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RESEARCH ARTICLE

Genomic responses to climate: Understanding local adaptation in the Andean tree species *Nothofagus pumilio* and implications for a changing world

Jill Sekely[1,2] | Paula Marchelli[3] | Verónica Arana[3] | Benjamin Dauphin[4] | María Gabriela Mattera[3] | Mario Pastorino[3] | Ivan Scotti[5] | Carolina Soliani[3] | Katrin Heer[2] | Lars Opgenoorth[1,4]

[1]Plant Ecology and Geobotany, Philipps-Universität Marburg, Marburg, Germany

[2]Eva Mayr-Stihl Professorship for Forest Genetics, Albert-Ludwigs Universität Freiburg, Freiburg, Germany

[3]INTA Bariloche, Instituto de Investigaciones Forestales y Agropecuarias Bariloche IFAB (INTA-CONICET), San Carlos de Bariloche, Argentina

[4]Swiss Federal Research Institute WSL, Birmensdorf, Switzerland

[5]Institut national de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE), Avignon, France

Correspondence
Jill Sekely, Plant Ecology and Geobotany, Philipps-Universität Marburg, Karl-von-Frisch-Straße 8, 35032 Marburg, Germany.
Email: jtsekely@gmail.com

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Societal Impact Statement

Forest trees tend to be strongly genetically adapted to their local environments, but climate change will probably subject trees to novel combinations of precipitation, temperature, and photoperiod. Local adaptation was investigated in the ecologically and economically important Patagonian tree species *Nothofagus pumilio* by characterizing its genetic diversity in relation to the varied environmental conditions across its range. These insights are useful for conservation and management decisions, for example by identifying suitable populations to establish seed source plantations for restoration and characterizing relationships with environmental drivers of selection to better understand how this species will respond to climate change.

Summary

Nothofagus pumilio is a foundation tree species that inhabits a 2000-km-long range in the southern Andes, a region with two perpendicular environmental gradients: temperature and photoperiod (North–South), and precipitation (West–East). We investigated local adaptation patterns by searching for relationships between environmental clines and signatures of adaptation in candidate genes related to stress response, growth, and phenology. Using a paired site sampling design within a landscape genome analysis, we analyzed 493 adult *N. pumilio* trees in 20 sampling sites across the species' latitudinal range. We screened 47,336 single nucleotide polymorphism (SNP) loci in 1632 contigs (i.e., coding regions along the genome). Population structure and genetic diversity analyses preceded four genome scan analyses using genetic and environmental data. Population structure and genetic diversity are mainly oriented along the latitude axis. Genome scans identified 445 outlier SNPs, which are loci showing signatures of selection. Temperature and photoperiod variables were associated with notably more outliers than precipitation. However,

Katrin Heer and Lars Opgenoorth contributed equally to this work

the most frequent biological functions among genes were water deprivation response and cold response, suggesting that stress response is comprised of complex and polygenic traits that are affected by many environmental variables. Our findings suggest that *N. pumilio* shows signatures of local adaptation to extant climate conditions, including temperature, photoperiod, and precipitation. However, climate change is likely to alter existing relationships among environmental conditions to which this species is currently adapted. These changes may have unpredictable consequences for the species' future survival, adaptation potential, and the people who depend upon these forests.

KEYWORDS
climate change, genome scan, genotype-environment association, lenga, local adaptation, *Nothofagus*, outlier loci

1 | INTRODUCTION

Genetic diversity within primary producer species has strong influence on community and ecosystem dynamics (Raffard et al., 2019), and the ongoing loss of this intraspecific diversity is a hidden biodiversity crisis that can affect the people and other organisms that depend upon forests (Des Roches et al., 2021). An acute threat to genetic diversity is climate change, which is expected to have myriad impacts on forests, including shifts in species ranges, tree growth rates, and phenology (IPBES, 2019). Tree populations typically have high levels of standing genetic diversity due to their widespread distributions across diverse habitats and large effective population sizes, and therefore they may have high local adaptation potential even when faced with rapidly changing conditions (Kremer et al., 2012; Milesi et al., 2023; Savolainen et al., 2007). However, the extraordinary challenge of contemporary climate change is that it will likely create novel combinations of precipitation, temperature, and photoperiod that neither occur within the current range nor have occurred for millions of years (Burke et al., 2018; Williams & Jackson, 2007). By decoupling current relationships among environmental conditions, no-analog conditions could impose unique selection pressures that will challenge tree populations' ability to adapt and survive.

Temperature and precipitation patterns are forecast to shift across regions (Barros et al., 2015; Williams & Jackson, 2007), while photoperiod will be unaffected by climate change. The potential consequences are many. For example, phenology in temperate species is mediated by a combination of photoperiod and temperature cues (Howe et al., 1995; Singh et al., 2017), and climate change could disrupt these relationships. Indirect effects are also likely; pest and pathogen species may likewise experience range or phenology shifts due to climate change, thereby changing the timing or severity of infestations (Castex et al., 2018; Paritsis & Veblen, 2011). Climate shifts also have implications for drought, which is a direct consequence of water availability but its severity is influenced by temperature, since high temperatures can increase both evapotranspiration rates and drought stress during plants' growing season (Vicente-Serrano et al., 2010). Taken together, no-analog climate combinations will likely impose selection pressure on traits, thus affecting genes related to phenology (Hänninen & Tanino, 2011), extreme temperature and drought response (Niinemets, 2010), and defense response (Haynes et al., 2022). Selection upon these genes leaves signatures of adaptation along the genome, and characterizing these signatures can provide critical information about how tree populations might respond to climate change. An initial step is to establish whether these signatures are currently observed.

Forests in the Patagonia region of the southern Andes mountain range present an ideal study system due to the mountains' orientation, which creates two geographically perpendicular environmental gradients. One is a North–South gradient of day length and temperature that is driven by latitude, and the other is a West–East precipitation gradient driven by prevailing winds and a montane rain shadow. The most widespread native tree species in this region is the southern beech "lenga" (*Nothofagus pumilio* ([Poepp. & Endl.] Krasser)), a cold-tolerant deciduous tree that inhabits a nearly continuous range of more than 2000 km in length. Its range covers large parts of the aforementioned Andean gradients, encompassing a diverse climate space from 6000 mm of annual precipitation in the west to just 200 mm in the east (Veblen et al., 1996, Figure 1). In addition to being an ecologically important species, *N. pumilio* is also the most exploited local timber resource (Gea-Izquierdo et al., 2004). However, little is known about its local adaptation patterns at the fine geographical scale. Previous studies have examined its neutral genetic diversity and phenotypic plasticity using neutral markers (Mathiasen & Premoli, 2013, 2016; Mattera et al., 2020; Premoli, 2003; Soliani et al., 2012, 2015), but adaptive genetic variation along environmental clines using high-throughput single nucleotide polymorphisms (SNPs) has not yet been assessed.

N. pumilio is an abundant foundation species that defines an entire ecosystem, and characterizing and conserving its diversity are critical to maintaining the health of local Patagonian forests and supporting the people who depend upon it. The objective of this study was therefore to characterize the extent of this species' current local

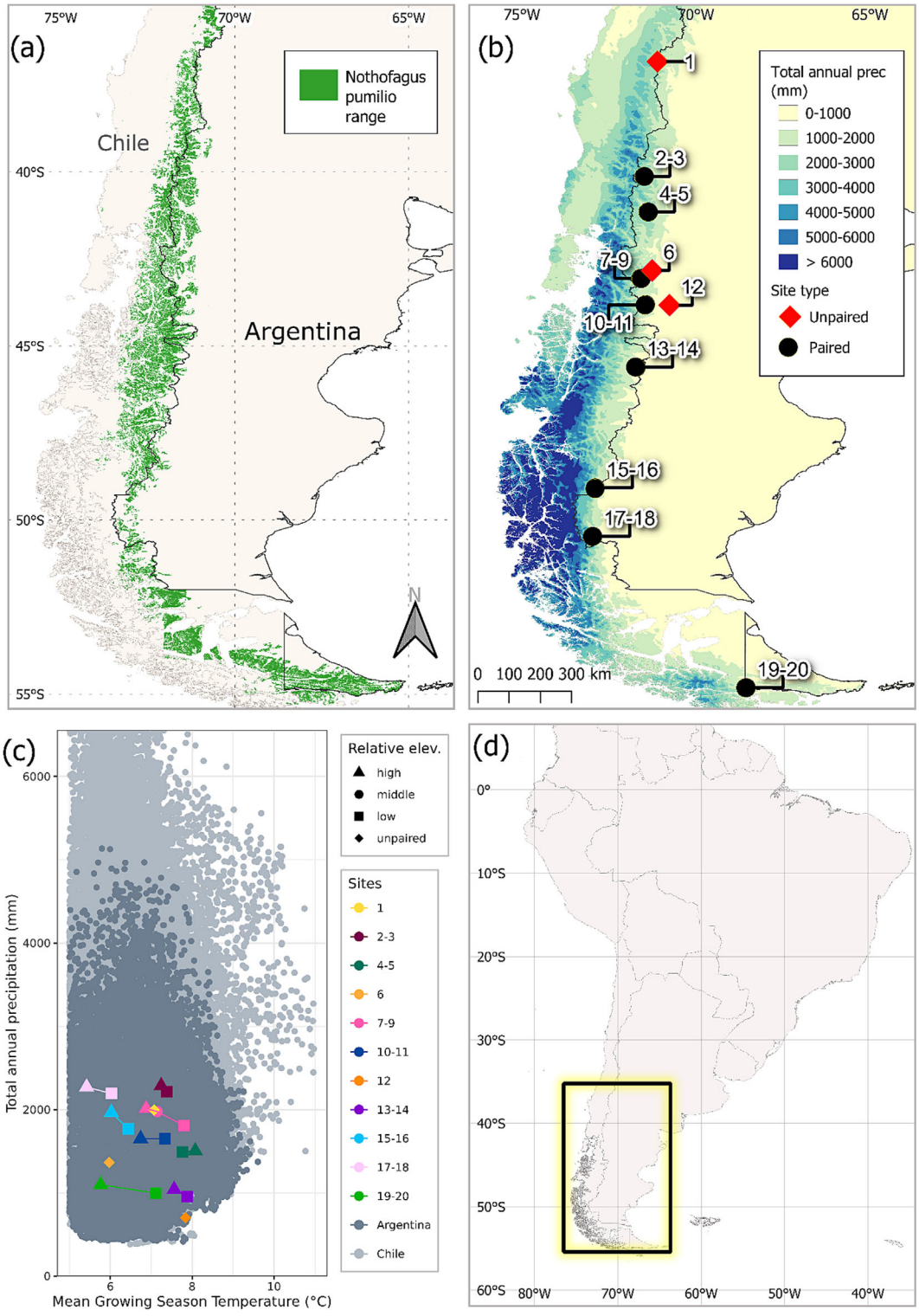

FIGURE 1 **Characterization of the full *Nothofagus pumilio* species distribution and study sampling sites therein.** (a) Species distribution map; (b) example climate raster layer (total annual precipitation) that also shows sampling site locations and whether the locality contains paired or unpaired sites; (c) climate space inhabited by *Nothofagus pumilio* in terms of mean temperature of all growing season days (°C) and mean annual total precipitation (mm). All climate values are the mean across the years 1981–2010. Values were extracted for the full distribution range in Argentina (dark gray) and Chile (light gray), and sampling localities are indicated by color, with sites within a pair differentiated by shape to show their local elevation class and connected by same-color lines. *Note*: Mean growing season temperature was truncated at 5°C to compensate for the low spatial resolution of CHELSA data (which showed erroneous low-temperature artifacts at high elevations due to sharp mountain slopes) and also truncated over 6000 mm of precipitation to improve graph clarity; (d) overview map of South America showing the focal area highlighted in panels a and b.

adaptation. We searched for signatures of local adaptation within candidate genes in situ, that is, in "natural" forests that are not under experimental control. We used a landscape genomics approach to assess how evolutionary processes and environmental variation have shaped genetic variation (Capblancq & Forester, 2021; Rellstab et al., 2015). Local adaptation depends on a balance among many factors whose individual effects can be difficult to differentiate, and choosing an appropriate sampling design and analysis methods is crucial for improving study power (Lotterhos & Whitlock, 2015; Meirmans, 2015). We used a paired-site sampling design, which aims to disentangle environmental effects from a neutral population structure by maximizing the climatic distance between pairs of sampling sites while minimizing the neutral genetic divergence (Lotterhos & Whitlock, 2015; Scotti et al., 2023). We distributed sampling site pairs along the two gradients on the eastern side of the Andes and assessed variation using univariate and multivariate genome scan methods. We hypothesize that the two gradients have exerted strong selection pressure on *N. pumilio* and have thus created signatures of local adaptation in candidate genes, which we addressed by quantifying the strength of correlations between environmental variables and genetic characteristics. We predicted that (i) allele frequencies in candidate genes that are linked to growth and stress response will correlate with temperature and photoperiod clines and (ii) allele frequencies in candidate genes linked to drought response will correlate with precipitation, albeit to a weaker degree given the geographically narrower precipitation gradient.

2 | MATERIALS AND METHODS

2.1 | Study species

N. pumilio, common name "lenga," is a deciduous and broad-leaved tree species native to the southernmost temperate forests of the Andes mountains, where it grows between latitudes 35° to 56°S (Veblen et al., 1996). It is a wind-pollinated, wind-dispersed, and strictly outcrossing species. Individuals can reach approximately 350 years of age and generation time is around 50 years (Veblen et al., 1996). It is relatively cold-tolerant and often forms monospecific stands up to the montane tree line. North of 41°S, lenga grows in the subalpine zone, but it also grows at sea level in the southernmost (i.e., poleward) parts of its range. Lenga is a member of the Nothofagaceae family, within the Fagales order, which predominantly inhabits the Northern Hemisphere and includes *Fagus*, *Quercus*, and *Betula* species (Vento & Agraín, 2018). *N. pumilio* is a non-model species (i.e., without a reference genome), but a de novo transcriptome is available (Estravis-Barcala et al., 2021).

2.2 | Sampling design

To disentangle neutral and adaptive genetic variation, we used a paired-site study design after Lotterhos and Whitlock (2015). According to the authors, this sampling design has greater power to detect signatures of local adaptation compared to transect or random sampling designs, particularly when combined with genome scan methods based on latent factor mixed models (LFMM) and Bayesian methods (see genome scan methods below). Sites within a pair should be geographically close enough to share a demographic history but distant enough that they experience different environmental selection pressures. We selected eight localities that were distributed along the species' full latitudinal range on the eastern slope of the Andes (Figure 1), and each locality contained two (or, in one locality, three) sampling sites. Linear distance among paired sites within a locality was always less than three kilometers, to stay within reasonable gene flow distance. Gene flow information is limited for *Nothofagus* species, but estimates from related species and natural post-fire regeneration suggest pollen and seed dispersal may be limited to 45–60 m from adult trees (Sola et al., 2020; Urretavizcaya et al., 2022). The elevation difference among the paired sites' centroids was at least 150 m to capture an approximate 1–2°C difference in mean annual temperature due to lapse rate (Whiteman, 2000). In other words, sites were physically as close to each other as possible while also capturing a wide portion of the local elevation gradient. The high-elevation sites were located well below the alpine tree line to avoid sampling trees with shrub-like krummholz formation (Table 1). In addition, we sampled three unpaired localities in geographically marginal habitats (i.e., located at the edge of the species distribution). Each unpaired locality has one sampling site (Epulaufquen [Site 1], La Hoya [6], and Jose de San Martin [12]). The latter two sites are located at approximately the same latitude as one of the paired localities, in an attempt to capture a wider portion of the East–West precipitation gradient.

We sampled between 21 and 25 adult trees per site, for a total of 493 individuals. Selected trees were dominant or co-dominant mature individuals, that is, at least 50 years old (Veblen et al., 1996), as confirmed by annual tree rings (Sekely et al, manuscript in progress). Intertree distances were at least 30 m to reduce the chance of sampling directly related individuals. Geographic coordinates for each tree were recorded with a handheld GPS device (Garmin model GPSMAP 64st). We collected fresh leaf buds for DNA extraction and stored them at −80°C. Immediately before extraction, buds were manually descaled, flash-frozen with liquid nitrogen, and ground with mortar and pestle. Samples were randomly assigned to extraction batches. Total genomic DNA was extracted from 0.1 g of plant material using the CTAB protocol by Doyle (1990) with minor modifications, since *N. pumilio* leaf buds have high levels of polysaccharides and polyphenols that can impact the quality and quantity of extracted DNA. Therefore, we added 1% soluble polyvinylpyrrolidone (PVP) and dithiothreitol (DTT) to the lysis buffer (Porebski et al., 1997). Extracted DNA quantity was measured with a QUBIT 1.0 Fluorometer (Invitrogen, Carlsbad, CA), and the quality was spot-checked with Nanodrop™ 2000 (ThermoFisher Scientific, catalog ND-2000). Extracted DNA samples were randomized among plates for downstream sequencing.

TABLE 1 **Geographic characteristics of the 20 *Nothofagus pumilio* sampling sites in Argentina, including the number of adult trees sampled per site.** Sites are numbered from north (Site 1) to south (Site 20).

Site number	Locality	Elevation class	Elevation (m a.s.l.)	Latitude (°)	Longitude (°)	No. of samples
1	Epulaufquen	Unpaired	1511	−36.8321	−71.1134	25
2	San Martín dl Andes	High	1478	−40.1263	−71.4886	25
3	San Martín dl Andes	Low	1253	−40.1281	−71.4799	25
4	Cerro Otto	High	1382	−41.1482	−71.3783	25
5	Cerro Otto	Low	1146	−41.1512	−71.3658	24
6	La Hoya	Unpaired	1442	−42.8341	−71.2592	25
7	Trevelin	High	1360	−43.0565	−71.5877	21
8	Trevelin	Middle	1312	−43.0548	−71.5847	25
9	Trevelin	Low	1085	−43.0663	−71.574	25
10	Lago Guacho	High	1314	−43.8121	−71.4513	25
11	Lago Guacho	Low	1162	−43.823	−71.4629	25
12	José de San Martín	Unpaired	1317	−43.8281	−70.757	24
13	El Triana	High	915	−45.6044	−71.7387	25
14	El Triana	Low	729	−45.6119	−71.7181	25
15	El Chaltén	High	670	−49.0749	−72.9001	25
16	El Chaltén	Low	505	−49.0986	−72.9007	25
17	El Calafate	High	614	−50.4683	−72.9687	24
18	El Calafate	Low	295	−50.4729	−72.979	25
19	Ushuaia	High	326	−54.8191	−68.5575	25
20	Ushuaia	Low	20	−54.8223	−68.5675	25

Note: Locality is the local site name. Elevation class refers to the site's relative elevation within that locality, either paired (high, middle, and low elevation) or unpaired. Elevation in meters above sea level, latitude, and longitude are in from coordinate reference system WGS84.

2.3 | Environmental data and covariate choice

Empirical climate data is limited for the Andes region, so environmental variables were extracted from the global public repository climate dataset CHELSA (v 2.1, Karger et al., 2017). CHELSA incorporates empirical climate data from 1981 to 2010, from which further variables were derived and extrapolated across the globe at a resolution of 30 arcsec (~1 km^2). We chose this dataset because it has been shown to represent orographic conditions more accurately than WorldClim (e.g., Bobrowski et al., 2021). We extracted tree-level data from climate layers with the raster package, using the extract() command for individual tree GPS locations and the "bilinear" option, which interpolates values from the four nearest raster cells to approximate finer-scale climate parameters (Hijmans et al., 2015).

Genome scans are sensitive to collinearity, so we first pruned environmental variables (Dormann et al., 2013; Rellstab et al., 2015). From the CHELSA dataset we first selected a short list of variables related to temperature and precipitation, calculated pairwise Spearman correlation values among these covariates using the psych package (Revelle, 2015), and finally selected variables that had correlation coefficients with other parameters less than |0.8| (Figure S1). Ultimately, we selected two CHELSA temperature parameters (isothermality and mean growing season temperature), one precipitation parameter (total annual precipitation), and one temperature-affected precipitation parameter (snow cover days) (Figure S2). We chose the latter variable because longer snow cover persistence, particularly in late spring, has been shown to reduce radial growth in *N. pumilio* adults at higher elevations (Villalba et al., 1997). Finally, since we also investigated circadian clock candidate genes, we calculated the average day length in the midsummer month of January using latitude and the geosphere package (Hijmans et al., 2017) to approximate day length (Gárate-Escamilla et al., 2019). All environmental variables were scaled prior to analysis.

2.4 | Probe design and filtering

Trees were genotyped with targeted sequencing (i.e., exome capture), for which we assembled a starting set of target candidate genes. A recent study investigated candidate gene orthogroups across seven European tree species including Fagales members (Milesi et al., 2023). Those authors selected candidate genes from various sources, including cold, heat, drought, and defense response among GO (Gene Ontology) terms, AmiGO (Carbon et al., 2009), and KEGG (Kyoto Encyclopedia of Genes and Genomes) gene regulation networks (Ashburner et al., 2000; Carbon et al., 2009; Kanehisa & Goto, 2000), convergent genes that were identified between distantly-related conifers (Yeaman et al., 2016), and cold-related genes (Miura & Furumoto, 2013). These orthogroups were represented by 1789 candidate genes in the model species *Arabidopsis thaliana*. We supplemented this list with

394 species-specific *N. pumilio* candidate genes, including some that were differentially expressed in a recent heat stress transcriptomic study (Estravis-Barcala et al., 2021) or are affiliated with wood growth or circadian clock rhythms (Estravis-Barcala et al., 2020). Our total starting candidate gene list therefore contained 2183 genes.

We identified the respective ortholog genes in the *N. pumilio* transcriptome using BLASTn (Altschul et al., 1990) (Methods S1). From the starting candidate gene list, 1913 had hits in the *N. pumilio* transcriptome (88%, Figure S3), and their respective sequences were used for the probe design. Library preparation, sequencing, and coarse quality filtering were performed by IGA Technology Services (Udine, Italy), and called variants were finely quality-filtered (Methods S1). After filtering, the dataset contained 116,136 SNPs in 1783 contigs (i.e., coding regions along the genome). Paralogous loci were pruned with the HDplot method (McKinney et al., 2017), then loci in linkage disequilibrium were pruned with plink (Chang et al., 2015), which retains the allele with the greater minor allele frequency. This pipeline created our "main dataset," which contained 47,336 SNPs (1632 contigs). Finally, we applied a minor allele frequency filter of 5% to create a "maf-filtered dataset" that contained 9601 SNPs (1437 contigs).

2.5 | Descriptive genetic diversity statistics and population structure

We calculated population structure and genetic diversity values using the main dataset. Population structure was analyzed using ADMIXTURE (Alexander et al., 2009) (see Table S1 for all software and package version numbers). We assessed every possible value of K (i.e., number of subpopulation clusters) from 1 to 20, to represent the 20 sampling sites. The main dataset contains singleton loci (i.e., alleles found within only one individual), which can confound model-based inference of population structure such as ADMIXTURE (Linck & Battey, 2019), so they were removed prior to analysis. Pairwise F_{ST} statistics were calculated in vcftools for every possible pair of sampling sites using the weighted θ correction (Weir & Cockerham, 1984). Nucleotide diversity was calculated with pixy software (Korunes & Samuk, 2021), which also uses invariant loci to calculate less-biased values. Therefore, our input dataset for nucleotide diversity contained the main dataset plus all called invariant loci, which were quality-filtered using the same thresholds as the main dataset. Following pixy user guidelines, we aggregated values within a sampling site by summing raw count differences and dividing by summed comparisons. We used R for all remaining analyses (R Core Team, 2023). The rarefied count of private alleles was calculated with the poppr package (Kamvar et al., 2014). We calculated heterozygosity and F_{IS} using hierfstat (Goudet, 2005).

2.6 | Genome scan method

To identify putative SNPs under selection, we applied multiple genome scan methods. Genome scans compare genetic variation across the targeted genome areas and identify over-differentiated loci, hereafter called outlier SNPs. We assessed the maf-filtered dataset, as is common practice, because GEA methods have low power to detect extremely rare alleles (De La Torre et al., 2019; Lasky et al., 2023; Pearson & Manolio, 2008). There is an ever-growing list of genome scan tools and algorithms (see Bourgeois & Warren, 2021), each of which has its own benefits and pitfalls (e.g., Rellstab et al., 2015; Waldvogel et al., 2020). Common practice is to analyze a dataset with multiple methods and inspect overlap among their results, since this provides stronger evidence that a locus is a true-positive outlier (de Villemereuil et al., 2014; Waldvogel et al., 2020). Further reasoning for using multiple methods and inspecting overlap is the inherent environmental collinearity within this study system. Isothermality and day length variables both have strong (but opposite) correlations with latitude and respectively very little or no differentiation within our paired sites (Figure S2), in contrast to the ideal orthogonal relationship between precipitation and each of these variables (Lasky et al., 2023). Considering this correlation, GEA may only be able to identify suites of collinear variables associated with outliers rather than individual predictors (Lasky et al., 2023). On a related note, while limited environmental distance contradicts an underlying argument for using paired sites (Lotterhos & Whitlock, 2015), the main goal of using this sampling design was to improve study power to find true positive outliers. Thus, choosing appropriate genome scan methods is critical.

Genome scans identify loci that are strongly differentiated among genetic clusters (e.g., subpopulations) and/or strongly associated with environmental gradients (Savolainen et al., 2013). We use both methods and classify them respectively as "population differentiation" (sensu Beaumont & Nichols, 1996) and "genotype-environment association" (sensu Hedrick et al., 1976). The advantage of population differentiation tests is that they require no prior knowledge about environmental selection pressures and therefore are less susceptible to errors related to missing environmental data or suboptimal choice of climatic variables. We used one population differentiation test, pcadapt (Duforet-Frebourg et al., 2014), as implemented in the pcadapt package (Privé et al., 2020). Meanwhile, genotype-environment associations (GEA) can provide evidence about which environmental variables are associated with adaptive differentiation, and they may have greater power to detect weakly selected loci, which are often critical for adaptation but may only show small allele frequency shifts (De La Torre et al., 2019). We used three GEA methods that each assess SNP frequency variations and environmental covariates in different univariate or multivariate configurations. The Bayesian hierarchical model BayPass is a univariate method for both genetic and environmental components (Gautier, 2015; Materials S1). The other two GEA methods assess multivariate environmental parameters, which can account for interaction among environmental factors. The first is LFMM (Caye et al., 2019), which we ran as univariate for genetic components but multivariate for environmental. As an aside, LFMM could have been used as univariate for environmental factors, but we chose to use its multivariate configuration to better reflect the real-world multidimensionality of environmental covariates. For this analysis, we

used the LEA package (Frichot & Francois, 2015) and the lfmm2() command. The second, redundancy analysis (RDA), is a multivariate approach for both environmental and genetic variables (Capblancq & Forester, 2021). We used the rda() command in the vegan package (Oksanen et al., 2007).

We curated a study-wide list of candidate outliers by first converting all calibrated *p*-values to *q*-values. For RDA, we used the custom command "rdadapt()" (Capblancq & Forester, 2021) to calculate *q*-values using $K = 3$ (Figure S4). For all other analyses, we used the p.adjust() command in the base R stats package with the Benjamini–Hochberg equation (Benjamini & Hochberg, 1995) and then applied a false discovery rate control threshold across tests (François et al., 2016). We chose a fairly lenient study-wide false discovery rate threshold of 0.01 (i.e., <1% false positives). Finally, we compared overlap among all four tests to determine evidence strength for true positive outliers. Gene functions were obtained during assembly of the target candidate gene set from the TAIR and UniProt databases (Milesi et al., 2023; Rhee et al., 2003; The UniProt Consortium, 2023). We ran a PANTHER GO-term statistical overrepresentation test on all associated GO terms using our starting candidate gene list as the background list (Thomas et al., 2022).

3 | RESULTS

3.1 | Genetic diversity and population structure

We found significant negative correlations between each genetic diversity parameter and latitude (Figure 2), meaning diversity values are highest in sites closest to the equator and decrease poleward. There are no consistent significant local elevation trends within paired sites, thus the locally higher sites do not always have lower diversity. In the north, diversity values tend to be greater in higher-elevation sites (e.g., Sites 2 and 4), but in the south, they tend to be lesser in higher-elevation sites (Sites 15, 17, and 19). The same patterns hold true for nucleotide diversity, which had the weakest correlation with latitude. Observed heterozygosity per sampling site was always greater than expected, meaning there is heterozygote excess (negative F_{IS}), as is expected in a self-incompatible (i.e., outcrossing) species. Epulaufquen (Site 1) had the greatest number of private alleles (i.e., endemic diversity); then, the values sharply decreased poleward.

Population structure is also oriented along the latitudinal gradient (Figures 3, S1, and S5), although the exact number of historical population clusters is ambiguous. According to cross-validation values in ADMIXTURE, the optimal number of genetic clusters (*K*) is 2 (Figure S6). However, principal component and snmf analyses suggested that $K = 3$ is optimal (Figures S4 and S7). We present *K* values from 2 to 4 (Figure 3a), since all are informative about the hierarchical population structure (Meirmans, 2015). Across *K*-values, a break consistently occurs between Sites 12 and 13 (i.e., between latitudes 43.8–45.6°S), with admixture appearing in Sites 13 and 14. At $K = 3$, Sites 2–5 show admixture (Figure 3b), and at $K = 4$, this region becomes its own cluster, with a break between Sites 5 and 6 (41.2–42.8°S). The unpaired northernmost Site 1 (Epulaufquen) also isolates into its own cluster at $K = 4$, whereas the other two unpaired sites (6 and 12) show similar cluster compositions as their counterpart paired sites at similar latitudes (7–9 and 10–11, respectively). Finally, paired sites generally had the lowest pairwise subpopulation differentiation (F_{ST}) values, and a linear regression analysis of genetic versus geographic distance suggests that there is isolation by distance (Figure 3c).

3.2 | Climate conditions per site

Climate variables often show strong clines along the local elevation gradient or the latitude axis (Figure S2). Locally higher elevation sites typically experience greater total annual precipitation and more snow cover days, reflecting that the majority of annual precipitation is received in winter as snow in many *N. pumilio* forests (Veblen et al., 1996, Figure S2). Growing season temperature is also lower at higher elevation sites, with the exception of Cerro Otto (Sites 4–5), possibly due to CHELSA resolution and the site's location at the crest of the local hill. The two unpaired localities in the central portion of the range (6 and 12) have low annual precipitation and high isothermality, also in relation to their counterpart paired localities at similar latitudes (Sites 7–9 and 10–11, respectively), demonstrating that these geographically marginal sites are also environmentally marginal. In terms of the latitude axis, photoperiod in January is a direct function of latitude and therefore its values do not differ within paired sites (Figures S1c and S2). Isothermality likewise has a strong negative correlation with latitude and relatively little differentiation within site pairs (Figures S1a and S2). The remaining three variables (annual precipitation, snow cover days, and growing season temperature) have fairly weak relationships with latitude (Figure S1). A principal components analysis of environmental variables indicated that 72% of the variance among sampling sites can be described by two axes: The first mainly comprises the photoperiod and two temperature variables, and the second mainly comprises annual precipitation (Figure S2f). Finally, the latitude axis had strong relationships with genetic diversity statistics (Figure 2) and population structure (Figure 3), in addition to the aforementioned environmental variables (Figure S1), which may have had a confounding effect. We attempted to address this from various methodological angles, including using genome scan methods that account for population structure, using two multivariate environment analyses, and assessing outlier overlap.

3.3 | Outlier-containing genes

A total of 445 loci in 320 contigs were identified as outliers by at least one genome scan method (4.6% of maf-filtered dataset) (Table S2). Among the subset of study-specific candidate genes, 48 contained outlier SNPs. From the perspective of individual genome scan analyses, the population differentiation method pcadapt identified the greatest number of outliers ($n = 256$, Figure 4), whereas the GEA test

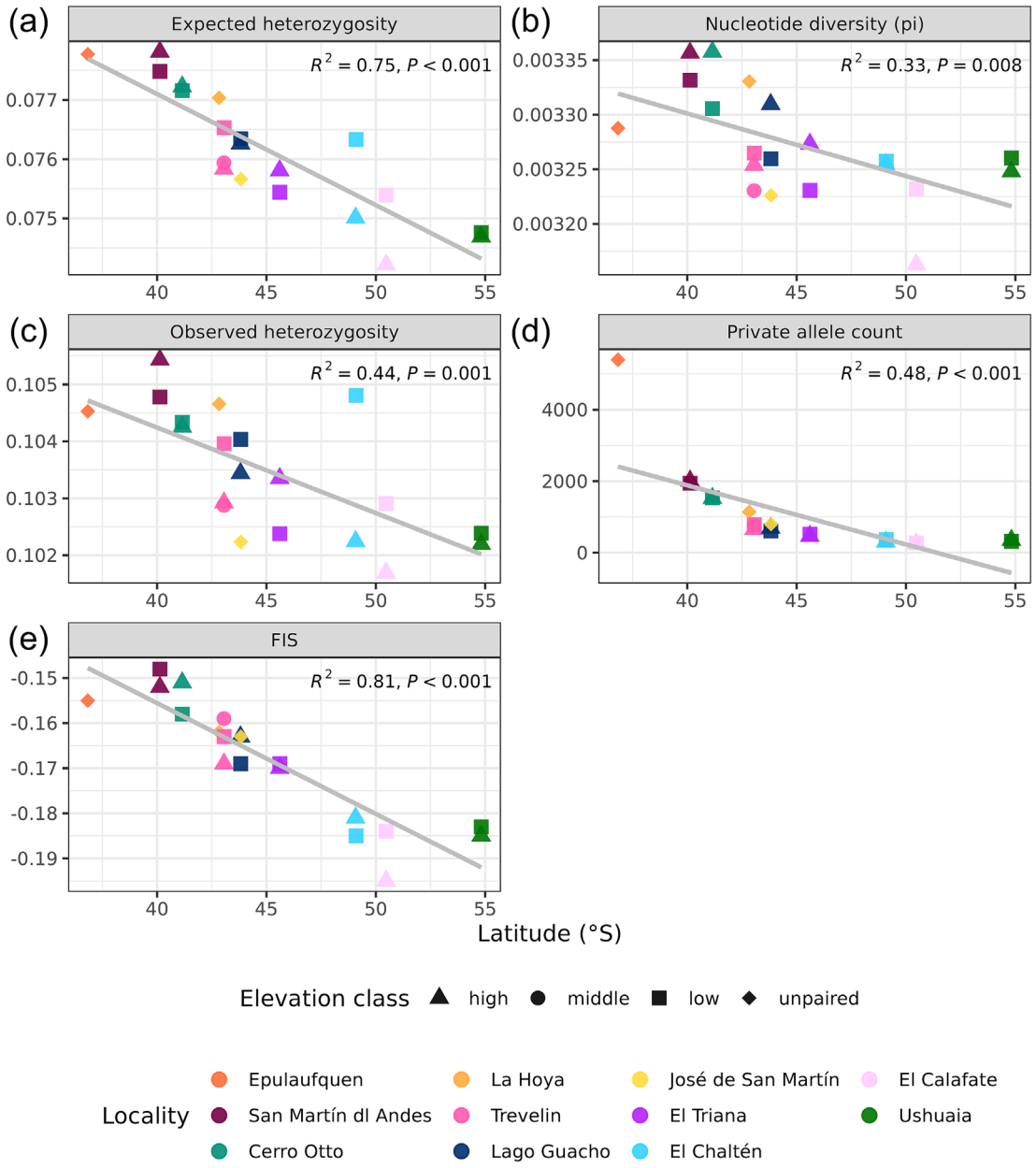

FIGURE 2 **Five measures of genetic diversity at *Nothofagus pumilio* sampling sites and their relationships with latitude.** Genetic diversity statistics are (a) expected heterozygosity, (b) nucleotide diversity (also including invariant called sites), (c) observed heterozygosity, (d) rarefied private allele count, and (e) fixation index F_{IS}. Colors indicate the sampling site, and shapes indicate the relative elevation class of that site within the locality (high, middle, and low [within paired localities] or unpaired). Coefficient of determination (R^2) and significance (p) values for linear regression models are included at the top right of each graph.

LFMM identified the fewest ($n = 94$). To determine which outliers had stronger evidence for being true positives, we compared overlap among methods. Convergence among results strongly differed (Figure 4). For example, RDA identified 122 outliers, and all but three were also identified by other methods, whereas over half of the 244 BayPass outliers were unique to that analysis. A total of 171 SNPs (1.8% of maf-filtered dataset) were identified by at least two methods, and 35 of these were identified by all four algorithms (Figure 4, Table 2, and Table S1). These two outlier subsets will hereafter be called moderate-evidence and strong-evidence outliers (i.e., for being true positives), respectively.

The BayPass algorithm assesses all genetic and environmental variables individually, so it is possible to identify the most important environmental factors per outlier SNP (Figure 5). Day length in January and temperature variables (isothermality and growing season) were significantly associated with the most SNP outliers ($n = 116$ and 110, respectively). Among the 35 strong-evidence outliers, all but six were associated with one or both of these variable groups. Precipitation variables ("annual precipitation" and "snow cover days") were associated with the fewest outliers ($n = 58$). Thirty-eight SNPs were associated with more than one variable.

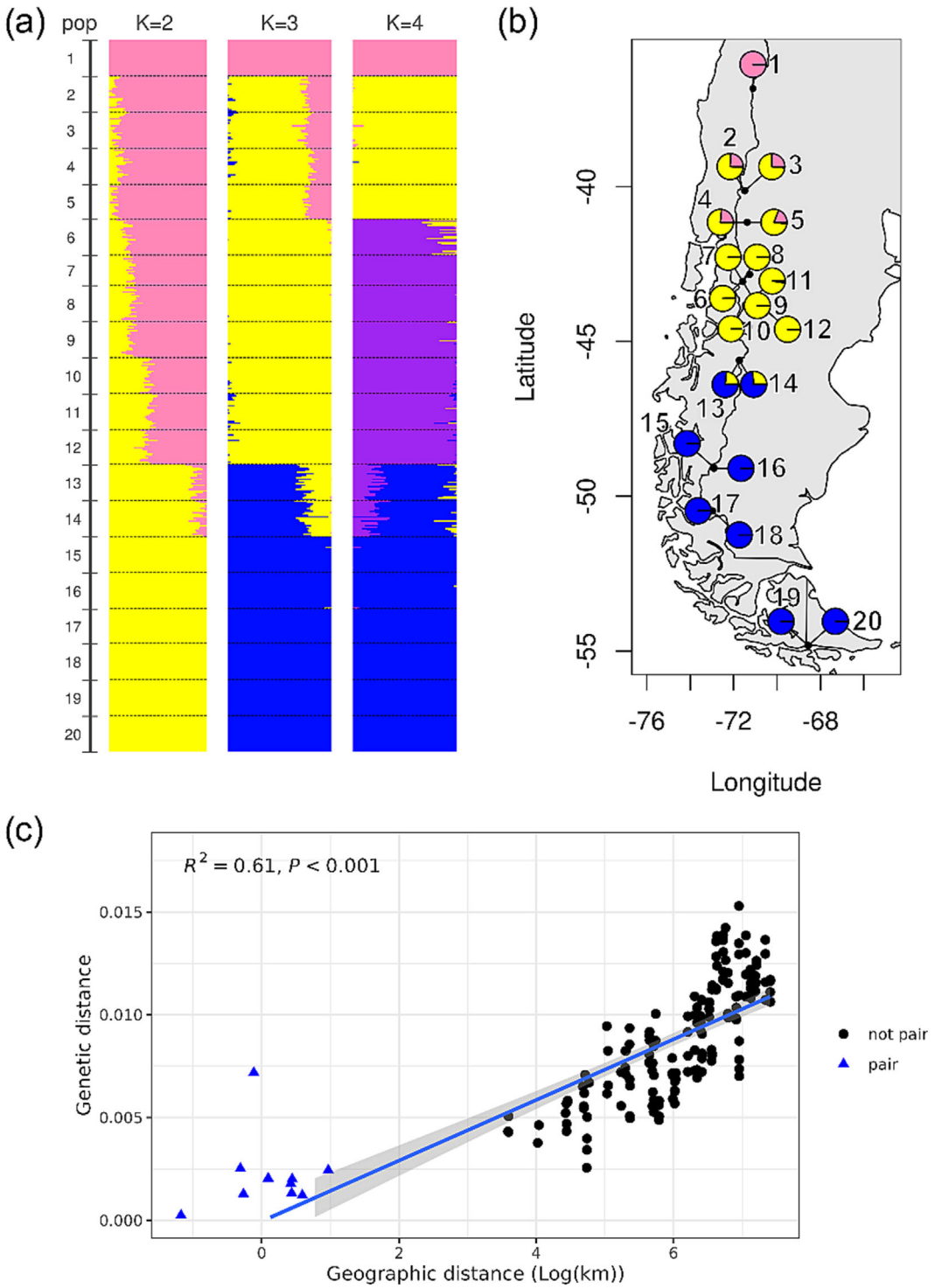

FIGURE 3 **Population genetic structure analysis of *Nothofagus pumilio* using single nucleotide polymorphism data at 20 Argentina sampling sites, including isolation by distance.** (a) Individual ADMIXTURE plots for *K* (ancestral population) cluster values of 2, 3, and 4, ordered from the north (sampling Site 1) at the top to the south (sampling Site 20) at the bottom. Each individual tree is represented by one line, and each color within lines indicates one population cluster. (b) Pie charts showing cluster percentages per sampling site using averaged $K = 3$ results from ADMIXTURE analysis shown in 3a. Pie charts have been jittered to avoid overlap and are connected to their actual geographic locations by a black line. (c) Isolation by distance was assessed by comparing logarithmic geographic distance with pairwise genetic distance ($F_{ST}/[1 - F_{ST}]$) among sampling sites. F_{ST} is the fixation index, or proportion of total genetic variation in that subpopulation compared to total population, and was quantified with Weir and Cockerham pairwise values. The graph does not include Site 1 because these values are inflated (>0.02). Blue triangles indicate within-pair values (e.g., Sites 4 and 5, the two sites in the Cerro Otto locality), and black circles indicate not-paired sites (e.g., Sites 4 and 20). Coefficient of determination (R^2) and significance (*p*) value class are reported in the upper left.

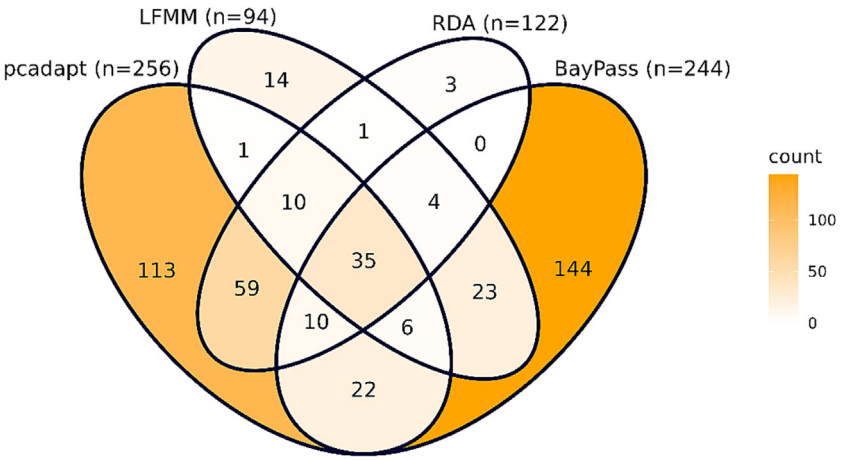

FIGURE 4 **Number of single nucleotide polymorphisms (SNPs) significant outliers that were found by individual genome scan methods, and the overlap among methods, for *Nothofagus pumilio* at 20 sampling sites in Argentina.** The name of each method is shown above the Venn section, where the acronym "LFMM" stands for latent factor mixed models, and "RDA" stands for redundancy analysis. Total number of loci found by a method is shown in parentheses after the method name. Numbers inside each Venn section indicate the number of loci found by the method(s), and color indicates relative count, from low (white) to high (dark orange).

The GO enrichment analysis indicated that no gene functions were significantly overrepresented in relation to the starting candidate gene list. However, PANTHER assesses all GO terms associated with each gene, while our study targeted only a subset thereof. Therefore, we report and discuss some results regarding only the targeted terms (Table S3). For the moderate-to-strong outliers, the top three most frequent targeted terms included response to water deprivation, response to cold, and starch and sucrose metabolism. Defense response was the most frequently targeted GO term from the candidate gene set, but a disproportionately small amount of outlier-containing genes were associated with this term.

4 | DISCUSSION

Forecasting the impact of climate change on forest trees such as *N. pumilio* requires knowledge about current local adaptation. We used a landscape genomics approach to determine which genes show signatures of adaptation and which environmental factors might be influencing that selection. Temperature, photoperiod, and precipitation were investigated alone and in combination with each other, since interplay among covariates can affect biological processes. We found that population structure and genetic diversity are mainly structured along the latitude axis, which also aligns with the predominantly north–south spine of the Andes. As predicted, temperature and photoperiod variables were significantly associated with the greatest numbers of SNP outlier loci, while precipitation variables were associated with fewer. However, many outliers were either identified only by multivariate analyses, associated with more than one environmental variable or were located in genes related to biological functions more diverse than their environmental associations, suggesting that target genes are affected by combinations of environmental variables. Thus, climate change may have unpredictable effects on *N. pumilio* survival and adaptation if it decouples relationships among environmental selection pressures to which its genes are currently adapted, warranting close monitoring and further study of this species in the future.

4.1 | Population structure and genetic diversity patterns follow latitude

Population structure follows latitude and we observed the strongest phylogenetic division at mid-latitudes, between 43 and 45°S (Sites 12 and 13) (Figures 3 and S5). At higher population cluster (*K*) values, we also observed a division further north, approximately between 41.1 and 42.8°S (Sites 5 and 6). Previous studies with neutral markers also found evidence for two geographically segregated *N. pumilio* lineages (Mathiasen & Premoli, 2010; Mattera et al., 2020; Soliani et al., 2012, 2015), although the exact location differed among studies. Some studies found the greatest division near 42°S (Mathiasen & Premoli, 2010; Mattera et al., 2020), whereas another suggested between 42 and 44°S (Soliani et al., 2015). Similar latitude-oriented phylogenetic divides have been observed across many Patagonian taxa of flora and fauna (Sersic et al., 2011), suggesting that these divides were driven by a shared biogeographic history in addition to species-specific biology. For example, divergent glacial patterns (Glasser et al., 2008) and paleobasins (Mathiasen & Premoli, 2010) have been postulated as shared drivers. Species-specific gene flow and expansion patterns following range contractions also play a role in population structure patterns, but it is difficult to directly study this factor since limited empirical gene flow information exists for *N. pumilio*. However, further clarification may come from the genetic diversity statistics.

All genetic diversity parameters also show significant negative relationships with latitude (Figure 2), which may both shed light on past demographics and help predict populations' resilience to climate change. Historical pollen and neutral genetic data have been used to suggest *Nothofagus* species responded to glaciations by migrating (Villagran, 1990) and retreating to refugia (Markgraf, 1993), although there is ongoing debate about refugia locations. Refugia locations are often identified by their high heterozygosity (Petit et al., 2003; Roberts & Hamann, 2015), and we found higher heterozygosity values in the north, providing support for refugia there. Higher nucleotide diversity values in the north imply historically greater effective population sizes, meaning northern populations were probably more numerous and/or larger (assuming a similar mutation rate

TABLE 2 Gene annotation information for a selection of strong-evidence outliers that were identified in genetic-environment association analysis for *Nothofagus pumilio* at 20 sampling sites in Argentina.

				Genome scan significance (q-values)			
Biological process	**Nothofaguscontig**	**TAIR**	**Gene name**	**Pcadapt**	**LFMM**	**RDA**	**BayPass**
Stress response	chain_4216	AT1G13960	WRKY DNA-binding protein 4 (WRKY4)	2.63E-07	2.16E-03	9.80E-04	3.64E-13
	chain_1793	AT1G56070	Ribosomal protein S5/elongation factor G/III/V family protein (LOS1)	3.53E-04	1.20E-04	7.87E-04	1.04E-06
	chain_2392	AT4G17880	Basic helix–loop–helix (bHLH) DNA-binding family protein	3.44E-08	8.94E-04	1.56E-04	2.86E-12
	chain_30204	AT2G18050	Histone H1	9.39E-04	2.02E-06	1.01E-04	3.89E-10
	chain_3297	AT4G34000	Abscisic acid-responsive elements-binding factor 3 (ABF3, DPBF5)	1.31E-04	3.59E-09	4.48E-04	6.49E-06
Synthesis-metabolism	chain_10152	AT5G42740	Sugar isomerase (SIS) family protein	1.18E-06	1.70E-09	2.08E-04	1.03E-07
	chain_1133	AT5G05340	Peroxidase superfamily protein	5.31E-04	1.78E-04	5.62E-03	6.63E-08
	chain_15308	AT1G26560	Beta glucosidase 40 (BGLU40)	7.19E-04	7.37E-03	4.01E-03	2.75E-08
	chain_37834	AT1G62660	Glycosyl hydrolases family 32 protein	2.16E-05	3.98E-04	3.17E-05	4.76E-10
	chain_2479	AT3G20040	Hexokinase (ATHXK4, HKL2)	1.40E-09	1.19E-08	4.00E-07	Infinite

Note: The table includes five genes each from "stress response" and "synthesis-metabolism" biological process groups. Nothofagus contig indicates where in the transcriptome these outliers occurred. "TAIR" is a unique gene identification code whose acronym and information are sourced from "The Arabidopsis Information Resource" database, which details gene function and gene name information in *Arabidopsis thaliana*. Genome scan significance (*q*) values signify that this gene contained a significant outlier in the four individual genome scan methods, which are pcadapt, "LFMM" (latent factor mixed models), "RDA" (redundancy analysis), and BayPass, and a blank cell indicates a value of 0, meaning this single-nucleotide polymorphism (SNP) was not significant in this test. "Environmental associations (BF)" contain Bayes factor values, which signify if environmental covariates were significantly associated with that outlier locus. Blank cells indicate the SNP was not significantly associated with that environmental parameter. Target gene functions and pathways indicate the source that was used to target this candidate gene. For details on remaining outliers, see Table S2.

TABLE 2 (Continued)

	Environmental associations (BF)					
Biological process	**Iso-therm**	**Ann. Prec**	**Gs. Temp**	**Snow Cover**	**Day length**	**Target gene functions and pathways**
Stress response	13.28				13.74	GO:0006952 (defense response)
				36.24		GO:0009409 (response to cold)|GO:0009631 (cold acclimation)
			16.46		16.13	GO:0006952 (defense response)
	25.45			52.96		GO:0009414 (response to water deprivation)
					39.11	Plant hormone signal transduction (KEGGmap04075)|stress ABA signalling pathway (PathwayStudio—TAIR)| GO:0009414 (response to water deprivation)
Synthesis-metabolism	13.63				19.49	Starch and sucrose metabolism (KEGGmap00500)| GO:0006955 (immune response)
	20.88				18.98	Phenylpropanoid biosynthesis (KEGGmap00940)
	12.79					

(Continues)

TABLE 2 (Continued)

Biological process	Environmental associations (BF)					Target gene functions and pathways
	Iso-therm	Ann. Prec	Gs. Temp	Snow Cover	Day length	
						Phenylpropanoid biosynthesis (KEGGmap00940)|starch and sucrose metabolism (KEGGmap00500)
				29.75		Starch and sucrose metabolism (KEGGmap00500)
				52.96		Starch and sucrose metabolism (KEGGmap00500)

Note: The table includes five genes each from "stress response" and "synthesis-metabolism" biological process groups. Nothofagus contig indicates where in the transcriptome these outliers occurred. "TAIR" is a unique gene identification code whose acronym and information are sourced from "The Arabidopsis Information Resource" database, which details gene function and gene name information in *Arabidopsis thaliana*. Genome scan significance (*q*) values signify that this gene contained a significant outlier in the four individual genome scan methods, which are pcadapt, "LFMM" (latent factor mixed models), "RDA" (redundancy analysis), and BayPass, and a blank cell indicates a value of 0, meaning this single-nucleotide polymorphism (SNP) was not significant in this test. "Environmental associations (BF)" contain Bayes factor values, which signify if environmental covariates were significantly associated with that outlier locus. Blank cells indicate the SNP was not significantly associated with that environmental parameter. Target gene functions and pathways indicate the source that was used to target this candidate gene. For details on remaining outliers, see Table S2.

across populations, Nei & Takahata, 1993) (Figure 2). This higher northern diversity could also mean these populations will be more resilient under climate change, since greater standing diversity has been linked to greater adaptation potential (Alberto et al., 2013). Meanwhile, southern sampling sites showed lower heterozygosity, but each southern sampling site had at least 250 private alleles, which could support the claim that there were also multiple southern refugia (Marchelli & Gallo, 2006; Mathiasen & Premoli, 2010; Premoli et al., 2010). Further supporting evidence comes from the strong north–south divide in our population structure analyses (Figure 3) and isolation by distance pattern (Figure 3c), which imply long-term population persistence in both areas (Carnaval et al., 2009). Concerning post-glacial expansion, high heterozygosity is also expected in admixture zones including places along recolonization routes where secondary contact occurred, a phenomenon that was previously observed in mid-latitude regions (e.g., Soliani et al., 2015). We observed relatively elevated heterozygosity near the first (northern) phylogenetic divide but not the second (southern), which could have been influenced by background evolutionary forces including genetic drift or gene flow. Finally, regarding the lack of consistent patterns between locally high and low elevation sites (Figure 2), we generally chose "high" sampling sites that were located well below the local tree line to avoid sampling krummholz, so these sites may be better classified as "intermediate" elevation sites, and these locations often contain the locally highest levels of diversity (Ohsawa & Ide, 2008).

4.2 | Population differentiation genome scan identified many unique outliers

The population differentiation genome scan method, pcadapt, identified the greatest overall number of SNP outliers, including 113 unique outliers that were not identified by any of the genetic environment association (GEA) analyses (Figure 4). Population differentiation methods may have more power than GEA analyses when demographic history has caused collinearity between neutral allele frequencies and environmental clines (Lasky et al., 2023; Lotterhos & Whitlock, 2015). This is likely the case in the orographic habitats of southern Patagonia, particularly along the aforementioned latitude axis that correlated with population structure, environmental clines, and overall genetic diversity (Figures 2, 3, and S2). The most frequent biological process terms among the pcadapt outlier-containing genes were related to stress response, including water deprivation, cold, and defense response (Table S3). The fact that many stress- and metabolism-related genes contained outliers according to population differentiation tests but not genetic environment tests suggests they might be either associated with unobserved climatic factors or influenced by non-climactic factors (e.g., Meirmans, 2015). The pcadapt-unique genes also had diverse biological functions, including phenology, photosynthesis, and lignin catabolism. These may be interesting candidate genes for investigation in further studies.

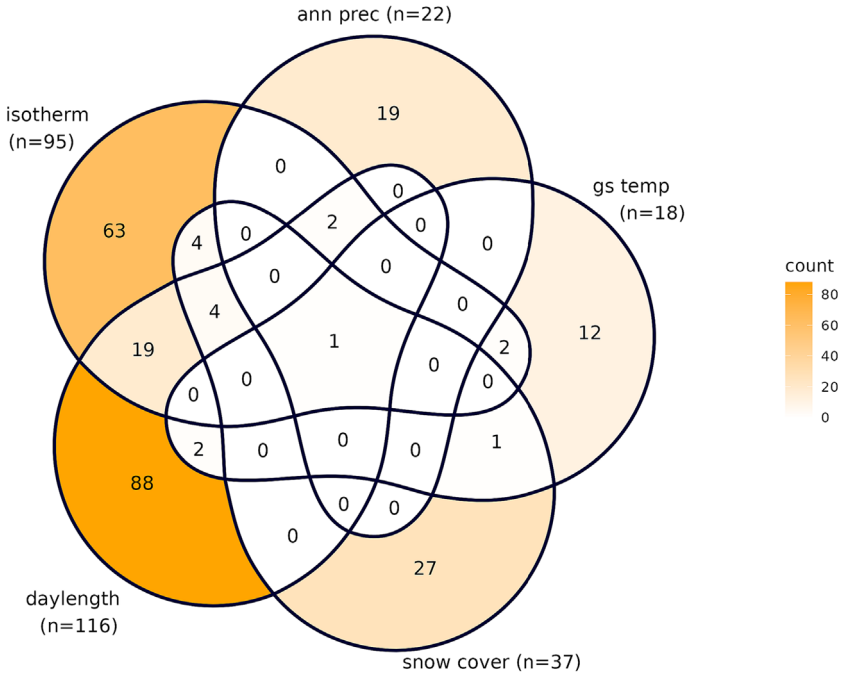

FIGURE 5 **Number of single nucleotide polymorphisms (SNPs) outliers that were significantly associated with each of the five individual environmental parameters in the gene-environmental association test BayPass for *Nothofagus pumilio* at 20 sampling sites in Argentina.** The name of each environmental parameter is shown outside the Venn section ("isotherm" = isothermality, "daylength" = day length in January, "snow cover" = total snow cover days, "gs temp" = growing season temperature, and "ann prec" = total annual precipitation). Total number of associated SNPs is shown in parentheses after the parameter name. The threshold for significance is a Bayes factor value greater than 10. Numbers inside each Venn section indicate the number of SNP(s) associated with the covariate(s). Color indicates relative count, from low (white) to high (dark orange).

4.3 | Temperature and photoperiod are the most important predictors in univariate GEA analysis

In the environment-univariate test BayPass, more SNP outliers were significantly associated with temperature and/or photoperiod than precipitation variables (Figure 5, Table 2, and Table S1). Therefore, it is possible that temperature and photoperiod have stronger effects on *N. pumilio* biology and selection than precipitation does, which aligns with some previous studies. Temperature was the main driver of intraspecific *Nothofagus* phenology differences along local elevation clines, albeit within a limited latitudinal range (Juri & Premoli, 2021). In seedlings, temperature governing germination phenology was a stronger factor than air humidity for mortality rate along elevation clines (Arana et al., 2016; Cagnacci et al., 2020). Temperature can also have indirect effects on selection. For example, insect folivory rates on *N. pumilio* have been shown to decrease with increasing latitude, possibly because lower temperatures suppress insect population sizes (Garibaldi et al., 2011); at the same time, increased frequency of defoliation events has already been observed in southern forests and was attributed to climate warming (Paritsis & Veblen, 2011). Photoperiod results are more complicated to assess, since our study is the first to explicitly assess its role in *N. pumilio* genetic adaptation. However, the 116 day length-associated outliers suggest a strong effect, for which there is supporting evidence from other plant species. Photoperiod is the most reliable predictor for oncoming seasonal change in temperate regions and is therefore a strong regulator of phenology (Roeber et al., 2022), that is, the timing of recurrent seasonal events including flowering and leaf senescence. For example, delayed spring bud burst in response to short photoperiod has been observed in related tree species *Fagus sylvatica* and *Quercus petraea* under common garden conditions (Basler & Körner, 2012; Vitasse & Basler, 2013). Notably, photoperiod has also been shown to influence plants' response to stress (Roeber et al., 2022, and references therein), including regulating signaling pathways and affecting freezing tolerance and drought response in *Arabidopsis thaliana* (Han et al., 2013). Our results include many photoperiod-associated SNPs in stress-related genes (Table S3). Finally, the combination of these two parameters is biologically relevant, as suggested by the overlap of SNPs that are associated with both temperature and photoperiod (Figure 5). Autumnal dormancy in perennial plants is largely initiated by the combination of shortened photoperiod and low temperature (Howe et al., 1995; Singh et al., 2017). These results highlight the importance of temperature, photoperiod, and their interactions in regard to growth, stress response, and phenology.

We found fewer precipitation-associated outliers, for which there are many possible implications and explanations. From a study design standpoint, our sampling area encompassed the drier portion of the species' range, namely, in Argentina, but we still captured a precipitation gradient from ~1400 mm to ~250 mm per year (Figures 1c and S2). Therefore, we should have captured populations that are at greater risk of drought stress and are more likely to show signatures of drought adaptation. The fact that BayPass identified relatively few SNPs could indicate that precipitation is a relatively less important environmental cue for *N. pumilio*. However, common garden studies performed on young *N. pumilio* that were sourced from local precipitation and/or elevation gradients consistently show trait differentiation in water use and morphology when those plants are grown under drought conditions, although there is little consensus about whether genetics or phenotypic plasticity is responsible. Some studies suggest a genetic basis (Ignazi et al., 2020; Mondino et al., 2019; Soliani & Aparicio, 2020), others suggested that responses are plastic (Ivancich et al., 2012), and still, others found supporting evidence for both

explanations (Mathiasen & Premoli, 2016; Premoli & Brewer, 2007; Soliani et al., 2021). Phenotypic plasticity is advantageous when physical conditions are highly variable, for instance, in northern Patagonian locations with a Mediterranean climate (Villalba et al., 2003). Plasticity can allow plants to evade temporarily suboptimal conditions, but sidestepping the selection pressures required for genetic adaptation may mean that fewer loci show adaptive signatures. Another explanation is that the complex physiological, biochemical, and morphological adjustments to water stress in trees (Estravis-Barcala et al., 2021) create complex polygenic trait architectures that are less easily detected with genome scans (Lasky et al., 2023). Taken together, these results suggest water stress response is a complicated process affected by many factors, which may be further supported by examining implicated candidate gene functions and their outliers' associations.

4.4 | Biological functions of outlier-containing genes

Although we predicted that drought response candidate genes would contain outlier SNPs associated with precipitation variables, we found this was not always the case. Although water deprivation response was indeed among the most frequent biological process terms for the outlier-containing genes (Table S3), these genes often contained outliers that were either associated only with univariate temperature and/or photoperiod variables (e.g., Table 2), identified by multivariate environment analyses, or identified only by the population differentiation test and therefore did not associate with any environmental parameters. At the same time, precipitation-associated SNPs were found in genes representing diverse biological process terms including biosynthesis, phenology, and metabolism (Table S3). Similarly, outliers associated with temperature and photoperiod are often found in genes related to expected processes such as stress response and phenology that were included in our prediction (i), but also more diverse genes involved in synthesis and metabolism (Tables 2 and S3). On the other hand, defense and immune response were the most frequent biological process terms in the starting candidate gene list but we found a relatively small number of outliers within these genes. Some outliers were found by the population differentiation test or were associated with temperature or photoperiod, but there were no associations with precipitation. One explanation is that temperature and photoperiod may have indirect effects, for example by affecting the pathogenic species themselves (Garibaldi et al., 2011). Finally, the numerous outliers identified by multivariate environment but not univariate analyses hint at the importance of variable interaction, although synthetic variables are difficult to interpret biologically, and in this case, it can be informative to compare gene functions among studies.

The previous study regarding *N. pumilio* transcriptome expression under heat stress found that many genes related to stress response genes were overrepresented under heat treatment, while photosynthesis and metabolism were underrepresented, indicating a trade-off between growth and survival (Estravis-Barcala et al., 2021). We identified 19 contigs that were both differentially expressed in the heat stress study and contained moderate-to-strong-evidence outlier SNPs in this study. For example, the gene ATC4H, putatively related to defense response against ultraviolet light and pathogens (Rhee et al., 2003), was promoted under heat stress, and we found it to contain a strong-evidence outlier that was associated with photoperiod (Table 2). Similarly, the transcriptome study found that genes related to signaling pathways were over-represented under heat stress. Abscisic acid is produced under water deficit and confers tolerance to water and salt stress (Abe et al., 2003), and MAPK is implicated in growth and stress response (Kumar et al., 2020). We identified 19 outlier-containing genes that were related to signaling, and among these, there were significant associations with all studied environmental parameters besides annual precipitation (Tables 2 and S1). Furthermore, three outlier-containing signaling genes were also identified as convergent genes in distantly related conifers (Yeaman et al., 2016), and finding such convergent evolution across continents may hint at broader implications for the findings of this study.

Nested within the growth–survival tradeoff is phenology. For example, we identified three outlier SNPs in dormancy-related phytochrome and cryptochrome genes (Table S2). Phytochromes have shown stark latitude clines in related *Populus* species (Ingvarsson et al., 2006), and both gene types have shown latitude clines in conifers, where variation in light quality was proposed as the main driver (Ranade & García-Gil, 2023). Outliers in phenology-related genes were predominantly identified by the multivariate methods, which provides further evidence that interactions among genes and/or environmental factors are important for phenology. Future disconnections among these variables could therefore disrupt phenological cues. Taken together, these results suggest that signaling pathways and phenology are affected by complex interactions that may be unpredictably impacted by climate change.

4.5 | Application and future steps

Genetic diversity information such as that presented here can be incorporated into forest management and conservation activities, for example, by informing reforestation seed source decisions (e.g., Mattera et al., 2020) or identifying conservation gaps, i.e., diversity hotspot areas that are unprotected. It is also possible to monitor changes in genetic diversity over time (Raffard et al., 2019), especially as genotyping methods become faster and cheaper. Our results provide important baseline information about current genetic diversity patterns and candidate genes putatively under selection. One promising next step to predict the species' response to climate change is to quantify genetic offset (reviewed in Capblancq et al., 2020), which characterizes the mismatch between extant allele compositions (i.e., those reported here) and compositions that might be required under future conditions. Our results could also be reassessed in combination with eventual results from ongoing common garden trials, for example, by validating results, disentangling correlated environmental conditions, and differentiating genetically controlled traits from phenotypic plasticity.

5 | CONCLUSION

Local adaptation patterns in *N. pumilio* are mainly structured along the latitude axis, and signatures of local adaptation in candidate genes are often significantly associated with temperature, photoperiod, and, to a lesser univariate extent, precipitation. Outlier-containing genes related to stress response and growth were often associated with temperature and photoperiod, which supports our prediction (i). However, against our prediction (ii), genes related to drought response were not always associated with precipitation variables and were just as likely to be associated with temperature or photoperiod. Additionally, many outliers were either identified only by multivariate analyses, associated with more than one environmental variable or had biological functions more diverse than their environmental associations, suggesting that target genes are affected by combinations of environmental variables. This suggests a complex response among environmental predictors and the genes upon which selection occurs. These results have many unpredictable implications for Patagonian forests and people under future climate change, particularly, if that change decouples the existing relationships that we have characterized here.

AUTHOR CONTRIBUTIONS

Paula Marchelli, Verónica Arana, Carolina Soliani, Katrin Heer, and Lars Opgenoorth conceptualized the study; Benjamin Dauphin, Ivan Scotti, Katrin Heer, and Lars Opgenoorth organized methodology; Jill Sekely, Paula Marchelli, Verónica Arana, María Gabriela Mattera, Mario Pastorino, Ivan Scotti, Carolina Soliani, Katrin Heer, and Lars Opgenoorth assembled project resources; Paula Marchelli, Katrin Heer, and Lars Opgenoorth were project administrators. Jill Sekely, Paula Marchelli, Verónica Arana, María Gabriela Mattera, Mario Pastorino, Carolina Soliani, and Lars Opgenoorth collected samples; Jill Sekely, Paula Marchelli, Verónica Arana, María Gabriela Mattera, and Carolina Soliani extracted DNA. Jill Sekely and Benjamin Dauphin performed data analysis with all authors contributing to its interpretation. Jill Sekely wrote the manuscript with editing contributions by all authors.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Zenodo at https://zenodo.org/doi/10.5281/zenodo.7930351.

Files included:

1. **Npumilio_specific_candidate_genes.xlsx** (Study-specific candidate genes)
2. **N_pumilio_06Nov2019_probe_coverage.txt** (Probe design file as provided by IGA Technology Services)
3. All called SNP variants. Data for 502 individual tree samples are included: 496 adult trees + 6 replicates. **variants-gatk_haplotypecaller_SNP_raw.vcf.gz** are raw called variants, **variants-gatk_haplotypecaller_SNP.vcf.gz** are coarsely quality-filtered and are the starting datasets for our downstream analyses.
4. **Npumilio_IDs_vcforder_all_info.xlsx** (Metadata for all samples in the .vcf files [in the same order], including site name, geographic coordinates, and elevation)
5. **Npumilio_orthogroups.csv** (Orthogroups, TAIR codes, and best-hit Nothofagus contigs for cross-referencing with orthogroup candidate gene set (Milesi et al., 2023 [https://doi.org/10.1101/2023.01.05.522822]).

ORCID

Jill Sekely https://orcid.org/0009-0002-3186-412X
Paula Marchelli https://orcid.org/0000-0002-6949-0656
Verónica Arana https://orcid.org/0000-0002-9483-7906
Benjamin Dauphin https://orcid.org/0000-0003-0982-4252
María Gabriela Mattera https://orcid.org/0000-0002-7062-050X
Mario Pastorino https://orcid.org/0000-0003-0120-7727
Ivan Scotti https://orcid.org/0000-0002-8951-2680
Carolina Soliani https://orcid.org/0000-0003-0388-2291
Katrin Heer https://orcid.org/0000-0002-1036-599X
Lars Opgenoorth https://orcid.org/0000-0003-0737-047X

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