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Impact of pleistocene glaciations and environmental gradients on *Embothrium coccineum* genetic structure

Francisco Sepúlveda-Espinoza[1], Ariana Bertin-Benavides[1], Rodrigo Hasbun[2], Oscar Toro-Nunez[3], Antonio Varas-Myrik[2], Diego Alarcón[4], and Marie Guillemin[5]

[1]Universidad de Concepción
[2]Universidad de Concepcion
[3]Universidad de Concepción Facultad de Ciencias Naturales y Oceanográficas
[4]Universidad de Chile Instituto de Ecologia y Biodiversidad
[5]Universidad Austral de Chile

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Abstract

The South American temperate forests have been subjected to drastic past topographic and climatic changes during the Pliocene – Pleistocene linked to Andean orogeny and glacial cycles. These changes are common drivers of genetic structure and adaptation process. *Embothrium coccineum*, a member of the Proteaceae family and an emblematic tree of the South American temperate forest with a distribution spanning 20° of latitude, has been strongly affected by these topographic and climatic changes. Previous studies have shown that the species presents a marked genetic structure with distinct ecotypes described; yet, little is known about their adaptive genetic responses. The main goal of this study was to investigate the effects of historical and contemporary landscape features affecting the genetic diversity and connectivity of *E. coccineum* throughout its natural distribution. Using more than 2000 SNPs, two genetic groups (North and Center-South) that have diverged some 2.8 million years ago were observed. The level of genetic structure was higher between populations within the North genetic group than within the Center-South group. We propose that these contrasting patterns of genetic structure are related to differences in pollinator's assemblage and evolutionary histories between genetic groups. Moreover, we observed the existence a strong patter of isolation by environment in *E. coccineum*, suggesting that selection could have leaded to adaptive divergence among localities. We propose that, within the Chilean temperate forest, the patterns of genetic variation in *E. coccineum* reflect both a Quaternary phylogenetic imprint and the impact of selection to the strong environmental gradient.

Abstract

The South American temperate forests have been subjected to drastic past topographic and climatic changes during the Pliocene – Pleistocene linked to Andean orogeny and glacial cycles. These changes are common drivers of genetic structure and adaptation process. *Embothrium coccineum,* a member of the Proteaceae family and an emblematic tree of the South American temperate forest with a distribution spanning 20° of latitude, has been strongly affected by these topographic and climatic changes. Previous studies have shown that the species presents a marked genetic structure with distinct ecotypes described; yet, little is known about their adaptive genetic responses. The main goal of this study was to investigate the effects of historical and contemporary landscape features affecting the genetic diversity and connectivity of *E. coccineum* throughout its natural distribution. Using more than 2000 SNPs, two genetic groups (North and Center-South) that have diverged some 2.8 million years ago were observed. The level of genetic structure was higher between populations within the North genetic group than within the Center-South group. We propose that these contrasting patterns of genetic structure are related to differences in pollinator's assemblage

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and evolutionary histories between genetic groups. Moreover, we observed the existence a strong patter of isolation by environment in*E. coccineum* , suggesting that selection could have leaded to adaptive divergence among localities. We propose that, within the Chilean temperate forest, the patterns of genetic variation in *E. coccineum* reflect both a Quaternary phylogenetic imprint and the impact of selection to the strong environmental gradient.

Keywords: Temperate forest - Population genomics - Proteaceae - Isolation by environment - Last Glacial Maxima - Divergence time

Editor in Chief

Ecology and Evolution

Dear Dr. Allen Moore,

I hope this cover letter finds you safe and healthy during these absolutely unprecedented pandemic times.

I wish to submit an original research article for publication in Tree Genetics and Genome, titled *"Impact of pleistocene glaciations and environmental gradients on Embothrium coccineum genetic structure"* . The paper was authored by Sepúlveda-Espinoza, F; Bertín-Benavides, A; Hasbún, R; Toro-Núñez, O; Varas-Myrik, A; Alarcón, D; and Guillemin, M-L. This study evaluated the effects of historical and contemporary landscape features affecting the genetic diversity and connectivity of *E. coccineum* throughout its natural distribution.*Embothrium coccineum* is an emblematic tree part of the Proteaceae familly and endemic to the South American temperate forests. Genotypes for more than 2000 SNPs were obtained for trees sampled throughout the complete natural distribution of the species. Results show the existence of two main genetic groups (North and Center-South) that diverged some 2.8 million years ago each characterized by distinct complex patterns of genetic divergence among populations with 1) a clear genetic isolation between populations located in the North and 2) a more homogeneous distribution of the genetic variation in the Center-South with a gradient of admixture between the North and Center-South genetic groups detected in the species center part of the distribution. These patterns reflect the clear impacts of past (Pliocene – Pleistocene) topographic and climatic changes, linked to Andean orogeny and glacial cycles, but also the possible importance of regional differences in pollinator's assemblage leading to contrasting level of gene flow in the North and Center-South on *E. coccineum* populations. A strong signal of isolation by environment was also observed in *E. coccineum* , suggesting that selection could have led to adaptive divergence among localities. We propose that local adaptation in this species could be linked in particular to differential access to water during the driest months.

We believe that our study makes a significant contribution to the topics addressed by your journal. Indeed, this study is the first to present estimations of time of genetic divergence in *E. coccineum* but also allow to disentangle the effects of local adaptation and isolation by environment (IBE) from neutral processes, such as isolation by distance (IBD) or co-ancestry linked to the species glacial history (IBA), in shaping among-population genetic differentiation of this species. The identification and quantification of the environmental variables structuring population genetic variation could inform management decisions for conservation, restoration or reforestation purposes.

This manuscript has not been published or presented elsewhere in part or in its entirety and is not under consideration by another journal. We have read and understood your journal's policies, and we believe that neither the manuscript nor the study violate any of these. We declare no conflicts of interest.

Thank you for your consideration.

Best regards.

Ariana Bertin-Benavides

ONG Conciencia Sur

Concepción

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Introduction

Due to their isolation and high degree of endemism, South American temperate forests are considered of great ecological and evolutionary importance (Smith-Ramirez et al., 2007). In Chile, temperate forests stretch from the 35°S down to the 55°S, forming a continuous area that can be considered a "biogeographic island" separated by impassable barriers (deserts, mountains, and oceans) from the rest of the ancestral sources of its biota (Villagrán 1991). As a result, the climate and topography characterizing the narrow strip (2000 km long and 120 km wide) of Chilean temperate forests generate high heterogeneity of forest and soil types, climatic conditions, and disturbance regimes (Loguercio et al., 2018). Adaptive processes linked to persistence in a mosaic of heterogeneous habitats may have contributed to plant species diversification in the temperate forests of southern South America (Johnson et al., 2014; Prunier et al., 2017). In the same way, within species genetic divergence of plants with extensive distribution under strong natural environmental gradients could also reflect the action of selection (Saldaña et al., 2007, Zeppel et al., 2014; Grossiord et al., 2004). The effect of contrasting climatic and edaphic features on phenotypic and genetic diversity has been observed in various temperate plants (Saldaña et al., 2007; Torres-Díaz et al., 2007). For instance, the existence of distinct ecotypes has been linked to contrasting access to water and snow winter coverage in *Embothrium coccineum* J.R. Forts. & G. Forst (Dimitri, 1972), a widespread tree that inhabits the temperate forest of South America (Zegers 1994). This species is an endemic tree of Gondwanan origin with a distribution range matching the extent of the temperate forest biome in Chile and Argentina, which exhibits different phenotypes throughout its distribution (Alberdi y Donoso, 2004; Chalcoff et al., 2008). The distribution of these ecotypes is concordant with the pattern of genetic structure observed using isoenzymatic genetic markers (Souto and Premoli, 2007), suggesting that*E. coccineum* populations across the species range may be locally adapted to distinct environmental conditions (Souto and Premoli, 2007).

Besides the possible effect of current selective regimes, climate shifts during the Pleistocene have also strongly altered the spatial patterns of genetic variation of many southern South American taxa (Vidal-Russell et al., 2011; Turchetto-Zolet et al., 2013). During the Last Glacial Maximum (LGM, 25.000 years Before Present, BP), due to the presence of ice caps, a large expanse of temperate forest located between the 42 °S and the 56 °S disappeared (Rabassa et al., 2005). Glacial maxima triggered a rapid range shift, with most temperate South American species surviving only in glacial refugia located north of the 42°S (Allnutt et al., 1999; Premoli et al., 2000, Sérsic et al., 2011). Some cold tolerant species, as *E. coccineum,* were also able to survive in refugia embedded within the main glaciated area in localities where microclimate, topography or geothermic activity hindered the formation of the ice cap (Allnutt et al., 1999; Premoli et al., 2000, Sérsic et al., 2011). These areas could represent sources of regional diversity recovery after deglaciation (Comps et al., 2001). The presence of 11.000 years BP cold tolerant plants pollen fossil in areas covered by ice during the LGM (Fesq-Martin et al., 2004, Steubing et al., 1983) and results of genetic studies based on sequences of chloroplast genes or genotyping of nuclear isoenzymatic markers (Vidal-Russel et al., 2011; Souto and Premoli, 2007) suggest a complex glacial-interglacial history in this species.

Recent developments in high throughput sequencing technologies and population genomics have allowed to distinguish the influence of past environmental changes and selection to the current environmental conditions on the spatial patterns of genetic variation in both South American plants and animals (Turchetto-Zolet et al., 2013; Hasbún et al., 2016; Varas-Myrik et al., 2021). If divergent natural selection has led to genetic differentiation between populations of *E. coccineum* along environmental gradients characterizing South American temperate forests, a pattern of isolation by the environment (IBE; Wang and Bradburd, 2014) should be expected. Indeed, when selection against immigrant limits gene flow among distinct environments, genetic differentiation increases with environmental differences (Wang and Summer, 2010; Wang and Bradburd, 2014). To test for the existence of IBE, the level of correlation between genetic differentiation and environmental distances should be contrasted against the one observed between genetic differentiation and geographic distances (isolation by distance; IBD; Slatkin, 1993). IBD could be considered as a null model for which genetic differentiation increases with geographical distance, given the local accumulation of genetic differences when the dispersion between populations is geographically restricted. In *E. coccineum* , gene flow could be limited by constraints on pollen transport that is linked to the distribution and behavior of

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pollinators (i.e., more than 20 species including birds and insects are reported as pollinators of *E. coccineum* ; Chalcoff et al., 2008) and anemochorous seed dispersal (Rovere and Premoli, 2005).

The main goal of this study was to evaluate the effects of historical and contemporary landscape features affecting the genetic diversity and connectivity of *E. coccineum* throughout its natural distribution using SNPs obtained by genotyping-by-sequencing (GBS). Patterns of genetic structure were evaluated independently using SNPs found in genomic regions that may be subject to selection (i.e., outlier loci) and all loci. We hypothesized that patterns of genetic variation in*E. coccineum* will reflect both the impact of historical processes (i.e., glacial / interglacial cycles during the Pleistocene) and contemporary landscape features including the selective effect of environmental heterogeneity.

Materials and methods

Samples collection and DNA extraction

Locations were selected to cover a large range of climatic conditions across *E. coccineum* natural distribution; with three locations (Chillán, Nahuelbuta and Curacautín) representing the northern part of the distribution, four locations (Puerto Montt, Chiloé Norte, Chiloé Sur and Pumalín) the center part of the distribution, and three locations (Coyhaique, Chile Chico and Torres del Paine) the southern part of the distribution (Fig. 1a and Supplementary table 1). These three regions are subsequently named North, Center and South. Three to six individuals were sampled within each location and five mature leaves were collected per tree. A total of 42 trees were sampled. To calibrate divergence time among *E. coccineum* genetic groups, two individuals of*Lomatia hirsuta* Diels a Proteaceae species from Embothrieae tribe (Sauquet et al., 2009) were included as outgroup.

Whole genomic DNA extraction was carried out using the Qiagen DNeasy Plant kit (Qiagen Inc., USA) following manufacturer's instructions. Foliar tissue (50 mg) was kept in Precellys24 homogenizer (Precellys, USA) with two 1/4" ceramic spheres (MP BIOMEDICALS, USA) and AP1 buffer until processing in the laboratory. The genomic DNA integrity was evaluated using direct visualization in 1% agarose gels, and the DNA was quantified using a Qubit fluorometer (Invitrogen, USA).

Climatic data collection

To characterize patterns of spatial environmental variation among the localities, the historical values (1970-2000) of 19 environmental variables (bio 1 - bio 19) and elevation (Elv) were obtained using the package raster v.2.7.15 and the Wordclim data of 2.5 arcmins (available at *http://worldclim.org/*). We tested for covariation among environmental variables using the *vifcor* () function from the "usdm" R package (Naime et al., 2014). Variables presenting no strong covariation (R <0.8; see Fitzpatrick and Keller, 2014) were conserved for subsequent analyses (Supplementary table 1).

Preparation of the library, high throughput sequencing and GBS genotyping

Library preparation and high-throughput sequencing were performed at the University of Wisconsin Biotechnology Center (DNA Sequencing Facility). The preparation of the GBS genomic library was done following the protocol detailed by Elshire et al., (2011) using the ApeKI restriction enzyme and 44 specific barcodes. High throughput sequencing was performed using an Illumina HiSeq 2000 (Illumina, USA) and single-strand sequencing runs of 100 bp. The raw GBS dataset generated and analyzed during the current study are available in the SRA repository (*https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA783610*). Quality of the sequenced raw data was assessed using FastQC (*https://www.bioinformatics.babraham.ac.uk/projects/fastqc/*).

SNP calling was made using Stacks v.2.2 pipeline (Catchen et al., 2013). Values of the two main parameters (-M: number of mismatches allowed between stacks within individuals; and -n: number of mismatches allowed between stacks between individuals) were chosen following the optimization procedure described in Paris et al. (2017).

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GBS data sets

To test for possible effects of parameters assembly and missing data on patterns of population genetic structure, distinct sets of parameters (-p: minimum number of populations where a locus must be present for it to be included; and -r: percentage of individuals in a population that must possess a particular locus for it to be included) were examined before choosing optimal filtering options for further analysis. Eight combinations of parameters -p and -r were tested (-r= 0.6 and 0.7; -p= 6, 7, 8 and 9). For each combination the % of missing data, % of heterozygosity and number of SNPs were estimated (data not shown). The minimum minor allele frequency (min-maf) was set to 0.02 and maximum observed heterozygosity (max-obs-het) to 0.5. The "Population Genetic data set" (i.e., PG_dataset), was build using -p 9 and –r 0.7.

Furthermore, another data set was built to estimate the divergence time among the *E. coccineum* genetic groups, using the Bayesian multispecies coalescent model (Stange et al., 2018). In order to reduce calculation time, we selected a subset of sampling locations from the North (Nah and Cu), the Center (ChlN and ChlS) and the South (Coy and TP). Then, a subset of 12 *E. coccineum* individuals (i.e., two per location) were selected to maximize the number of SNPs available and minimize the amount of missing data. These 12 *E. coccineum*individuals were processed jointly with the samples of *L. hirsuta* , included as outgroup. A new SNP calling was conducted following the same parameters than those used for the PG_dataset in Stacks v.2.2 pipeline (Catchen et al., 2013), eventually generating the "divergence time data set" (i.e., DT_dataset). In this case, optimal values for -p and -r parameters were chosen in order to decrease the percentage of missing data: only the loci present in all localities, including the outgroup (-p 7), and present in a least 80 % of the individuals (-r 0.8) were kept for further analyses. Putative outliers loci were removed from the DT_dataset. For each data set, one SNP was randomly selected per locus to aproximate unlinked loci variation.

Populations diversity statistics

To explore distribution of genetic diversity, we calculated for each location the observed (Ho) and expected (He) levels of heterozygosity and the inbreeding coefficient F_{IS} for each location with GenoDive v.3.02. The percentage of polymorphic loci and of private alleles were estimated using the R package "hierfstat v. 0.04-22" (Goudet 2005).

Genetic structure

Four complementary analyses were conducted to characterize the pattern of genetic structure within *E. coccineum* using the PG_dataset. First, we calculated the pairwise FST values among all sampling localities using the function *stamppFst* (), using 1000 bootstrap replicates across loci and 95% of confidence intervals with the R package StAMPP v.1.5.1 (Pembleton et al., 2013). Second, a principal component analysis (PCA) was performed using the *glPCA* () function with the R package adegenet v.2.1.1 (Jombart, 2008). Only the first three components were retained to estimate genetic groups. Third, a model-based STRUCTURE clustering analysis was performed using STRUCTURE v.2.3.4. (Falush et al., 2003). Ten independent simulations allowing admixture were run for each K (K=1-10), each with 200.000 Markov chain Monte Carlo replicates (MCMC) and 100.000 samples discared as burnin. STRUCTURE HARVESTER (*http://taylor0.biology.ucla.edu/structureHarvester/*) was used to determine the optimum K based on L(K) and ΔK parameters (Zhang et al., 2018). Fourth, to describe the relationships between individuals and genetic groups among *E. coccineum* , the Nei's genetic distance between individuals was calculated within the R package StAMPP v.1.5.1 (Pembleton et al., 2013). Unrooted Neighbour-Joining (NJ) distances trees were constructed using the individual Nei's distance matrix, as implemented by the R package ape v .5.3 (Paradis and Schliep, 2018).

Outlier detection and association of allele frequency with environmental variables

To identify candidate loci potentially influenced by selection within*E. coccineum* PG_dataset, two complementary approaches were used. First, we used a Principal Component Analysis (PCA) to generate a "model-free" null distribution of individual genetic distances to detect outlier loci. This analysis was conducted using the R packages PCAdapt v.4.0.3 (Luu et al., 2017) and qvalue v.2.16.0 (Storey et al., 2004) with

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default parameters and applying a search with K=10. The detection of outliers was confirmed by plotting the histograms of$p$ -values and the Mahalanobis (D^2) test statistic, with a False Discovery Rate (FDR) set at 5%. Second, we implemented a Bayesian test to detect outlier loci using the BAYESCAN 2.1 software (Foll et al., 2008). All simulations were performed using the default parameters with 20 pilot runs of 5,000 iterations followed by 50,000 sampling iterations and using a FDR of 5%. Outlier loci detected by both PCAdapt and BAYESCAN were selected as candidate adaptive loci.

To search for candidate adaptive loci with allelic frequency potentially influenced by the environmental variation characterizing our study area (see Supplementary table 1), a GradientForest (GF) analysis was implemented in the R package gradientForest (Ellis et al., 2012). GF provides a ranked list of the relative predictive power (R^2) of all environmental variables allowing the identification of those that best explaining the observed genetic variation. Allele frequency of candidate adaptive loci (i.e., detected in both PCAdapt and BAYESCAN) was used as response variable. GF was fitted using 2,000 regression trees per SNP and a variable correlation of 0.5 (Fitzpatrick and Keller, 2014)

Isolation by distance and isolation by environment

To better understand the drivers potencially explaining the observed patterns of spatial genetic variation in *E. coccineum* , we performed distance-based redundancy analysis (db-RDA) and partial db-RDA at the individual level on the complete PG_dataset and on loci potentially influenced by selection. RDA is a multiple linear regression method performed between a matrix of dependent variables and matrices of independent variables, which has been demonstrated as more appropriate than other methods (i.e., Mantel test) when multiple variables are analyzed to identify drivers of population genetic structure (Nadeu et al., 2016; Orsini et al., 2013).

A dependent matrix, calculated with a Bray-Curtis dissimilarity index (Bray and Curtis, 1957), of co-dominant variant call format of each individual was used as a response variable. Predictor matrices were geographic distance (IBD), environmental disparity (IBE) and degree of shared co-ancestry (IBA). As environmental data, we used seven environmental variables with low level of covariation (see Supplementary table 1). For the distance matrix, we used a Principal Coordinates of Neighbourhood Matrix (PCNM), using a truncation threshold of 0.05 for long distances with the function pcnm() within the R package vegan v.2.5.7 (Oksanen et al., 2007). PCNM is commonly used to transform spatial distances into rectangular data matrices suitable for constrained ordination or regression analyses (Borcard and Legendre 2002). Populations Q-values obtained with STRUCTURE K = 2, which separated populations into North and Center-South ancestry linages (see results for more details), were used as co-ancestry variable. Prior to the analysis, all three independent matrices were scaled to a mean of zero and variance of one with the scale() function in R.

Among populations variation in *E. coccineum* was partitioned into components explained by geographic distance (IBD), ecological gradients (IBE) and co-ancestry (IBA), or their combination (i.e., constrained by the effects of the remaining two independent matrices), using the vartpart() function within R package vegan v.2.5.7 (Oksanen et al., 2007). Significance of each partition was tested with the anova.cca() function through 1,000 permutations on the R package vegan v.2.5.7 (Oksanen et al., 2007).

Divergence time among *Embothrium coccineum* genetics groups

To estimate divergence times among *E. coccineum* genetic groups, we implemented a Bayesian multispecies coalescent model (Rannala and Yang, 2003) using the DT_dataset. For this purpose, we calibrated the molecular clock using the following data: 1) a crown age of the Embothrieae tribe of 66 millions of years (My), with a lower limit of 56 My and an upper limit of 75 My (Sauquet et al., 2009) using a lognormal distribution; 2) a generation time of 10 years per individuals (following A. Bertin, personal observation). The analyses were conducted with the coalescent simulator SNAPP v.1.3.0 (Bryant et al., 2012), included in the package BEAST v.2.4.5 (Bouckaert et al., 2014). Species trees were inferred directly with no prior grouping assignment. We used SNAPP with four independent runs of 2,000,000 generations with a sampling every 200 generations each. The results (i.e., ESS $>$ 300) were checked for mixing for all parameters with Tracer v1.7 (Rambaut et al., 2018) and convergence of split frequencies among runs (Supplementary Figure

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2) with the R package rwty (Warren et al. 2019). The final trees from each run (corresponding to the 95% highest posterior density (HPD), after discarding a 10% burn-in of tree topologies) were combined and subsequently summarized using a maximum credibility tree with TreeSetAnalyser v.2.4.5. This species tree was visualized as a cloudgram with DensiTree v.2.2.7 (Bouckaert, 2010).

Results

To evaluate population structure, divergence time among genetics groups and local adaptation in *E. coccineum,* 38 trees were genotyped using GBS. A total of 116.606.122 reads were generated and *de novo* assembled into 1,303,750 loci, from which 473,461 SNPs were called. The application of filtering criteria resulted in a final PG_dataset of 2,155 SNPs. For the DT_dataset, composed by 12 individuals of *E. coccineum* and two individuals of *L. hirsuta* , a total of 320 SNPs were called.

Genetic diversity

The Expected and observed heterozygosity (He and Ho, respectively), inbreeding coefficient (F_{IS}), the percentages of polymorphic loci (PPL) and the number of private alleles (PA), are reported in the Table 1 for each sampling locality. Except for the low value observed in Chile Chico (ChCh; PPL=12,668 %), PPL were fairly homogeneous throughout the species range, varying from 18.794% in Chillán (Ch) to 42.367% in Coyhaique (Coy) (Table 1). Four sampling localities, Puerto Montt (PM), Chiloé norte (ChlN), Chiloé sur (ChlS) and ChCh presented a lower PA (PA[?]30) than the rest of the sampled range (44<PA<83). Ch and Pumalin (Pu) were the only two localities with a negative F_{IS} value while four localities (i.e., PM, ChlN, ChlS and ChCh) show positive values of F_{IS} superior to 0.1.

Table1: Genetic diversity of *Embothrium coccineum* in each sampling locality.

ID	n	He	Ho	F_{IS}	PPL	PA
Ch	3	0.1	0.105	-0.049	18.794	51
Nah	4	0.138	0.132	0.037	32.715	65
Cu	4	0.128	0.126	0.012	30.998	73
PM	3	0.152	0.135	0.114	29.791	30
ChlN	3	0.163	0.139	0.15	36.241	29
ChlS	3	0.152	0.128	0.155	33.132	29
Pu	3	0.134	0.154	-0.148	27.425	83
Coy	6	0.143	0.137	0.043	42.367	62
ChCh	3	0.124	0.106	0.146	12.668	10
TP	6	0.139	0.127	0.086	42.088	44

Patterns of population genetic differentiation

The highest pairwise values of FST (Table 2) were observed between North and South localities ($0.319 < FST < 0.424$), followed by those calculated between North and Center localities ($0.222 < FST < 0.429$) and then by the ones calculated between Center and South localities ($0.070 < FST < 0.193$). Lower levels of divergence were observed between localities sampled in the same macro region (North: $0.089 < FST < 0.174$; Center: $0.010 < FST < 0.152$; South: $0.043 < FST < 0.061$) (Table 2). All FST values were significant.

Table2: Average FST values for pairwise comparisons among sampling locations of *Embothrium coccineum* in Chile based on a set of 2,155 SNPs.

	Ch	Cu	Nah	PM	ChlN	ChlS	Pu	Coy	ChCh	TP
Ch	-									
Cu	0.163	-								
Nah	0.174	0.089	-							

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PM	0.312	0.263	0.222	-						
ChlN	0.309	0.260	0.225	0.024	-					
ChlS	0.345	0.292	0.257	0.041	0.010	-				
Pu	0.429	0.369	0.338	0.152	0.126	0.135	-			
Coy	0.382	0.340	0.319	0.117	0.083	0.071	0.168	-		
ChCh	0.424	0.362	0.341	0.133	0.089	0.070	0.193	0.061	-	
TP	0.384	0.345	0.322	0.133	0.094	0.085	0.185	0.061	0.043	-

The PCA showed that *E. coccineum* was divided in two major genetic groups: North and Center-South (Fig. 1b). The northern more locations were retrieved separated from those corresponding to the Center-South along the first axis of the PCA (PC1, representing 21.54% of the genetic variance), while samples from the Center and the South were separated along the second axis of the PCA (PC2, representing 5.82% of the genetic variance).

Results from the model-based Bayesian clustering approach showed a K = 2 (highest LnP(D) and ΔK) as optimal and K = 4 as a potential secondary level of substructure (Supplementary Fig. 1a-b). The STRUCTURE clustering (K = 2) revealed that all individuals sampled from the North were assigned to the blue genetic cluster while all individuals sampled from Center and South were assigned to the orange genetic cluster (Fig. 2a). In North and South samples, almost no admixture was observed. Individuals from PM, ChlN and ChlS (Center) were majorly assigned to the orange genetic cluster but exhibiting low levels of admixture from the blue genetic cluster. The level of assignation to the blue genetic cluster tends to recede across latitude (from 20% of the genome assigned to the blue genetic cluster in PM to less than 10% in ChlS; Fig. 2a). The STRUCTURE clustering using K = 4 showed two new genetic clusters within the Center-South part of the species range (Fig. 2b). Individuals from the South were assigned to the orange genetic cluster, individuals from Pumalín (PU, Center) were mostly assigned to the new purple genetic cluster and the rest of the individuals from the Center (PM, ChlN and ChlS) were admixed between the orange genetic cluster and the newly detected green genetic cluster (Fig. 2b).

The dendogram reconstructed with the Nei's distances tree calculated with the PG_dataset of 2.155 SNPs allowed to separate the North and Center-South samples (Fig. 3a), being concurrent with the results obtained by the PCA and the STRUCTURE clustering analyses. When compared with the results obtained with the whole data set (i.e., PG_dataset), the dendogram retrieved with the 59 outlier loci putatively under selection (see below for more details) revealed a much deeper separation between the North and Center-South samples; yet, most sub clustering within major genetic groups was lost (Fig. 3b).

Genome scan for outlier loci detection

Whithin the 2,155 loci included in the PG_dataset, PCAdapt detected 132 outlier loci and BAYESCAN 89 outlier loci. The two methods concurr with the detection of 59 loci (2.73%) that were then identified as potentially adaptive loci in *E. coccineum* .

The gradient forest revealed four environmental factors with the highest impact on *E. coccineum* populations (i.e., estimated as the variables presenting the highest values of R^2 weighted importance), which correspond to: Precipitation of driest month (bio 14), Mean temperature of wettest quarter (bio 8), Elevation, and the Mean temperature of driest quarter (bio 9) (Fig. 4). The value of the R^2 weighted importance for bio 14 (R^2 weighted importance > 0.05) was at least two-times higher than those observed for the other variables.

Isolation by distance and isolation by environment

The full RDA model using the PG_datset explained 59.4 % of the total genetic variation and supported an influence of environmental (Env), geographic (Geo) and coancestry (Anc) variables in shaping allelic variation (Env: adjusted $R^2 = 0.420$, $p < 0.001$; Geo: adjusted $R^2 = 0.226$, $p < 0.001$; Anc: adjusted $R^2 = 0.105$, $p < 0.001$; Table 3). With the partial db_RDA, the contribution of the environmental variables to genetic divergence was much higher than the contribution of the geographic distance or the co-ancestry (Env

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| Geo+Anc: adjusted R^2 = 0.288; Geo | Env+Anc: adjusted R^2 = 0.118; Anc | Env+Geo: adjusted R^2 = 0.011; Table 3). In total, 0.178 of the explained variation was confounded between the effects of IBE, IBD and IBA, and 0.405 of the variation remained unexplained (Table 3). Very similar results were obtained for the subset of 59 loci potentially influenced by selection (i.e., outlier loci). The partial db-RDA highlights the important contribution of the environmental variables to potential local adaptation in *E. coccineum* (Env | Geo+Anc: adjusted R^2 = 0.244; Table 3).

Table 3 : Redundancy analysis (RDA) partitioning among-population genetic variation in *Embothrium coccineum* into three components: environmental (Env), geographic (Geo) and North / Center-South coancestry (Anc).

Combined fractions	All loci Adjusted R^2	Outlier loci Adjusted R^2	Individual fractions	All loci Adjusted R^2	Outlier loci Adjusted R^2
Env	0.420	0.569	Env | Geo+Anc	0.288	0.244
Geo	0.226	0.378	Geo | Env+Anc	0.118	0.111
Anc	0.105	0.259	Anc | Env+Geo	0.011	0.002
Env+Geo	0.582	0.719	Total confounded	0.178	0.360
Env+Anc	0.476	0.606	Unexplained	0.405	0.283
Geo+Anc	0.305	0.473			
Env + Geo + Anc	0.594	0.717			

Divergence time

The divergence time between the North and Center-South genetic groups was estimated at 2.81 My (1.64 – 4.17 My), near the end of the Pliocene and the beginning of the Pleistocene. The localities within the North genetic group (Nah and Cu) or within the Center-South genetic group (ChlN and Coy, TP, ChlS) seem to have diverged much more recently, towards the end of the Pleistocene (0.33 / 0.37 My ago; Fig. 5).

Discussion

Previous studies based on allozymes genotypes or chloroplast sequences (Mathiasen et al., 2007; Souto and Premoli, 2007; Vidal-Russell et al., 2011; Souto and Smouse, 2014) have reported genetic structure in*E. coccineum* . However, the limited variability and genome coverage of the markers used in previous studies has led to a lack of clarity regarding the species genetic structure as well as the processes that has shaped it. In the present study, samples of *E. coccineum* , including localities from its whole distribution range along the Chilean temperate forest, were genotyped through GBS to investigate patterns of genetic variation and their possible drivers using genome-wide data in this emblematic Chilean tree. The thousands of loci successfully genotyped provided a much better resolution of *E. coccineum* population genetic structure than the one obtained for the previously used molecular markers. First, our data showed the existence of two main lineages, North and Center-South. The existence of two genetic groups was already reported in the study of Souto and Premoli (2007), but the GBS data set also uncovered fine-scale genetic differentiation, especially in the northern part of the distribution. Inference of divergence time between these two genetic groups suggested that intraspecific divergence occurred some 2,8 Myrs ago, at the beginning of the Pleistocene, driven by climatic changes linked to glacial cycles. Moreover, the heterogeneous environment characterizing the South American temperate forests have been recognized as major driver explaining the actual genetic makeup of various plants and animals (Premoli et al., 2000) and could also have leaded to adaptive divergence among localities in *E. coccineum* . The strong IBE pattern detected in *E. coccineum* could indeed reflect the impact of actual gradient of temperature, precipitation and elevation, characteristic of the species distribution range, on its genetic diversity. These results suggest that selection, linked to the strong environmental gradient, could have leaded to adaptive divergence among localities.

Genetic differentiation within *Embothrium coccineum:* two genetic groups separated since the early Pleistocene

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Our result confirms previous studies in *E. coccineum* documenting the presence of two genetic groups, one in the northern and one in the southern part of the species distribution (Souto and Premoli, 2007; Vidal-Russell et al, 2011); yet, these datasets exhibit different levels of spatial resolution. Alloenzymes revealed only a weak geographic structure, with some northern and southern localities clustered together (Vidal-Russell et al, 2011). The authors explained this pattern by proposing a possible effect of glacial survival in multiple refugia followed by recolonization and / or the existence of actual gene flow blurring the trace of the historical geographic structure. Contrastingly, chloroplast markers revealed a clearly distinct southern genetic group characterized by a very low genetic variation (i.e., only a few haplotypes were observed in the South). In this last case, two hypotheses could explain this pattern: 1) the existence of both northern and southern glacial refugia and a recent colonization from the southern to the northern part of the country or 2) a unique refugium in the north and a recent recolonization to the south (Souto and Premoli, 2007). Nonetheless, due to the lack of marker resolution, no robust evidence is provided to support the conclusions of both studies. Contrastingly, given the higher resolution provided by genomic SNPs, the present study not only unambiguously supports the existence of the two proposed genetic groups, but also shows that individuals from the center part of the species distribution are more genetically similar to those from the south. A divergence time of 2,8 My was estimated between the North and Center-South genetic groups, corresponding to the Late Pliocene and Early Pleistocene, while the divergence between populations within each North and Center-South group was much more recent (i.e., some 0.35 My ago). These results emphasize the possible role of the glacial cycles and associated environmental changes as a possible motor of divergence in *E. coccineum* . Our study provides then a refined picture of the probable divergence time between the North and Center-South genetic groups.

Pleistocene glaciations and associated climate change have dramatically altered the landscape of southern South America (Ramos and Ghiglione, 2008; Martínez and Kutscher, 2011; Ponce et al., 2011). The numerous glacial advances and retreats have impacted the abundance and distribution of the local biota (Rabassa et al., 2011). Most phylogeographic studies have shown that populations located at southern latitudes and impacted by glaciations have experienced a recent and rapid expansion after glacial retreat, whereas northern populations have remained more stable (Koszak et al., 2006; Fontanella et al., 2008). Indeed, various studies (e.g., Markgraf, 1983; Vidal et al., 2005) propose the existence of refugia north of the area heavily impacted by ice (i.e., ranging between 36°S - 40°S and 54°S). For example, the absence of some native plants pollen records as *Eucryphia codifolia* and *Caldcluvia paniculata* during the LGM at 41°S (Moreno, 1997; Heusser et al., 1999; Moreno et al., 1999; Moreno and León, 2003) and in Chiloé (Villagrán, 1988; Abarzúa et al., 2004; Heusser and Heusser, 2006) suggests a contraction north of the ice-sheet line for these species during glacial maxima. Furthermore, the presence of rare private haplotypes and the high levels of genetic diversity observed in populations located at 39 °S – 40 °S suggest the existence of northern glacial refugia and a southern expansion after glacial retreat in *E. cordifolia* (Segovia et al., 2012). However, Sérsic et al., (2011) reported genetic patterns strongly supporting the existence of *in situ* refugia located south of 42°S in both plants and animals. Comparative phylogeographic studies in southern South America (Bruno et al., 2016; Perez 2017; Sérsic et al., 2011) propose the existence of at least six areas where both terrestrial plant and animal species survived during glacial maxima. These areas show higher genetic diversity than areas where post-glacial colonization occurred, as in some parts of the southern Patagonian Steppe (Hewitt 2001; Pinceel et al., 2005; Huck et al., 2009).

In the case of *E. coccineum* , information based on cpDNA haplotypes suggested that southern populations of *E. coccineum*had been affected by glacial cycles, declining gradually in size since the Pleistocene (100 ky - 12 ky ago), and then rapidly increasing after the LGM (Vidal-Rusell et al., 2011). Distribution of chloroplast haplotypes also provides evidence of local expansion in the north. The existence of various microrefugia located in Nahuelbuta (37°S - 72°W), Manzanar (38°S - 71°W), Alerce Costero (40°S - 73°W), Laguen Ñadi (41°S - 73°W) and Lago Verde (42°S - 71°W) was suggested by the authors. In our study, relatively high genetic diversity and percentage of polymorphic loci were found in Nahuelbuta, Curacautín, Chiloe Norte (nearby Laguen Ñadi), Coyhaique (nearby Lago Verde) and Torres del Paine, and the number of private alleles was also high in these same locations. These results, combined with the observed pattern of genetic

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structure, support the hypothesis of *E. coccineum* survival during glacial periods of the Pleistocene glacial cycles in various refugia, including at least two refugia located south of 42°S. The existence of southern local refugia in *E. coccineum* is also supported by fossils records of cold-tolerant plant species characteristic of the austral forest reported south of 42°S (Villagran et al., 1986; Markgraf 1995). High levels of population divergence and phylogeographic structure attributed to isolation in multiple refugia have been detected in other cold-tolerant native trees such as *Fitzroya cupressoides*(Allnutt et al., 1999; Premoli et al., 2000; Premoli et al., 2003),*Pilgerodendron uviferum* (Premoli et al., 2001; Premoli et al., 2002; Ausin et al., 2003), *Araucaria araucana* (Bekessy et al., 2002), *Podocarpus nubigena* (Quiroga and Premoli, 2010),*Nothofagus alpina* (Marchelli et al., 1998; Marchelli and Gallo, 2006), and *Nothofagus pumilio* (Premoli, 2004; Mathiasen and Premoli, 2010; Premoli et al., 2010).

Possible regional differences in pollen and seeds dispersal: effect on *Embothrium coccineum* gene flow

Our analysis of the genetic differentiation drivers in *E. coccineum* identified a significant pattern of isolation by distance (IBD). The existence of IBD indicates that gene flow is limited as geographic distance increases (Wright, 1943), however some level of connectivity was observed between nearby populations, mostly in the Center and South. In other plants, as *Dactylis glomerata* (Sun et al., 2017) and *Protea rapens* (Prunier et al., 2017), IBD patterns were detected at similar geographic scale, pointing out opportunities for increased allelic exchange between neighboring populations. Moreover, STRUCTURE, PCA and NJ distance tree analyses, and estimation of pairwise-FST values, showed that populations from the North genetic group of *E. coccineum* are highly structured. We hypothesize that this population structure differences between localities within the North and the Center-South genetic groups could be, in part, linked to the existence of a more restricted gene flow in the northern part of the distribution.

Plant gene flow may occur via pollen and/or seed dispersal and is directly affected by the species reproductive system (Martins, 1987). The Proteaceae family is mostly composed by outcrossing shrubs and/or small trees presenting patchy, naturally fragmented distribution and moderate levels of genetic diversity (Collins and Rebelo 2006, Souto and Premoli, 2007). These features have led Mathiasen et al., (2007) to consider *E. coccineum* as a species with low sensitivity to the effects of forest fragmentation due to a predominantly outcrossing breeding system, which may favor pollen exchange among nearby fragments (Rovere and Chalcof 2010). Indeed, genetic erosion by drift in forest patches could be counteracted by pollinator's movements, reducing inbreeding, and allowing the populations to retain sufficient heterozygosity through a continuous influx of alleles from locally distinct gene pools (Mathiasen et al., 2007). *Embothrium coccineum* , gene flow is highly dependent on pollinating agents (Mathiasen, 2004; Rovere and Chalcof 2010) and his flowers are visited by more than 20 species, including birds of the orders Passeriformes and Apodiformes, and insects of the orders Hymenoptera, Diptera, Lepidoptera and Coleoptera (Chalcoff, 2008).

The composition of the pollinator assemblage affects both, pollen transport and seed production. In regions with low number of *E. coccineum* pollinators, pollen limitation can be high, leading to low reproductive efficiency (i.e., few fruits produced in relation to the available flowers) (Chalcoff, 2008). This could translate in populations presenting reduced genetic diversity and high genetic structure due to the lack of genetic exchange (Rovere and Chalcof 2010). Pollinating insects, generally characterized by low migration capacities, are distributed throughout the whole *E. coccineum* distribution range even if they are more abundant and visit the plants more frequently in populations located in drier and sunnier climates (i.e., North) (Rovere and Chalcof, 2010). Contrastingly, birds, as the hummingbird*Sephanoides sephaniodes* , are very abundant in southern populations located in areas characterized by cool and humid conditions where they act as major pollinators (Chalcoff, 2008). We propose that differences between North and Center-South of *E. coccineum*pollinators distribution and behavior could be one of the drivers leading to the observed genetic structure, with a lower dispersal capacity in the north leading to a higher level of structure.

However, other differences between regions, as the level of landscape fragmentation, could also generate the pattern observed in our data set and need to be further tested. In *Protea rapens* , a widespread Proteaceae from the Cape Floristic Region in South Africa, a genetic split between eastern and western population was

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observed (Prunier et al., 2017). Changes in the timing of rainfall, from a predominantly winter rainfall in the west, to a more evenly distributed rainfall, during the year in the east were reported in the study region (Schultze, 2007). Prunier et al. (2017) proposed that differences in *P. rapens* flowering time (Heeleman et al., 2008), linked to rainfall patterns, could hinder gene flow between populations, reinforcing the east-west divide. Differences in climatic conditions along the species distribution range also affect *E. coccineum* flowering period, with a flowering period beginning later (i.e., November – December) in populations located at high latitude and altitude when compared to the rest of the distribution (i.e., September- October) (Hoffmann, 1997; Chalcoff, 2008; Rovere and Chalcof, 2010). Limited gene flow between populations presenting differences in flowering phenology could also lead in part to the observed pattern of divergence between and within genetic groups, by limiting exchange between close by populations located at contrasting altitude.

Signatures of local adaptation in *Embothrium coccineum*

One of our objectives was to disentangle the effects of local adaptation and isolation by environment (IBE) from neutral processes, such as isolation by distance (IBD) or co-ancestry linked to the species glacial history (IBA), in shaping among-population genetic differentiation of*E. coccineum* . Our results show that the observed pattern of genetic variation is, at least in part, the result of adaptation to the strong environmental gradients characterizing the species distribution range (Danield and Veblen 2000; Souto and Premoli 2007; Souto et al., 2009; Souto and Smouse, 2013). Indeed, results from the redundancy analysis (RDA) show that IBE was a significant driver of population structure and explained the largest amount of among-population variation in *E. coccineum* , even after controlling for IBD and IBA. These results were corroborated by the much higher divergence detected between the North and Center-South genetic groups in the NJ tree based on genetic distance calculated using only the outlier loci putatively under selection than the one observed when using all the loci genotyped.

Our gradient forest analysis identifies precipitation of the driest month as the most important explanatory environmental variable explaining *E. coccineum* pattern of genetic variation. The level of precipitation of the driest month distinguishes the northern neighboring Mediterranean biome and the Patagonian steep close to the east, both with very low summer precipitation, from the temperate biome inhabited by *E. coccineum* (Escobar et al., 2006). The second explanatory environmental variable was the mean temperature of the wettest quarter. This result is consistent with studies focused on other Proteaceae; with summer rainfall associated with differences in functional traits in *P. rapens* (Carlson et al., 2016) and in*Protea* section *Exsertae* (i.e., a group of six*Protea* species; Carlson et al., 2011; Prunier et al., 2012, Prunier et al., 2017). Differences in morphology associated with environmental gradients have already been reported in *E. coccineum* (Chalcof, 2008; Souto and Premoli, 2007), supporting the idea of possible local adaptation in the species. Access to water seem to be key in determining the ecotypes observed in each region with individuals from the northern and driest part of the distribution developing as short plants, of 0.5-2.5 m in height, with small leaves while the individuals in wet temperate forest of the center part of the distribution can reach more than 10 m in height and develop very large leaves (Souto et al., 2009; Souto and Smouse 2013). Plants in areas characterized by steady and high rainfall may be on average less heat and water-stressed, favoring the production of leaves that are larger and broader and maximizing the photosynthetic area when risks of overheating and drought stress are low (Givnish, 1987; Hallik et al., 2009). Indeed, a reduction in leaf size has been proposed as one of the key traits allowing plants to withstand water deficit (B and Xu, 2007, Peguero-Pina et al., 2014). For example, a reduction in *Quercus faginea* leaf size has been proposed as one of the key traits allowing Mediterranean oaks to withstand the water deficit characteristic of the region (Baldocchi and Xu, 2007, Peguero-Pina et al., 2014).

To complement the results of the present study it would be interesting to investigate in detail the possible genetic adaptations of *E. coccineum* to different soil chemical properties. Indeed, the species cluster roots are highly specialized in phosphor mobilization and uptake (Delgado et al., 2021) and it has been described that energy allocation to cluster roots varies between regions with a lower cluster roots growth in plants from the northern part of the species distribution (Bertin Benavides et al. 2020; Zuñiga-Feest et al., 2015).

Conclusion and prospects

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Known patterns of morphological variations coupled with new results of genetic diversity and structure obtained during the present study revealed a long history of geographic isolation and local adaptation in *E. coccineum* in South American temperate forests. Here, we observed a strong genetic structure in *E. coccineum* in part linked to local adaptation, especially to differential access to water during the driest months. These results support previous studies that have reported the existence of distinct ecotypes along the species natural distribution (Souto and Premoli, 2007). The identification and quantification of the environmental variables structuring population genetic variation could inform management decisions for conservation, restoration or reforestation purposes (Gugger et al., 2017). For example, knowledge about the association between genotype and environment is important for selecting proper seed when tree populations are locally adapted (Aitken and Whitlock, 2013) as is the case of *E. coccineum*. However, as the environment changes, nonlocal seed sources may be considered for restoration or reforestation purpose based on their match to the novel environment. Therefore, seed transfer guidelines can benefit from knowledge on factors structuring the landscape of genetic variation. Many conservation efforts rely on delineating distinct populations for management but often ignore the continuous nature of landscape variation and its potential relationship with local adaptation or other processes that lead to genotype–environment associations (Frankham, 2010; Rodríguez-Quilón et al., 2016). Concerning land management decisions, the present results advocate for seed transfer guidelines that should be restricted within each ecological zone (Dudley et al., 2017). The strong genetic structure also offers an opportunity to further explore local adaptation among groups and exploit these for agroforestry.

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DATA AVAILABILITY STATEMENT

The authors declare that all the work presented in this manuscript was done according to the standards of the Ecology and Evolution. Raw GBS data used in this study has been already published in ncbi-SRA database and is available at http://www.ncbi.nlm.nih.gov/sra/?term=PRJNA783610.

CONFLICT OF INTEREST

On behalf of all authors, the corresponding author states that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors contributed to the design of the study. Material preparation, data collection and analysis were carried out by Francisco Sepulveda-Espinoza, Ariana Bertin-Benavides, Rodrigo Hasbun, Oscar Toro-Nunez, Antonio Varas-Myrik, Diego Alarcon and Marie-Laure Guillemin. The first draft of the manuscript was written by Francisco Sepulveda-Espinoza, Ariana Bertin-Benavides and Marie-Laure Guillemin and all authors commented on earlier versions of the manuscript. All authors read and approved the final manuscript.

Figure 1 : a) Sampling locations of *Embothrium coccineum* . Circles, triangles and diamonds represent samples from the northern, center and southern part of the species distribution, respectively.*Embothrium coccineum* distribution area is highlighted in light grey and hatched lines represent ice limits from UMG. **b)Principal component analysis (PCA) based on a 2,155 SNPs data set of *Embothrium coccineum* samples.** Symbols and colors used for each locality are as in Figure 1a.

Figure 2: Structure analyses, for K = 2 (a) and K = 4 (b) of 38*Embothrium coccineum* individuals based on a 2.155 SNPs data set . Each individual is displayed from north to south represented by a vertical bar. Sampling location codes are as in Supplementary table 1. Color corresponds to genetic group and proportion of the genome assigned to each genetic group is given along the Y-axis.

Figure3: NJ tree reconstructed based on Nei's distance between *Embothrium coccineum* individuals. NJ reconstruction based on (a) the PG_dataset of 2.155 SNPs, and (b) the 59 outlier loci putatively under selection. Sampling location codes are as in Supplementary table 1. Nodes with high support ($> 0{,}95$) are filled in black.

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Figure4: Predictor overall importance plot. The R^2 weighted importance of environmental variables with low level of covariance explaining the genetic variation across sampling locations as obtained from GF analysis. Environmental variables used in the analyses are the same as in Supplementary table 1; bio 14, Precipitation of the driest month (mm); bio 8, Mean temperature of the wettest quarter (degC); elevation, Elevation (masl); bio 9, Mean temperature of the driest quarter (degC); bio 4, Temperature seasonality (degC); bio 3, Isothermality (%); bio 13, Precipitation of the wettest month (mm).

Figure5: Estimation of divergence time in *Embothrium coccineum* based on Bayesian coalescent analysis using SNAPP. Nodes with high support (posterior probability > 0.99) are filled in black. Median ages are provided above nodes with 95% highest posterior densities (HDP) below. The divergence time was inferred only for the nodes showing high support (posterior probability > 0.99). Sampling location codes are as in Supplementary table 1; colored symbol for each sampling location match Fig. 1a.

Table 1: Genetic diversity of *Embothrium coccineum* in each sampling locality. Localities ID are as in Supplementary table 1. n, number of genotyped individuals; He, expected heterozygosity; Ho, Observed heterozygosity; FIS, inbreeding coefficient; PPL, percentage of polymorphic loci; PA, private alleles.

Table 2: Average FST values for pairwise comparisons among sampling locations of *Embothrium coccineum* in Chile based on a set of 2,155 SNPs. All *p-values* are significant (<0.05). Sampling location codes are as in Supplementary table 1. North: Ch, Cu and Nah; Center: PM, ChlN, ChlS and Pu; South: Coy, ChCh and TP.

Table 3: Redundancy analysis (RDA) partitioning among-population genetic variation in *Embothrium coccineum* into three components: environmental (Env), geographic (Geo) and North / Center-South coancestry (Anc). All p values <0.001.

Supplementary Table 1 : Environmental characteristics of each sampling location of *Embothrium coccineum.*

GPS coordinates are given for each sampling location. Environmental variables: bio 14, Precipitation of the driest month (mm); bio 8, Mean temperature of the wettest quarter (degC); Elv, Elevation (mamsl); bio 9, Mean temperature of the driest quarter (degC); bio 4, Temperature seasonality (degC); bio 3, Isothermality (%); bio 13, Precipitation of the wettest month (mm).

Locations	ID	Latitude	Longitude	bio 14	bio 8	Elv	bio 9	bio 4	bio 3	bio 13
Chillán	Ch	-36.908	-71.504	23	3.9	1216	14.0	4.1	55	257
Curacautín	Cu	-37.642	-73.099	28	4.4	989	11.2	2.8	57	297
Nahuelbuta	Nah	-38.455	-71.734	53	5.5	797	14.7	3.7	56	390
Puerto Montt	PM	-41.521	-72.753	95	7.9	81	14.9	2.9	49	267
Chiloe Norte	ChlN	-42.296	-73.459	90	7.7	129	13.5	2.4	47	333
Chiloe Sur	ChlS	-42.783	-73.806	85	8.0	97	13.4	2.3	45	303
Pumalín	Pu	-42.536	-72.496	96	7.4	22	15.4	3.3	49	309
Coyhaique	Coy	-46.505	-73.066	98	4.4	175	9.9	3.2	44	181
Chile Chico	ChCh	-46.649	-72.362	35	2.6	428	11.8	3.7	44	101
Torres del Paine	TP	-51.361	-72.799	37	9.1	33	9.7	3.4	48	67

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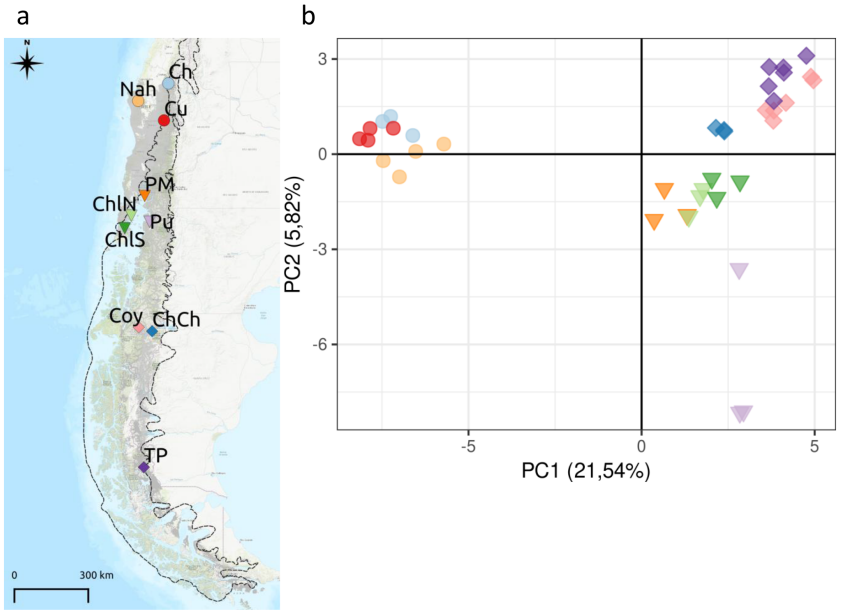

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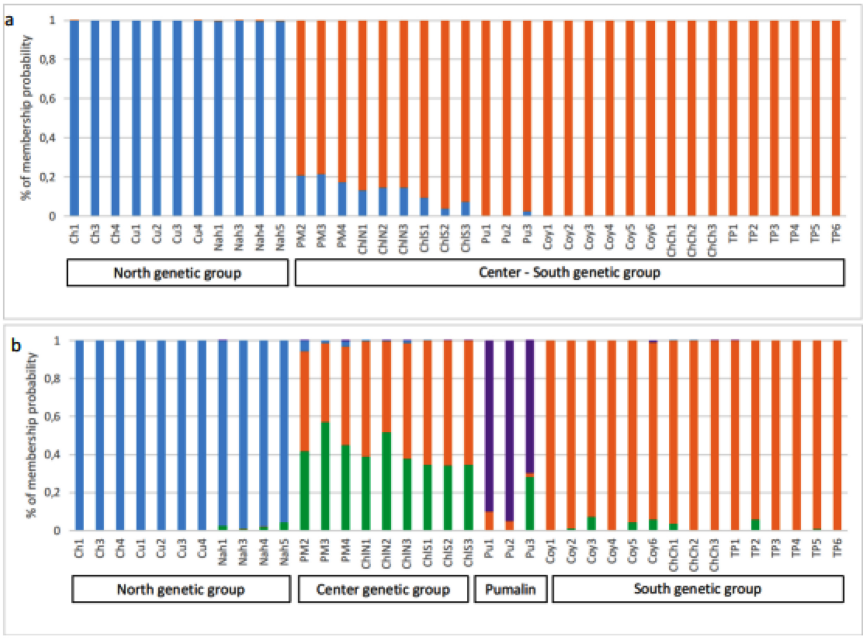

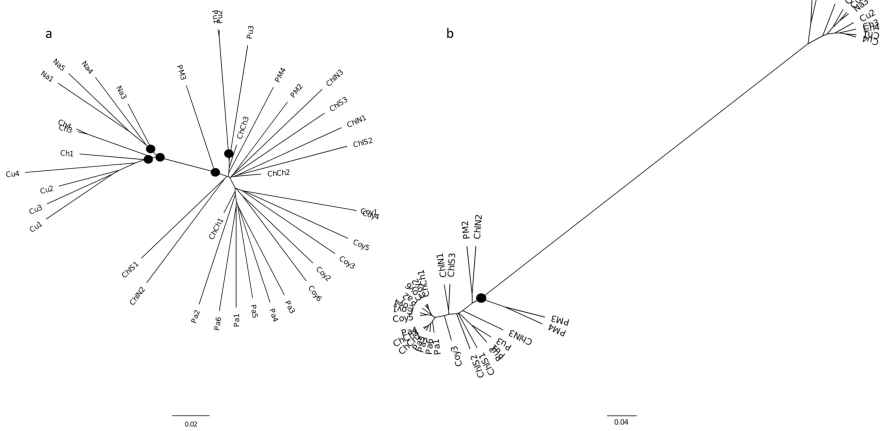

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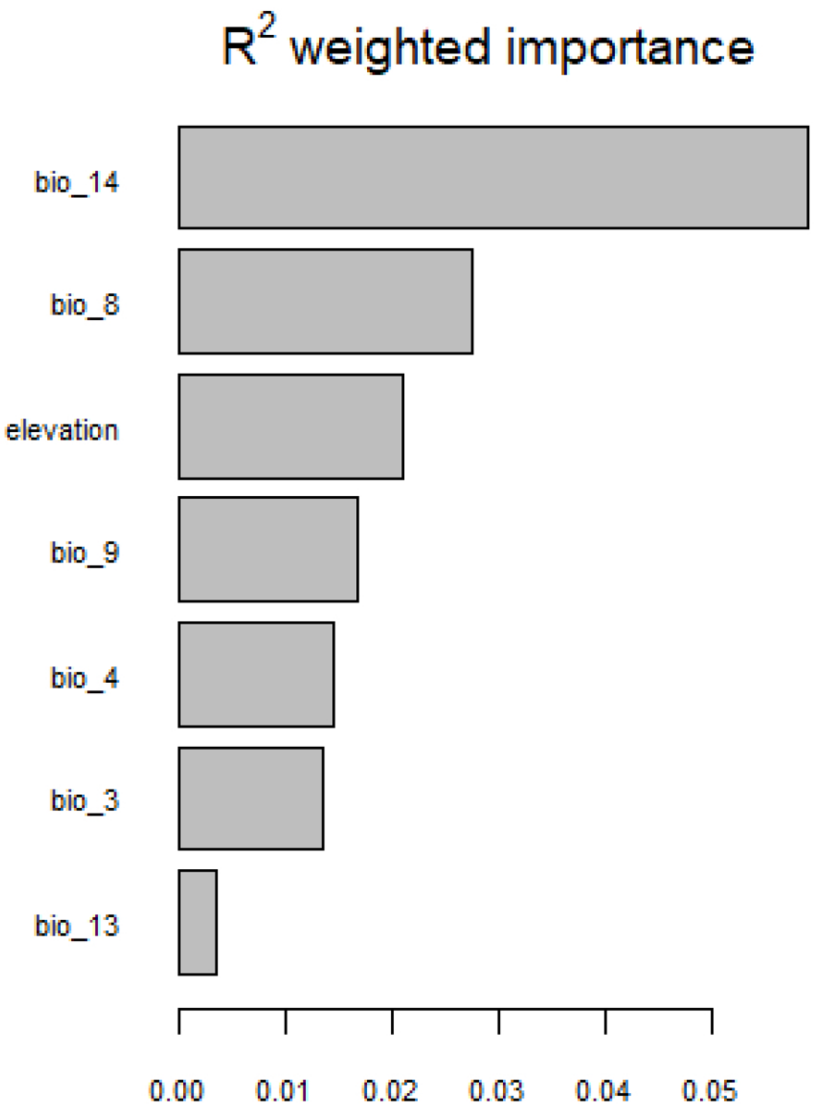

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