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Predicting climate change-related genetic offset for the endangered southern South American conifer *Araucaria araucana*

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ABSTRACT

Understanding adaptive genetic variation is key for predicting the evolutionary response of species and populations to climate change, decisively influencing management and conservation decisions. Landscape genomics provides a framework to disentangle the effects of local adaptation from those of geographic distance and demographic history, through genomic analysis and the modeling of genotype-environment relationships. This approach can inform how evolutionary forces shape the neutral and adaptive genetic structure, helping to identify those populations subject to a greater risk of maladaptation due to anthropogenic climate change, i.e., the "genetic offset". Using restriction-site associated DNA sequencing (RAD-Seq) and more than 49,000 single nucleotide polymorphisms screened from 12 locations of *Araucaria araucana* in Chile, we assessed the genetic structure and predicted the genetic offset of this emblematic tree species under two future climate scenarios. Using generalized dissimilarity modeling (GDM) we found that the temperature annual range was the most important variable shaping the observed patterns of adaptive divergence. Our results show that populations living in the piedmont of the southern Andes Mountain range are at the greatest risk of maladaptation, while populations living in the high elevation zones in the Andes Mountain range are at the lowest risk. This study constitutes an important tool for forestry management and conservation of *A. araucana* forests.

1. Introduction

Anthropogenic climate change is expected to cause shifts in the distribution of species, populations declines and extinctions. Species can cope the threats that arise from new environmental conditions in current habitats either migrating, adapting in situ or through phenotypic plasticity (Sultan, 2000; Hoffmann and Sgrò, 2011; Gougherty et al., 2021). During adaptive responses, genetic variation increases, causing maladaptation in the short-term, but higher adaptability in the long-term (Urban, 2015; Wiens, 2016; Derry et al., 2019). While maladaptation causes suboptimal population fitness, adaptation can usually restore populations to optimal fitness levels in the long-term. Nonetheless, the normal continuum from maladaptation to adaptation can be disrupted by rapid climate change, causing a breakdown in the genetic-environmental relationship faster than the species' capability to migrate or adapt in situ, with a consequent loss of fitness (Jump and Penuelas, 2005; Aitken et al., 2008). The measure of the amount of adaptive genetic change between present and future climate conditions required to maintain species current genetic–environmental association has been coined as the species' "genetic offset" index (Fitzpatrick and Keller, 2015). Among other applications, this risk index can improve conservation strategies by gathering spatially explicit genetic information that helps to build more resilient and better adapted populations (Fitzpatrick and Keller, 2015; Jia et al., 2020). For example, the genetic offset can guide assisted gene flow programs, supporting the use of seed lots composed by local and non-local—but pre-adapted—genotypes, thus increasing adaptive diversity and resilience (Aitken and Whitlock, 2013; Aitken and Bemmels, 2016). The implementation of genetic offset indexes could represent a modern solution to support the local adaptation of natural populations under rapidly changing environments.

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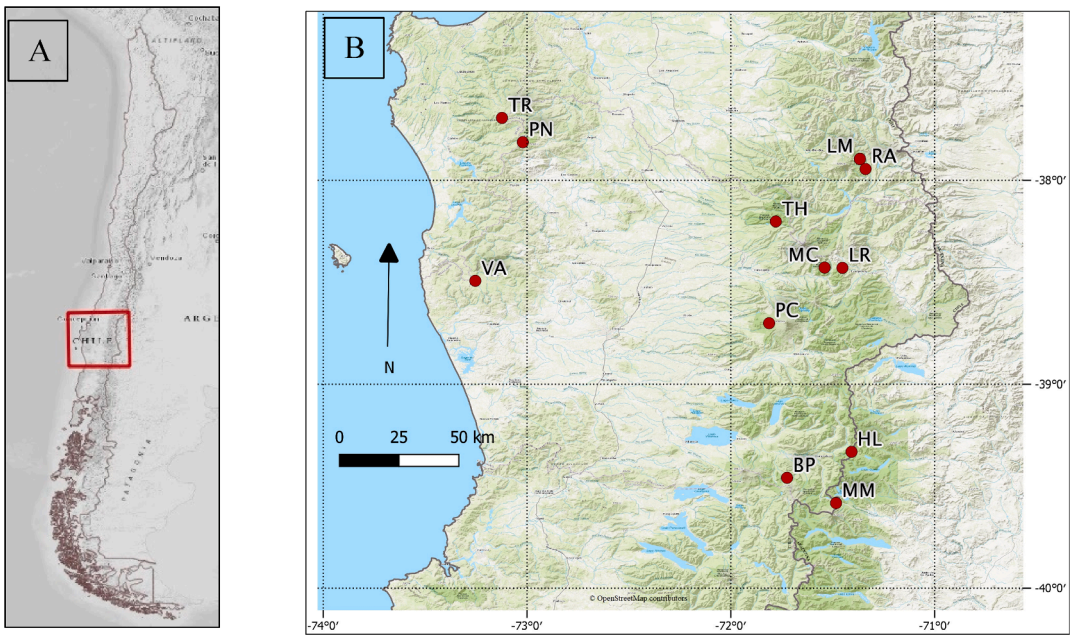

Fig. 1. Sampling locations map of *A. araucana*. Panel A): regional map. The red box indicates sampling area. Panel B): detailed map. Red dots show sampling locations and its code.

The first step to assessing genetic offset is to determine the strength and structure of adaptive genetic variation, i.e. the variation found between the genomes of individuals due to natural selection (Schoville et al., 2012). Landscape elements that impose dispersal limitations (e.g., geographic distance, physical barriers, etc.) and modulate ecological processes (e.g., colonization) act concomitantly with natural selection to shape adaptive divergence (Hoban et al., 2016). Elucidate the singular and combined effects of each of these factors in adaptive divergence imposes an analytical challenge, since the influence of neutral and adaptive processes are usually confounded in natural populations (Ahrens et al., 2018). Landscape genomics —a synthetic discipline that combines concepts and tools from population genetics, landscape ecology, geography and spatial statistics (Manel et al., 2003; Holderegger and Wagner, 2008)—provides an robust theoretical framework to face this challenge through the identification of loci putatively under selection based on associations between genetic data and environmental variables hypothesized to drive selection, while controlling for neutral genetic structure (Rellstab et al., 2015). To gain insight into the processes that shape adaptive divergence, redundancy analysis (RDA) can aid in the quantification of the specific and combined effects of landscape elements on adaptive divergence, partitioning genomic variation into components explained by geographic distance, ecological gradients, and their combinations (Capblancq and Forester, 2021). Thus, adaptive divergence can be modeled using this subset of adaptive loci as response matrix and landscape elements, mainly environment and geography, as predictors. These models, which we will call from now on "adaptive divergence models", can be harnessed to evaluate genetic offset under future conditions by measuring the distance between current and future predictions (Fitzpatrick and Keller, 2015; Holliday et al., 2017).

While studies dealing with genetic offset have initially been implemented for different species across several ecosystems (Fitzpatrick and Keller, 2015; Bay et al., 2018; Gugger et al., 2018; Jia et al., 2020), its implementation has not yet achieved a comprehensive use in several geographic areas that require immediate assistance for conservation. One case is the temperate forests of southern South America, characterized by their complex evolutionary and biogeographic history, sustaining large ecosystems with high endemism, which are regarded of great biological conservation value (Sersic et al., 2011; Villagrán, 2018). While some interest has been placed on the use of genetic tools in conservation studies of Patagonian tree species, (Premoli et al., 2000; Marchelli et al., 2010; Souto et al., 2015; Mattera et al., 2020), landscape genomics has not been widely used to identify adaptive loci and to model adaptive divergence, (Martín et al., 2014; Hasbún et al., 2016), leaving this useful tools and its applications (e.g. genetic offset prediction) unexplored for species growing in temperate forests of southern South America. Given its potential to usefully inform conservation and forest management programs, the prediction of climate change-related genetic offset could aid in defining empirical criteria for conservation of valuable and threatened local tree species.

An emblematic and endangered tree species living in South America temperate forests is *Araucaria araucana* (Molina) K. Koch, commonly referred to as "Pewen" (indigenous name) or "Monkey Puzzle Tree" (English name) (Premoli et al., 2013), which is distributed in restricted and fragmented patches of Chile and Argentina. In Chile, this species occurs in both the Costa and Andes Mountain ranges (37.5° to 38.5° and 37.5° to 39.7° south latitude, respectively). Recent studies in population genetics of *A. araucana* have described weak patterns of population structure, most likely as a result of bird-mediated seed dispersal and the human consumption of its starch-rich seeds (Martín et al., 2014; Tella et al., 2016). Currently, this species is being increasingly affected by a dieback called "*Araucaria leaf damage*" (Velez et al., 2018; Puchi et al., 2021). Provided that we are dealing with an iconic species, this dieback has prompted public and private conservation efforts, which have included assisted migration. However, there is a dearth of knowledge regarding the genetic-environmental relationship, which could scientifically inform conservation programs, reducing the risk of maladaptation in novel and changing environments.

With the purpose to provide empirical information for conservation and forest management programs, the main goal of this study was to predict the genetic offset of populations of *A. Araucana* due to climate change. Besides, two complementary objectives were addressed: (a) to assess *Araucaria araucana*'s population genetic structure, and (b) to evaluate the effect of ancestry, environment and geographic distance on whole and adaptive genetic variation.

Table 1
Sampling locations, geographic coordinates (degrees of south latitude and west longitude), sample size (N) and Mountain Range of the 12 *A. araucana* sampled sites.

Map code (Fig. 1)	Site name	Latitude	Longitude	N (Valid)	Mountain range
BP	Bosque Pehuén	−39.46	−71.72	12	Andes
HL	Hualalafquén	−39.33	−71.41	7	Andes
LM	La Mula	−37.90	−71.37	11	Andes
LR	Las Raíces	−38.43	−71.45	14	Andes
MC	Malalcahuelo	−38.43	−71.54	8	Andes
MM	Mamuil Malal	−39.58	−71.48	10	Andes
PC	Conguillío	−38.70	−71.81	9	Andes
PN	Parque Nahuelbuta	−37.81	−73.02	14	Costa
RA	Ralco	−37.94	−71.34	14	Andes
TH	Tolhuaca	−38.20	−71.78	9	Andes
TR	Trongol	−37.69	−73.12	11	Costa
VA	Villa Araucarias	−38.49	−73.25	15	Costa

2. Materials and methods

2.1. Sampling, RAD-Seq library preparation and sequencing

Tissue samples of *A. araucana* were collected from 12 different locations in both the Coastal and Andes Mountain Ranges in Chile (Fig. 1, Table 1), to cover the range of environmental variation of its habitat. For each location, we traversed a 2 km transect where sampled trees were separated by at least 100 m. Eleven to 20 trees were sampled per transect; we selected fresh, healthy-looking leaves from the tips of the branches. Trees 3 to 5 meters height were selected to study trees of roughly the same age group. Samples were maintained in sterile conical tubes stored in coolers with ice packs until arriving to the laboratory, where they were stored at −80 °C until DNA extraction. Tissue samples were washed sequentially with 1.5 g/liter Captan (PubChem CID 8606), 70% ethanol, 1% sodium hypochlorite, and sterile water to remove epiphytic microbes. Then, plant material was ground manually before being flash frozen for 1 min (with liquid nitrogen). After one cycle of tissue disruption in a TissueLyser II for 1 min at 30 hz, samples were frozen in liquid nitrogen again to repeat the disruption step. Fifty milligrams of disrupted plant material were used for DNA extraction, using the DNeasy PowerPlant Pro kit (Qiagen).

DNA was quantified in a Qubit 3.0 instrument (Thermo Fisher Scientific) using the Qubit double-stranded DNA (dsDNA) high-sensitivity (HS) assay kit. Genomic DNA from 190 individuals was extracted using a standardized protocol (Doyle, 1991). Libraries for RAD-Seq were prepared by digesting DNA with a *PstI* restriction enzyme. Sequencing was carried out by Floragenex Inc. (Portland, USA), using Illumina HiSeq2500 technology. Reads were filtered and processed with ipyrad v.0.9.58, a pipeline usually employed in phylogenetic and population genetic analyses given its handling of variable indels during the clustering process (Eaton and Overcast, 2020).

The data were assembled *de novo*, following the ipyrad's default parameters, which considered filters for readings with an average *phred* value lower than 33, barcode removal, and a clustering threshold of 85%. The clusters obtained were selected with a ratio of 0.5 maximum heterozygosity per locus, 0.2 Single Nucleotide Polymorphism (SNP) per locus, two maximum alleles per site, and 8 indels per locus. The final consensuses were filtered to retain those present in at least 4 individuals per locus. Subsequently, the data set was additionally filtered with VCFtools software (Danecek et al., 2011). Sites with heterozygosity greater than 50%, minor allele frequency less than 5%, and missing data greater than 50%, were excluded. Individuals with more than 50% missing data were also discarded. After these filtering steps, a genotypic matrix of 134 individuals and 49,122 biallelic SNPs was obtained and later used for population structure analyses. In addition, a second matrix of 18,988 SNPs was obtained by taking only one SNP per fragment to conduct the detection of loci putatively under selection, in order to avoid some of the problems caused by linkage among markers (Hoban et al., 2016). Sequence data can be found in NCBI's Sequence Read Archive database under BioProject PRJNA634877.

2.2. Environmental predictors

To detect environmental drivers of adaptive divergence, we considered a comprehensive set of 25 edaphoclimatic predictors, which were later subjected to a variable selection procedure to build a set of few meaningful and uncorrelated variables. This initial dataset considered 19 bioclimatic variables plus elevation from the WorldClim version 1.4 database (Hijmans et al., 2005), representative of the average conditions for the years 1960–1990. In addition, five soil variables obtained from the Soilgrids 2.0 database (de Sousa et al., 2020) were considered. Then, we conducted a two-step variable reduction procedure (Fitzpatrick et al., 2011).

First, we reduced the full set of variables by selecting ten bioclimatic and soil variables that minimized correlation ($r < 0.7$), retaining those variables of correlated pairs that were, in our opinion, the most biologically significant. These included: annual mean temperature (°C), isothermality (%), precipitation of the wettest month (mm), precipitation of the driest month (mm), temperature annual range (°C), precipitation seasonality (%), elevation (m), cationic exchange capacity (CEC, mmol kg^{-1}), total soil nitrogen (cg kg^{-1}), and soil pH. This set, which includes annual trends, seasonality, limiting environmental factors and soil variables, was employed for the environmental association analysis to detect potentially adaptive loci, which are described below.

Second, to fit the adaptive divergence model, we further reduced this set of ten variables by using a backward-elimination technique (Fitzpatrick et al., 2011), as implemented in the *gdm.varImp* function in the *gdm* R-package (Fitzpatrick et al., 2021). Briefly, this algorithm started removing less important variables, assessing the significance of each through permutation tests. This procedure continued until the difference in deviance between the model with and without the variable became significant and no more variables could be discarded without decreasing the model performance. After this step, temperature annual range (°C), precipitation of the wettest month (mm), precipitation of the driest month (mm), elevation (m) and soil pH were retained.

To project the bioclimatic variables used to fit the adaptive divergence model to future climate conditions, we considered four scenarios composed by two Representative Concentration Pathways (RCP4.5 and RCP8.5), reflecting moderate and extreme future conditions, respectively; and two projection periods (2050 and 2070). In order to account for uncertainty in climate projections (Sanderson et al., 2015), we chose two general circulation models (GCM) for each of the four scenarios: ACCESS1-0 (Bi et al., 2013) and HadGEM2-ES (Jones et al., 2011), from the fifth phase of the Coupled Model Intercomparison Project (CMIP5), which were ensemble using average. Future climate data were obtained from the Worldclim 1.4 (Hijmans et al., 2005). Lastly, the genetic offset was mapped using a mask corresponding to previous niche-based species distribution model reported for *A. araucana* (Alarcón and Cavieres, 2015), covering an area of 28.576 km^2. All raster data had a resolution of 30 arc-second.

2.3. Building the adaptive divergence model

The spatially explicit distance-based adaptive divergence model was fitted prior to a population structure analysis and detection of potentially adaptive loci, detailed below.

2.3.1. Population structure

To evaluate the population structure, the genetic ancestry coefficients and the number of ancestral populations (*K*), the sNMF

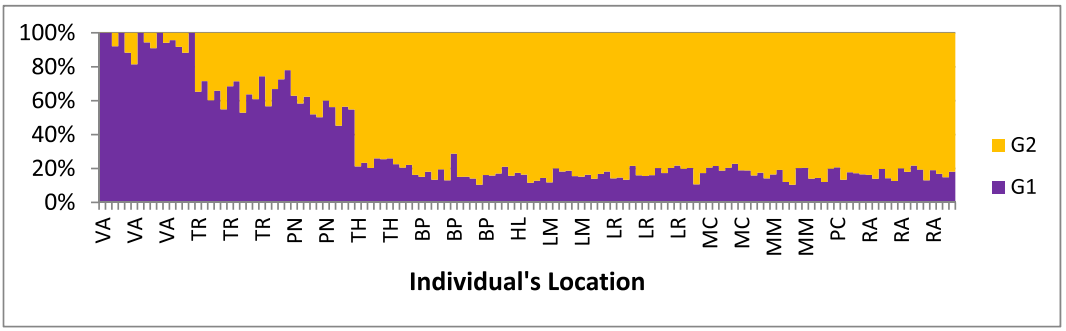

Fig. 2. Population memberships ($K = 2$) of the 134 individuals of *A. Araucana*, based on sNMF algorithm. Vertical bars represent the percentage of individual's genome that belonged to each of the two ancestral groups (G1 and G2). Note that individuals from the VA location represent a distinctive class of admixture, showing a high presence of G1. Individuals from TR and PN constituted a second class of admixture, with a relatively balanced presence of G1 and G2, and the remaining individuals from Andes Mountain Range made up a third admixture class, with a low presence of G1.

algorithm (scattered non-negative matrix factorization algorithm (Frichot et al., 2014)) was used as in the *LEA* R-package (Frichot and François, 2015). This approach provides estimates of ancestry coefficients through least squares and incorporates a cross-entropy criterion to evaluate the model fit. *K* values from 1 to 5 were tested with 20 repetitions, selecting the optimal *K* value as the one corresponding to the run showing the lowest cross entropy.

2.3.2. Identification of potentially adaptive loci

Potentially adaptive loci were identified following a consensus approach between two environmental association analysis methods (EAAs). The first one was a latent factor mixed model (LFMM), which identifies loci showing significant correlations with environmental gradients, while controlling for neutral population structure by integrating *K* as a random component (Frichot et al., 2013). This model was fitted using the *lfmm_ridge* function in the *lfmm2* R-package (Caye et al., 2019). As the number of latent factors, *K,* was considered as the optimal number of ancestral populations identified through sNMF. The second EAA was the redundancy analysis (RDA), a multivariate constrained ordination technique, frequently used in landscape genomic analyses (Hecht et al., 2015; Capblancq et al., 2018; Shryock et al., 2020). RDA is an extension of multiple regression modelling to multivariate response data (Legendre and Legendre, 2012), which has been shown to be more effective in detecting potentially adaptive loci than other techniques (Capblancq et al., 2018; Forester et al., 2018). To perform RDA, a biallelic genotypic matrix was used as the response matrix Y, and a set of environmental (climate and soil) variables was used as the predictor matrix X. After this constrained ordination step, we employed the methodology implemented in the *pcadapt* R-package to find outlier loci (Luu et al., 2017), based on the Mahalanobis distance. For both methods (LFMM and RDA), a false discovery rate of 5% was adopted.

2.3.3. Fitting adaptive divergence model

To model adaptive divergence, a generalized dissimilarity model (GDM) (Ferrier et al., 2007) was fitted at the individual level, using all of 134 individuals described in Section 2.1. GDM is a non-linear distance-based approach that models genotype-environmental associations and adapts to a variable rate of change in allele frequencies along environmental gradients. For accomplishing this, we used the *gdm* R-package (Fitzpatrick et al., 2021), adopting 6 knots per spline for all predictors. As response variable, a distance matrix containing pairwise Jaccard's dissimilarity index among individuals was used, which was obtained by applying the *vegdist* function of the *vegan* R-package (Oksanen et al., 2020) on the biallelic matrix containing loci putatively under selection. As predictors, the set of environmental variables retained after the backward elimination process, in addition to the geographic distance, were used. Once the model was fitted, and in order to identify specific zones where genetic offset could be higher, we first created a continuous adaptive divergence map (Fig. S4) obtained according to Fitzpatrick and Keller (2015). Then, we grouped this continuous raster into six adaptive homogeneous zones by using the *clara* function of the *cluster* R-package (Maechler et al., 2021). These zones (Fig. 4) represent areas with similar adaptive allelic compositions and were used as spatial units of genetic offset quantification.

2.4. Distance-based redundancy analysis

For better understanding the structure of whole and adaptive genomic variation, we assessed the degree to which both are influenced by environmental variables, geographic distance and genetic ancestry. To achieve this goal, we performed a distance-based redundancy analysis (db-RDA) (Legendre and Anderson, 1999) at the individual level using both the adaptive and the full set of SNPs. To do this, we use the function *varpart* function of the *vegan* R-package (Oksanen et al., 2020), which partitioned the variation of genomic dissimilarities in components explained by three explanatory tables (environment, ancestry and geography) and their combinations. To evaluate the significance of each partition, functions *capscale* and *anova* from *vegan* R package were applied running 999 permutations. For assessing the effect of geographic distance, we utilized a principal coordinates of neighborhood matrices (PCNMs), also known as Moran's eigenvector maps (MEM). PCNMs are a set of orthonormal variables calculated through eigenvalue decomposition of a spatial weighting matrix based on geographic coordinates (Legendre and Legendre, 2012). PCNMs were calculated by using the *pcnm* function of the *vegan* R-package. The environmental matrix employed for db-RDA was the same as used for selective loci detection, which was scaled and centred.

2.5. Genetic offset predictions

We performed the genetic offset predictions by using the *predict.gdm* function of the *gdm* R-package (Fitzpatrick et al., 2021), which directly calculates the adaptive distance among two environmental raster stacks (present and future), and outputs a single raster layer containing genetic offset values. We assumed soil characteristics were invariable over time, so they had no influence on the genetic offset prediction. Given that two RCPs were evaluated in 2 different periods, the result was a set of four genetic offset maps. Each of these four rasters was overlaid onto the

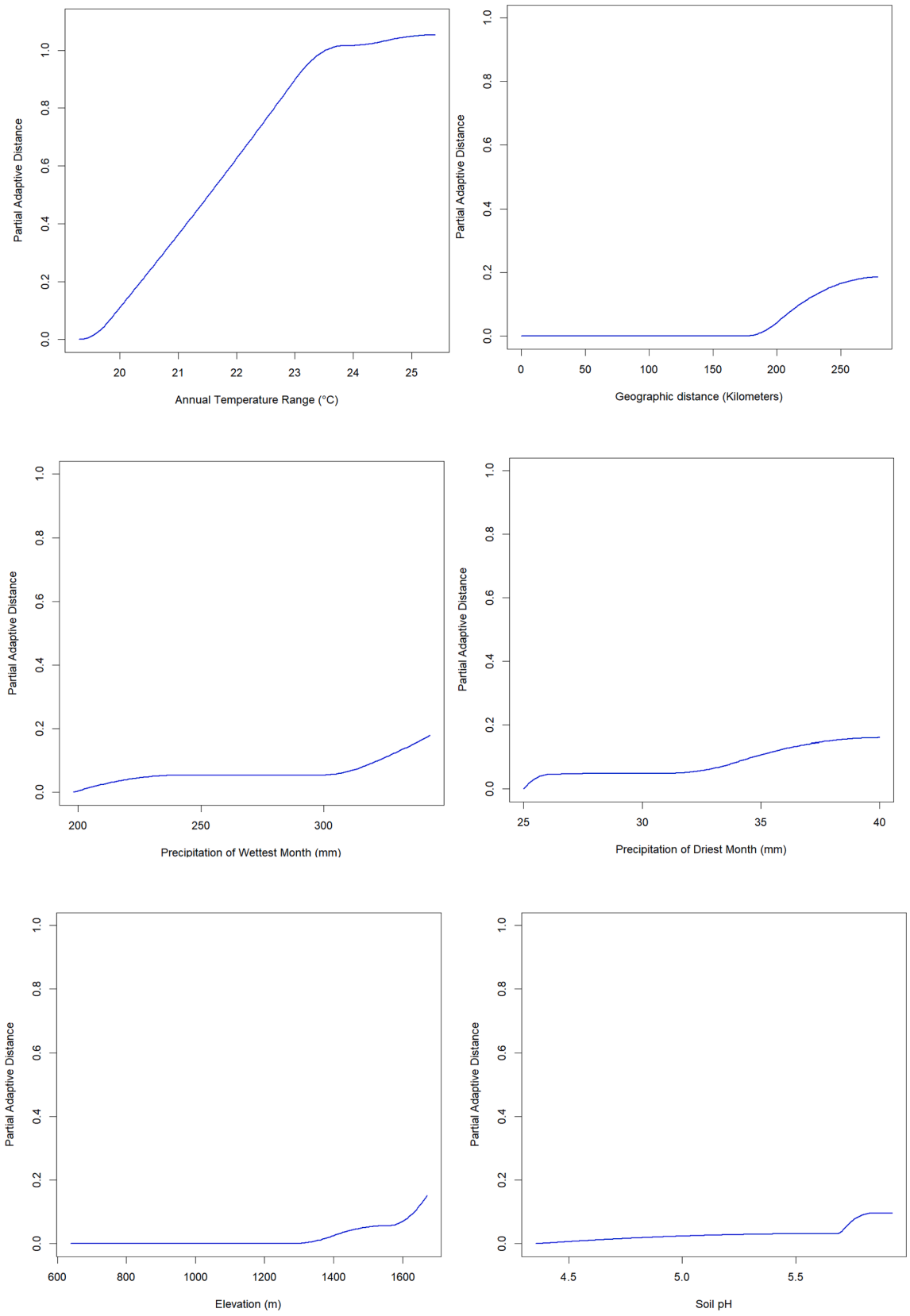

Fig. 3. GDM *I-splines* for each environmental predictor and geographic distance. The maximum height of each curve indicates the total amount of change in allele frequencies associated with that predictor (variable importance). The shape of each curve indicates how the rate of change in allele frequencies varies along the predictor gradient.

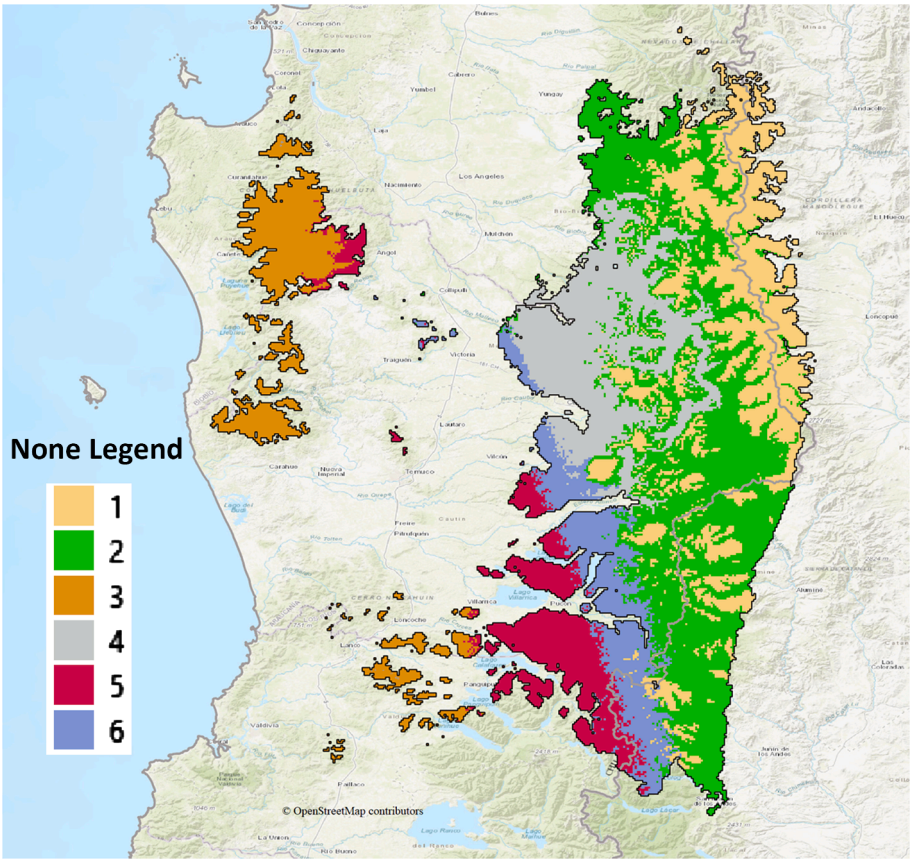

Fig. 4. Map showing six zones of similar adaptive allelic compositions for *A. araucana*, built with the *K-means* clustering algorithm applied to GDM transformed predictor's data frame. These zones were used as genetic offset quantification units.

adaptive raster zones described earlier, and a pixel-level data frame containing genetic offset, area in km^2 and adaptive zone number was assembled for summarize the results.

3. Results

3.1. Adaptive divergence model

3.1.1. Population structure

The sNMF algorithm showed a minimum cross entropy value with the parameter $K = 2$, which was used as a surrogate for the number of ancestral populations in the subsequent analyses. The ancestry coefficients revealed a clear differentiation between the populations in the Andes and the Costa Mountain Ranges (Fig. 2). Regarding ancestral admixtures, the Andes populations exhibited homogeneous levels of admixture, close to 20% of cluster G1 and 80% of cluster G2. In contrast, coastal populations showed heterogeneous levels of admixture. While individuals from locations PN and TR accounted for about 60% of cluster G1 and 40% of cluster G2, individuals from VA exhibited admixture levels about to 90% of cluster G1 and 10% of cluster G2.

3.1.2. Potentially adaptive loci

After EAAs modelling, the LFMM detected 1,113 loci as potentially adaptive. The predictor that returned the highest number of adaptive loci was temperature annual range, followed by soil pH and cationic exchange capacity. On the other hand, RDA returned 2,760 loci as potentially under selection. Observing the projection of each SNP in the RDA space, we were able to detect associations between adaptive SNPs and environmental predictors (Fig. S3). The RDA1 axis, which explained almost 50% of the variation, was strongly correlated with the temperature annual range, cationic exchange capacity and elevation, while the RDA 2 axis, which explained a minor amount of variation (9.7%), was mainly associated with the annual mean temperature and precipitation seasonality. Finally, 896 loci were detected as potentially adaptive by both methods.

Table 2
Distance-based redundancy analysis (db-RDA). The amount of variance explained by each component or its combination is expressed as adjusted R^2, in addition to its significance level. **Note:** F: Dependent matrix of individual genetic distances; the db-RDA tests are in the form of independent matrices F ~ | covariate matrices. env.: environment; geo.: geographic distance; anc.: ancestry. Total Explained: Adjusted R^2 total of individual fractions. Abbreviation: ns: not significant. * p < .05; ** p < .01; *** p < .001.

	All SNPs			Adaptive SNPs		
Combined Fractions	**Adj. R^2**	**P(>F)**		**Adj. R^2**	**P(>F)**	
F ~ env.	0.12379	0.001	***	0.50592	0.001	***
F ~ geo.	0.12396	0.001	***	0.50247	0.001	***
F ~ anc.	0.09680	0.001	***	0.43595	0.001	***
Individual Fractions						
F ~ env. | (geo. + anc.)	0.00735	0.001	***	0.00838	0.001	***
F ~ geo. | (env. + anc.)	0.00853	0.001	***	0.00953	0.002	**
F ~ anc. | (env. + geo.)	0.00062	0.137	ns	0.00000	0.466	ns
F ~ env. + geo. | anc.	0.02129			0.06163		
F ~ geo. + anc. | env.	0.00103			0.00003		
F ~ env. + anc. | geo.	0.00203			0.00464		
F ~ env. + anc. + geo	0.09312			0.43128		
Total explained	**0.13397**			**0.51549**		
Total unexplained	**0.86603**			**0.48451**		
Total	**1.00000**			**1.00000**		

3.1.3. Adaptive divergence model (GDM) fit

The GDM explained 85.1% of the variation in individual adaptive

Table 3
Genetic offset values for each adaptive zone, according to RCP and year of projection. Values correspond to area-weighted average of each of 6 adaptive zones overlaid onto 4 genetic offset raster (2 RCPs and 2 Years). Genetic offset values correspond to individual adaptive genomic distance computed as Jaccard index.

		Year	
RCP	**ZONE**	**2050**	**2070**
	1	0.51	0.51
	2	0.53	0.53
	3	0.61	0.55
4.5	4	0.57	0.57
	5	0.66	0.64
	6	0.61	0.61
	SubTotal	**0.56**	**0.55**
	1	0.51	0.51
	2	0.53	0.54
	3	0.60	0.65
8.5	4	0.59	0.61
	5	0.67	0.70
	6	0.62	0.64
	SubTotal	**0.57**	**0.58**
	Total	**0.56**	**0.57**

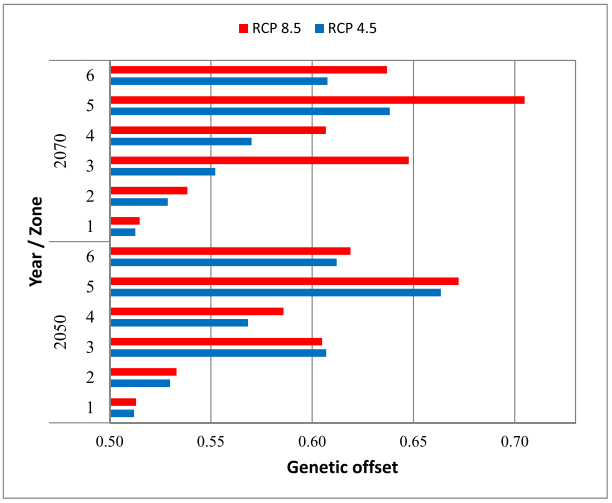

Fig. 5. Genetic offset predictions per year and adaptive zone, for each RCP. Bars represent an area-weighted average of each of 6 adaptive zones overlaid onto 4 genetic offset raster (2 RCPs and 2 Years). Genetic offset values (axis x) correspond to individual adaptive genomic distance computed as Jaccard index.

genetic distance. The maximum height of each *I-Spline* (Fig. 3) indicates the total amount of change in allele frequencies associated with that predictor (variable importance). Temperature annual range proved to be the most important predictor, followed by geographic distance, precipitation of the wettest month, precipitation of the driest month, elevation and soil pH. Regarding the shape of the *I-Splines* (Fig. 3), the temperature annual range exhibited a linear pattern between 19 and 23 °C, beyond which it showed a sharp decrease in slope, suggesting a plateau behaviour after this threshold. Geographic distance showed a different pattern: between 0 and 160 km. its importance was reported null, but over 160 km. its importance increased steadily. Adaptive zones map showed a differentiation among Andes and Costa Mountain ranges (Fig. 4). While almost the entirety of zone 3 is located in the Costa Mountain range and central valley, the remaining zones were located in the Andes Mountain Range, following a pattern of diagonal bands oriented from the northeast to the southwest. This suggests that environmental drivers that are adaptively significant have a wider range in the Andes Mountain range than the Costa Mountain range.

3.2. Distance based redundancy analysis

The results of the db-RDA, using both adaptive (896) and full set of SNPs (18,889), revealed a high degree of environmental adaptation (Table 2) in *A. araucana*, showing a great amount of total variance explained by adaptive SNPs (51.5%), regarding all SNPs (13.4%). Concerning the combined fractions in the full set of SNPs, environment and geography explained 12.4% of the total variance, while genetic ancestry explained only 9.7%. For the adaptive SNPs, environment and geography explained a much higher amount (50.6% and 50.2%, respectively), while the lowest value was returned by genetic ancestry (43.6%). Regarding individual fractions (i.e., the variation explained exclusively by a factor, excluding combined effects), the total amount of variance explained was low, with the greatest proportion being confounded among the three factors. As in the combined fractions, genetic ancestry explained the lowest amounts of variance in all SNPs, and in adaptive SNPs (0.06 and 0.00% respectively), while geographic distance explained the highest amounts, 0.85 and 0.95%, respectively.

3.3. Genetic offset

Predicted mean genetic offset was 0.56 for 2050 and 0.57 for 2070 (Table 3). The RCP8.5–2070 scenario showed the highest genetic offset (0.58), while RCP4.5–2070 exhibited the lowest risk of maladaptation (0.55) (Fig. 5). Zone 5, corresponding to the piedmonts of the Andes and the southern border of the range of *A. araucana* (Fig. 6), showed it will be most affected by genetic offset in both 2050 and 2070 in RCP4.5 (0.66 and 0.64, respectively), similar to RCP 8.5 (0.67 and 0.70, respectively). High elevation zones in the Andes Mountain range (Zone 1) appeared to be at the lowest risk (0.51 for both years and RCPs). Zone 3, containing mainly Coastal Mountain range populations, presented an intermediate risk level. It can also be observed that the Bío-Bío river basin is a zone of higher genetic offset than the surrounding areas (Fig. 6).

4. Discussion

This study confirms the presence of adaptive genetic variation in the genome of *A. araucana*, which was highly correlated with environmental variation. Based on our adaptive divergence model (GDM), we predicted that populations living in the piedmonts of southern Andean distribution will be most affected by maladaptation due to future climatic conditions and such populations should be prioritized for conservation. To the best of our knowledge, this is the first study dealing with genetic offset, not only for this emblematic species, but in the southern South American region, which may provide valuable information to ecological restoration, assist gene flow and assisted migration programs.

4.1. Population structure

Our population structure analysis revealed two ancestral clusters in sampled populations ($K = 2$), both depicting a disjoint distribution of genetic groups between Costa and Andes Mountains ranges (Fig. 2). This pattern is concurrent with biogeographic studies which propose an important role of glaciation cycles shaping current species distribution and genetic structure of Patagonian temperate forests. Long glacial periods of areal expansion alternated with shorter periods of retraction and isolation during the interglacial warmer phases would have produced the present disjoint distribution of *A. araucana* populations (Villagrán, 2018). According to pollen and charcoal records, colonization route could have been from glacial refuges located in the Costa Mountain range toward the Andes Mountain range (Sersic et al., 2011; Nanavati et al., 2020).

4.2. Local adaptation

We found a high degree of local adaptation in *A. Araucana* whose

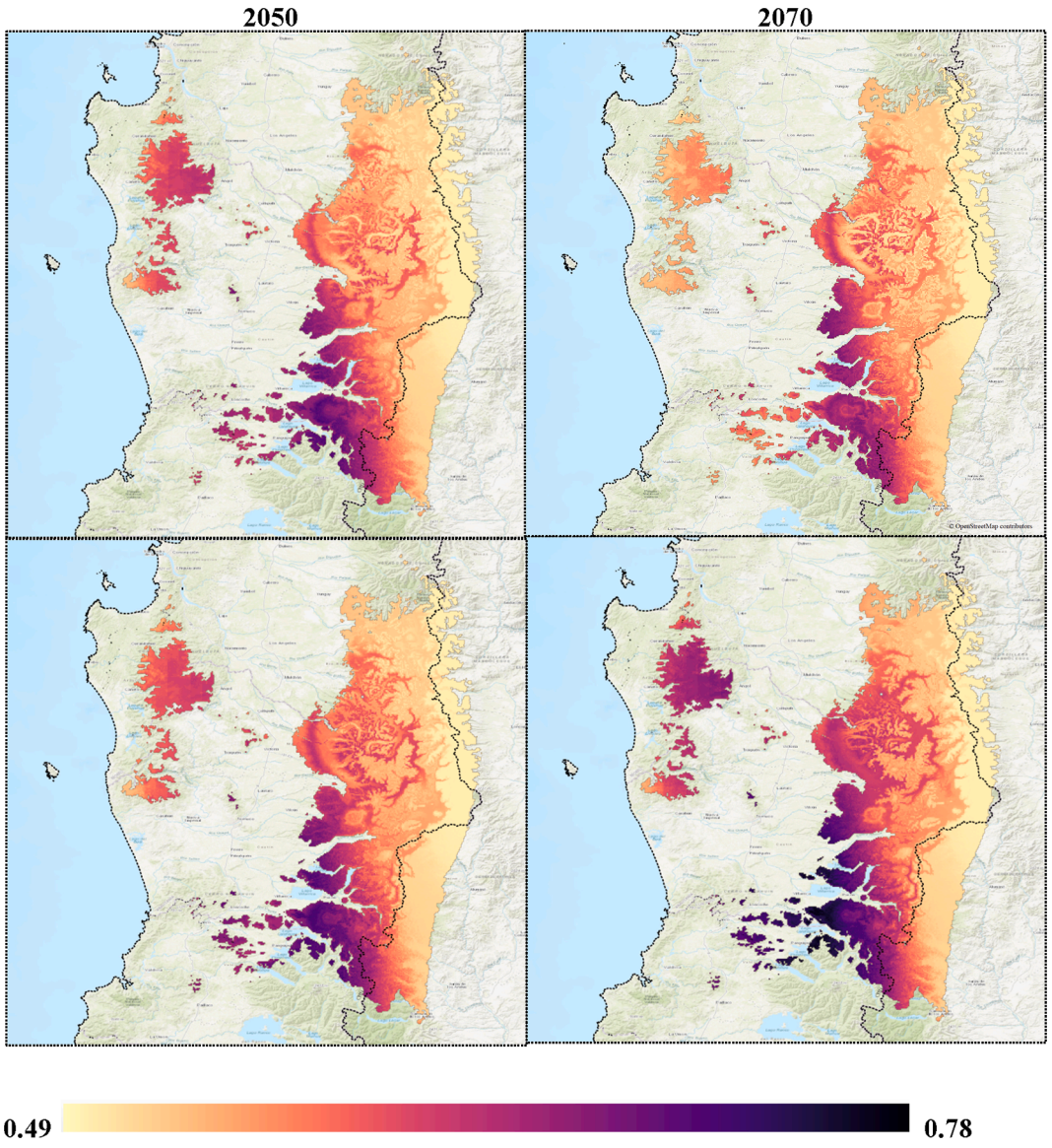

Fig. 6. Continuous genetic offset maps of *A. araucana* for the years 2050 and 2070 under the RCP4.5 (up) and RCP8.5 (down) scenarios. Black and yellow represent high and low offset levels, respectively.

main environmental driver was temperature annual range. Coincidentally, this environmental driver has been also detected as most important in a common garden trial study (McIntosh et al., Unpublished results) reinforcing the power of landscape genomic approach to detect and to model adaptive divergence. In addition, we detected limiting factors influencing adaptive genomic variation (i.e., precipitation of the wettest month and precipitation of the driest month) probably reflecting physiological trade-offs associated with an aridity gradient. This is not surprising, since water stress has formerly been shown to be an active driver of adaptation and possibly the most globally selective force acting on trees, causing mortality in vegetation from arid zones to the understory of tropical rainforests (Engelbrecht et al., 2007; Allen et al., 2010). More importantly, a global population decline has been documented in Araucariaceae species during the Cretaceous, which has been attributed to climatic drying and cooling (Kershaw and Wagstaff, 2001).

One key functional trait of this species is its remarkable isohydry, a physiological feature present principally in the basal clades of Gymnosperms (Pinaceae, Araucariaceae). Isohydric species reduces stomatal conductance rates as soil water potential decreases and atmospheric conditions dry, maintaining a strict control over the leaf water potential and closing stomata by steadily increasing abscisic acid (ABA) concentrations, showing a high sensibility to drought (Zimmer et al., 2016). Furthermore, according to Brodribb et al. (2014), tolerance to xylem failure due to water shortage in Araucariaceae was the least among conifer families. This costly and inefficient strategy to cope with water shortage stress could enhance intra-specific natural selection in abiotic gradients associated with drought in *A. araucana*. In a work that directly supports this hypothesis, Rafii and Dodd (1998), using foliar epicuticular wax alkanes as genetic markers, found longer chains of this wax in populations living in dryer habitats, consistent with a genetic adaptation to resist water loss in more arid conditions (Papú et al., 2021). In addition, Puchi et al. (2021) conducted a retrospective analysis for the 1800–2017 period, measuring tree ring growth, wood anatomical traits and $\delta^{18}O$ stable isotopes to assess dieback causes. They found that drought severity, expressed as SPEI (Standardized Precipitation-Evapotranspiration Index), was the main climatic driver of tree growth.

Regarding of local adaptation structure given by db-RDA analyses, we found that a large amount of the explained among-individual genetic variation co-varied with the effects of environment, geography and ancestry (Table 2, Fig. S5) and a small, yet significant, proportion of

genetic divergence was explained exclusively by environmental conditions in both full and adaptive sets of SNPs. Such confounding effects of local adaptation with neutral processes are frequent and not mutually excluding in natural landscapes (Engelbrecht et al., 2007). Two main drivers could explain such confounding effects: a) the presence of selective gradients spatially correlated with demographic history (e.g., postglacial colonization along climatic gradients); and b) the simultaneous action of natural adaptive and neutral processes shaping genetic variation and gene flow among populations. The presence of both drivers is supported by our genetic structure analysis, which showed different levels of ancestral admixtures in three ecologically divergent and distant areas.

4.3. Genetic offset and conservation strategy

Our results predict a high risk of maladaptation in the whole range of *A. araucana*, particularly in southern Andean piedmonts, which means that the amount of change in adaptive allele frequencies for preserving the observed genetic–environmental association will be high. Therefore, we propose that these populations be prioritized for conservation. In contrast, high elevations zones in Andes Mountain range will be the least affected by climate change. This could be interpreted as the continuation of demographic processes described in point 4.1: while the environment warms up, the cooler highlands become refuges for this species. However, in this anthropogenic era this process is happening at a much faster rate.

Unfortunately, longevity of this species slows down the rate of emergence and spread of new adaptive alleles in populations through *de novo* mutations, making this species particularly vulnerable to rapid climate change (Fajardo et al., 2019). To cope this, we propose an assisted gene flow conservation strategy to help *A. araucana* adapt to novel environments. Assisted gene flow is a controlled migration of individuals or gametes among populations within the species range, which can be effective in accelerating adaptation to future climate conditions (Aitken and Whitlock, 2013). We suggest adopting a predictive seed sourcing strategy, mixing local with pre-adapted non-local seed lots to increase diversity and resilience. Candidate donor populations could be prospected by using an adaptive divergence model like the one proposed in this study, finding areas where current environmental conditions are similar to the future conditions in the recipient population. In addition, common garden experiments must be simultaneously conducted to test donor genotypes before implementing operational conservation programs, in order to evaluate key traits and phenotypic plasticity.

Assisted gene flow have unintended risks. One of these is the outbreeding depression, which consists of a reduction in the reproductive fitness in the first or subsequent generations following attempted crossings of genetically distant populations (Frankham et al., 2011). This risk can, however, be empirically estimated and could therefore be manageable.

4.4. Methodological challenges

There is a consensus in the fact that loci under selection and maladaptation predictions by landscape genomic approach must be functionally validated in common garden and reciprocal transplant experiments (Li et al., 2017). According to this, we adopted conservative controls for putatively adaptive loci identification in all stages of this analysis. First, we reduced the initial dataset to a set containing only one SNP per fragment, minimizing the risk of linkage among markers. Then, we applied a consensus approach for two GEA analyses (LFMM and RDA), which required the loci to be identified by both genome scan methods, minimizing type I errors. This led us to exclude more than 1,800 loci identified by the RDA. As a result, we retained 4.7% of the full set of SNPs as potentially adaptive; less than the 8.9 % reported by Strasburg et al. (Strasburg et al., 2012). Therefore, our findings were based on a high confidence adaptive genotypic matrix. In the second stage, to unravel the structure of local adaptation, we adopted two approaches. First, a nonlinear distance-based model was fitted (GDM), and second, a distance-based redundancy analysis (db-RDA) was performed on both full and adaptive sets of SNPs.

The variation explained by the db-RDA was lower than that explained by the GDM, possibly due to the linear nature of db-RDA, which weakens its power to reproduce non-linear patterns of allelic turnover along environmental gradients. Despite this, both approaches were equally able to detect the most important predictor (Temperature annual range). We believe that the development of novel statistical tools that combine non-linear approaches with ordination techniques could aid in disentangling the evolutionary forces driving adaptive divergence. Finally, a major challenge is to build a reference genome for *A. Araucana* to map adaptive loci for functional trait studies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

AVM and RH planned and designed the research; ECN designed sampling, collected tissue samples and executed the RAD-Seq preparation; OTN, RH and FSE performed *ipyrad* analyses for provide SNPs data; DA provided environmental data and habitat suitability model; AVM perform statistical analyses, wrote R scripts and wrote mainly the manuscript; all authors contributed to the writing of the manuscript; AF edited and improved the manuscript; all authors approved the final manuscript.

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