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Original Article

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Common garden experiments and SNP genotyping at the extremes of a steep precipitation gradient suggest local adaptation in a Patagonian conifer

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ABSTRACT

Plants inhabiting contrasting physical conditions might develop local adaptations overriding the homogenizing effects of gene flow. Hypotheses of local adaptation on phenotypic, genomic, and environmental variation under extreme precipitation regimes were tested in the Patagonian conifer *Austrocedrus chilensis*. Common garden experiments on progeny and genotype-to-environment association analysis on adults were conducted. Samples consisting of seeds and leaves from adult trees were collected from contrasting dry (DF) and humid (HF) forests along a steep but short precipitation gradient. Seeds were germinated and seedlings were grown under common garden conditions for 24 months. DNA was extracted from 75 randomly selected trees from DF and HF, and genotyped by sequencing to obtain single-nucleotide polymorphisms (SNPs). Seedlings from HF outgrew DF ones suggesting genetically based differences. Twenty-four outlier SNP loci differed between DF and HF, whereas neutral genes (3242) showed high levels of admixture. Eight out of the 24 outlier SNPs aligned with transcripts, half of them related to drought stress responses, and principal component analysis identified four precipitation-related marker–climate associations. Quantitative and genomic traits suggested that natural selection maintains divergence under contrasting climatic conditions regardless high gene flow. This underscores the importance of dry forests as reservoirs of drought-tolerant variants to cope with forecasted climate change.

Keywords: *Austrocedrus chilensis*; dryland forests; genotype-to-environment association; phenotypic traits

INTRODUCTION

Drylands cover about 41% of the Earth's land surface (Sorensen 2007), with more than half of dryland forests (52%) located in the least arid regions such as inland South America (Spinoni *et al.* 2021). In particular, drylands of Patagonia consist mainly of semi-arid and dry subhumid zones (Paruelo *et al.* 1998) and contain 10-fold greater tree canopy cover than those of the hyperarid zones (FAO 2019). Yet, forested dryland areas are understudied, despite the fact that woodlands provide numerous ecosystem services such as biodiversity habitats, valuable shade, and wood products, as well as protection against water and wind erosion and desertification (Newton and Tejedor 2011). Drylands have expanded significantly over the last 60 years and are predicted to keep to this trend (Feng and Fu 2013). Thus, dryland forests can increase landscape resilience particularly under global change trends (FAO 2015).

In the face of climate projections, it has become crucial to investigate how natural populations will respond to changing environments. Increased severity, duration, and frequency of drought events are important manifestations of climate change (Dai 2013, Haslinger *et al.* 2016). These processes might have global effects on a wide range of forest ecosystems (Allen *et al.* 2010, Bradford *et al.* 2021, McNellis *et al.* 2021) including Patagonian temperate forests (Suarez *et al.* 2004, Suarez and Kitzberger 2008, Rodriguez Catón *et al.* 2016). For trees, drought is a major limitation for establishment, development, growth, and survival, especially in long-generation tree species (Adams and Kolb 2005). Moreover, the increased tree mortality observed, and forest dieback as consequences of large-scale worldwide drought periods raised concerns on how, and if, forests will cope with climate change (Batllori *et al.* 2020, Ogaya *et al.* 2020, Senf *et al.* 2020, Gazol and Camarero 2022).

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Ongoing climate change impose novel selection regimes to populations, and particularly sessile species as woody taxa, may adjust through distinct mechanisms to elude local extinction (Anderson *et al.* 2012). They may either disperse to suitable habitats elsewhere or they can respond locally by phenotypic plasticity and/or through genetically based adaptation to the changed conditions (Gienapp *et al.* 2007). Evidence of local adaptation to climate exist in many trees even under high gene flow (Aitken *et al.* 2008). However, microevolutionary responses may occur in relatively short time-scales to cope with the velocity of climate changes. Therefore, due to time constraints, species with long life cycles cannot rapidly adapt through the accumulation of novel mutations (Potvin and Tousignant 1996). Instead, adjustments via changes in allele frequencies or genotypic recombination, might be the key process for local rapid adaptation, stressing the relevance of substantial intraspecific genetic variation to adapt to unprecedented conditions (Bell and Gonzalez 2009, Anderson and Song 2020).

Different approaches can be used to disentangle potential responses to variation in climate that may include common garden and molecular genetic change studies (Merilä and Hendry 2014) or a combination of them. Common gardens are powerful tools to study tree adaptation to climate (Capblancq *et al.* 2023), and large genomic datasets are increasingly being used to analyse local adaptation (Savolainen *et al.* 2013; Whitlock and Lotterhos 2015) to identify genes of known adaptive value and their divergent trait expression (Wadgymar *et al.* 2017), and the association of genetic-environmental variation along gradients in a landscape genomics framework (Sork *et al.* 2013, Tiffin and Ross-Ibarra 2014, Hoban *et al.* 2016, Martins *et al.* 2018). Combined analysis of quantitative, genomic, and climate associations has provided evidence of adaptation in diverse tree species as *Eucalyptus microcarpa* (Jordan *et al.* 2020), *Picea rubens* (Capclancq *et al.* 2023), and *Nothofagus dombeyi* (Diaz *et al.* 2022). In sum, study designs applying experimental gardens and genomic data are widely used to explore the evolution of local adaptation in plant populations (Sork 2017).

In particular, common garden experiments allow the exploration of the degree of phenotypic plasticity and/or the evidence for local adaptation of long-lived species. Several authors found significant regional differentiation, sowing seeds from opposing environments under homogenous conditions and measuring traits related to growth, survival as well as whole plant and leaf morphological traits (Alía *et al.* 1997, Correia *et al.* 2008, Ignazi *et al.* 2020) at early stages of their development (de Villemereuil *et al.* 2016, Berend *et al.* 2019, Schwinning *et al.* 2022). Also, the broad availability of detailed climatic data offers valuable information on the potential intraspecific variation of climatic adaptation, which is essential to understand species' responses to climate change. This in combination with next-generation sequencing and single-nucleotide polymorphisms (SNPs) provide the opportunity to investigate signs of local adaptation and identify candidate genes under selection (Nadeem *et al.* 2018). For example, an association between a SNP and aridity may indicate that the gene or its regulatory region affect tree performance in dry versus wet environments (Eckert *et al.* 2010a, b). Since SNPs are abundant and spread over the entire genome, they can be associated with non-synonymous substitutions in coding regions, changes in the transcription level of functional genes, and in the regulation processes underlying adaptive phenotypes (González-Martínez *et al.* 2006, Sork *et al.* 2013). The large economic and ecological interests of the gymnosperms provide the necessary incentive to try and tackle these complex genomes, which markedly differ from leaf-bearing species in their massive size (Nystedt *et al.* 2013).

Under novel climatic regimes, intraspecific genetic variation in drought response is expected to play an important role in determining tree populations' persistence, as it allows for spontaneous selection and local adaptation. Estimating the potential of such adaptation requires quantitative genetic knowledge of drought sensitivity across significant parts of species distributions including environmentally extreme conditions of ecologically dominant tree species. Dry transition forests of northern Patagonia are dominated by the conifer *Austrocedrus chilensis* (D. Don) Florin & Boutelje, hereafter *Austrocedrus*. Eastern-most dry margin populations have been considered of lesser value and genetically impoverished due to genetic bottlenecks acting on isolated populations. Nonetheless, these marginal populations of *Austrocedrus* have shown both unique variants and increased genetic diversity at neutral loci, despite high gene flow via wind pollen and seed dispersal (Arana *et al.* 2010, Souto *et al.* 2012). So, the hypothesis that eastern-most dry margin populations consist of individuals holding potentially adaptive traits maintained by selection was tested. These traits might be of great value for the long-term persistence of tree populations particularly under forecasted increased droughts in Patagonia.

This study aims to investigate the genetic basis of phenotypic variation by means of early growth of progeny, and genomic variability of adult *Austrocedrus* trees at the extremes of a steep precipitation gradient. Steep gradients may serve as natural 'laboratories' for studying the response of species to environmental changes, including climate change. The study also aims to identify candidate genes and genotypes that are adapted to expected future climatic conditions, which can be of value for restoration and assisted migration trials.

MATERIALS AND METHODS

Study species

Austrocedrus is one of the three native monotypic genera of the Cupressaceae family, and occurs on both slopes of the Andes, in Argentina and Chile between 32° 39′ S and 43° 44′ S, respectively. This tree species is dioecious, although occasionally diclino monoecious, with wind-borne pollen and winged-seed dispersal although 95% of seeds disperse < 43 m from a given mother tree (Markgraf *et al.* 1981, Kitzberger 1994). *Austrocedrus* experiences Mediterranean-type climates, from mean annual precipitation (MAP) > 1700 mm in the Andes to < 400 mm in the Patagonian steppe of Argentina (Donoso 1982, Souto *et al.* 2015), and up to 6 months of summer water deficit, reflecting its drought tolerance (Veblen *et al.* 1995). In areas of higher rainfall (west of the Andes) *Austrocedrus* forms continuous mixed forests with *Nothofagus* species. In xeric environments, particularly on the eastern slopes, *Austrocedrus* forms mostly scattered populations in rocky outcrops, being almost the only tree species inhabiting such microsites of low productivity. *Austrocedrus* occupies mild topographic positions such as northerly aspects, and it is virtually absent from cold-air drainage valley bottoms, as it is intolerant of cold frost-prone conditions, making it a species with relatively mesothermal requirements (Donoso 1982). *Austrocedrus* is listed as Vulnerable with high risk of extinction (IUCN 2014). It is the only conifer of the steppe-forest ecotone on drier eastern slopes of the Andes, and is considered a keystone element of this temperate dry ecosystem.

This study was carried out in *Austrocedrus*' central range (40 to 42° S latitude) in north-western Patagonia, Argentina. The sampling scheme was based on two previously published papers (Souto *et al.* 2012, 2015) that showed the central range of *Austrocedrus* as a genetic unit, subdivided in east and west, associated with dry (DF) and humid (HF) forests, respectively. Thirty-seven populations were sampled along this range at the extremes of a steep precipitation gradient. Towards the eastern-most dry edge, DF occurs as naturally scattered populations, with mean annual precipitation (MAP) of 706.47 mm, and mean annual temperature (MAT) of 7.48°C. Meanwhile, HF are found westward in continuous mesic stands under MAP of 932.33 mm and MAT of 7.64°C. At this latitude, these climatic conditions are the result of westerly air masses that bring substantial amounts of moisture, which is deposited as rainfall on the windward and adjacent Andean forested areas (hereafter, humid forests, HF), while more eastern extra-Andean forests and woodland experience drier conditions due to the rain shadow effect of the Andean Cordillera (dry forests, DF) (Fig. 1).

Environmental characterization

Climatic characterization of environmental conditions of sampled *Austrocedrus* populations sampled was performed by using a combination of 72 monthly, seasonal, and annual variables: 30 variables generated with the ClimateSA v.1.0 software package (available at http://tinyurl.com/ClimateSA) based on the methodology described by Hamann *et al.* (2013), 19 bioclimatic variables with 30 arc-second spatial resolution from WorldClim 2 (www.worldclim.org; Fick and Hijmans 2017), and 16 environmental variables from the ENVIREM database (Title and Bemmels 2018) representing summaries of means and variation in temperature and precipitation, and seven soil quality variables from the Harmonized World Soil Database v.1.2 (http://www.fao.org/soils-portal/data-hub/soil-maps-and-databases/harmonized-world-soil-database-v12/en/; Fischer *et al.* 2008). All variables were standardized. To reduce our 72-trait dataset to an even more parsimonious representation, a principal component analysis (PCA) was conducted on a norotated variance-covariance matrix using STATISTICA v.10 software (StatSoft 2011; StatSoft, Inc.) (Supporting Information, Table S1).

Cultivation under common garden

To assess genetically based morphological differences between provenances, seeds were collected during April–May 2020 from five populations under contrasting precipitation regimes located in dry and humid extremes. The seeds were obtained from 5 to 10 randomly selected mother trees from each location (Fig. 1; Supporting Information, Table S2). Seeds were physically scarified with sand and were kept under cold humid stratification for 40 days until seedlings started to emerge. During mid spring in October 2020, 224 seedlings from three DFs and 91 seedlings from two HFs were individually placed in 0.35 L pots, properly tagged, and identified by site. Each seedling was cultivated using a mixed substrate containing vermiculite and natural soil. Randomly arranged seedlings were grown in a naturally lighted greenhouse at the Instituto de Investigaciones en Biodiversidad y Medio Ambiente in Bariloche (INIBIOMA-UNCOMA-CONICET), under the same regional climate of sampled provenances. The pool of 315 seedlings were used to monitor plant mortality, calculated as the percentage of seedlings from the total number of emerging seedlings in October 2020 that had died by October 2022. In winter 2021 a randomly selected set of 55 and 52 seedlings from DF and HF, respectively, were transplanted to 6 L pots that were used for morphological measurements under common garden conditions. During the growing seasons, seedlings' traits were measured four times, when seedlings were 6, 18, 20, and 24 months old. Variables measured were: seedling height and width measured with a ruler (error 1 mm), number of branches, and basal stem diameter measured with a digital electronic calliper 723Z-6/150MM (Starrett, USA). Shoot and root dry biomass were quantified twice on two randomly selected set of 12 month-old (first growing season) ($N = 17$ and 11, from DF and HF, respectively) and 24 month-old seedlings (second growing season) ($N = 10$ from DF and 10 from HF). This rather small sample size was used to avoid seedling´s massive destructive methods given that plants are being used in follow-up drought manipulative trials and reciprocal transplant experiments. Plants were oven dried for 120 h at 66°C and weighted using a digital balance (Mettler AJ150) with precision of 0.0001 g. Additionally, before oven drying, all these seedlings were measured their root and shoot length with a ruler (error 1 mm). To explore the explanatory power of morphological variables of seedlings in predicting their provenance hydric environment (DF vs. HF) *k*-means clustering based on four morphological variables (seedling height, seedling width, number of branches, and basal stem diameter) were performed, based on measures from 81 seedlings at 24 months old. Each of our 81 observations was assigned to either one of two clusters based on the nearest cluster centroid. This classification was evaluated with the observed provenance using a confusion matrix and calculated the Accuracy and the Kappa index of reliability.

Sample collection and SNP calling

To perform genotype-to-environment association (GEA) fresh leaf tissue from a total of 75 randomly selected adults of *Austrocedrus* were sampled, 45 of which were collected from DF and 30 from HF (Fig. 1). Vouchers of individuals of sampled extreme populations were deposited in the Herbario de Plantas Vasculares de la Universidad Nacional del Comahue, Argentina (Fasanella_1 and Fasanella_2, BCRU). Total genomic DNA from 100 mg of fresh leaves was extracted from each individual following a modified protocol from Doyle and Doyle (1990). The concentration of extracted DNA was quantified using a Qubit spectrophotometer (Thermo Fisher Scientific), and DNA integrity was assessed by electrophoresis in 1% agarose gels at INIBIOMA-UNCOMA-CONICET. Single-end sequencing generated 100 base-pair reads on a single lane of an Illumina HiSeq 2000 flow cell. Library preparation and

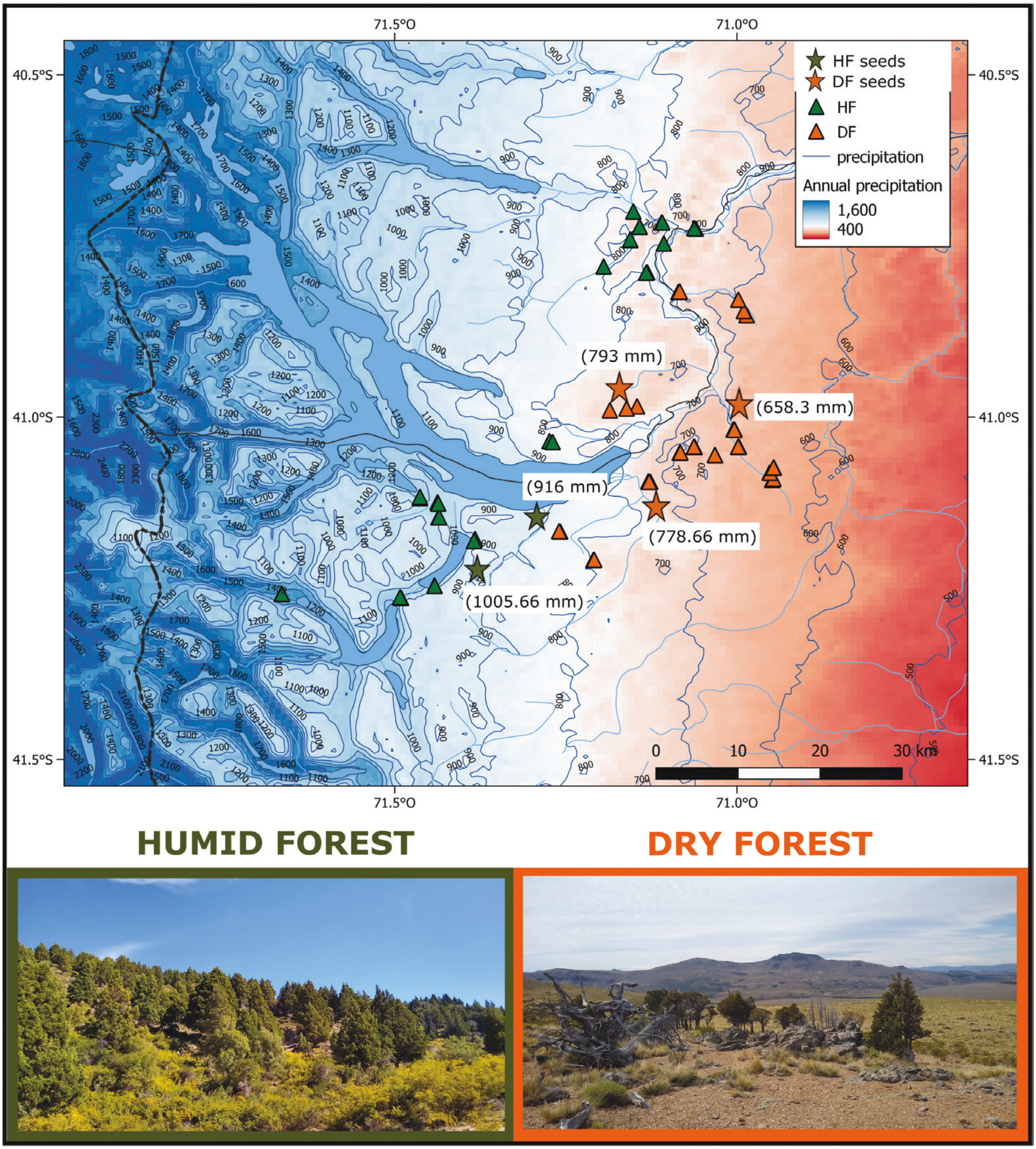

Figure 1. Sampling locations of *Austrocedrus* individuals collected from dry (DF in orange) and humid forests (HF in green) in northern Patagonia. Stars depict seed collection sites and their corresponding mean annual precipitation (MAP) are shown in brackets. Isolines represent MAP. Photographs illustrate that HF and DF are identifiable in the field.

high-throughput GBS (Genotyping-by-Sequencing) were performed at the University of Wisconsin Biotechnology Center (DNA Sequencing Facility, Madison, WI, USA). Raw data bases consisted of 25.9 Gb sequences from 75 individual samples that yielded 413 millions of 100 base-pair-long reads. *De novo* assembly of the sequences was performed using the genomic analysis tools available in UNEAK pipeline (Universal Network Enabled Analysis Kit) included in TASSEL v.5.0 (Trait Analysis by aSSociation, Evolution, and Linkage; Bradbury *et al.* 2007). Base calling was performed in Casava v.1.8 (Illumina, San Diego, CA, USA). The UNEAK pipeline works by first trimming reads to 64 base pairs and collapsing identical reads into tags. This pipeline does not depend on a reference genome, so is suitable for *Austrocedrus*. SNPs genotypes were configured using the enzyme APeKI, with error parameters of 3%, a minimum of five coverage depth, and zero minimum frequency of alleles. After trimming low quality reads (QC < 30), 208 061 non-redundant SNPs were identified with 57.7% missing data and 12.65 average coverage depth (range 10.91–15.55). After removing SNPs that did not pass the genotyping quality control criteria [minor allele frequency (MAF) > 0.03 and individuals containing 70% or more of loci where no alleles were distinguished], 68 individuals (38 from DF and 30 HF) with 3266 SNPs with no missing data were kept. SNP functional annotations were obtained from the species' reference genome annotations in the TreeGenes database (https://treegenesdb.org) and by sequence alignment against the NCBI non-redundant protein sequences database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) with an e-value $< 1 \times 10^{-10}$.

Outlier test, population structure, and diversity estimates

Putative adaptive loci, i.e. considered hereafter outlier SNPs, were those showing higher F_{st} than neutral loci that were identified using R packages by three approaches: (i) DETSEL identifies markers that show deviation from neutral expectation in pairwise comparisons of diverging populations, and the significance level was set at 95% (Vitalis *et al.* 2003); (ii) OutFLANK infers the null distribution by removing loci in the top and bottom of the distribution, and a 5% as the trim points and a minimum expected heterozygosity of 0.10 was chosen to infer the distribution (Whitlock and Lotterhos 2015); and (iii) PcAdapt performs PCA and computes *P*-values to test for outliers based on the correlations between genetic variation and the first *K* principal components (Luu *et al.* 2016). On the basis of the 'scree plot' representing the percentage of variance explained by each principal component $k = 2$ was used (Supporting Information, Fig. S1). Venn diagrams were generated using an R package (VennDiagram v.1.7.3) to overlap the three outlier methods. Outlier SNPs will be those that are found in at least two out of the three analyses.

To analyse potential genetic differences between DF and HF, a Bayesian model–based clustering approach in STRUCTURE v.2.3 (Pritchard *et al.* 2000) was implemented for all three groups of SNPs: outliers, neutral, and combined outlier + neutral. To infer lambda values, simulations were performed for each dataset, obtaining the following values of lambda: 0.65, 0.42, and 0.42 for 24 outlier loci, 3242 neutral, and all 3266 SNP loci, respectively. The model was run with the following parameter settings: correlated allele frequencies, no population information, and the value of λ for each data set. Ten separate runs at each *K* (1–5) and a burn-in period of 10 000 and 100 000 Markov chain Monte Carlo iterations for each run were performed. The number of distinct clusters was obtained using STRUCTURE HARVESTER (Earl and von Holdt 2012) based on the conservative Evanno's method (Evanno *et al.* 2005). Results from the STRUCTURE analysis were summarized by creating a histogram plot, in which each individual was represented by a single vertical line broken into *K* coloured segments, with lengths proportional to each of the *K* inferred clusters (Pritchard *et al.* 2000).

Standard genetic diversity parameters were estimated for outlier and neutral SNPs (total SNPs minus outliers) for DF and HF using GenAIEx v.6.5 (Peakall and Smouse 2012). These were: number of alleles (NA), number of private alleles (A_p), percentage of polymorphic loci (P%), observed heterozygosity (H_o), expected heterozygosity (H_e), and inbreeding coefficient (F_{is}) including 95% confidence intervals. Also, for both groups of SNPs (outliers and neutral), PCA analyses using the R package Adegenet v.2.1.3 were performed to identify distinct population clusters (Jombart 2008). Such genetic clusters identified by population structure analyses (STRUCTURE and PCA) were used for AMOVA (Analysis of Molecular Variance) to estimate genetic divergence (F_{st}) and 95% confidence interval between DF and HF (Peakall and Smouse 2012).

Genotype-to-environment associations (GEA)

To detect possible associations between genotype and environment, three different methods were used: linear mixed model regressions (MLM) implemented in GAPIT, latent factor mixed models implemented in LFMM, and a Bayesian approach implemented in Bayenv2, using as raw data each individual SNP genotype and 23 climatic variables that were obtained from a PCA performed in the environmental characterization section. SNPs were considered candidates for divergent selection if MAF was higher than 3%, have a threshold value of $-\log10(P\text{-value}) > 2$, a Z-score > 4 (following Frichot *et al.* 2013), and BF > 4 (following De la Torre *et al.* 2014), for MLM, LFMM, and Bayenv, respectively. SNPs functional characterization were obtained from the species' reference genome annotations in TreeGenes (https://treegenesdb.org) and by sequence alignments against the NCBI non-redundant protein sequences database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) with an e-value $< 1 \times 10^{-10}$. Complete schematic summary of methods is depicted in Fig. 2.

RESULTS

Environmental characterization

The first two principal components, which explain 85.34% of the variation, were used to define the environmental space occupied by the samples. Twenty-three bioclimatic variables showed high loadings with these axes (Fig. 3; see Supporting Information, Table S2). DF samples tend to occupy more positive values on PC2 axis, associated with lower precipitation and higher aridity, while HF samples formed two groups, one towards higher annual precipitation and lower temperatures (negative PC1 and PC2 values) and the other with lower annual precipitation and higher temperatures (negative PC1 and positive PC2 values).

Seedling growth in common garden

Greenhouse seedlings growth and mortality were significantly different when classified according to their origin (DF or HF). When grown in soils at field capacity for two years (October 2020-October 2022) DF provenance seedlings showed higher mortality (38.39%) compared to seedlings originated from HF parent trees (17.58%). DF seedlings developed smaller and less branched roots and shoots (Figs. 4a, b; $P < 0.01$), than HF ones. DF attained significantly lower basal stem diameter, number of branches and plant height than in HF seedlings in first and second

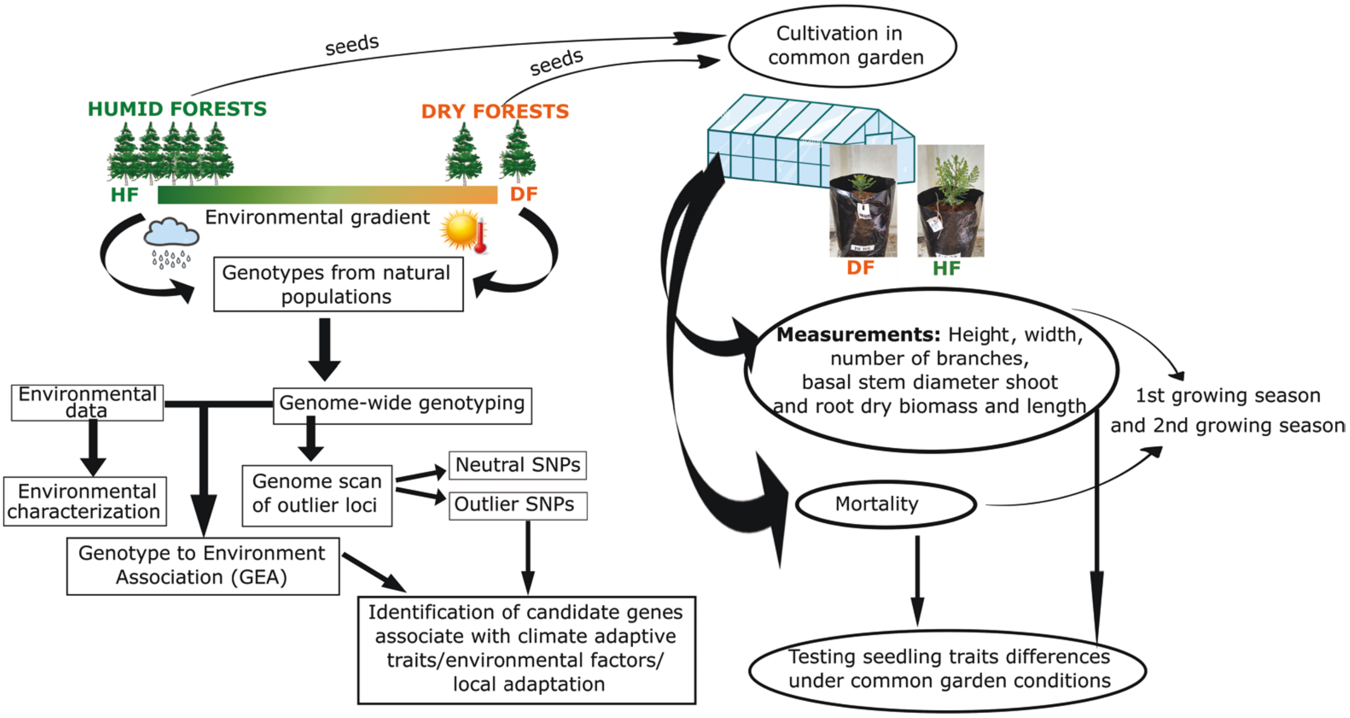

Figure 2. Schematic summary of methods. More details can be found in the Materials and Methods section.

growing season in the common garden ($P < 0.01$; Fig. 4c). Only root/shoot length and root/shoot weight showed to be constant ratios for *Austrocedrus* from DF and HF in both growing seasons after 12 and 24 months old, respectively. K-means clustering based on morphological variables of seedlings led to a reliable DF vs. HF provenance classification (Kappa = 0.76) with lower misclassifications in DF (4.0%) than in HF (21.8%) (Supporting Information, Table S3).

Outlier test, population structure, and diversity estimates

Local adaptation based on outliers (i.e adaptative loci) was assessed using 3266 SNPs in 68 adult individuals. OutFLANK identified 24 SNPs with moderate *Fst* values (between 0.10 to 0.16), PcAdapt identified 74 SNPs with *P*-values < 0.01, and DETSEL identified 27 SNPs with moderate *Fst* values (between 0.10 to 0.16). Overlapping the three univariate GEA analyses, 24 SNPs were identified as outliers in at least two out of the three analyses (Fig. 5 and Table 1), only one SNP (19743) was identified with the three methods. From now on, these 24 SNPs are called 'outliers', and the rest 3242 SNPs are considered as 'neutral' the latter calculated as the total SNPs minus outliers. Eight out of the 24 outliers aligned with transcripts to known proteins at the NCBI and Treegenes databases (Table 1) and could be identified and associated to a gene ontology class (biological process, cellular component, or molecular function). Half of them (SNPs 9316, 96798, 129284 and 142672) are located in genes with a potential function related to stress response (Table 1).

Standard population genetic diversity parameters yielded no statistically significant differences between DF and HF, neither for outliers nor for neutral SNPs (Supporting Information, Table S4). As expected, PCA for neutral SNPs showed no apparent clustering, only explaining 5% of the total observed variation (Supporting Information, Fig. S2). Instead, a PCA analysis using outlier SNPs separated dry from HFs, and the first two components explaining 27% of the total observed variation (PC1 explain 17% while PC2 9.6%; Fig. 5b), with five SNPs being responsible for the first component (PC1) that yielded genotype differences between DF and HF (Fig. 5c). For example, SNP142672 and SNP129284 presents almost all individuals from DF with homozygous AA and TT, respectively.

Population structure analyses showed two *K* groups for outlier SNPs (the best k value according to Evanno´s method; Supporting Information, Fig. S3a) that almost unambiguously separated DF individuals from HF ones (Fig. 6), while no structure was found for neutral SNPs and the combined dataset (Supporting Information, Fig. S3b and S3c). Also, an AMOVA for outliers SNPs showed a significant differentiation between DF and HF ($F_{st} = 0.123$; CI = 0.117–0.130, $P = 0.001$), whereas differences between DF and HF were almost nil for neutral SNPs (*Fst* = 0.001, $P = 0.268$).

Genotype–environment associations (GEA)

GEA analyses using a reduced set of 23 climatic variables (Supporting Information, Table S1) yielded significant associations with 70 SNPs (Supporting Information, Table S4), 12 of which were outlier SNPs (Table 1), and 25 matched with transcripts aligned to known proteins at the NCBI and Treegenes databases (Supporting Information, Table S5). Ten of them are located in genes with a potential function related to stress response (SNPs 3380, 9316, 1949, 23919, 44241, 71346, 92737, 96798, 135874 ,and 197451) (Supporting Information, Table S5). From 70 SNPs yielding significant association with climatic variables, 14 showed statistically significant differences in allele frequencies between DF and HFs and were mostly associated with precipitation variables (nine SNPs) and aridity index (four

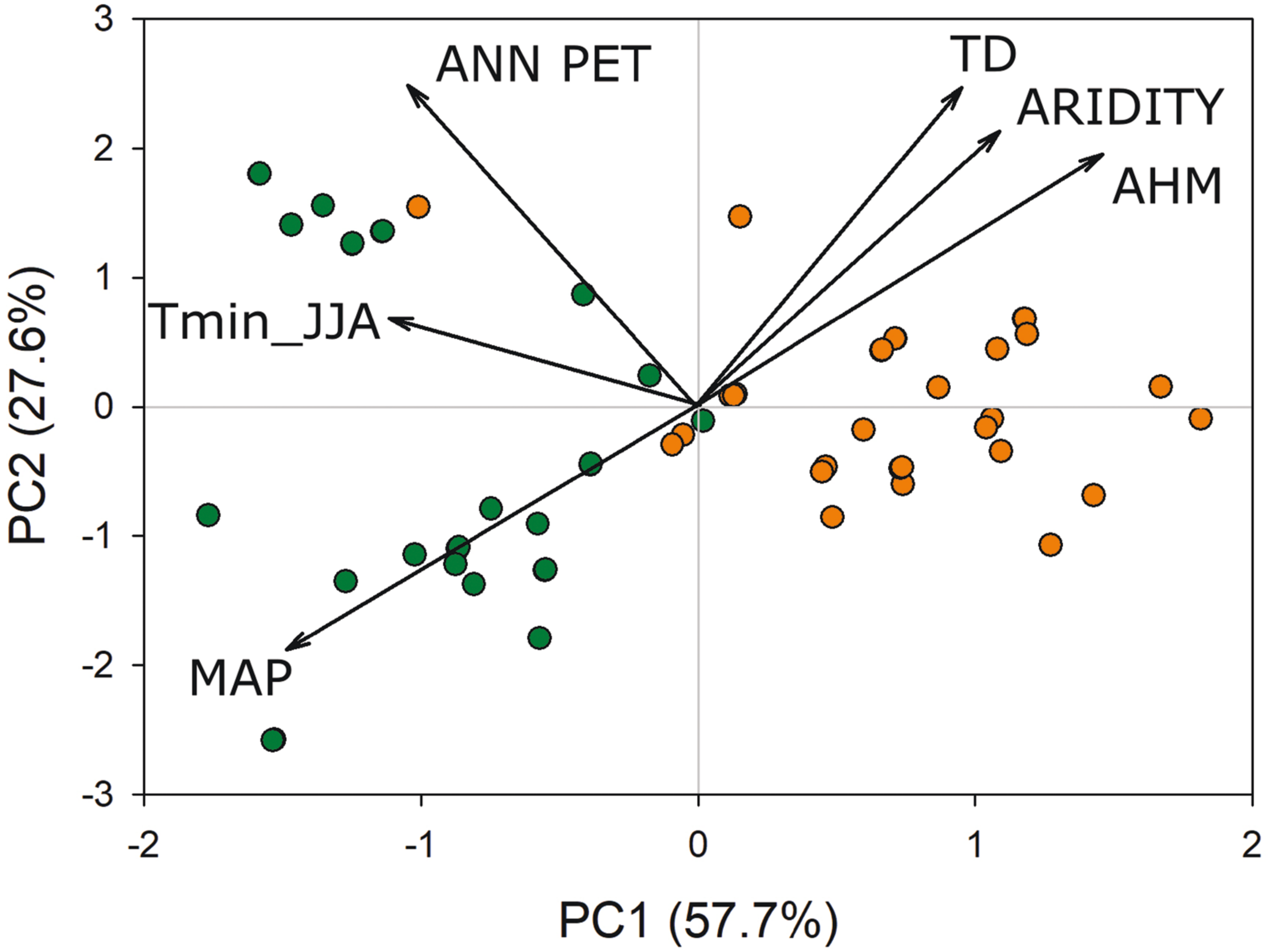

Figure 3. PCA based on 23 climatic variables for 68 sampled *Austrocedrus* individuals. Orange and green dots represent individuals from dry (DF) and humid (HF) forests (N = 38 and 30, respectively). Black lines indicate projection of the variables in the factor-plane of distinct metrics: annual heat-moisture index (AHM); annual potential evapotranspiration (ANN PET); aridity index (ARIDITY); MAP; continentality (TD); and winter mean minimum temperature (Tmin_JJA).

SNPs), except for SNP 60571, which was significantly associated with annual mean temperature (BIO1) (Fig. 7). DF associated with higher aridity index, while HF linked with higher values of precipitation [MAP, PPT_JJA (winter precipitation in South America), PPT_DJF (summer precipitation in South America), and BIO14 (precipitation of the driest month)] (Fig. 3; see Supporting Information, Table S4).

DISCUSSION

The evidence presented here based on common garden grown seedlings and genomic analysis of adult *Austrocedrus chilensis* suggests a strong genetic differences between individuals and progenies from environmentally contrasting sites located only a few tens of kilometres apart along a steep precipitation gradient. Common garden experiments yielded sharp differences of progeny for traits related to above and below ground growth, which are a reflection of genetically based adaptations to distinct environmental envelops. Similarly, adult trees showed a significant genetic structure by means of outlier SNPs associated to contrasting precipitation conditions whereas neutral ones demonstrated a great deal of admixture. Therefore, adaptive differences between provenances of *Austrocedrus* are maintained in the face of gene flow. Seedlings originated from parental trees from dryer and thermally more rigorous climates, e.g. colder winters and hotter summers, as eastern-most locations tended to be half the aerial and underground growth of seedling originated from parent trees located ~30 km to the west growing on wetter and thermally more stable conditions. Nonetheless, seedlings from distinct progenies yielded similar root/shoot allocation reflecting that such traits are species-specific on the resource acquisition/conservation spectrum (Husáková *et al.* 2018).

A significant portion of plant trait variation can be linked to their ability to adapt to environmental factors such as climate and soil composition (Wright *et al.* 2000, Oliveira *et al.* 2019, Caruso *et al.* 2020). The direction of quantitative trait changes along ubiquitous environmental gradients such as aridity (Ivanova *et al.* 2018) is often repeated within species, providing fertile ground for the development of general principles of environmental adaptation (Chesson *et al.* 2004). Dry and humid

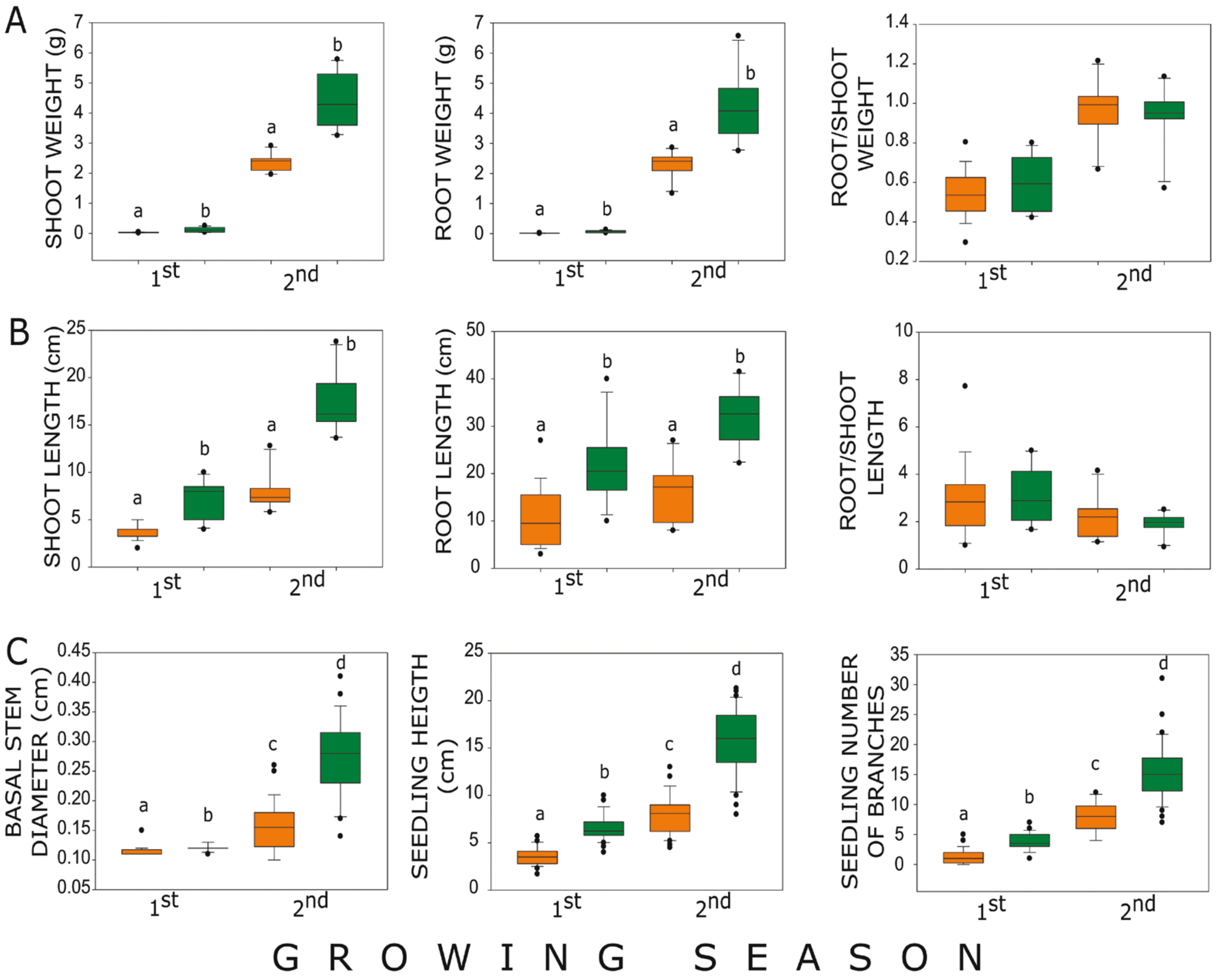

Figure 4. Growth traits measured on seedlings cultivated under common garden from dry (orange) and humid (green) forests at two growing seasons (12 and 24 months old). A, Shoot, root, and root/shoot weight; B, Shoot, root, and root/shoot length; C, Basal stem diameter, height, and number of branches of seedlings. Different letters depict significant differences ($P \leq 0.001$) estimated by means of Kruskal–Wallis One-Way Analysis of Variance on Ranks and *post hoc* pairwise multiple comparison procedure using Dunn's method.

forests of *Austrocedrus* show climatic differences. Dry forests are associated with lower precipitation and higher aridity environments while HFs are so with relatively benign settings. Under common garden conditions, differences in seedling growth reflected the climate of parental origin. Dry forests seedlings have lower seed survival, number of branches, basal diameter, and height than HF ones. Other common garden studies in conifers also found that trees from drier climates often show lower height (de la Mata *et al.* 2014), less aboveground biomass (Kerr *et al.* 2015), and lower seedling survival (Castanha *et al.* 2013) than trees from humid climates. Genetically based differences at the leaf and entire plant levels were also found in *Nothofagus dombeyi* and *N. pumilio* seedlings from the extremes of the precipitation gradient from northern Patagonia growing in common gardens and those from mesic sites outgrew dry-most ones (Diaz *et al.* 2020, Ignazi *et al.* 2020). Similarly, other studies from seasonally dry biomes showed greater growth potential with increased precipitation provenance in common gardens (Schwinning *et al.* 2022) reflecting genetic differences. The evidence presented here contrasts with no among-provenance differences to experimental drought found in *Austrocedrus*, despite high heritability for survival (Aparicio *et al.* 2012) as well as reduced morphological differences of seedlings that were interpreted mostly as plastic responses (Pastorino *et al.* 2010). In the same way, other plants growing in controlled environments showed that growth-related traits are highly plastic (Münzbergová *et al.* 2017). Nonetheless, *Austrocedrus* traits analysed in the previously mentioned studies included provenances along latitudes that may have override the effects of steep precipitation variance tested along longitudes that were detected here.

Water shortage and nutrient-poor soils under drier conditions impose stressful environments, limiting plant growth, therefore above and below ground biomass was also lower in dry *Austrocedrus* forests. Previous work in this species shows faster

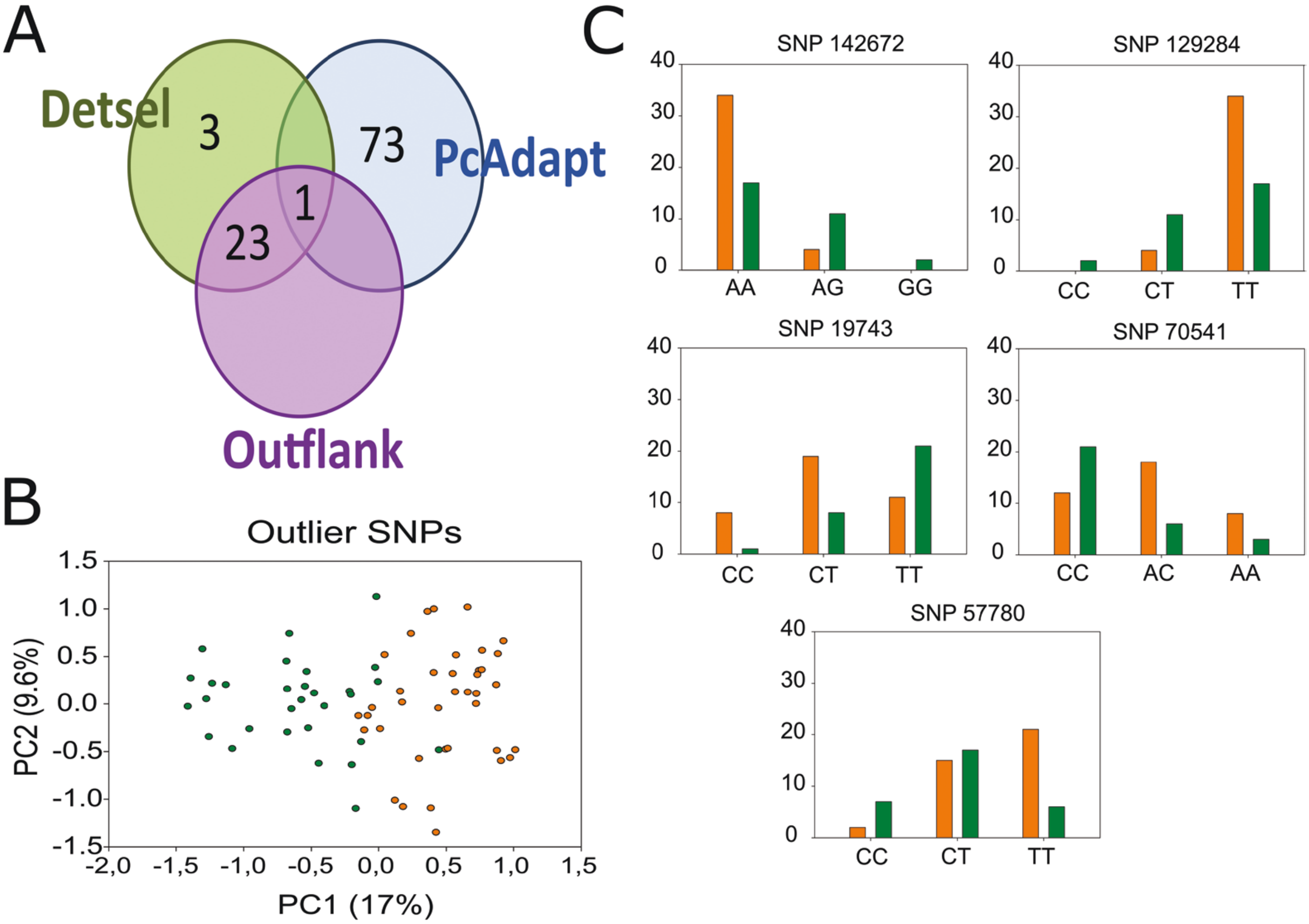

Figure 5. Outlier tests by F_{st} and PCA analysis for 24 SNPs. Depicting dry (orange) and humid (green) forests. A, Venn diagram illustrating the overlap among outlier loci of *Austrocedrus* identified using the three different approaches (OutFLANK, PcAdapt, Detsel). B, PCA based on 24 outliers SNPs from 68 *Austrocedrus* individuals. C, Absolute genotype frequency for the five SNPs that best explained the first PCA axis. All chi-squared tests of association between forest origin (dry or humid) and genotypic frequencies for each SNPs were significant ($P < 0.005$).

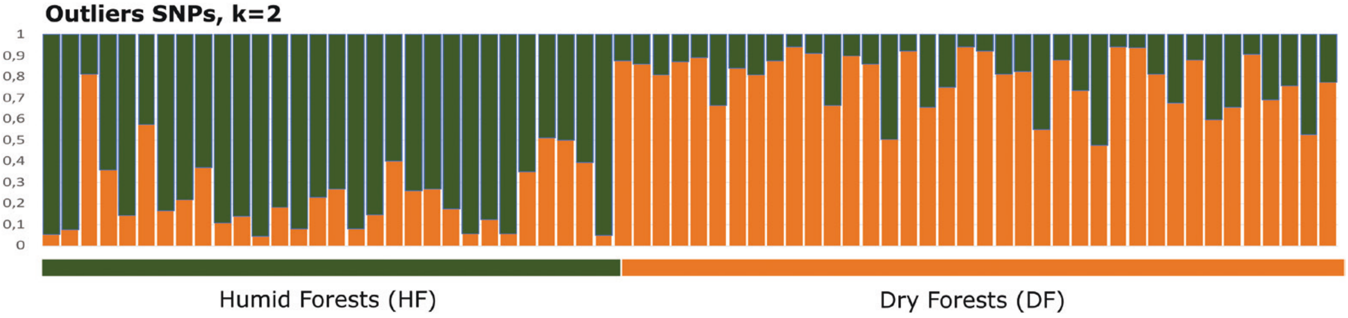

Figure 6. STRUCTURE results showing the assignment of 68 *Austrocedrus* individuals from DF and HFs for 24 outlier SNPs and two assumed clusters ($k = 2$). Each single vertical bar represents the individual's estimated proportion of membership to a given genetic cluster.

radial growth rates (Villalba and Veblen 1997), larger tree sizes (Oddi *et al.* 2022), and higher growth resilience (Marcotti *et al.* 2021) towards the wetter end of the rainfall gradient. Although the growing differences found in this work might stem from genetic differences between DF and HF, they may also be influenced by epigenetic variation and/or maternal effects. So, further experiments would be needed to elucidate how much of these growing differences are under either genetic or environmental control. Even though it is well documented that tree species adapted to dry climatic regimes have higher root-to-shoot ratios and deeper root systems to optimize water uptake than mesic species (Brunner *et al.* 2015, Kolb *et al.* 2016) in *Austrocedrus*, we found non-significant differences. Patagonian dry forests (where *Austrocedrus* grows) are characterized by rocky outcrops (refuges), where a more extensive root system could be adaptive. In these environments, rainfall is scarce, and trees have very shallow roots (Fasanella Pers. Obs.). A possible explanation is that superficial roots allow them to capture the limited precipitation of the

Table 1. Functional annotation of outlier SNPs that differentiate Austrocedrus dry (DF) an humid forests (HF). Including: SNP ID, allele frequencies in DF and HF, function and related biological process, and references (including in brackets in which species was observed).

SNP	MAF		Functional annotation	GO-biological process	References and species
	DF	HF			
3886	0.316	0.583			
9316	0.184	0.450	Retrovirus-related, polyprotein from transposon TNT 1-94	Present in tree roots. A common feature of most retrotransposons activated by stress and environmental factors.	Todorovska (2007) (*Tobacco*)
19038	0.197	0.017			
19743	0.461	0.167			
26808	0.197	0.033	Poly(3-hydroxybutyrate) depolymerase	Significantly increase plant growth.	Silveira Alves *et al.* (2021) (*Setaria viridis*)
51637	0.118	0.333			
52150	0.053	0.233	Transcription factor MYB57-like	Key in processes such as vegetative and reproductive development.	Abdur Rahim *et al.* (2019) (*Tobacco*)
57780	0.250	0.517			
60571	0.566	0.300	GTP-binding protein; clone: CSFL018_I03	Expressed in reproductive shoots.	Molendijk *et al.* (2008) (*Cryptomeria japonica*)
70541	0.447	0.200			
73311	0.132	0.367			
76858	0.579	0.300			
78956	0.145	0.367			
79109	0.039	0.200			
92012	0.237	0.033			
93067	0.605	0.333	PIN/TRAM domain-containing protein; clone: CLFL038_I18,	Expressed in needles.	Matelska *et al.* (2017) (*Cryptomeria japonica*)
96798	0.355	0.117	Ethylene-responsive transcription factor 2-like	Ethylene response factors (ERFs) are a superfamily of proteins which participate in multiple abiotic stress tolerance response such as salt, drought, heat, and cold.	Thirugnanasambantham *et al.* (2014) (*Camelina sativa*)
122681	0.276	0.067			
123625	0.171	0.433			
129284	0.053	0.250	RING-type E3 ubiquitin transferase	Plays a positive role in drought tolerance. Positively regulates the drought stress response via ABA-mediated signalling.	Joo *et al.* (2018) (*Capsicum annuum, Arabidopsis thaliana, Nicotiana benthamiana*)
129334	0.329	0.117			
142672	0.053	0.250	Serine/threonine-protein kinase HT1	Activator of the ABA signalling pathway that regulates numerous ABA responses, such as stomata closure in response to drought, darkness, high CO_2, plant pathogens, or decreases in atmospheric relative humidity (RH).	Sierla *et al.* (2018)
150970	0.329	0.117			
154410	0.197	0.433			

region that falls in the form of rain or snow. Having a deeper root system in seedlings growing in extremely dry soil may not necessarily result in tapping into higher moisture soil and could be more of a burden. In the light of this, genetic differences are likely to play an important role in geographical variation in these drought-tolerance traits (McDowell *et al.* 2008, Shu and Moran 2023).

Genome scans of adult *Austrocedrus* trees growing along this steep gradient showed a strong genetic two-group clustering based on potentially adaptive genes but complete lack of structure when analysing neutral ones. This suggests local adaptation to contrasting climatic conditions and limiting resources despite high gene flow in this airborne pollinated and wind dispersed conifer (Arana *et al.* 2010, Souto *et al.* 2015). According to Aitken *et al.* (2008), there is evidence of local adaptation to climate in many trees even with high gene flow. *Austrocedrus* showed genetic differences in allele frequencies between DF and HF in a naturally steep precipitation gradient. GEA analyses identified

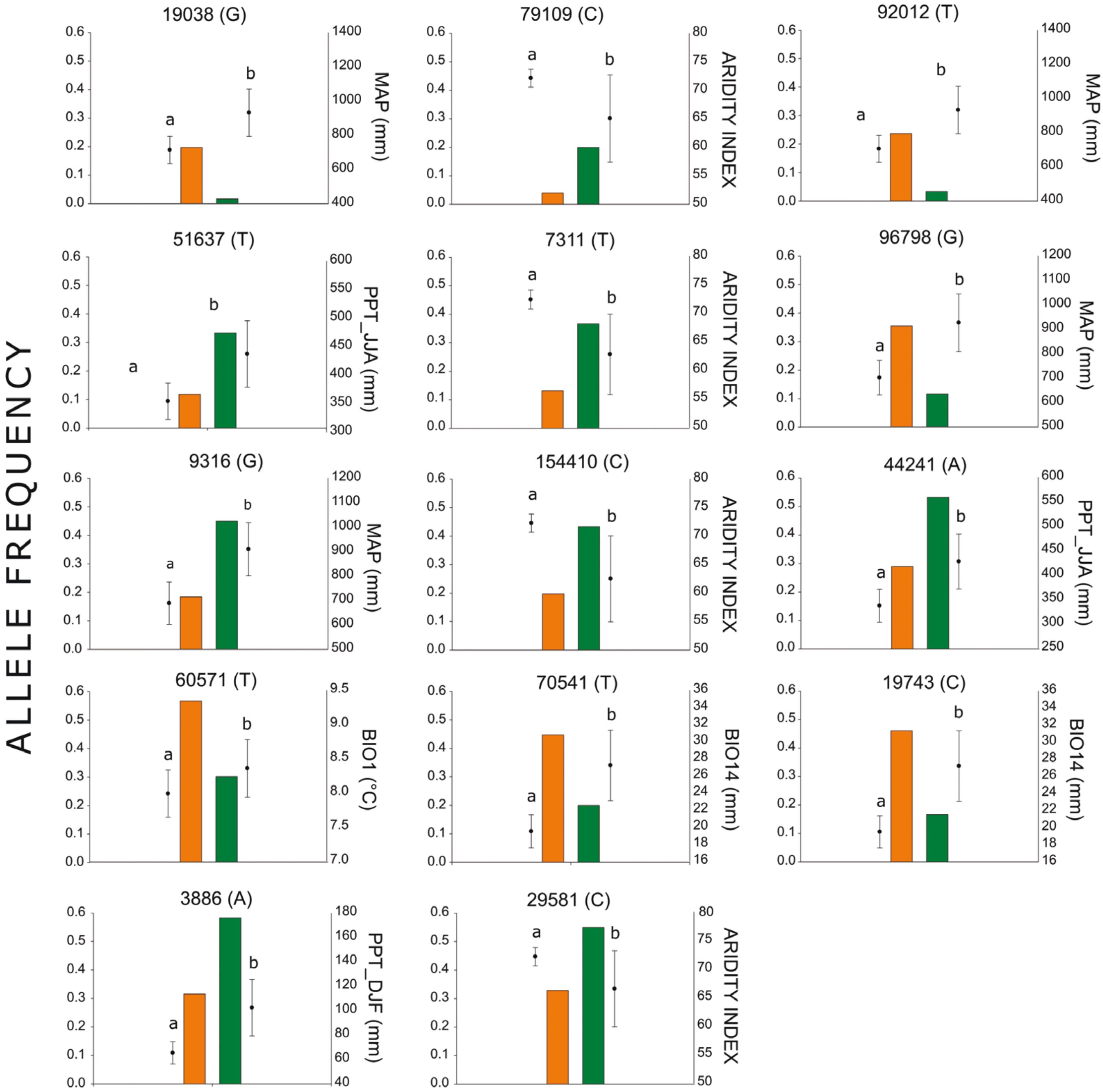

Figure 7. Significantly different allele frequencies for DF (orange) and HFs (green) of SNPs associated with climatic variables. In parentheses, the more frequent allele for each SNP is shown. Different letters indicate statistically significant differences of climatic variables (mean and SD) between DF and HFs (Student's *t*-test). See Supporting Information, Table S5 for detailed information on GEA association.

four statistically significant marker–climate associations driving genetic differentiation. Climate variables best explaining differentiation between DF and HF for outlier genes involved in leaf development and drought tolerance were: precipitation of wettest quarter, precipitation of coldest quarter, MAP, annual heat-moisture index, and summer precipitation. The association between a SNP and aridity may indicate that these genes or their regulatory regions affect trees performance in dry versus moister environments, but do not reveal what traits are under selection in a given environment. Observed genomic patterns for outlier loci and the lack of it for neutral ones suggest that despite gene flow depicted by the latter, selection has favoured divergence by adaptation.

Twenty-four outlier SNPs that differ in allele frequencies and separate DF from HF were found; eight of which were located in genes with a potential function related to stress response. Considering that no reference genome is available for *Austrocedrus chilensis*, the fact that 33% of the outlier SNPs aligned with transcripts on NCBI and Treegenes databases represents a significant number of potentially adaptive loci. Plants have developed different mechanisms to cope with abiotic stress: changes in abiotic conditions exert different biological effects at the cellular level that trigger the stress-sensing mechanism (Estravis-Barcala *et al.* 2019). For example, ethylene response factors, ubiquitin transferase, and serine/threonine-protein kinases are proteins that participate during multiple abiotic stress tolerance. The last two regulate the drought stress response via abscisic acid (ABA)-mediated signalling. ABA is produced in roots as well as in leaves. When plants are exposed to drought, ABA is transported from roots to leaves, where it acts as a long-distance signal inducing the closure stomata and triggers the expression of ABA biosynthesis genes, increasing the ABA content in leaves (Brunner *et al.* 2015). Moran *et al.* (2017) showed that conifers in the pine family respond to drought through ABA-dependent control of water transpiration. A reduced number of candidate genes previously associated in the literature to drought stress adaptations were identified as explaining this environmentally associated genetic structuring. Strikingly, the two SNPs with greatest load between genetic variants and water availability conditions (serine/threonine-protein kinase HT1 and RING-type E3 ubiquitin transferase) are both involved in the regulation of ABA-mediated signalling. The virtual absence of the minor allele C of the RING-type E3 ubiquitin transferase and minor allele G of Serine/threonine-protein kinase HT1 in dry forests individuals suggests that these genetic variants may have been selected under strong water stress conditions conferring most individuals in this populations with more strict ABA-mediated stomatal control at the expense of less growth of above and underground organs, even if stress factors are removed, as shown in our common garden experiment.

With more frequent and intense drought episodes occurring due to climate change (IPCC 2021), it is imperative to understand the genomic and physiological basis of drought tolerance to be able to predict how species will respond in the future (De la Torre *et al.* 2021). This is especially important in conifers since genomic studies have been limited due to their large genome sizes and long-generation times (Van Ghelder *et al.* 2019, De la Torre *et al.* 2021, Depardieu *et al.* 2021, Schueler *et al.* 2021). Also, the capacity of trees to adjust growth to the prevailing climatic conditions is gaining importance in drought prone areas (Vieira *et al.* 2020).

Genetic variation observed in *Austrocedrus* individuals from opposing environments at the plant and genomic levels is expected to play an important role in the survival of populations in the face of the frequent droughts expected in northern Patagonia (IPCC 2021). This genetic component would allow: (i) rapid adjustments and local adaptation, (ii) assisted seed transfer from drought resistant populations (DF) to other drought-affected populations, and (iii) the detection of drought resistant genes in tree improvement. Nonetheless, hydroclimatological modelled conditions for South America suggest a shift towards increasing drought for most of the continent, including more humid areas as Patagonia (Zaninelli *et al.* 2019). Therefore, restoration efforts of *Austrocedrus* stands particularly at the dry-most end should not only considered local germplasm sources but also future climatic trends (Jones 2013).

Our study points to dry forests as reservoirs for drought-tolerant genetic variants adapted to cope with forecasted climate change and thus reducing extinction risks that are occurring faster than expected. So, these adaptive genes could provide potential drought adaptations to the species. Also, these populations could be candidate seed sources for assisted migration. Our results contribute to understand the genomic basis of drought tolerance in long-generation conifers and provide information on adaptation that can be used to guide management measures and restoration efforts in the context of forecasted changes in climate.

SUPPLEMENTARY DATA

Supplementary data is available at the *Botanical Journal of the Linnean Society* online.

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AUTHOR'S CONTRIBUTIONS

M.F., C.S., K.T., and A.C.P. conceived ideas, collected samples, analysed data, interpreted results, and wrote the manuscript. M.F. and C.S. performed the laboratory and common garden work.

CONFLICT OF INTEREST

None declared.

DATA AVAILABILITY

The data underlying this article are available in Universidad Nacional del Comahue Institutional Digital Repository RDI Unco at http://rdi.uncoma.edu.ar/, and can be accessed with accession number 17699, http://rdi.uncoma.edu.ar/handl e/uncomaid/17699.

REFERENCES

Adams HD, Kolb TE. Tree growth response to drought and temperature in a mountain landscape in northern Arizona, USA. *Journal of Biogeography* 2005;**32**:1629–40.

Aitken SN, Yeaman S, Holliday JA *et al.* Adaptation, migration or extirpation: climatic changes outcomes for tree populations. *Evolutionary Applications* 2008;**1**:95–111.

Alía R, Moro J, Denis JB. Performance of *Pinus pinaster* provenances in Spain: interpretation of the genotype by environment interaction. *Canadian Journal of Forest Research* 1997;**27**:1548–59.

Allen CD, Macalady AK, Chenchouni H *et al.* A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management* 2010;**259**:660–84.

Anderson J, Lee CR, Rushworth C *et al*. Genetic tradeoffs and conditional neutrality contribute to local adaptation. *Molecular Ecology* 2012;**22**:699–708.

Anderson JT, Song BH. Plant adaptation to climate change—where are we? *Journal of Systematics and Evolution* 2020;**58**:533–45.

Aparicio A, Zuki S, Pastorino M *et al*. Heritable variation in the survival of seedlings from Patagonian cypress marginal xeric populations coping with drought and extreme cold. *Tree Genetics & Genomes* 2012;**8**:801–10.

Arana MV, Gallo LA, Vendramin GG *et al*. High genetic variation in marginal fragmented populations at extreme climatic conditions of the Patagonian cypress *Austrocedrus chilensis*. *Molecular Phylogenetics and Evolution* 2010;**54**:941–9.

Batllori E, Lloret F, Aakala T *et al*. Forest and woodland replacement patterns following drought-related mortality. *Proceedings of the National Academy of Sciences of the United States of America* 2020;**117**:29720–9.

Bell G, Gonzalez A. Evolutionary rescue can prevent extinction following environmental change. *Ecology Letters* 2009;**12**:942–8.

Berend K, Haynes K, McDonough MacKenzie C. Common garden experiments as dynamic tool for ecological studies of alpine plants and communities in northeastern North America. *Rhodora* 2019;**121**:174–212.

Bradbury PJ, Zhang Z, Kroon DE *et al*. TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics* 2007;**23**:2633–5.

Bradford JB, Shriver RK, Robles MD *et al*. Tree mortality response to drought-density interactions suggests opportunities to enhance drought resistance. *Journal of Applied Ecology* 2021;**59**:549–59.

Brunner I, Herzog C, Dawes MA *et al*. How tree roots respond to drought. *Frontiers in Plant Science* 2015;**6**:547.

Capblancq T, Lachmuth S, Fitzpatrick MC *et al*. From common gardens to candidate genes: exploring local adaptation to climate in red spruce. *The New Phytologist* 2023;**237**:1590–605.

Caruso CM, Mason CM, Medeiros JS. The evolution of functional traits in plants: is the giant still sleeping? *International Journal of Plant Sciences* 2020;**181**:1–8.

Castanha C, Torn MS, Germino MJ *et al*. Conifer seedling recruitment across a gradient from forest to alpine tundra: effects of species, provenance, and site. *Plant Ecology & Diversity* 2013;**6**:30718.

Chesson P, Gebauer RLE, Shwinning S *et al*. Resource pulses, species interactions, and diversity maintenance in arid and semi-arid environments. *Oecologia* 2004;**141**:236–53.

Correia I, Almeida MH, Aguiar A *et al*. Variations in growth, survival and carbon isotope composition (13C) among *Pinus pinaster* populations of different geographic origins. *Tree Physiology* 2008;**28**:1545–52.

Dai A. Increasing drought under global warming in observations and models. *Nature Climate Change* 2013;**3**:52–8.

De La Torre AR, Roberts DR, Aitken SN. Genome-wide admixture and ecological niche modelling reveal the maintenance of species boundaries despite long history of interspecific gene flow. *Molecular Ecology* 2014;**23**:2046–59.

De La Torre AR, Sekhwal MK, Puiu D *et al*. Genome-wide association identifies candidate genes for drought tolerance in coast redwood and giant sequoia. *Plant Journal* 2021;**109**:7–22.

Depardieu C, Gérardi S, Nadeau S *et al*. Connecting tree-ring phenotypes, genetic associations and transcriptomics to decipher the genomic architecture of drought adaptation in a widespread conifer. *Molecular Ecology* 2021;**30**:3898–917.

Diaz DG, Ignazi G, Mathiasen P *et al*. Climate-driven adaptive responses to drought of dominant tree species from Patagonia. *New Forests* 2022;**53**:57–80.

Diaz DG, Mathiasen P, Premoli AC. Subtle precipitation differences yield adaptive adjustments in the mesic *Nothofagus dombeyi*. *Forest Ecology and Management* 2020;**461**:117931.

Donoso C. Reseña ecológica de los bosques mediterráneos de Chile. *Bosque* 1982;**2**:117–46.

Doyle JJ, Doyle JL. Isolation of plant DNA from fresh tissue. *Focus* 1990;**12**:13–5.

Earl DA, Vonholdt BM. Structure Harvester: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* 2012;**4**:359–61.

Eckert AJ, Bower AD, Gonzalez-Martinez SC *et al*. Back to nature: ecological genomics of loblolly pine (*Pinus taeda*, Pinaceae). *Molecular Ecology* 2010a;**19**:3789–805.

Eckert AJ, van Heerwaarden J, Wegrzyn JL *et al*. Patterns of population structure and environmental association to aridity across the range of loblolly pine (*Pinus taeda L*, Pinaceae). *Genetics* 2010b;**185**:969–82.

Estravis-Barcala M, Mattera MG, Soliani C *et al*. Molecular bases of responses to abiotic stress in trees. *Journal of Experimental Botany* 2019;**71**:3765–79.

Evanno G, Regnaut S, Goudet J. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 2005;**14**:2611–20.

FAO. *Global Forest Resources Assessment 2015: Desk Reference*. Roma, Italy: Food and Agriculture Organization of the United Nations, 2015.

FAO. *Trees, Forests and Land Use in Drylands: The First Global Assessment – Full Report*. FAO Forestry Paer No. 184. Rome, Italy: Food and Agriculture Organization of the United Nations (FAO), 2019.

Feng S, Fu Q. Expansion of global drylands under a warming climate. *Atmospheric Chemistry and Physics* 2013;**13**:10081–94.

Fick SE, Hijmans RJ. WorldClim 2: new 1km spatial resolution climate surfaces for global land areas. *International Journal of Climatology* 2017;**37**:4302–15.

Fischer G, Nachtergaele F, Prieler S *et al*. *Global Agro-ecological Zones Assessment for Agriculture (GAEZ 2008)*. IIASA, Laxenburg, Austria and Rome, Italy: FAO, 2008.

Frichot E, Schoville SD, Bouchard G *et al*. Testing for associations between loci and environmental gradients using latent factor mixed models. *Molecular Biology and Evolution* 2013;**30**:1687–99.

Gazol A, Camarero J. J. Compound climate events increase tree drought mortality across European forests. *Science of the Total Environment* 2022;**816**:151604.

Gienapp P, Teplitsky C, Alho JS *et al*. Climate change and evolution: disentangling environmental and genetic responses. *Molecular Ecology* 2007;**17**:167–78.

González-Martínez SC, Ersoz E, Brown GR *et al*. DNA sequence variation and selection of tag SNPs at candidate genes for drought-stress response in *Pinus taeda* L. *Genetics* 2006;**172**:1915–26.

Hamann A, Wang T, Spittlehouse DL *et al*. A comprehensive, high-resolution database of historical and projected climate surfaces for western North America. *Bulletin of the American Meteorological Society* 2013;**94**:1307–9.

Haslinger K, Schöner W, Anders I. Future drought probabilities in the Greater Alpine Region based on COSMO-CLM experiments – spatial patterns and driving forces. *Meteorologische Zeitschrift* 2016;**25**:137–48.

Hoban S, Kelley JL, Lotterhos KE *et al*. Finding the genomic basis of local adaptation: pitfalls, practical solutions, and future directions. *The American Naturalist* 2016;**188**:379–97.

Husáková I, Weiner J, Munzbergová Z. Species traits and shoot–root biomass allocation in 20 dry-grassland species. *Journal of Plant Ecology* 2018;**11**:273–85.

Ignazi G, Bucci SJ, Premoli AC. Stories from common gardens: water shortage differentially affects *Nothofagus pumilio* from contrasting precipitation regimes. *Forest Ecology and Management* 2020;**458**:117796.

IPCC. Climate Change 2021: the physical science basis. In: Masson-Delmotte V, Zhai P, Pirani A *et al*. (eds), *Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press, 2021. In press, https://doi.org/10.1017/9781009157896.

IUCN. Red List of Threatened Species. Version 2014.3. International Union for the Conservation of Nature. 2014. www.iucnredlist.org (September 2023, date last accessed).

Ivanova LA, Ivanov L, Ronzhina DA *et al*. Leaf traits of C3- and C4-plants indicating climatic adaptation along a latitudinal gradient in Southern

Siberia and Mongolia. *Flora – Morphology Distribution Functional Ecology of Plants* 2018;**254**:122–34.

Jombart T. Adegenet: an R package for the multivariate analysis of genetic markers. *Bioinformatics* 2008;**24**:1403–5.

Jones TA. When local isn't best. *Evolutionary Applications* 2013;**6**:1109–18.

Jordan R, Prober SM, Hoffmann AA *et al.* Combined analyses of phenotype, genotype and climate implicate local adaptation as a driver of diversity in *Eucalyptus macrocarpa* (grey box). *Forest* 2020;**11**:495.

Kerr KL, Meinzer FC, McCulloh KA *et al.* Expression of functional traits during seedling establishment in two populations of *Pinus ponderosa* from contrasting climates. *Tree Physiology* 2015;**35**:535–48.

Kitzberger T. Fire regime variation along a northern Patagonian forest-steppe gradient stand and landscape response. PhD dissertation, University of Colorado, Boulder, United States, 1994.

Kolb TE, Grady KC, McEttrick MP *et al.* Local-scale drought adaptation of ponderosa pine seedlings at habitat ecotones. *Forest Science* 2016;**62**:641–51.

Luu K, Bazin E, Blum MGB. Pcadapt: an R package to perform genome scans for selection based on principal component analysis. *Molecular Ecology Resources* 2016;**17**:67–77.

Marcotti E, Amoroso MM, Rodríguez-Catón M *et al.* Growth resilience of *Austrocedrus chilensis* to drought along a precipitation gradient in Patagonia, Argentina. *Forest Ecology and Management* 2021;**496**:119388.

Markgraf V, D'antoni HL, Ager TA. Present pollen dispersal in Argentina. *Palynology* 1981;**5**:43–63.

de la Mata R, Merlo E, Zas R. Among-population variation and plasticity to drought of Atlantic, Mediterranean, and interprovenance hybrid populations of maritime pine. *Tree Genetics & Genomes* 2014;**10**:1191–203.

Martins K, Gugger PF, Llanderal-Mendoza J *et al.* Landscape genomics provides evidence of climate-associated genetic variation in Mexican populations of *Quercus rugosa*. *Evolutionary Applications* 2018;**11**:1842–58.

McDowell N, Pockman WT, Allen CD *et al.* Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *The New Phytologist* 2008;**178**:719–39.

McNellis BE, Alistair S, Hudak AT *et al.* Tree mortality in western U.S. forests forecasted using forest inventory and Random Forest classification. *Ecosphere* 2021;**12**:1–24.

Merilä J, Hendry AP. Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. *Evolutionary Applications* 2014;**7**:1–14.

Moran EV, Lauder J, Musser C *et al.* The genetics of drought tolerance in conifers. *New Phytologist* 2017;**216**:1034–48.

Münzbergová Z, Hadincová V, Skálová H *et al.* Genetic differentiation and plasticity interact along temperature and precipitation gradients to determine plant performance under climate change. *Journal of Ecology* 2017;**105**:1358–73.

Nadeem MA, Nawaz MA, Shahid MQ *et al.* DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing. *Biotechnology and Biotechnological Equipment* 2018;**32**:261–85.

Newton AC, Tejedor N (eds), *Principles and Practice of Forest Landscape Restoration: Case Studies from the Drylands of Latin America*. Gland, Switzerland: IUCN, 2011, xxvi + 383.

Nystedt B, Street N, Wetterbom A *et al.* The Norway spruce genome sequence and conifer genome evolution. *Nature* 2013;**497**:579–84.

Oddi FJ, Casas C, Goldenberg M *et al.* Modeling potential site productivity for *Austrocedrus chilensis* trees in northern Patagonia (Argentina). *Forest Ecology and Management* 2022;**524**:120525.

Ogaya R, Liu D, Barbeta A *et al.* Stem mortality and forest dieback in a 20-years experimental drought in a Mediterranean Holm Oak Forest. *Frontiers in Forests and Global Change* 2020;**2**:89.

Oliveira RS, Costa FRC, van Baalen E *et al.* Embolism resistance drives the distribution of Amazonian rainforest tree species along hydro-topographic gradients. *The New Phytologist* 2019;**221**:1457–65.

Paruelo J, Beltrán AB, Jobbágy E *et al.* The climate of Patagonia: general patterns and controls on biotic processes. *Ecología Austral* 1998;**8**:85–101.

Pastorino MJ, Ghirardi S, Grosfeld J *et al.* Genetic variation in architectural seedling traits of Patagonian cypress natural populations from the extremes of a precipitation range. *Annals of Forest Science* 2010;**67**:508.

Peakall R, Smouse PE. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. *Bioinformatics* 2012;**28**:2537–9.

Potvin C, Tousignant D. Evolutionary consequences of simulated global change: genetic adaptation or adaptive phenotypic plasticity. *Oecologia* 1996;**108**:683–93.

Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. *Genetics* 2000;**155**:945–59.

Rodríguez-Catón M, Villalba R, Morales M *et al.* Influence of droughts on *Nothofagus pumilio* forest decline across northern Patagonia, Argentina. *Ecosphere* 2016;**7**:1–17.

Savolainen O, Lascoux M, Meriäa J. Ecological genomics of local adaptation. *Nature Reviews Genetics* 2013;**14**:807–20.

Schueler S, George JP, Karanitsch-Ackerl S *et al.* Evolvability of drought response in four native and non-native conifers: opportunities for forest and genetic resource management in Europe. *Frontiers in Plant Science* 2021;**12**:648312.

Schwinning S, Lortie CJ, Esque TC *et al.* What common-garden experiments tell us about climate responses in plants. *Journal of Ecology* 2022;**110**:986–96.

Senf C, Buras A, Zang CS *et al.* Excess forest mortality is consistently linked to drought across Europe. *Nature Communications* 2020;**11**:6200.

Shu M, Moran E. Identifying genetic variation associated with environmental variation and drought-tolerance phenotypes in Ponderosa Pine. *Research Square* 2023;**13**:1–16. e10620.

Sorensen L. *A Spatial Analysis Approach to the Global Delineation of Dryland Areas of Relevance to the CBD Programme of Work on Dry and Subhumid Lands*. Cambridge: UNEP-WCMC, 2007.

Sork V. Genomic studies of local adaptation in natural plant populations. *Journal of Heredity* 2017;**109**:3–15.

Sork VL, Aitken SN, Dyer RJ *et al.* Putting the landscape into the genomics of trees: approaches for understanding local adaptation and population responses to changing climate. *Tree Genetics & Genomes* 2013;**9**:901–11.

Souto CP, Heinemann K, Kitzberger T *et al.* Genetic diversity and structure in *Austrocedrus* populations: implications for dryland forest restoration. *Restoration Ecology* 2012;**20**:568–75.

Souto C, Kitzberger T, Arbetman M *et al.* How do cold-sensitive species endure ice ages? Phylogeographic and paleodistribution models of postglacial range expansion of the mesothermic drought-tolerant conifer *Austrocedrus chilensis*. *New Phytologist* 2015;**208**:960–72.

Spinoni J, Barbosa P, Cherlet M *et al.* How will the progressive global increase of arid areas affect population and land-use in the 21st century? *Global and Planetary Change* 2021;**205**:103597.

StatSoft, Inc. STATISTICA (Data Analysis Software System), version 10. 2011. www.statsoft.de

Suarez ML, Ghermandi L, Kitzberger T. Factors predisposing episodic drought-induced tree mortality in Nothofagus – site, climate sensitivity and growth trends. *Journal of Ecology* 2004;**92**:954–66.

Suarez ML, Kitzberger T. Recruitment patterns following a severe drought: long-term compositional shifts in Patagonian forests. *Canadian Journal of Forest Research* 2008;**38**:3002–10.

Tiffin P, Ross-Ibarra J. Advances and limits of using population genetics to understand local adaptation. *Trends in Ecology and Evolution* 2014;**29**:673–80.

Title PO, Bemmels JB. ENVIREM: an expanded set of bioclimatic and topographic variables increases flexibility and improves performance of ecological niche modeling. *Ecography* 2018;**41**:291–307.

Van Ghelder C, Parent GJ, Rigault P *et al.* The large repertoire of conifer NLR resistance genes includes drought responsive and highly diversified RNLs. *Scientific Reports* 2019;**9**:11614.

Veblen TT, Burns BR, Kitzberger T *et al.* The ecology of the conifers of southern South America. In: Enright NJ, Hill RS (eds), *Ecology of the Southern Conifers*. Melbourne, Victoria, Australia: Melbourne University Press, 1995, 120–55.

Vieira J, Carvalho A, Campelo F. Tree growth under climate change: evidence from xylogenesis timings and kinetics. *Frontiers in Plant Science* 2020;**11**:90.

Villalba R, Veblen TT. Spatial and temporal variation in Austrocedrus growth along the forest-steppe ecotone in northern Patagonia. *Canadian Journal Forest Research* 1997;**27**:580–97.

de Villemereuil P, Gaggiotti OE, Mouterde M *et al*. Common garden experiments in the genomic era: new perspectives and opportunities. *Heredity (Edinb)* 2016;**116**:249–54.

Vitalis R, Dawson K, Boursot P *et al*. DetSel 1.0: a computer program to detect markers responding to selection. *The Journal of Heredity* 2003;**94**:429–31.

Wadgymar SM, Lowry DB, Gould BA *et al*. Identifying targets and agents of selection: innovative methods to evaluate the processes that contribute to local adaptation. *Methods in Ecology and Evolution* 2017;**8**:738–49.

Whitlock MC, Lotterhos KE. Reliable detection of loci responsible for local adaptation: inference of a null model through trimming the distribution of $F(ST)$. *The American Naturalist* 2015;**186**:S24–36.

Wright IJ, Clifford HT, Kidson R *et al*. A survey of seed and seedling characters in 1744 Australian dicotyledon species: cross-species trait correlations and correlated trait-shifts within evolutionary lineages. *Biological Journal of the Linnean Society* 2000;**69**:521–47.

Zaninelli PG, Menéndez CG, Falco M *et al*. Future hydroclimatological changes in South America based on an ensemble of regional climate models. *Climate Dynamics* 2019;**52**:819–30.