al. 2013).

Plants cannot escape environments in which they germinate. They adapt over time to local environmental conditions through natural selection, resulting in genetic and phenotypic differentiation across their ranges (Leimu & Fischer 2008; Anderson et al. 2011). Thus, population differentiation is widespread in plants (Leimu & Fischer 2008), occurring at spatial scales from meters (Lekberg et al. 2012) to hundreds of kilometers (Liepe et al. 2016; Supple et al. 2018). Population differentiation has been documented 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). Conversely, moving plants outside their range of local adaptation can reduce fitness and negatively affect adjacent populations through founder effects, genetic swamping, or outbreeding depression (Lesica & Allendorf 1999; McKay et al. 2005; Broadhurst et al. 2008). Understanding genetic differentiation among and within populations in key adaptive traits is essential for informing conservation and restoration efforts (Broadhurst et al. 2008; Breed et al. 2013; Gann et al. 2019).

Beyond characterizing patterns of population differentiation, research aims to identify environmental drivers that explain these patterns (Reich et al. 1997; Aitken & Whitlock 2013; Alberto et al. 2013). Climate gradients are commonly implicated (Alberto et al. 2013; Bower et al. 2014), as plant distributions are strongly influenced by climate (Webb 1986; Woodward 1987; Woodward et al. 2004). Provenance studies, which have been conducted for multiple centuries, show that climate variability can explain population differentiation depending on species, traits, and the magnitude of climate gradients (Alberto et al. 2013; Leites & Benito Garzón 2023). Soil variables can have an equal or greater role in driving population differentiation (Lekberg et al. 2012; Siefert et al. 2014; Gibson et al. 2019). In this study, we evaluate which climate and soil variables best explain multivariate genetic trait differentiation among populations.

The importance of maintaining genetic and phenotypic variation is increasingly recognized in species conservation and restoration strategies, especially under rapid climate change (Gann et al. 2019; Fremout et al. 2022; Nelson et al. 2024). Genetic variation is a record of past natural selection and the raw material for future adaptation to environmental change (Kramer & Havens 2009; Kremer et al. 2012). Understanding the distribution of variation within and among populations has practical implications for sourcing plant material for restoration. For example, in a study of the threatened species *Eucalyptus melliodora* in Australia, most genetic variation occurred within versus among populations, suggesting that seeds could be sourced broadly versus locally 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). Substantial research supports these case studies to show that for trees (particularly those that are wind pollinated), this pattern of population differentiation across large spatial scales 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 it remains unknown how species with restricted and fragmented ranges like *A. araucana* vary among and within populations.

Understanding drivers and spatial patterns of genetic and phenotypic variation is important for pewen given considerable interest in the restoration of this species. Although restoration programs are in progress (see Ipinza & Müller-Using 2021), lack of information on genetically based phenotypic variation (rather than neutral genetic variation, which has been largely resolved; see Bekessy et al. 2002a; Martín et al. 2014; Varas-Myrik et al. 2022) limits understanding of genetically appropriate material for restoration and the 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. This mortality adds urgency to the need for information on regional and population differentiation in relation to the environment for this species.

We implemented a common garden study to assess patterns of among- and within-population genetic variation of pewen across its range in Chile. When plants are grown in a common setting,

differences in phenotypical traits among different provenance locations are generally interpreted as genetic differences associated with natural selection in different environments of origin (see Leites & Benito Garzón 2023 for additional discussion); thus, we have described these phenotypic differences as "genetic variation" within this study. Our study is one of only a handful that addresses within-species genetic variation in a suite of traits rangewide in South American conifers (see Aparicio et al. 2010; Pastorino et al. 2010). For *A. araucana*, we build on previous phenotypic and genetic studies in this species (see Rafii & Dodd 1998; Bekessy et al. 2003; Martín et al. 2014; Varas-Myrik et al. 2022) to assess among- and within-population variation in a broad suite of traits and relate this variation to climate and soil variables. First, we assessed whether plants from different provenances show trait variation among or within populations and regions (Andes vs. coastal mountain ranges) (Q1). Given strong regional genetic differentiation and within-population variation found in previous studies (Bekessy et al. 2002a, 2002b; Martín et al. 2014; Varas-Myrik et al. 2022), we expected traits would differ by region (coastal vs. Andes ranges) but also retain substantial variation within regions and populations. Additionally, we assessed which plant traits drive overall differences in phenotypes among and within populations and regions (Q2). Because there is no information on adaptive traits in this species, we did not have expectations for which traits would vary most. Finally, we assessed which climate and soil variables drive overall differences in phenotypes among and within populations and regions (Q3). Given previous evidence that pewen differed regionally in carbon isotope discrimination in relation to precipitation (Bekessy et al. 2002b), we expected variables related to water availability to best predict trait variation.

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; Fig. 1). The range of pewen, although relatively small, spans substantial elevation (664–1227 m), precipitation (1100–2219 mm annually), and temperature (6.1–9.6°C mean annual temperature) gradients (Table S1). *Araucaria araucana* is a dioecious and wind-pollinated masting species (Sanguinetti & 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 pine nut-like seeds of pewen) are important for subsistence (Herrmann 2006). Pewen has been listed as "Endangered" on the International Union for Conservation of Nature (IUCN) Red List since 2011 (Premoli et al. 2015) due to historic deforestation (although it is now protected), 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 et al. 2015; Tella et al. 2016). Because of its significant climate gradient in Chile, pewen is an excellent study system to assess 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 S1, Fig. 1). Populations were located within five genetic clusters (two coastal, three Andean) identified by Martín et al. (2014) using a landscape genetic approach. Ten populations were located in the Andes. We were able to include only two populations in the coastal range, which is limited in this area due to fragmentation from agriculture and deforestation (see Section 4.5). Coauthors collected the seeds for this effort (Ipinza and Muller-Using 2021). Seeds from each site were grown in a common garden.

Seed Collection, Seedling Growth, and Trait Measurements

For each population, we collected seeds from trees that were at least 150 m apart and had produced 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 from nearby roads or trails because of convenient access (see Section 4.5). For each population, we collected seeds from 20 individuals, except in two populations (Lonquimay and Marimenuco), where seeds from only seven individuals were obtained. Additionally, one individual was not measured by accident in our common garden, reducing the sample size to 19 for this population (Table S1).

After cold stratification at 4°C for 2 months, we cut the end of each seed and submerged them in water for 2 days at 4°C. Seeds were planted in 125 mL pots containing composted pine bark substrate, put in plastic flats, and germinated in a commercial greenhouse in Yumbel, Chile (−37.098090, −72.562230) and then grown for 1 year. Seedlings experienced ambient light conditions and were well-watered (at least once and sometimes more than twice per day, depending on temperature). Temperature was not controlled within the greenhouse, but we assume all seedlings experienced similar temperatures. Seedlings were rotated throughout the growing period to limit greenhouse effects. Germination rate was measured at 30 weeks, and seedling survival was measured after 1 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 performance traits (e.g. growth and survival) commonly used in the literature to develop seed transfer zones for trees (Beaton et al. 2022; Leites and Benito Garzón 2023), as well as functional traits related to stress tolerance, architecture, and leaf economics that address resource use strategies across taxa (Wright et al. 2004; Reich 2014; Song et al. 2022). We intentionally selected traits that are measured without the use of specialized equipment so that our methods could be used by additional studies with the goal of informing restoration and

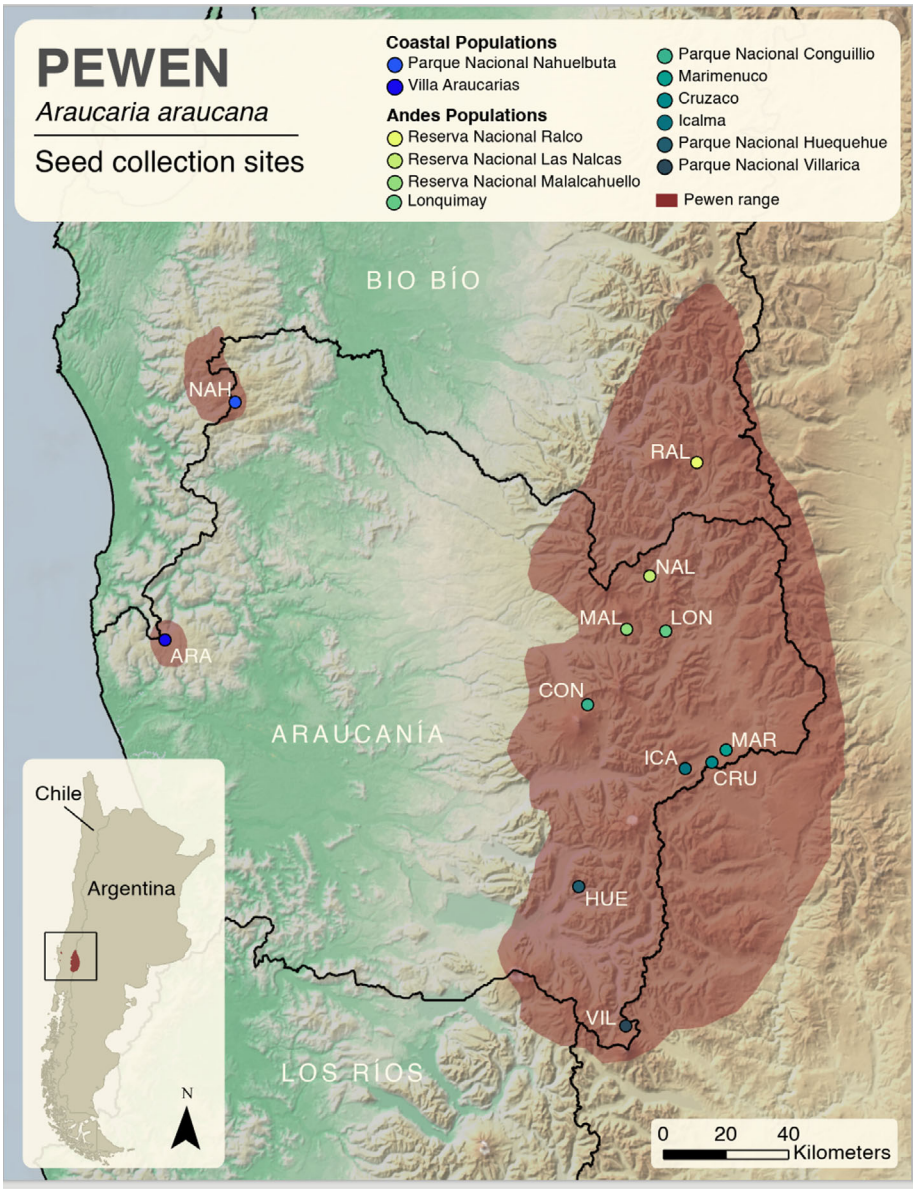

Figure 1. Map of seed collection sites in Chile. The range of pewen is shown in brown in the map and its inset. Study populations are indicated by dots, with colors corresponding to populations as used in subsequent figures. The common garden (CG) site (Yumbel, Chile) is labeled with a triangle. Codes for each population are presented in Table S1. Map created by Shira Ellenson.

conservation where resources are limited. For these types of projects, the specialization, time, and funding needed to measure physiological traits, which may more directly relate to resource use or stress tolerance, are limiting. Additional explanation for the selection of traits is included in Table S2.

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 maximum length and width of the three longest needles to calculate maximum needle lengths and widths (referred to as needle length and width throughout). Stem length was the distance between the differentiation of above- and belowground tissue to the tallest needle. Basal diameter was measured at the same point of tissue differentiation. For aboveground and belowground biomass, we weighed dried tissue. We measured needle area using the app LeafByte (Getman-Pickering et al. 2020) and calculated needle mass per area (dried mass per unit fresh area; leaf mass per unit area [LMA]). For needle density, we measured fresh needle volume using the water displacement method (Hughes 2005) and divided fresh volume by dry needle mass. Needle thickness was calculated by dividing fresh needle volume by dry needle area. Needle succulence was calculated by subtracting needle dry weight from needle fresh weight and dividing it by fresh needle area. Germination rate was the

proportion of seeds that germinated for a given maternal family, and survival was the proportion of seedlings that survived to 1 year. Additional descriptions of trait measurements and units are included in Table S2.

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 coordinates of each tree from which seeds were collected (see Table S3 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). Data resolution was 1 km. 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. Data resolution was 4 km. For SoilGrids data, we used Google Earth Engine to access variables listed in Table S3. Data resolution was 250 m. SoilGrid data were the only soil data available for our region of interest.

Statistical Analysis

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 two factors: region and population nested within region to address the relative contribution of region and population and to identify traits that varied among populations and should be included in additional analysis (Tables S1–S9). Assumptions of ANOVA (normality and homogeneity of variance) were checked using residual and normal quantile plots. For count traits only (number of whorls and number of branches, which both contained zeros), we used a generalized linear model using function lme4 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 because our analyses sought to identify and emphasize the ways in which populations differed (see Tables S2 & S4 for a list of the eliminated traits).

To address multivariate trait differences among regions and populations (Q1), we used principal coordinates analysis (PCoA) with Gower distances using the daisy() function in the cluster package in R (Maechler et al. 2025). A Gower distance matrix was used to account for mixed data types (continuous trait data and count data zeros). A PCoA was used to address the non-Euclidean nature of Gower's dissimilarity matrix (Gower 1971, 2015). We used multiple regressions with PCoA scores as response variables and traits as predictors to address which traits best explained overall differences in phenotypes (Q2). A separate model was created for each of the first two PCoA axes (which explained 78% of variation; axes where the eigenvalues less than 1 were not used following the Kaiser Rule). 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 $p \geq 0.05$ (Table S5). We then excluded traits that covaried with other traits using the cutoff of $|r|$ greater than 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 version 2024.12.0 + 467 (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 PCoA axis using climate and soil variables as predictors. For each of the first two PCoA axes, we used Spearman's r to identify the traits most correlated with each axis where $|r| \geq 0.20$ and $p \geq 0.05$ (Table S5). We used backwards selection to remove additional traits that did not add predictive power to the model ($p > 0.05$; Table S6). Overall contribution of climate variables in explaining trait variation across axes was assessed using permutational multivariate analysis of variance (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 ArcGIS Pro.

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 (Table 1; Fig. 2). Across the 15 measured traits, 11 differed significantly to varying degrees among regions and populations (Table S4; Fig. 2A). Generally, traits related to growth did not differ among populations or regions, while traits related to plant architecture and needle economics did. Across all retained traits, regions were highly distinct (Fig. 2B), explaining 11.8% of overall trait variation. Populations within each region also varied significantly in their traits, with population accounting for 22.3% of overall trait variation.

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 PCoA1, the number of whorls, needle length, and LMA explained overall trait variation (adjusted $r^2 = 0.94$, $p < 0.001$; Table 1). The number of branches and branch length were both highly correlated with the number of whorls (and thus not included in the model; Tables S5 & S7) and showed similar patterns among populations as the number of whorls (shown in Fig. 2C). For PCoA2, needle succulence, branch length, and needle length best explained overall trait variation (adjusted $r^2 = 0.81$, $p < 0.01$; Table 1, Fig. S1). No other traits that were not

Table 1. Multiple regression models for traits that explain variation in the first two PCoA axes. Additional traits correlated with these traits that were not included in the model are shown in Tables S1–S9. Preliminary full models before elimination of variables using backward selection are shown in Table S3. Est., estimate; SE, standard error.

	Coefficient	*Est.*	*SE*	t	$p_{coeff.}$	*Adj.* r^2	F (df)	p_{model}
PCoA1 (62%)	Intercept	-2.7×10^{-2}	2.0×10^{-2}	−11.0	<0.001	0.93	985.3 (3, 193)	<0.001
	Number of whorls	-1.5×10^{-2}	4.5×10^{-3}	−33.3	<0.001			
	Mean needle length	5.1×10^{-2}	3.9×10^{-3}	13.0	<0.001			
	LMA	1.2×10^{-1}	8.9×10^{-3}	13.2	<0.001			
PCoA2 (16%)	Intercept	-4.3×10^{-1}	1.6×10^{-2}	26.9	<0.001	0.82	268.2 (3, 192)	<0.001
	Succulence	7.3×10^{-2}	3.5×10^{-3}	20.6	<0.001			
	Mean branch length	1.3×10^{-2}	6.2×10^{-4}	20.8	<0.001			
	Mean needle width	1.6×10^{-2}	2.2×10^{-3}	7.1	<0.001			

collinear with needle width were correlated with this axis (where $|r| > 0.20$).

The first two PCoA axes primarily differentiated Andes and coastal populations (regions) in their traits (Fig. 2B). On average, compared to Andes populations, coastal populations had more whorls and branches (nearly twice as many). Branches were on average 1.5× as long (Table S7; Fig. 2C). The number of branches and branch length show similar patterns among regions and populations as the number of whorls (shown in Fig. 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 (Fig. 2D). Needle trait effect sizes were smaller compared to branch architectural traits (Table S7).

Temperature Annual Range and Vapor Pressure Deficit Best Explained Overall Region and Population Trait Differences (Q3)

Overall trait differences between regions and among populations (variation in PCoA1 and PCoA2) were best explained by temperature annual range (TAR), the difference between maximum temperature in the warmest month and minimum temperature in the coldest month, and mean vapor pressure deficit, a measure of air "dryness" (Table 2). However, much trait variation remained unexplained by environmental variables.

For PCoA1, TAR explained 17.8% of the variation in PCoA1 scores ($p < 0.001$, Table 2). Our initial model included mean soil water equivalent, but this was not significant in the model (Table S8). Variation in PCoA2 was best explained by mean vapor pressure deficit (adjusted $r^2 = 0.112$, $p < 0.001$, Table 2). For PCoA2, our initial model included mean maximum temperature, but this was also not significant in the model (Table S8).

TAR explains 13.4% of all trait variation ($p = 0.001$) and varies significantly among regions and populations (Table S9; Fig. 3). Additional climate and soil variables that covaried with TAR ($|r| > 0.60$) were not included in the multiple regression models (Table S5). Coastal populations tend to have smaller TARs than Andes populations (Fig. 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, $p < 0.001$).

Mean vapor pressure deficit explains 11.5% of overall trait variation ($p = 0.001$) and varies significantly among regions and populations (region and population explain >98% of variation in mean vapor pressure deficit). Coastal populations tend to have lower vapor pressure deficits (0.34 ± 0.001 kPa) than do Andes populations (0.53 ± 0.003 kPa), consistent with the more moderate coastal climate.

Discussion

Our findings contribute to the growing research on among- and within-population variation in trees. We asked how populations across the range of *Araucaria araucana*, 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. To our knowledge, our study assesses the largest number of populations and traits in a common garden of any study of this 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 partly explained by climate variables. TAR, which explained the most trait variation, also explains putatively adaptive genomic differentiation in this species (Varas-Myrik et al. 2022). Our results highlight the importance of conserving genetic variation among and within regions, informing conservation strategies and seed sourcing guidelines for restoration. Our results are complemented by the work of Varas-Myrik et al. (2024), which suggests using genomic approaches to identify pre-adapted genotypes for assisted migration in Andes populations.

Pewen Shows Differentiation Between Regions and Populations, With High Within-Population Variation

Consistent with expectations, we found clear differentiation in traits between regions. Traits that explained the most differentiation among populations were related to branch architecture and leaf economics. Coastal populations generally had smaller, less succulent leaves and more branches, while Andes populations had larger, more succulent leaves and fewer branches. Coast–

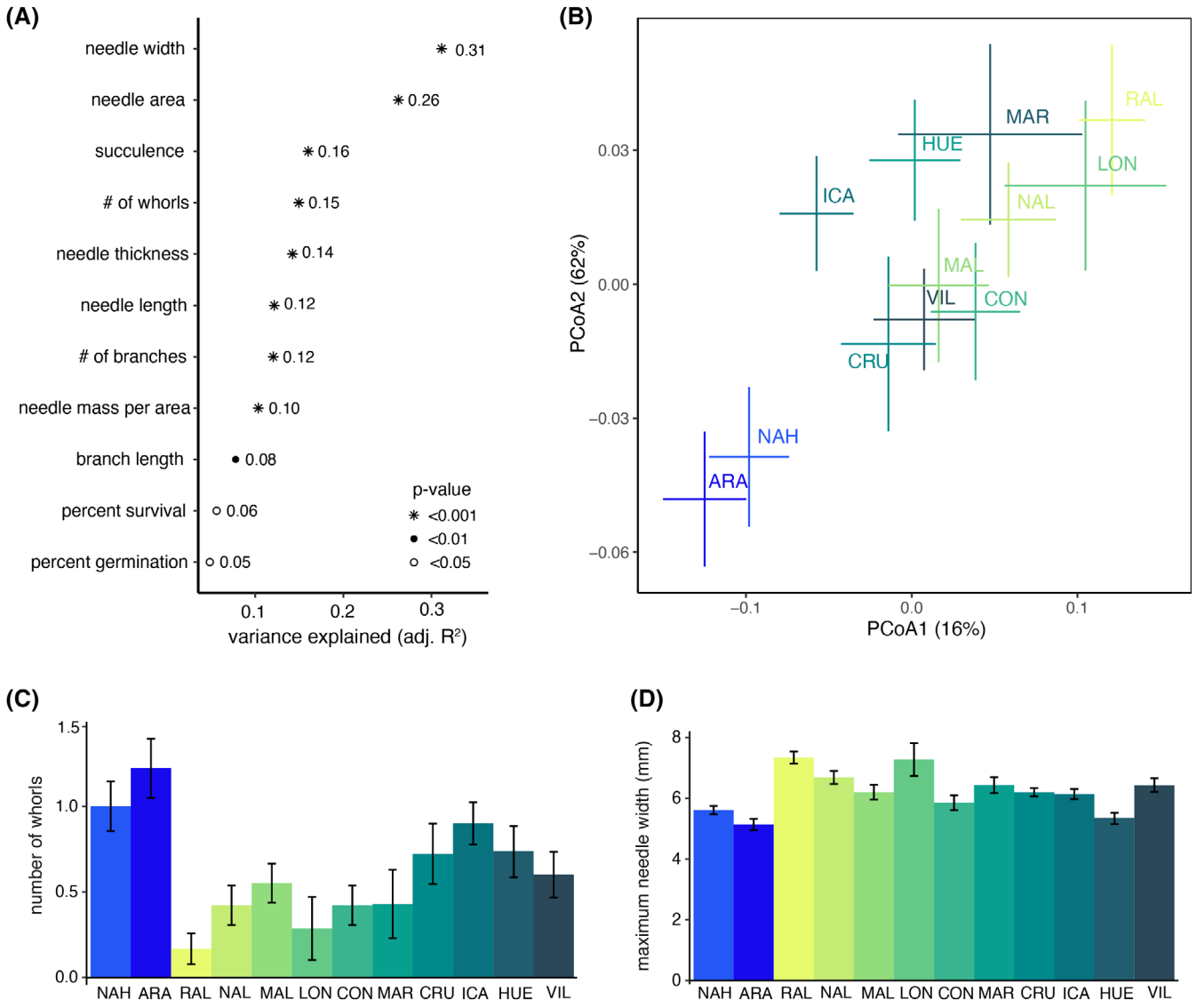

Figure 2. (A) A suite of traits varies significantly among regions and populations across the range of pewen in Chile. Adjusted r^2 values (numbers) and p values (symbols, see legend) for ANOVA models of plant traits by populations indicate variance explained by and statistical significance of each trait assessed. Only traits for which p less than 0.05 are included (see Table S2 for excluded traits). See Table S2 for trait units. (B) Traits of pewen vary by region and population. Mean PCoAA scores for PCoAA axes 1 and 2 (percent variation explained in parentheses) for each population (three-letter codes, see Table S1, Fig. 1). Bars show ± SE. (C & D) 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 color gradients by latitude from north (light) to south (dark). Population codes are printed (see Table S1 for more information on populations).

Table 2. Multiple regression models for environmental variables that explain variation in the first two PCoA axes. 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 eliminating variables using backwards selection are shown in Tables S1–S9. mean VPD, mean vapor pressure deficit (kPa); TAR, temperature annual range (°C).

	Coefficient	*Est.*	*SE*	*t*	p_{coeff}	*Adj.* r^2	*F* (df)	p_{model}
PCoA1 (62%)	Intercept	-7.0×10^{-1}	1.0×10^{-1}	−6.7	<0.001	0.18	45.72 (1, 211)	<0.001
	TAR	3.0×10^{-3}	4.4×10^{-4}	6.8	<0.001			
PCoA2 (16%)	Intercept	-1.3×10^{-1}	2.7×10^{-2}	−4.8	<0.001	0.10	24.16 (1, 211)	<0.001
	Mean VPD	2.7×10^{-1}	5.5×10^{-2}	4.9	<0.001			

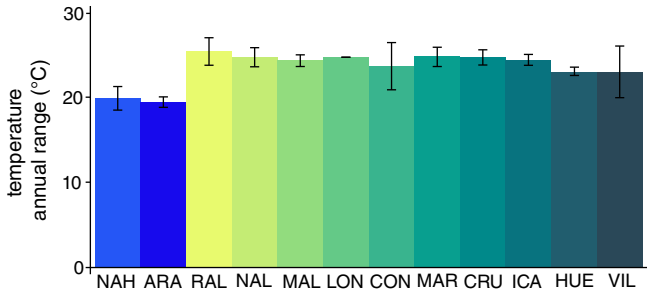

Figure 3. Temperature annual range (TAR, °C) varies significantly among regions and populations. Bars show ± SE. Color categories correspond to region (green = Andes, blue = coast), with color gradients by latitude from north (light) to south (dark). Population codes are printed (see Table S1 for more information on populations).

Andes region differences were best explained by TAR and vapor pressure deficit, with higher and lower temperature extremes and vapor pressure deficit 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 differentiation should be considered in plant material selection.

Regional Differentiation and High Within-Population Variation Are Consistent With Previous Assessments of Phenotypic and Genetic Variation in Pewen

Our results are consistent with our expectations and two previous assessments of trait and genetic variation in pewen, which also showed differentiation between coastal and Andes regions and high within-population variation. A phenotypic study of alkenes in foliar epicuticular wax, which contribute to reducing cuticular water loss, revealed differences between coastal and Andes populations (Rafii & Dodd 1998). Although only four populations were used, these authors 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 the variation in carbon isotope discrimination and 14% of the variation in root: shoot ratio was explained by region (coast, Chilean Andes, Argentinian Andes; Bekessy et al. 2002b). These patterns were also corroborated by a study of neutral genetic variation, which found 16% of total variation explained by the region (coast vs. Chilean Andes; Martín et al. 2014, Varas-Myrik et al. 2022). 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, although Varas-Myrik et al. (2022) propose possible divergent effects due to local adaptation. 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. Similar patterns of regional variation were seen in other South American conifers (*Fitzroya cupressoides* and *Austrocedrus chilensis*), likely as a result of glaciation (Pastorino & Gallo 2009; Aparicio et al. 2010; Arana et al. 2010). We also found significant variation unexplained by region or population (78% and 89% for PCoA1 and PCoA2, respectively; 69–95% depending on the trait). In other studies, unexplained trait variation is commonly assumed to be variation maintained within populations (see Section 4.5 for further discussion). Within-population variation may be highly important for adaptation to future climate 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 common for forest trees (Kremer et al. 2012; Alberto et al. 2013). For *A. chilensis*, another South American conifer, two studies of genetic variation in growth traits across precipitation and aridity gradients found little population differentiation but substantial variation within populations (Aparicio et al. 2010; Pastorino et al. 2010). Genetic marker studies identified a large population across most of the species' range, and smaller, more fragmented, and differentiated populations in its eastern margins (Arana et al. 2010). For a small section of the range of wind-pollinated 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* was differentiated between regions and showed significantly higher within-population variance compared to among-population variance (Sorensen & Weber 1994). In addition, similar patterns of high regional differentiation and high within-region variation in morphological, phenological, and physiological traits were found in two Northern hemisphere spruces (*Picea glauca* and *P. engelmannii*) and lodgepole pine (*Pinus contorta*) (Liepe et al. 2016).

Temperature Annual Range and Vapor Pressure Deficit Best Explain Overall Trait Variation

Although TAR and vapor pressure deficit explained a limited amount of overall trait variation (13.4 and 11.5%, respectively), this is a substantial variation for single climate variables. These findings are consistent with another study, where TAR best explained genomic differentiation (Varas-Myrik et al. 2022). In conifers, relationships between genetic trait variation and the environment, specifically temperature and precipitation-related variables, are extremely common (Leites & Benito Garzón 2023). 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 PCoA 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. Seedlings growing in Andes populations experiencing significant frosts (particularly those in the northern part of the range) may be unable to support large branches due to potential frost damage. Temperature maximums could explain regional differences in leaf succulence, with drier Andes populations having increased succulence (and leaf size) to store water under drought conditions. Greater leaf succulence may also represent an adaptation to higher vapor pressure deficits of the Andes region, as succulence can increase a plant's ability to maintain physiological function during drought by reducing desiccation (Guo et al. 2024).

Limitations

We note several potential limitations of our study. First, the selection of adult trees from which seeds were collected was not randomized, as seed collection was limited due to the availability of seed and access. However, our minimum distance between trees (150 m) was greater than that of other studies (50 and 100 m; Rafii & Dodd 1998, Bekessy et al. 2002a, 2002b) and our collection sites within populations varied considerably with respect to topography and microclimate. We do not feel that this limitation has biased results.

Second, we were only able to collect seeds from two coastal populations (compared to 10 Andes populations). The two sampled coastal populations represent the majority of the remaining populations in the coastal region of Chile, where the range of *A. aruacana* is extremely fragmented. However, the analyses we conducted where region was included are robust to differences in group sizes. ANOVA is robust to moderate departures from balanced design, especially when variances are equal, and sample sizes are not extremely unequal (Quinn & Keough 2002). Similarly, mixed models are well suited for analyzing data from unbalanced designs, which frequently occur in ecological data (Zuur et al. 2010). Additionally, other studies found substantial differences between coastal and Andes regions.

Third, we did not replicate within families (trees) in our populations and, therefore, cannot differentiate between within-population variation and error (although it is common practice in the literature to attribute variance unexplained by population to within-population variation; see Alberto et al. 2013). If possible, future studies should further replicate within families to account for within-population variation.

Fourth, there is no information on which plant traits might be adaptive for this species, so we selected traits related to performance that are commonly measured in conifer provenance studies (e.g. height, survival, growth, biomass, and basal diameter; Song et al. 2022), traits related to the leaf economics spectrum (e.g. needle length, width, mass per unit area, and succulence), or traits related to plant architecture (e.g. branch length and number of branches or whorls). Many of these are commonly measured in studies using phenotypes to delineate seed transfer zones (Johnson et al. 2013; Gibson et al. 2019; Beaton et al. 2022, Song et al. 2022). Additionally, because our study addresses patterns of variation relevant to restoration, we selected traits that were easily measured without expensive equipment, thus making them accessible for future studies with this aim. Because we don't have information on reproductive or survival outcomes (beyond survival at 1 year), we cannot necessarily conclude that the variation we identified is adaptive. This is a limitation of studies of trait variation and seed transfer in general. The work of Varas-Myrik et al. (2022) uses a genomic approach to develop an initial approximation of adaptive variation, which could be complemented by the traits informative in this study.

Finally, a limitation of many common garden studies is maternal effects (Gibson and Nelson 2017), 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. 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) share this limitation.

Considerations for Management

As ecological restoration commitments ramp up in Chile and beyond, developing science-based resources to guide the selection of plant materials is key to maximizing outcomes. In Chile, the lack of genetically appropriate seed supply for restoration is a barrier to achieving restoration goals. Here, we provided trait-based information to complement information on patterns of genomic differentiation and assisted migration being developed by colleagues to guide restoration for this species. Our work sets the stage for developing seed transfer zones for this species, maps that identify ecotypes to guide seed sourcing for restoration. Seed transfer zones will directly build capacity for restoration in Chile, where managers can put them to use. Our results, however, among the other studies referenced above, suggest that regional and population variation should be considered in restoration efforts for this species. We recommend that regional and, if possible, local seed sources be used for restoration sites where this species is being seeded or planted, to avoid potential issues with maladaptation of seeds to restoration sites. Additionally, we recommend collecting widely from different individuals within sites to capture within-population variation and maximize genetic diversity. Future studies could assess the efficacy of this approach.

Acknowledgments

We acknowledge the Sistema de Monitoreo de Ecosistemas Forestales Nativos and the Instituto Forestal in Chile for financial and logistical support. We are grateful to our Mapuche Pewenche partners, who contributed to seed collection for many of our populations. Finally, we thank Compañía Manufacturera

de Papeles y Cartones (CMPC) and the Carlos Douglass Nursery for their assistance in germinating and growing seedlings. We greatly appreciate the support of Dr. P. S. Delgado and her lab for providing lab space for data collection. M.M. was supported by National Science Foundation DGE-184053 and additional scholarships from the University of Montana Franke College of Forestry and Conservation.

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Supporting Information

The following information may be found in the online version of this article:

Table S1. Collection sites covering the range of pewen in Chile vary in altitude and climate variables.
Table S2. Traits measured in common garden seedlings.
Table S3. Bioclimatic and soil variables used for multiple regressions with trait PC scores extracted for the latitude and longitude coordinates of each tree from which seeds were sampled.
Table S4. ANOVA tables for traits that varied significantly among regions or populations.
Table S6. ANOVA outputs from full PC score-trait models before nonsignificant traits were excluded.
Table S7. Means ± standard errors for key branch and needle traits that are differentiated regionally.
Table S8. ANOVA outputs from full PC score-environmental variable models before nonsignificant traits were excluded.
Table S9. ANOVA model outputs for differentiation in temperature annual range (TAR, °C) by region and population.
Table S5. Correlations between PC scores and traits or environmental variables.

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Received: 29 December, 2024; First decision: 25 March, 2025; Revised: 4 November, 2025; Accepted: 10 November, 2025